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An axonemal intron splicing program sustains *Plasmodium* male development

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Jiepeng Guan^{1,4}, Peijia Wu^{1,4}, Xiaoli Mo^{1,4}, Xiaolong Zhang^{2,4}, Wenqi Liang¹, Xiaoming Zhang¹, Lubin Jiang² , Jian Li [®] ¹ , Huiting Cui ¹ & Jing Yuan [®] ^{1,3}

Differentiation of male gametocytes into flagellated fertile male gametes relies on the assembly of axoneme, a major component of male development for mosquito transmission of the malaria parasite. RNA-binding protein (RBP)mediated post-transcriptional regulation of mRNA plays important roles in eukaryotic sexual development, including the development of female *Plas*modium. However, the role of RBP in defining the Plasmodium male transcriptome and its function in male gametogenesis remains incompletely understood. Here, we performed genome-wide screening for gender-specific RBPs and identified an undescribed male-specific RBP gene *Rbpm1* in the *Plasmodium.* RBPm1 is localized in the nucleus of male gametocytes. RBPm1deficient parasites fail to assemble the axoneme for male gametogenesis and thus mosquito transmission. RBPm1 interacts with the spliceosome E complex and regulates the splicing initiation of certain introns in a group of 26 axonemal genes. RBPm1 deficiency results in intron retention and protein loss of these axonemal genes. Intron deletion restores axonemal protein expression and partially rectifies axonemal defects in RBPm1-null gametocytes. Further splicing assays in both reporter and endogenous genes exhibit stringent recognition of the axonemal introns by RBPm1. The splicing activator RBPm1 and its target introns constitute an axonemal intron splicing program in the post-transcriptional regulation essential for *Plasmodium* male development.

Malaria is a worldwide infectious disease caused by the protozoan parasite *Plasmodium*¹. The spread of *Plasmodium* depends on the transition between the mammal host and the *Anopheles* mosquito. In mammal hosts, a small proportion of intraerythrocytic asexual parasites undergo sexual development, irreversibly differentiating into the sexual precursor gametocytes, which are transmission-competent for the mosquito vector². Within 10 min after being ingested into the mosquito midgut, the gametocytes escape from host erythrocytes and develop into fertile gametes, a process known as gametogenesis³. A flagellated motile male gamete fertilizes with a female gamete to form a zygote. After the zygote-ookinete-oocyst-sporozoite development in

mosquitoes, the parasites are finally injected from the salivary gland into a mammal host, completing the transmission of the malaria parasite⁴.

Sexual development plays a central role in malaria transmission^{5,6}. When activated by two joint environmental stimuli (a temperature drop⁷ and a metabolite xanthurenic acid⁸) in the mosquito midgut, a female gametocyte produces a haploid gamete, while a male gametocyte gives rise to 8 haploid gametes³. Female gametogenesis undergoes minor morphological changes, while male gametogenesis involves fast and spectacular changes^{9,10}. During the male gametogenesis, two spatially distinct components are coordinated. One is the

¹State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Faculty of Medicine and Life Sciences, Xiamen University, Xiamen, China. ²Shanghai Institute of Immunity and Infection, Chinese Academy of Sciences, Shanghai, China. ³Department of Infectious Disease, Xiang'an Hospital of Xiamen University, School of Medicine, Faculty of Medicine and Life Sciences, Xiamen University, Xiamen, China. ⁴These authors contributed equally: Jiepeng Guan, Peijia Wu, Xiaoli Mo, Xiaolong Zhang. e-mail: lbjiang@siii.cas.cn; jianli_204@xmu.edu.cn; cuihuiting@xmu.edu.cn; yuanjing@xmu.edu.cn

cytoplasmic assembly of 8 basal bodies and axonemes, and the other is the 3 successive rounds of genome replication without nuclear division, resulting in an octoploid nucleus. Subsequently, 8 axonemes with chromosomes attached are released from the cell body of male gametocytes, resulting in 8 flagellated daughter gametes, which is the process termed "exflagellation".

Parasite stage transition during the *Plasmodium* life cycle requires a fine-tuned multilayer regulation of gene expression¹¹⁻¹³. Previous studies have identified transcriptional and epigenetic programs critical for the sexual commitment and development of the gametocytes¹⁴⁻²³. However, how the Plasmodium establishes distinct repertoires of transcripts between male and female gametocytes remains incompletely illustrated. RNA-binding proteins (RBPs) can interact with transcripts in all manner of RNA-driven processes²⁴. RBPs regulate all aspects of the life cycle of mRNA, including mRNA transcription, splicing, modification, trafficking, translation, and decay^{25,26}. RBPcontaining ribonucleoprotein complexes, such as the DOZI (development of zygote inhibited) complex and CITH (CARI/Trailer Hitch homolog) complex, had been shown to repress the translation of multiple mRNAs in female gametocytes²⁷⁻²⁹. So far, our understanding of post-transcription control is still limited in the male gametogenesis. Recent transcriptome studies in both human malaria parasite P. falciparum and mouse malaria parasite P. berghei revealed that certain RBPs are specifically or preferentially expressed in male gametocytes30,31, implying gender-specific roles of RBPs in the posttranscriptional regulation for male development. However, systematic identification of male RBPs for male gametogenesis and their precise roles in defining the gender distinct transcriptome via the posttranscription regulation have not been reported.

In this work, we perform comparative transcriptome analysis on male and female gametocytes and obtain a list of gender-specific RBPs in the rodent malaria parasite *P. yoelii*. From this list, we identify a functionally unknown gene (PYI7X_0716700, named as *Rbpm1* in this study), which is specifically transcribed in male gametocytes. We demonstrate that RBPm1 is a nuclear RBP essential for male gametogenesis and mosquito transmission of parasite. RBPm1 interacts with the spliceosome E complex and initiates the splicing of certain introns in a group of axonemal genes. RBPm1-deficient parasites cannot express these axonemal proteins and fail to assemble the axoneme. These findings reveal an RBPm1-mediated intron splicing program of the axonemal genes essential for *Plasmodium* male development.

Results

RNA-binding protein RBPm1 is expressed in the nucleus of male gametocytes

Approximately 180 putative Plasmodium RBPs had been predicted in silico³². To identify the key RBPs for male gametogenesis, we searched the male gametocyte-specific RBPs in the rodent malaria parasite P. yoelii. Using the fluorescence-activated cell sorting, highly purified male and female gametocytes were collected from a P. yoelii reporter line DFsc7 (Fig. 1A and Supplementary Fig. 1A), in which fluorescent proteins GFP and mCherry are expressed in male and female gametocytes, respectively33. We performed RNA-seq and obtained genderspecific gametocyte transcriptome (Supplementary Fig. 1B and Supplementary Data 1). Among the 179 P. yoelii RBPs, an unstudied gene (PY17X_0716700) was identified with the greatest enrichment in male compared to female gametocytes (Fig. 1B, left panel). This gene was named as Rbpm1 for RBP in male gametocyte. Notably, the Rbpm1 orthologs PBANKA 0716500 and PF3D7 0414500 are also among the top male RBP genes of P. berghei and P. falciparum, respectively, (Fig. 1B, middle and right panels) based on the gender gametocyte transcriptomes30,31.

To investigate RBPm1 expression during the parasite life cycle, we tagged endogenous RBPm1 with a sextuple HA (6HA) at the carboxyl (C)-terminus in the *P. yoelii* 17XNL strain (wild type or WT) using CRISPR-

Cas9^{34,35}. The tagged parasite *Rbpm1::6HA* developed normally in mice and mosquitoes, indicating no detectable detrimental effect of tagging on protein function. Immunofluorescent assay (IFA) showed that RBPm1 was expressed only in gametocytes, but not in asexual blood stages, ookinetes, oocysts, or sporozoites (Fig. 1C, upper panel). Immunoblot also confirmed the gametocyte-restricted expression of RBPm1 (Fig. 1D). Gametocyte-specific expression of RBPm1 was observed in another parasite line Rbpm1::gfp, in which RBPm1 was tagged with GFP from the 17XNL (Fig. 1C, lower panel). To dissect whether RBPm1 expression is male-specific, the Rbpm1::6HA gametocytes were co-stained with antibodies against HA and α-Tubulin II (a highly expressed protein in male gametocytes³⁶. RBPm1 was only detectable in the male gametocytes (Fig. 1E). Additionally, we tagged RBPm1 with 6HA in the reporter line DFsc7 and observed the male-specific expression of RBPm1 (Fig. 1F). We noticed the nuclear localization of RBPm1 in all the male gametocytes tested (Fig. 1C, E, F), which was further confirmed by immunoblot of nuclear and cytoplasmic fractions from the Rbpm1::6HA gametocytes (Fig. 1G). Last, we analyzed the localization dynamics of RBPm1 throughout the process of gametogenesis (0, 2, 8, and 15 min post activation, mpa) in the Rbpm1::6HA parasites. Both IFA and immunoblot revealed consistent protein expression profile and nuclear localization of RBPm1 during gametogenesis (Fig. 1H, I). Together, these results demonstrated that RBPm1 was a nuclear protein specifically expressed in the male gametocytes.

RBPm1 is essential for male gametogenesis and mosquito transmission of parasite

P. yoelii Rbpm1 gene encodes a protein of 361 amino acid (aa) residues, with two RNA recognition motifs (RRM1 and RRM2). To investigate its function, we generated a mutant line, $\Delta Rbpm1$, by deleting the entire genomic sequence (1904 bp) of Rbpm1 gene in P. yoelii 17XNL strain using CRISPR-Cas9 (Fig. 2A). ΔRbpm1 produced normal level of male and female gametocytes in mice (Fig. 2B), indicating that RBPm1 is not essential for asexual blood stage proliferation and gametocyte formation. We next measured the male gametogenesis by counting exflagellation centers (ECs) in vitro after stimulation with 50 µM xanthurenic acid (XA) at 22 °C. ΔRbpm1 showed a striking deficiency in the EC formation (Fig. 2C, D) and male gamete release (Fig. 2E). In contrast, RBPm1 disruption had no impact on female gamete formation in vitro (Fig. 2F), which corresponded with no RBPm1 expression in female. △Rbpm1 produced no ookinetes in vitro (Fig. 2G) or midgut oocysts and salivary gland sporozoites in the infected mosquitoes (Fig. 2H, I), indicating transmission failure in mosquito. Additionally, we deleted each of the RNA recognition motifs RRM1 (119-190 aa) and RRM2 (203-274 aa) of endogenous RBPm1 in the 17XNL (Fig. 2A). Both mutants, $\Delta rrm1$ and $\Delta rrm2$, displayed similar defects as those observed in ΔRbpm1 (Fig. 2B, D), suggesting essential role of both two RNA recognition motifs in RBPm1 function.

To further confirm that the ΔRbpm1 phenotype was caused by Rbpm1 deficiency, we introduced a sequence consisting of the coding region of Rbpm1 and a N-terminal quadruple Myc epitope (4Myc) back to the Rbpm1 locus in the ΔRbpm1 line, generating the complemented line, referred to as rescue (Fig. 2A). The 4Myc-tagged RBPm1 was detected in the rescue gametocytes (Fig. 2J) and localized in the nucleus of male gametocytes (Fig. 2K). The rescue parasites restored the formation of ECs (Fig. 2C, D), male gametes (Fig. 2E), ookinetes (Fig. 2G), midgut oocysts (Fig. 2H), and salivary gland sporozoites (Fig. 2I).

Lastly, we performed genetic crosses between $\Delta Rbpm1$ mutant and the male-deficient line $\Delta map2$ or the female-deficient line $\Delta mek4$. As expected, the cross between $\Delta map2$ and $\Delta nek4$ produced the ookinetes in vitro (Fig. 2L). The ookinete formation was restored in the $\Delta Rbpm1$ parasites that were crossed with $\Delta nek4$ but not $\Delta map2$, further confirming the defective male gamete formation in the $\Delta Rbpm1$. Together, these results demonstrated that RBPm1 is essential for male gametogenesis and mosquito transmission of parasites.

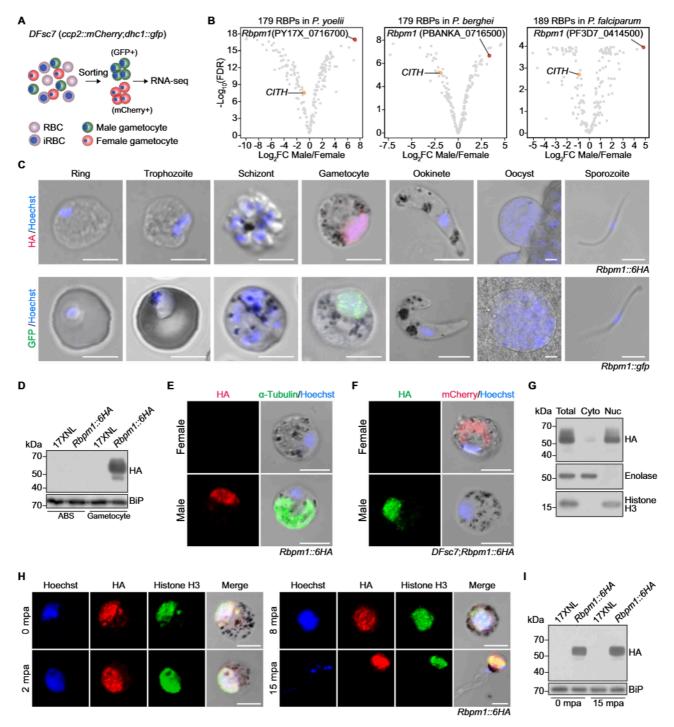


Fig. 1 | **RNA-binding protein RBPm1** is **expressed in the nucleus of male gametocytes. A** Flowchart showing the purification and transcriptome analysis of male (green, GFP+) and female (red, mCherry+) gametocytes from a *P. yoelii* parasite reporter line *DFsc7*. **B** Gender analysis of gene transcription for the *Plasmodium* genome-wide putative RBPs between male and female gametocytes. The top male gene PY17X_0716700, *RBPm1*, is marked in red. CITH (orange dot) is a known female RBP. The results of *P. berghei* (middle panel) and *P. falciparum* (right panel) were based on the published gametocyte transcriptomes contributed by Yeoh, L.M. 2017 and Lasonder, E. 2016. The *p*-values were calculated by quasilikelihood F-test and adjusted by false discovery rate (FDR). **C** Stage expression of RBPm1 during the *P. yoelii* life cycle. Immunofluorescence assay (IFA) of RBPm1 expression in the *Rbpm1::GFP* protein in the *Rbpm1::gfp* parasites (bottom panel). Nuclei were stained with Hoechst 33342. Three independent experiments with similar results. Scale bars: 5 μm. **D** Immunoblot of RBPm1 in the asexual blood

stage (ABS) and gametocyte of the *Rbpm1::6HA* parasites. BiP as a loading control. Three independent experiments with similar results. E IFA of HA-tagged RBPm1 and α-Tubulin (male gametocyte marker protein) in *Rbpm1::6HA* gametocytes. Three independent experiments with similar results. Scale bars: 5 μm. F IFA of HA-tagged RBPm1 and mCherry (expressed in female gametocytes) in the *DFsc7;Rbpm1::6HA* gametocytes. Three independent experiments with similar results. Scale bars: 5 μm. G Immunoblot of RBPm1 in cytosolic and nuclear fractions of *Rbpm1::6HA* gametocytes. Enolase (cytoplasmic/Cyto) and histone H3 (nuclear/Nuc) proteins used as controls respectively. Two independent experiments with similar results. H IFA of HA-tagged RBPm1 and histone H3 during male gametogenesis of the *Rbpm1::6HA* parasites. mpa: minute post activation. Three independent experiments with similar results. Scale bars: 5 μm. I Immunoblot of RBPm1 expression in the *Rbpm1::6HA* parasites during male gametogenesis. Two independent experiments with similar results.

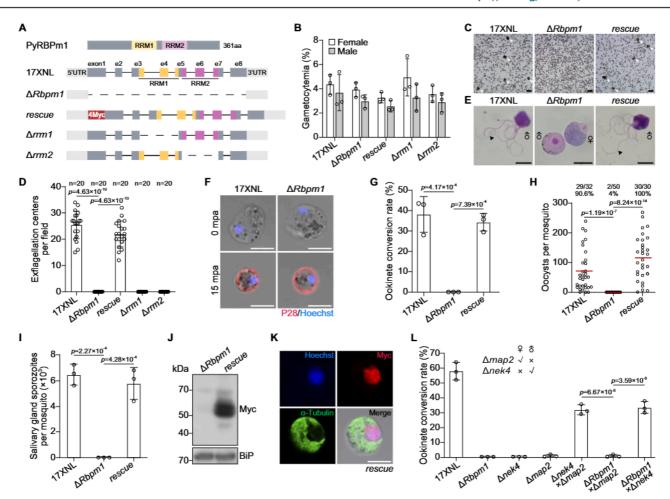


Fig. 2 | RBPm1 is essential for male gametogenesis and mosquito transmission of parasite. A A schematic showing genetic modification at the Rbpm1 locus in the P. yoelii parasite. The top panel depicts the protein structure of RBPm1 with two RNA recognition motifs RRM1 (residues 119-190, yellow) and RRM2 (residues 203-274, purple). ΔRbpm1, deletion of the whole coding sequence from the 17XNL (wild type) strain; rescue, the ΔRbpm1 line complemented with Rbpm1 fused with a 4Myc tag; Δrrm1 and Δrrm2, deletion each of RRM1 and RRM2 from the 17XNL. B Female and male gametocyte formation in mice for the modified parasite. Data are means ± SEM of three independent experiments. C Exflagellation center (EC) formation of activated male gametocytes at 10 mpa. Cell clusters representing the EC are marked with white arrows. Four independent experiments with similar results. Scale bars: 20 um. D Quantification of EC formation. The ECs were counted within a 1×1-mm square area in the hemocytometer under a light microscope. n represents the number of fields counted. Means ± SEM, one-way ANOVA with Tukey multiple pairwise-comparisons. Three independent experiments. E Light microscope images of the exflagellated male gametes (black arrow) after Giemsa staining. Four independent experiments with similar results. Scale bars: 5 µm. F Female gamete formation assayed by P28 staining. P28 is a female gamete plasma membrane protein. n = 32 and 35 female gametocytes in 17XNL and $\Delta Rbpm1$ respectively. Scale bars: 5 µm. G Ookinete formation in vitro. Data are means ± SEM from three

independent experiments, one-way ANOVA with Tukey multiple pairwisecomparisons. H Midgut oocyst formation in mosquitoes at 7 days after blood feeding. x/y at the top represents the number of mosquitoes containing oocysts/ the number of dissected mosquitoes, and the percentage represents the infection prevalence of mosquitoes. Red lines show the mean value of oocyst numbers, oneway ANOVA with Tukey multiple pairwise-comparisons. Three independent experiments with similar results. I Salivary gland sporozoite formation in mosquitoes at 14 days after blood feeding. At least 20 infected mosquitoes were counted in each group. Data are means ± SEM of three independent experiments, one-way ANOVA with Tukey multiple pairwise-comparisons. J Immunoblot analysis of RBPm1 expression in gametocytes of the complemented line rescue. BiP as a loading control. Two independent experiments with similar results, K IFA of Myc-tagged RBPm1 and α-Tubulin in gametocytes of the rescue parasite. Three independent experiments with similar results. Scale bars: 5 µm. L Gender gamete fertility assay of the \(\Delta Rbpm1 \) by parasite genetic cross. Fertility was determined by ookinete development of $\Delta \textit{Rbpm1}$ gametes after cross-fertilization with mutant lines that are defective in either female ($\Delta nek4$) or male ($\Delta map2$) gametes. Data are means \pm SEM of three independent experiments, one-way ANOVA with Tukey multiple pairwisecomparisons.

Defective axoneme assembly in RBPm1-deficient male gametogenesis

Next, we delineated more detailed defects of $\Delta Rbpm1$. During male gametogenesis, the parasites undergo axoneme assembly, genome replication, rupture of the parasitophorous vacuole membrane (PVM) and erythrocyte membrane (EM), and finally releasing eight uniflagellated male gametes. We first assessed the axoneme assembly. At 0 mpa, both α - and β -Tubulin were evenly distributed in the cytosol of male gametocytes of WT and $\Delta Rbpm1$ (Fig. 3A, upper panel). Immunoblot also detected comparable level for both Tubulins in gametocytes between WT and $\Delta Rbpm1$ (Fig. 3B). At 8 mpa, the axonemal

microtubules (MTs) were observed to be coiled around the enlarged nucleus in the WT gametocytes. However, aberrant axonemes were formed in $\Delta Rbpm1$ (Fig. 3A, middle panel). At 15 mpa, $\Delta Rbpm1$ failed to produce flagellated male gametes (Fig. 3A, lower panel). Under ultrastructure expansion microscopy (U-ExM)³⁷, the axonemes lost bundled structures at 8 mpa in $\Delta Rbpm1$ compared to the organized axonemes in WT (Fig. 3C). We used electron microscope to dissect the ultrastructural defects of axoneme in $\Delta Rbpm1$ male gametocytes at 8 mpa. The majority of axonemes (93%, 150 axonemes from 43 section images) displayed 9 + 2 arrangement of MTs in WT (Figs. 3D). In contrast, no intact axonemes (from 69 section images) were detected in either

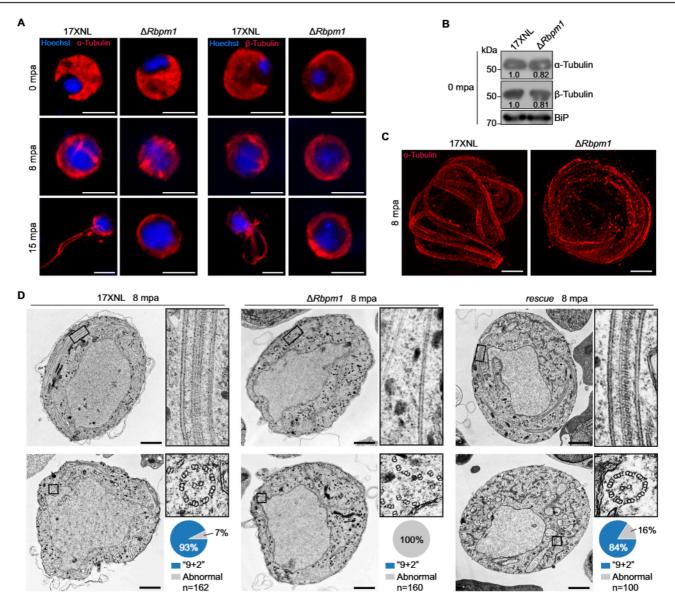


Fig. 3 | Defective axonemal assembly in RBPm1-null male gametogenesis. A Detection of formation and exflagellation of axonemes during male gametogenesis (0, 8, and 15 mpa) by staining α -Tubulin (left panels) and β -Tubulin (right panels). Nuclei were stained with Hoechst 33342. Four independent experiments with similar results. Scale bars: $5 \, \mu m$. B Immunoblot of α - and β -Tubulins in gametocytes. The numbers indicate the relative intensities of the bands in the immunoblots. BiP as a loading control. Two independent experiments with similar results. C Ultrastructure expansion microscopy (U-ExM) of the axonemes in male

gametocytes stained with α -Tubulin antibody at 8 mpa. Three independent experiments with similar results. Scale bars: 5 μ m. **D** Transmission electron microscopy of axoneme architecture in male gametocytes at 8 mpa. Inset panels show longitudinal sections (top panels) and cross sections (bottom panels) of axonemes. The enclosed area (black box) was zoomed in. Pie charts show the quantification of axoneme ("9 +2" microtubules) in the mutant parasites. n is the total number of intact and defective axoneme structures observed in each group. Three independent experiments with similar results. Scale bars: 1 μ m.

longitudinal or cross sections of $\Delta Rbpm1$ (Figs. 3D). All the axonemes in $\Delta Rbpm1$ showed severe defects with loss of either central singlet MTs or peripheral doublet MTs (Fig. 3D), consistent with the observation of Tubulin staining in Fig. 3C. In the complemented line *rescue*, the axoneme assembly restored to normal as in WT (Figs. 3D). These results demonstrated that RBPm1 is required for axoneme assembly during male gametogenesis.

We additionally analyzed genome replication and erythrocyte rupture during male gametogenesis. Flow cytometry analysis of male gametocytes at 8 mpa detected a comparable increase in DNA content in both parental *DFsc7* and its derivative mutant *DFsc7*;Δ*Rbpm1* parasites (Supplementary Fig. 2A). These results indicated normal genome replication in the absence of RBPm1, consistent with the enlarged nucleus observed in the activated Δ*Rbpm1* male gametocytes from both the fluorescence and electron microscope images (see Fig. 3A, D).

In addition, immunostaining of SEP1 (parasite PVM protein) and TER119 (mouse EM protein) showed that RBPm1 deficiency had no notable effect on parasite rupture from the gametocyte-residing erythrocytes (Supplementary Fig. 2B, C).

RBPm1 deficiency causes defective intron splicing of axonemal genes

To investigate the mechanism of RBPm1 in regulating the axoneme assembly, we performed RNA-seq to examine the changes in male transcriptome due to the loss of RBPm1 (Fig. 4A). To purify the RBPm1-null male gametocytes for comparison, we deleted *Rbpm1* in the *DFsc7* line. The mutant line *DFsc7*;Δ*Rbpm1* displayed the same phenotypes as Δ*Rbpm1* (Supplementary Fig. 3A–D). Purified male gametocytes of the *DFsc7*;Δ*Rbpm1* were collected by fluorescence-activated cell sorting for RNA-seq (Supplementary Fig. 3E, F). We analyzed the differentially

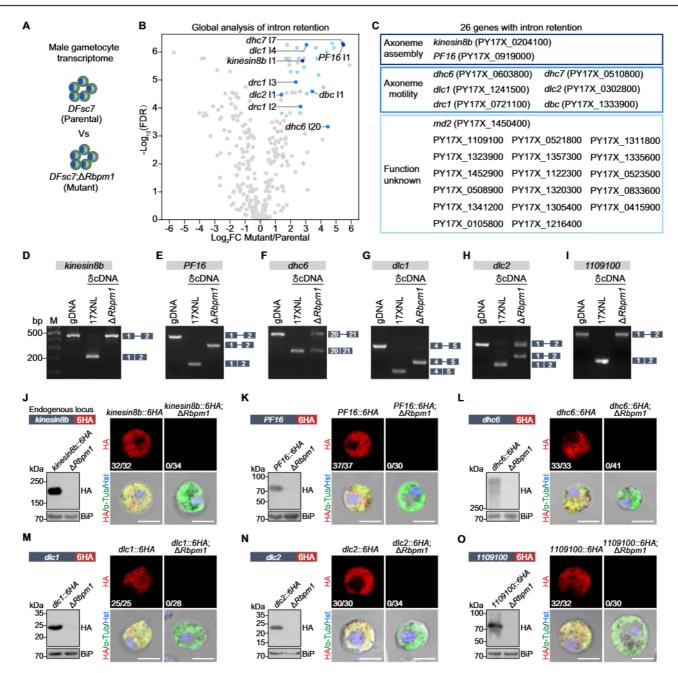


Fig. 4 | RBPm1 deficiency causes intron retention and protein loss of axonemal genes. A A schematic showing the transcriptome analysis of RBPm1-null male gametocytes. *DFsc7*;Δ*Rbpm1* is a *DFsc7*-derived RBPm1 mutant line. *DFsc7* (parental) and *DFsc7*;Δ*Rbpm1* (mutant) male gametocytes were sorted by FACS for RNA-seq. **B** Global analysis of differential intron retention identified 30 retained introns (blue dots) in the mutant versus the parental line. Retained introns with $\log_2 FC \ge 1$ and $p \le 0.05$ were further verified manually by visualization in IGV. The *p*-values were calculated by quasi-likelihood F-test and adjusted by FDR. These introns were originated from 26 genes. Detailed information of these genes and introns is provided in Supplementary Fig. 4A. **C** List of the 26 genes with intron retention from (**B**), categorized by protein function. **D–I**. RT-PCR confirmation of intron retention in 6 selected genes *kinesin8b*, *PF16*, *dhc6*, *dlc1*, *dlc2*, and *PY17X_1109100*. Genomic DNA (gDNA) from 17XNL parasite, complementary DNA

(cDNA) from male gametocytes of parental and mutant parasites were analyzed. Exons are indicated by boxes and introns by lines. Three independent experiments with similar results. RT-PCR analysis for all 26 genes is presented in Supplementary Fig. 5A. **J-O**. Protein expression analysis of the 6 genes shown in (**D-I**) in male gametocytes after loss of RBPm1. Each endogenous gene was tagged with a 6HA at the C-terminus in both 17XNL and $\Delta Rbpm1$ parasites (the schematic in the top left panel), generating two tagged lines. Immunoblot of the 6HA-tagged protein in gametocytes with and without RBPm1 (bottom left panel). IFA of the 6HA-tagged protein in male gametocytes with and without RBPm1 (right panel). x/y at bottom-left represents the number of HA-positive male gametocytes/the total number of male gametocytes tested. Three independent experiments with similar results. Scale bars: 5 µm.

expressed genes between *DFsc7* and *DFsc7*; \$\Delta Rbpm1\$ (Supplementary Data 2). As expected, the *Rbpm1* transcripts were undetectable in the *DFsc7*; \$\Delta Rbpm1\$ (Supplementary Fig. 3G, H). RBPm1 deficiency led to changed expression of several genes (Supplementary Fig. 3G), but none of the differentially expressed genes was known to be implicated in axoneme assembly during male gametogenesis.

We found 30 intron retention (IR) events in transcripts of 26 genes after loss of RBPm1 (Fig. 4B, C) by bioinformatic analysis of global intron retention and manual examination on Integrative Genomics Viewer³⁸. These genes were specifically or preferentially transcribed in the male gametocytes (Supplementary Fig. 4A). Among them (Fig. 4C), the orthologs of *kinesin8b* and *PF16* had been reported essential for

axoneme assembly of male gametogenesis in P. berghei³⁹⁻⁴¹. Six putative dynein motor-associated genes, dhc6 (dynein heavy chain, PY17X 0603800), dhc7 (dynein heavy chain, PY17X 0510800), dlc1 (dynein light chain, PY17X 1241500), dlc2 (dynein light chain, PY17X 0302800), drc1 (dynein regulatory complex protein, PY17X 0721100), and dbc (dynein beta chain, PY17X 1333900), were included. The md2 (male development protein 2, PY17X_1450400), a male gene recently identified42, was also included. The rest 17 IR genes had not been previously described in the *Plasmodium*. Gene Ontology (GO) enrichment analysis of these IR genes found significant GO terms that are associated with MT or cytoskeleton (Supplementary Fig. 4B). RT-PCR using the primers anchored in the flank exons of each of 26 introns further confirmed that these introns were retained in the transcripts in the absence of RBPm1, while their neighboring introns were correctly removed (Fig. 4D-I and Supplementary Fig. 5A). Using RT-qPCR, we further confirmed the IR of kinesin8b intron1 and PF16 intron1 in the RBPm1-null male gametocytes (Supplementary Fig. 5B). Interestingly, the whole part of intron was retained in the transcripts for most IR genes, while only a N-terminal part of intron was retained for three IR genes, including PF16 intron1, dlc1 intron4, and PY17X 1311800 intron5 (Fig. 4D-I and Supplementary Fig. 5A). Therefore, RBPm1 is required for the splicing of selective introns in certain male genes, especially MT or cytoskeleton-related genes.

We speculated that the RBPm1-regulated IR genes are axonemal given the following facts: (1) RBPm1 depletion causes defective axoneme assembly; (2) All IR genes are male-specific; (3) 8 IR genes are axoneme-related. To test it, we selected 12 out of the 26 genes, including 6 annotated (*kinesin8b*, *PF16*, *dhc6*, *dhc7*, *dlc1*, *dlc2*) and 6 unannotated (PY17X_1109100, PY17X_0521800, PY17X_1311800, PY17X_1323900, PY17X_1357300, PY17X_1335600). Each gene was endogenously tagged at the N- or C-terminus with a 6HA in the 17XNL. All 12 proteins were specifically expressed in male gametocytes during parasite life cycle (Supplementary Fig. 6A), in agreement with their transcript profile. In the inactivated gametocytes, these proteins were distributed in the cytoplasm, while after activation, 11 of 12 proteins displayed axoneme localization in the flagellating male gametes (Supplementary Fig. 6B–M). These results suggested that RBPm1 controls intron splicing for a group of the axonemal genes.

Intron retention leads to loss of axonemal protein in RBPm1-null male gametocytes

Nucleotide sequence analysis revealed that IR would result in premature translation and thus cause loss of protein expression for the axonemal genes (Supplementary Fig. 7). To analyze the effect of IR on the axonemal proteins after RBPm1 loss, we deleted *Rbpm1* gene in each of two tagged lines *kinesin8B::6HA* and *PF16::6HA* (Fig. 4J, K). In the absence of RBPm1, 6HA-tagged Kinesin8B, and PF16 were not detected or under detectable thresholds in male gametocytes compared to the parental counterparts in both IFA and immunoblot (Fig. 4J, K). To further confirm the protein loss, we analyzed 4 other IR genes *dhc6*, *dlc1*, *dlc2*, and PY17X_1109100. Endogenous *Rbpm1* gene was deleted in all the 4 tagged lines (*dhc6::6HA*, *dlc1::6HA*, *dlc2::6HA*, and 1109100::6HA) (Fig. 4L–O). These 6HA-tagged proteins lost expression in the RBPm1-null male gametocytes (Fig. 4L–O), similarly as Kinesin8B and PF16 did. These results demonstrated that RBPm1 deficiency causes expression loss of target axonemal proteins.

To confirm the essential roles of *P. yoelii* Kinesin8B and PF16 in axoneme assembly as reported in *P. berghei*^{39–41}, we disrupted *kinesin8b* and *PF16* genes in the 17XNL, obtaining mutant lines $\Delta kinesin8b$ and $\Delta PF16$ (Supplementary Fig. 8A). As expected, depletion of *kinesin8b* or *PF16* either blocked or severely impaired male gamete formation, respectively (Supplementary Fig. 8B). Neither mutant produced any midgut oocysts in the infected mosquitoes (Supplementary Fig. 8C). Ultrastructure analysis of male gametocytes at 8 mpa revealed that the $\Delta kinesin8b$ mutant failed to develop "9 + 2" axoneme,

with loss of both central and peripheral MTs, while most of the axonemes lost central MTs (shown as "9+0" or "9+1") in the $\Delta PF16$ (Supplementary Fig. 8D, E), in line with gene disruption phenotypes in *P. berghet* ^{39–41}. Therefore, depletion of Kinesin8B or PF16 phenocopies RBPm1 deficiency in axoneme assembly.

Intron deletion restores axonemal proteins and partially rectifies axoneme assembly defects in RBPm1-null gametocytes

Since IR disrupted the axonemal proteins expression, we tested whether enforced genomic deletion of the retained intron could restore protein expression by bypassing intron splicing at the transcripts in RBPm1-null male gametocytes. The endogenous kinesin8b intron1 (239 bp) was removed in the kinesin8b::6HA;ΔRbpm1 parasite by CRISPR-Cas9 (Fig. 5A), generating the intron-null mutant kinesin8bΔintron1 (kinesin8bΔII). Both IFA and immunoblot revealed that the deletion of intron1 restored Kinesin8B::6HA expression to WT level in the RBPm1-null gametocytes (Fig. 5B, C). To further confirm the restoration effect, we tested 3 other retained introns (PF16 intron1, dlc1 intron4, and PY17X 1109100 intron1). Compared to the parental RBPm1-null parasites, the expression of PF16::6HA and 1109100::6HA in male gametocytes were fully restored (Fig. 5D-F, J-L), while the Dlc1::6HA was partially restored after removal of the corresponding intron (Fig. 5G-I). Expression restoration of these axonemal proteins (Kinesin8b, PF16, Dlc1, and PY17X_1109100) via intron deletion strongly confirmed the causative effect of IR on axonemal protein loss in the absence of RBPm1.

We next tested whether genomic deletion of the retained introns could rescue or rectify the defective axoneme assembly in the $\Delta Rbpm1$ mutant. We deleted the *kinesin8b* intron1 in the $\Delta Rbpm1$ line, but this deletion of single intron failed to restore any EC formation in the ΔRbpm1;kinesin8b∆intron1 parasites. However, compared to complete lack of axonemes showing "9 + 2", "9 + 1", or "9 + 0" MTs in the parental $\Delta Rbpm1$, some axoneme-like structures ("9 + 2": 1%, "9 + 1": 3%, and "9 + 0": 15%) were detected in the $\Delta Rbpm1$; kinesin8b $\Delta intron1$ (Fig. 5M, N), indicating that deletion of kinesin8b intron1 could partially rescue the defective axoneme assembly caused by RBPm1 deficiency. Notably, additional deletion of the PF16 intron1 in the $\Delta Rbpm1$; kinesin8b\(\Delta\)intron1 parasite further mitigated axoneme defects in the resulted $\Delta Rbpm1$; kinesin8b Δ intron1; PF16 Δ intron1 parasite line ("9 + 2": 1%, "9 + 1": 11%, and "9 + 0": 32%) (Fig. 5M, N). These results demonstrated that RBPm1 regulates axoneme assembly by controlling intron splicing of a group of axonemal genes. Without RBPm1, deletion of 2 introns (kinesin8b intron1 and PF16 intron1) was insufficient to restore axoneme assembly to the WT level ("9 + 2": 93%) (Fig. 5M, N). Therefore, in addition to kinesin8b and PF16, other axonemal genes targeted by RBPm1 may also play important roles in axoneme assembly during male gametogenesis.

RBPm1 interacts with spliceosome E complex and introns of axonemal genes

To investigate whether RBPm1 associates with the spliceosome responsible for intron splicing, we used the biotin ligase TurbolD-based proximity labeling to identify RBPm1-interacting proteins in the gametocytes. The endogenous RBPm1 was tagged with a HA::TurbolD motif in the 17XNL, generating the line *Rbpm1::TurbolD* (Fig. 6A). A control parasite *Rbpm1::T2A::TurbolD* was generated by fusing endogenous RBPm1 with a "ribosome skip" T2A peptide, a NLS (nuclear localization signal), and a HA::TurbolD (Fig. 6A), permitting separated expression of RBPm1 and biotin ligase. Gametocytes expressing the ligase were incubated with 50 μM biotin for 20 min at 37 °C. Staining with fluorescent-conjugated streptavidin and anti-HA antibody detected a nuclear distribution of biotinylated proteins in both TurbolD-modified gametocytes (Supplementary Fig. 9A), indicating biotinylation of the potential RBPm1-interacting proteins in the nucleus. Mass spectrometry of the streptavidin affinity purified proteins from the

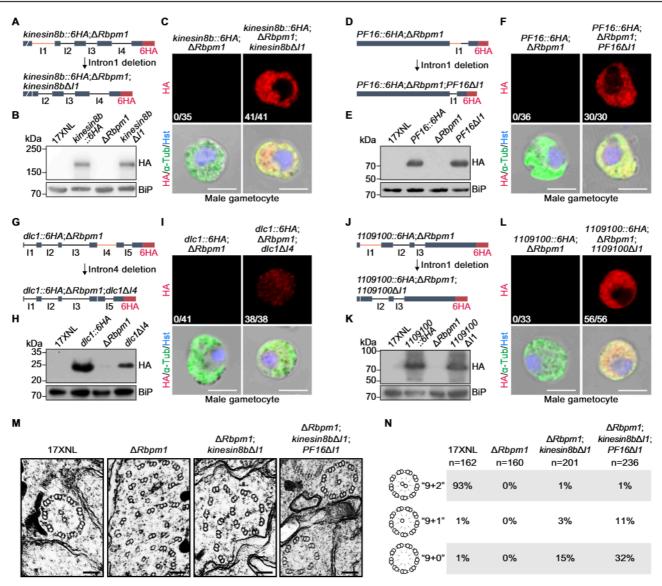


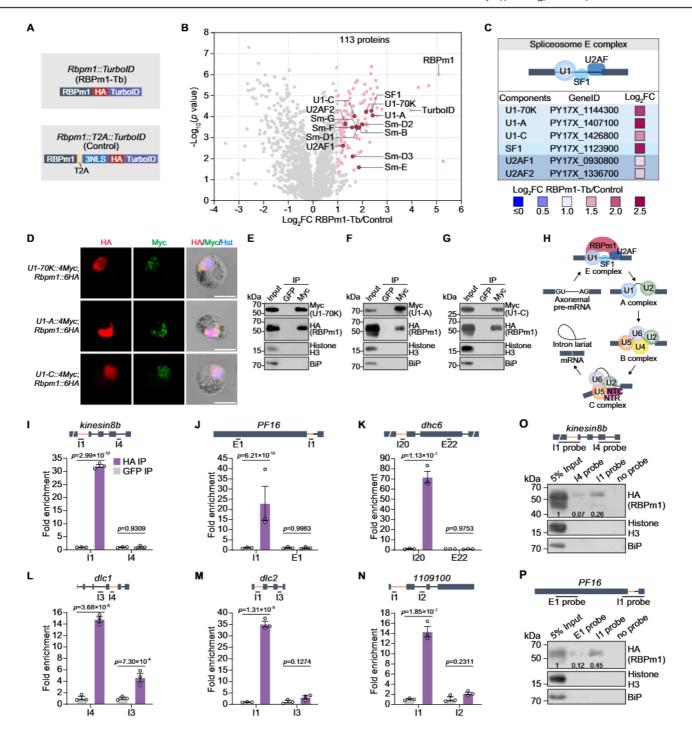
Fig. 5 | Intron deletion restores axonemal protein expression and partially rectifies axoneme assembly defects in RBPm1-null male gametocytes. A A schematic showing the genomic deletion of retained intron (kinesin8b intron1, orange) in the kinesin8b::6HA;ΔRbpm1 parasite (abbreviated as ΔRbpm1), generating the mutant kinesin8b::6HA;ΔRbpm1;kinesin8bΔintron1 (abbreviated as kinesin8bΔi1). B Immunoblot of 6HA-tagged Kinesin8B protein in gametocytes. Three independent experiments with similar results. C IFA of 6HA-tagged Kinesin8B in male gametocytes. x/y represents the number of HA-positive male gametocytes/the total number of male gametocytes tested. Three independent experiments with similar results. Scale bars: 5 μm. D, E, F Effect of intron deletion (PF16 intron1) on the restoration of PF16 protein in RBPm1-null male gametocytes. Similar analysis as in

(A, B, C). G, H, I Effect of intron deletion (*dlc1* intron4) on the restoration of Dlc1 protein in RBPm1-null male gametocytes. J, K, L Effect of intron deletion (*PY17X_1109100* intron1) on the restoration of PY17X_1109100 protein in RBPm1-null male gametocytes. M Transmission electron microscopy of axoneme architecture in male gametocytes at 8 mpa. Δ*Rbpm1;kinesin8b*ΔII is a Δ*Rbpm1*-derived modified line with deletion of *kinesin8b* intron1. Δ*Rbpm1;kinesin8b*ΔII;*PF16*ΔII is a Δ*Rbpm1* derived modified line with deletion of both *kinesin8b* intron1 and *PF16* intron1. Scale bars: 100 nm. N Quantification of axoneme formation from parasites in (M). n is the total number of the intact and defective axoneme structures observed in each group. Three independent experiments with similar results.

Rbpm1::TurbolD resulted in a list of 113 proteins enriched with high confidence compared to the control (Fig. 6B and Supplementary Data 3). RBPm1 was the top hit, confirming cis-biotinylation of RBPm1 (Fig. 6B). Among the significantly enriched proteins, we found the components of the spliceosome earliest assembling E complex^{43–45}, including the U1 small nuclear ribonucleoproteins (snRNP) U1-70K, U1-A, U1-C, Sm-B, Sm-D1, Sm-D2, Sm-D3, Sm-E, Sm-F and Sm-G (Fig. 6B, C), and three E complex key factors SF1, U2AF1, and U2AF2 (Fig. 6B, C). Tagging the endogenous U1-70K, U1-A, and U1-C proteins with 4Myc in the Rbpm1::6HA parasite showed that these three U1 snRNPs colocalized with RBPm1 in the nucleus (Fig. 6D). Co-immunoprecipitation also confirmed the interaction between RBPm1 and these U1 snRNPs (Fig. 6E–G). Spliceosome A, B, and C complex are formed after the

assembly of splicing initiating E complex^{46,47}. However, the components of A, B, and C complex were not detected (Supplementary Fig. 9B, C). Therefore, RBPm1 interacted only with spliceosome E complex, possibly helping to initiate splicing for certain introns in the axonemal genes (Fig. 6H).

Nuclear localization and interaction with spliceosome E complex imply that RBPmI may bind to the target introns in the pre-mRNA of axonemal genes. We performed UV crosslinking RNA immunoprecipitation (UV-RIP) followed by RT-qPCR with primers recognizing the target pre-mRNAs. In the *Rbpm1::6HA* gametocytes, RBPm1 bound to the intron1 of the *kinesin8b* transcripts using anti-HA nanobody (Fig. 6I). As a control, RIP using anti-GFP nanobody detected no binding (Fig. 6I). Additionally, we analyzed the interaction between



RBPm1 and five other target introns (*PF16* intron1, *dhc6* intron20, *dlc1* intron4, *dlc2* intron1, and *PY17X_1109100* intron1). As expected, RBPm1 bound these target introns. As expected, RBPm1 bound these target introns but not the neighboring introns or exons since each intron is individually excised as a lariat RNA during the splicing (Fig. 6J–N).

Furthermore, we used RNA pull-down to validate the interaction of RBPm1 with the *kinesin8b* intron1 and *PF16* intron1. A biotinylated 500nt RNA probe *kinesin8b* I1 and a control probe *kinesin8b* I4 were synthesized (Fig. 60, upper panel) and incubated with the *Rbpm1::6HA* gametocyte lysate. The potential RNA-interacted proteins were precipitated using the streptavidin beads and detected by immunoblot. The *kinesin8b* I1 probe retrieved more RBPm1 protein than the *kinesin8b* I4 probe (Fig. 60). Similarly, the *PF16* I1 probe captured more RBPm1 protein than the probe *PF16* E1 (Fig. 6P). Both RIP and RNA pull-

down experiments supported that RBPm1 binds the *kinesin8b* intron1 and *PF16* intron1.

RBPm1 directs splicing of axonemal introns inserted in a reporter gene

To further investigate the interaction between RBPm1 and the axone-mal introns, we test whether RBPm1 could direct splicing of target introns when inserted into a reporter gene. We developed a blue fluorescence protein (BFP) reporter assay that allows an easy splicing readout in male and female gametocytes of the *DFsc7* parasite. The intact *bfp* transcript driven by the *hsp70 5'-*UTR and the *dhfr 3'-*UTR was integrated into the *p230p* locus of *DFsc7* using CRISPR-Cas9, generating the control line *BFP* (Fig. 7A). The *kinesin8b* intron1 (*Kin8b*II, 239 bp) was inserted to the *bfp* gene at the nucleotides

Fig. 6 | RBPm1 interacts with spliceosome E complex and introns of

axonemal genes. A A schematic showing two modified parasite lines generated for searching RBPm1-interacting proteins by TurboID-based proximity labeling and mass spectrometry. The motif of HA::TurboID and T2A::3NLS::HA::TurboID, respectively, was inserted at the C-terminus of the endogenous RBPm1, generating the line Rbpm1::TurboID and the control line Rbpm1::T2A::TurboID. B Volcano plot displaying 113 significantly enriched proteins (pink dot, cutoffs log₂FC≥1 and $p \le 0.05$) in the *Rbpm1::TurbolD* versus *Rbpm1::T2A::TurbolD*. Among them, 13 subunits (red dot) of the early spliceosome E complex were included. The p-values were calculated by two-sided t-test and adjusted by FDR. C Protein interaction analysis between RBPm1 and six spliceosome E complex subunit proteins (U1-70K, U1-A, U1-C, SF1, U2AF1, and U2AF2) from (B). D IFA of 6HA-tagged RBPm1 and 4Myctagged U1 snRNP proteins (U1-70K, U1-A, and U1-C) in male gametocytes of three double-tagged parasites. Three independent experiments with similar results. Scale bars: 5 µm. E Co-immunoprecipitation of RBPm1 and U1-70K in gametocytes of the double-tagged parasite U1-70K::4Myc;Rbpm1::6HA. Anti-Myc nanobody was used. Bip as a loading control. Three independent experiments with similar results. F Coimmunoprecipitation of RBPm1 and U1-A in gametocytes of the double-tagged parasite U1-A::4Myc;Rbpm1::6HA. Three independent experiments with similar results. G Co-immunoprecipitation of RBPm1 and U1-C in gametocytes of the double-tagged parasite U1-C::4Myc;Rbpm1::6HA. Three independent experiments

with similar results. H Proposed model showing the interaction between RBPm1 and early spliceosome E complex for intron splicing of axonemal genes. I-N. UV-RIP detection of RBPm1 interaction with the retained introns of 6 axonemal genes (kinesin8b, PF16, dhc6, dlc1, dlc2, and PY17X 1109100). A top schematic shows the exon-intron structure of the RBPm1 target axonemal genes. The retained introns are indicated with orange lines, and the genomic regions for qPCR amplicon are shown, UV-RIP was performed in Rbpm1::6HA lines using anti-HA nanobody, Anti-GFP nanobody was used as a control. Bound RNA was analyzed by RT-qPCR. Means ± SEM from three independent experiments, two-sided t-test. O RNA pulldown assay detecting RBPm1 interaction with kinesin8b intron1. A top schematic shows the exon-intron structure of the kinesin8b gene. The retained introns are indicated in orange lines. A biotinylated 500 nt RNA probe II (comprising intron1 and its flanking sequences) and a control probe 14 (comprising intron4 and its flanking sequences) were used. Proteins via RNA pull-down were immunoblot with anti-HA antibody. The numbers are the relative intensities of bands in the blot. Histone H3 and Bip were used as negative controls. Two independent experiments with similar results. P RNA pull-down assay detecting RBPm1 interaction with PF16 intron1. A top schematic shows the exon-intron structure of the PF16 gene. The retained introns are indicated in orange lines. A biotinylated 500 nt RNA probe I1 (comprising intron1 and its flanking sequences) and a control probe E1 in exon1 were used. Two independent experiments with similar results.

396-397, generating the line BFP-Kin8bl1 (Fig. 7B). The inserted kinesin8b intron1 would result in premature translation of the bfp transcript if it is not spliced. In the control BFP line, BFP was expectedly detected in both male (GFP+) and female (mCherry+) gametocytes (Fig. 7A). However, in the BFP-Kin8bI1 line, BFP was detected only in male gametocytes (Fig. 7B), indicating that splicing of kinesin8b intron1 in the bfp transcripts occurred only in male gametocytes. To prove that splicing of kinesin8b intron1 in male was RBPm1-dependent, we deleted Rbpm1 in the BFP-Kin8bI1 line and obtained the mutant line BFP-Kin8bl1;ΔRbpm1 (Fig. 7C). RBPm1 deletion disrupted BFP expression in the BFP-Kin8bl1;ΔRbpm1 male gametocytes (Fig. 7C). We parallelly analyzed the kinesin8b intron2 (Kin8b12, 148 bp), whose splicing from the native gene transcript required no RBPm1 (Supplementary Fig. 5A). In both transgenic line BFP-Kin8bl2 (Fig. 7D) and its derivative mutant line BFP-Kin8bl2;ΔRbpm1 (Fig. 7E), BFP was detected in both male and female gametocytes, confirming RBPm1-independent splicing of kinesin8b intron2 from the bfp transcript.

Using the reporter assay, we tested 3 other target introns, including PF16 intron1 (Fig. 7F, G), dlc1 intron4 (Supplementary Fig. 10A, B), and *PY17X_1109100* intron1 (Supplementary Fig. 10C, D). The *PF16* intron1 (276 bp) was inserted to the *bfp* at the nucleotides 500-501 (Fig. 7F), the *dlc1* intron4 (193 bp) at the nucleotides 455–456 (Supplementary Fig. 10A), while the PY17X_1109100 intron1 (353 bp) at the nucleotides 390-391 (Supplementary Fig. 10C). As expected, these introns were spliced from the bfp transcript only in male gametocytes (Fig. 7F, Supplementary Fig. 10A, C). Similarly, these introns were not spliced at male gametocytes in the RBPm1-null parasites compared to their parental parasites (Fig. 7G, Supplementary Fig. 10B, D). Additionally, we analyzed the PY17X 1109100 intron2 (272 bp), which could be spliced in the RBPm1-null parasites (Supplementary Fig. 5A), and found that RBPm1 was not required for splicing of this intron from bfp transcript in both male and female gametocytes (Supplementary Fig. 10E, F).

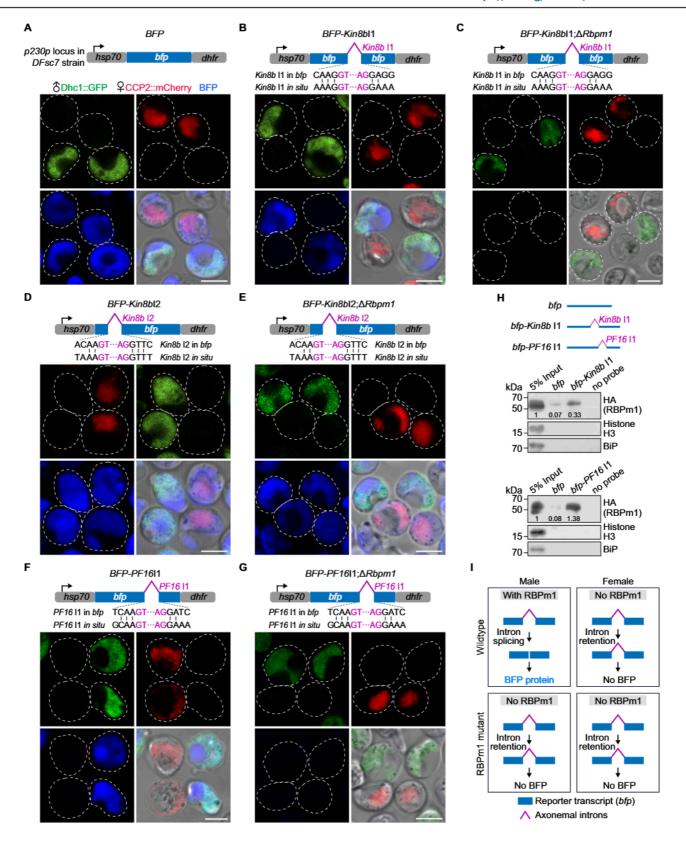
Furthermore, we analyzed the RBPm1 interaction with the *kinesin8b* intron1 and *PF16* intron1 in the *bfp* transcript by RNA pull-down. A biotinylated RNA probe *bfp-Kin8b*11, corresponding to the *kinesin8b* intron1-inserted *bfp* transcript (Fig. 7H, upper panel), retrieved significantly more RBPm1 from the *Rbpm1::6HA* gametocyte lysate compared to the control probe *bfp* (Fig. 7H, middle panel). Similarly, the probe *bfp-PF16*11, corresponding to the *PF16* intron1-inserted *bfp* transcript, captured more RBPm1 than the control probe *bfp* (Fig. 7H, lower panel). Therefore, RBPm1 could recognize the axonemal introns in the reporter transcript for splicing (Fig. 71).

RBPm1 directs splicing of axonemal introns inserted in an endogenous gene

In addition to the reporter gene, we also tested whether RBPm1 could direct splicing of target introns when inserted into an endogenous gene which does not require RBPm1 for intron splicing. We chose the gep1, a 4-exon gene expressed in both gender gametocytes and essential for initiating both genders' gametogenesis⁴⁸. We analyzed male and female gametogenesis by measuring EM rupture (TER119 staining), genome replication (DNA staining), and axoneme assembly (α-Tubulin staining). Compared to 17XNL, the gep1-deleted parasite line $\Delta gep1$ expectedly lost ability in EM rupture, genome replication, and axoneme assembly in activated male gametocytes, as well as EM rupture in activated female gametocytes (Fig. 8A, B, E, F, G, and H). Using CRISPR-Cas9, the kinesin8b intron1 was inserted into the exon3 of gep1 locus at the nucleotides 273-274 in the 17XNL (Fig. 8C), while the PF16 intron1 inserted into the exon1 at the nucleotides 885-886 (Fig. 8D). In both intron-inserted lines gep1-Kin8bl1 and gep1-PF16l1, normal male gametogenesis and defective female gametogenesis was speculated because GEP1 is not expressed in female due to no RBPm1mediated intron splicing from the gep1 transcript. Notably, both the gep1-Kin8b I1 and gep1-PF16 I1 parasites underwent EM rupture only in male (Fig. 8C-F). These results supported that both kinesin8b intron1 and PF16 intron1 were spliced from the gep1 transcript only in male gametocytes with RBPm1 expression. Consistent with GEP1 expression in male gametocytes, normal genome replication and axoneme assembly were detected during male gametogenesis in both gep1-Kin8b II and gep1-PF16 II parasites (Fig. 8C, D, G, H). Collectively, the results from the reporter and the endogenous gene assays (Fig. 81) indicated that the tested axonemal introns themselves could be specifically recognized by RBPm1 for splicing.

Intron retention prevents expression of axonemal proteins in female gametocytes

Despite the male-biased transcription, the axonemal genes still displayed low-level transcripts in female gametocytes (Supplementary Fig. 11A, C, E). However, no axonemal proteins are expressed in female gametocytes (Supplementary Fig. 11B, D, F). The facts of no protein product of low-level transcripts for the axonemal genes observed in this study are consistent with results from previous transcriptomic and proteomic studies^{30,31,49,50}, suggesting a post-transcription regulation for the axonemal genes in female gametocytes. Enforced genomic deletion of the retained intron could restore expression of the axonemal proteins Kinesin8b, PF16, Dlc1,



and PY17X_1109100 in the RBPm1-null male gametocytes (Fig. 5A–L). Strikingly, we found that deletion of these introns (*PF16* intron1, *dlc1* intron4, and *PY17X_1109100* intron1) unexpectedly resulted in low-level expression of PF16, Dlc1, and PY17X_1109100 in the counterpart female gametocytes (Supplementary Fig. 11B, D, F). Kinesin8B was not detected in female gametocytes after deletion of *kinesin8b intron1* (Supplementary Fig. 11H), fitting with the extremely low

transcription of *kinesin8b* in female gametocytes (Supplementary Fig. 11G). Furthermore, RT-PCR not only detected the transcripts of these axonemal genes (*PF16*, *dlc1*, and *PY17X_1109100*), but also IR in these transcripts from the purified female gametocytes (Supplementary Fig. 11I–L). These results indicated a role of the RBPm1-target introns in preventing the expression of axonemal proteins in female gametocytes.

Fig. 7 | RBPm1 directs splicing of axonemal introns inserted in a reporter gene. A A top schematic shows a transgenic line *BFP* with a *bfp* reporter expression cassette integrated at the *p230p* locus of the *DFsc7* reporter line. The intact *bfp* is driven by the *S'*UTR of *hsp70* and the 3'UTR of *dhfr*, allowing expression of BFP in both male (GFP+) and female (mCherry+) gametocytes. Live cell imaging was shown. Three independent experiments with similar results. Scale bars: 5 μm. **B.** A transgenic line *BFP-Kin8b*II with a *kinesin8b* intron 1 (*Kin8b* II, purple line)-inserted *bfp* cassette integrated at the *p230p* locus of the *DFsc7* line. *Kin8b* II (purple) was inserted into the *bfp* gene at the nucleotides 396-397 to mimic the splice site (vertical lines) of in situ *Kin8b* II. BFP expression was detected specifically in male gametocytes of the *BFP-Kin8b*II parasites. Three independent experiments with similar results. Scale bars: 5 μm. **C** A *BFP-Kin8b*II derived RBPm1 mutant line *BFP-Kin8bII*;Δ*Rbpm1*. No BFP expression was detected in male gametocytes of the *BFP-Kin8bII*;Δ*Rbpm1* parasites. Three independent experiments. Scale bars: 5 μm. **D** Effect of the *kinesin8b* intron 2 (*Kin8b* I2) insertion on the gametocyte expression

of BFP. Similar analysis as in (B). BFP expression was detected in both male and

female gametocytes of the BFP-Kin8bl2 parasites. E A BFP-Kin8bl2 derived RBPm1

mutant line BFP-Kin8bl2;ΔRbpm1. Similar analysis as in (C). BFP expression was detected in both male and female gametocytes of the BFP-Kin8b12;ΔRbpm1 parasites. F Effect of the PF16 intron1 (PF16 I1) insertion on the gametocyte expression of BFP. Similar analysis as in (B). BFP expression was detected specifically in male gametocytes of the BFP-PF16I1 parasites. G A BFP-PF16I1 derived RBPm1 mutant line BFP-PF16I1;ΔRbpm1. Similar analysis as in (C). No BFP expression was detected in male gametocytes of the BFP-PF16I1;ΔRbpm1 parasites. H RNA pull-down assay detecting RBPm1 interaction with the Kin8b I1 and PF16 I1 -inserted bfp transcripts from BFP-Kin8bl1 and BFP-PF16l1 gametocytes, respectively. Three biotinylated RNA probes bfp, bfp-Kin8bl1 (corresponding to the Kin8b l1-inserted bfp transcript), bfp-PF1611 (corresponding to the PF16 11-inserted bfp transcript) were used. Proteins via RNA pull-down were immunoblotted with anti-HA antibody. The numbers are the relative intensities of bands in the blot. Histone H3 and Bip were used as negative controls. Two independent experiments with similar results. I A schematic of RBPm1-dependent splicing of axonemal introns inserted in the reporter transcript.

Discussion

For efficient transmission, the malaria parasites in the vertebrate host differentiate into sexual precursor gametocytes that are poised to rapidly activate to fertile gametes upon entering into the mosquito midgut for fertilization and further development. So far, a limited number of transcription and epigenetic factors have been identified during gametocyte and gamete development sl.52. Among -180 putative *Plasmodium* RBPs, about one-third of RBP genes exhibit stage specific or elevated expression in the gametocyte and especific nuclear RBP, RBPm1, which is essential for male gametogenesis and mosquito transmission of the *Plasmodium*. RBPm1 operates as a stage- and gender-specific splicing factor for spliceosome assembly initiation and regulates the protein expression of a group of 26 male genes, most of which are axoneme-related.

Recent studies had discovered several RBPs playing roles in the developmental programs of gametocyte and gametes. During gametocytes development, UIS12 contributes to the development of gametocytes of both genders⁵³. Disrupting *Puf1* led to a reduction in gametocytes, especially female gametocytes⁵⁴, while ccr4-1 gene deletion obstructs male gametocyte development55. Puf2 knockout, on the other hand, promotes male gametocyte development⁵⁶. The CCCH zinc finger protein MD3 regulates the gametocyte maturation and male gametocytogenesis⁵⁷. In female gametocytes, the DOZI/ CITH/ALBA translation repressor complex and PUF2 hold the stored mRNAs for translation repression until their proteins were needed during the development of post fertilization^{28,58}. Additionally, the CAF1/CCR4/NOT complex also plays a role in safeguarding the stored mRNAs from degradation. In male gametocytes, two functional RBPs have been identified. The alternative splicing factor SR-MG promotes the establishment of sex-specific splicing patterns and knocking it out reduces the formation of male gamete⁵⁹; the ZNF4's knockout results in deregulation of 473 genes, including axonemal dynein-related genes⁶⁰. These documented RBPs and RBPm1 identified in this study may function together at the posttranscriptional regulation to shape the male transcriptome for gametocyte and gamete development.

During the manuscript preparation of this study, another work by ref. 42 demonstrated that deletion of the *Rbpm1* ortholog of *P. berghei* (PBANKA_0716500, named as *md5*) had no effect on female and male gametocyte formation, but resulted in male-specific infertility. These findings are consistent with the defective male gamete formation phenotype of the *ARbpm1* in *P. yoelii* in this study, indicating conserved function of RBPm1 in the rodent malaria parasites. As *P. falciparum* is the most lethal human malaria parasite, future studies are worthy to investigate whether the RBPm1 ortholog in the *P. falciparum* functions similarly in male gametogenesis.

The RBPm1-deficient parasites showed specific defects in axoneme assembly during male gametogenesis (Fig. 3). Axoneme is a MT cytoskeleton essential for the eukaryotic flagellar motility, consisting of a central pair of singlet MTs encircled by 9 outer doublet MTs. This 9+2 organization of axonemes is highly conserved in the eukaryotes, including Plasmodium⁶¹. However, the axoneme in Plasmodium differs from that in other model organisms in several aspects 10,62,63. First, the biogenesis of axoneme in Plasmodium male gametogenesis is extremely fast, taking only 6-8 min to assemble 8 axonemes^{9,10}. Second, location of basal body. In the canonical cilium, the basal body is localized under the plasma membrane. In *Plasmodium* male gametocytes, the basal bodies are residing at the nuclear membrane⁶³. Third, location for axoneme assembly. The canonical axoneme protrudes distally from the cell simultaneously when growing from the basal body. The *Plasmodium* assemblies the axoneme within the cytoplasm, independent of intraflagellar transport required for cilium formation^{10,62}. Last, each assembled axoneme associates with a haploid nuclei to progressively protrude from the parasite plasma membrane, resulting in a free motile flagellum¹⁰. Mechanisms underlying the cytoplasmic assembly and exflagellation of axonemes in Plasmodium remain largely unknown, although the involvement of some conserved basal body and axonemal proteins has been described, including armadillo repeat protein PF1641, motor protein Kinesin8B39,40,64, basal body proteins SAS4 and SAS663,65-67, and radial spoke protein RSP968. It is possible that the Plasmodium had evolved novel mechanisms to fulfill the requirement for the axoneme. In this study, we identified a group of 26 male genes targeted by RBPm1. Several known or putative axoneme-associated genes were included. Importantly, analysis of endogenous protein localization showed that most of the tested proteins encoded by RBPm1-target genes co-localizing with axoneme (Supplementary Fig. 6B-M), suggesting their roles in biogenesis, structure, regulation, or function of axoneme. For future studies, it will be intriguing to understand the roles of these 26 RBPm1-regulated genes, especially 17 previously undescribed ones, during male gametogenesis in the Plasmodium.

In the RBPm1 deficient male gametocytes, 30 IR events were detected in 26 male genes. One intron was retained in each of 22 genes while two introns were retained in each of 4 other genes, respectively (Fig. 4B and Supplementary Fig. 4A). Mechanistically, RBPm1 not only bound to the intron-retained transcripts, but also interacted with spliceosome E complex. U1 snRNPs recognize and pair with the 5' splice site of intron. SF1, U2AF1 and U2AF2 form a complex and bind to branch point, 3' splice site, and polypyrimidine tract respectively^{69,70}. These above factors assemble the E complex as a spliceosome earliest stage. After that, the spliceosome dynamically releases and recruits different snRNPs to establish the assembly for further stages, including spliceosome A, B, and C complex. RBPm1 was detected to interact

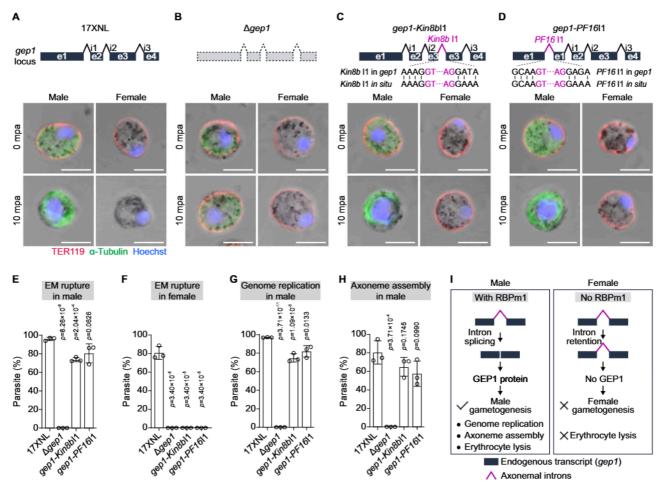


Fig. 8 | **RBPmI** directs splicing of axonemal introns inserted in the endogenous gene. A A top schematic shows the genomic locus of a 4-exon gene *gep1*, which is expressed in both gender gametocytes and essential for both genders' gametogenesis. Erythrocyte plasma membrane (EM) rupture, genome replication, and cytoplasmic assembly of the axoneme were analyzed in gametocytes of the 17XNL parasites at 10 mpa. The parasites were co-stained with anti-TER-119 and anti-α-Tubulin antibodies and Hoechst 33342. TER-119 (red) negative gametocytes were recognized as EM rupture. Enlarged nuclei represent the genome replication in male gametocytes. Enhanced α-Tubulin (green) signal represents cytoplasmic assembly of the axoneme in male gametocytes. Three independent experiments with similar results. Scale bars: $5 \,\mu \text{m}$. B. A top schematic shows a modified line $\Delta gep1$, in which the endogenous gep1 gene was deleted in the 17XNL. Similar analysis for the $\Delta gep1$ parasites as in (A). C A top schematic shows a modified line gep1-Kin8b11, in which the *kinesin8b* intron1 (Kin8b11) was inserted into the exon3 of gep1 locus at the nucleotides 273-274 in the 17XNL. Similar analysis for the gep1-Kin8b11

parasites as in (A). **D** A top schematic shows a modified line *gep1-PF16*11, in which the *PF16* intron1 (*PF16*11) was inserted into the exon1 of *gep1* locus at the nucleotides 885-886 in the 17XNL. Similar analysis for the *gep1-PF16*11 parasites as in (A). **E** Quantification of EM rupture in male gametocytes of the four parasites tested. Data are means ± SEM from three independent experiments, two-sided *t*-test. **F** Quantification of EM rupture in female gametocytes of the four parasites tested. Data are means ± SEM from three independent experiments, two-sided *t*-test. **G** Quantification of genome replication in male gametocytes of the four parasites tested. Data are means ± SEM from three independent experiments, two-sided *t*-test. **H** Quantification of axoneme assembly in male gametocytes of the four parasites tested. Data are means ± SEM from three independent experiments, two-sided *t*-test. **I** Schematic of RBPm1-dependent splicing of axonemal introns inserted in the endogenous gene *gep1*. Male-specific RBPm1 could recognize and splice the axonemal introns (*kinesin8b* intron1 or *PF16* intron1) inserted in the *gep1* transcript, allowing male-specific GEP expression and thus male gametogenesis.

exclusively with the components of spliceosome E complex, but not those of the A, B, and C complex (Supplementary Fig. 9C). Therefore, RBPm1 likely function as a splicing activator, linking spliceosome E complex with the selective introns of axonemal genes for splice site recognition. In mammals and plants, a RBP of Dek played a similar role and promoted the splicing of certain introns by bridging the intron with the UI/U2 snRNPs^{71,72}. At this stage, the data support an association of RBPm1 and spliceosome E complex, but it is not yet clear if it is a direct association.

Both RIP and RNA pull-down assays demonstrated that RBPm1 interacts with the target introns in transcripts of the axonemal genes, suggesting the presence of signals recognized by RBPm1 in these introns. To investigate if the signals for RBPm1 recognition are imparted by the introns themselves but not the adjoining exons, we analyzed the splicing capability of these introns when they were inserted in either a reporter gene (bfp) or an irrelevant endogenous

gene (gep1). The results from both intron splicing assays establish stringent dependencies of splicing on RBPm1 for these axonemal introns, suggesting intrinsic signals within the introns for RBPm1 recognition. In addition, the adjoining exons may play less modulatory role in the RBPm1 recognition of the axonemal introns. We attempted to search for the common features, such as length, GC content, splice sites, and motif enrichment, but unfortunately observed seemingly no shared features among these 30 RBPm1 target introns. The molecular basis for the axonemal intron recognition by RBPm1 is still unknown. One possibility is that RBPm1 target introns may possess the sequenceindependent features, such as RNA structures or epigenetic modifications, for RBPm1 recognition. The structure of RBPm1 is not available yet. To understand the recognition or interaction between RBPm1 and its target introns, future studies into an atomic resolution structure of the protein (RBPm1)-RNA (intron) complex will be required.

Axoneme is an essential cellular structure specifically required for male gametogenesis during the life cycle of Plasmodium. Consistent with this physiological requirement of male gametocytes, the axonemal genes display significant male-biased transcription 30,31,49. Interestingly, these axonemal genes also showed low-level transcripts in female gametocytes in many others and our studies30,31,49, likely due to the transcription leaking. However, no axonemal proteins are detected in female gametocytes50, suggesting a post-transcription regulation for the expression turn off of axonemal genes in female gametocytes. We found that genomic deletion of the retained intron (kinesin8b intron1, PF16 intron1, dlc1 intron4, and PY17X 1109100 intron1) could bypass the intron splicing and thus restore expression of the axonemal proteins (Kinesin8b, PF16, Dlc1, and PY17X_1109100) in the RBPm1-null male gametocytes. Notably, these introns deletion unexpectedly resulted in low-level expression of PF16, Dlc1, and PY17X_1109100 in female gametocytes (Supplementary Fig. 11B, D, F). The level of proteins restored was correlated with the level of transcripts for these axonemal genes in female gametocytes. These results confirmed the low-level transcripts of these axonemal genes in female gametocytes. In addition, no protein products of these low-level transcripts could be explained by IR and translation failure in female gametocytes. Based on these results, we proposed a dual role of RBPm1-target introns in axonemal gene expression in male and female gametocytes respectively (Supplementary Fig. 11M). In male gametocytes, RBPm1 (as a key)-directed splicing of axonemal intron (as a lock) allows protein expression of axonemal genes for axoneme assembly. In female gametocytes, dual blockage via weak transcription and IR shuts the protein expression of the axonemal genes. The splicing activator RBPm1 and its target introns constitute an intron splicing program, safeguarding the expression of axonemal proteins in male gametocytes while preventing the expression of these proteins in female gametocytes, to fulfill the sexually dimorphic protein profiles during sexual development of the Plasmodium.

Methods

Animals and ethics statement

The animal experiments conducted in this study were approved by the Committee for Care and Use of Laboratory Animals of Xiamen University (XMULAC20190001). Female ICR mice aged 5–6 weeks were acquired from the Animal Care Center of Xiamen University. The mice were housed in a controlled environment at 22–24 °C, relative humidity of 45–65%, and a 12-h light/dark cycle. They were used for parasite propagation, drug selection, parasite cloning, and mosquito feeding. The larvae of *Anopheles stephensi* mosquitoes (Hor strain) were maintained in an insect facility under controlled conditions of 28 °C, 80% relative humidity, and a 12-h light/12-h dark cycle. Adult mosquitoes were fed with a 10% (w/v) sucrose solution containing 0.05% 4-aminobenzoic acid and kept at 23 °C.

Plasmid construction

All genetically modified parasites in this study are listed in Supplementary Table 1. The CRISPR/Cas9 plasmid pYCm was used for gene editing^{34,73}. To construct plasmids for gene tagging, the 5'- and 3'-flanking sequences (300–700 bp) at the designed insertion site of target genes were amplified as homologous templates. DNA fragments encoding 6HA, GFP, or 4Myc were placed between them and in-frame with the target gene. To construct the plasmids for gene knockout, the left and right homologous arms consisted of 400–700 bp sequences upstream and downstream of the coding sequences of the target gene. To construct the plasmids for domain or intron deletion, the left and right homologous arms consisted of 200–700 bp sequences upstream and downstream of the domain or intron were PCR-amplified and inserted into specific restriction sites in pYCm. To construct the plasmids for intron insertion, the left and right homologous arms were

composed of gene genomic sequences ranging from 300–600 bp upstream and downstream of the insertion site, respectively. The left homologous arm, intron, and right homologous arm were connected by overlap PCR, and the fused fragment was inserted into specific restriction sites in pYCm. In each modification, at least two small guide RNAs (sgRNAs) were designed. To construct the plasmids for the *bfp* reporter assay, the intact *bfp* reporter (717 bp) driven by the 5'-UTR (1755 bp) of the *hsp70* gene and the 3'-UTR (561 bp) of the *dhfr* gene were inserted into specific restriction sites between the left and right homologous arms for transgenic integration in the *p230p* locus of *P. yoelii*⁷⁴. The *kinesin8b* intron1 (239 bp), *kinesin8b* intron2 (148 bp), *PF16* intron1 (276 bp), *dlc1* intron4 (193 bp), PY17X_1109100 intron1 (353 bp), and PY17X_1109100 intron2 (272 bp) were inserted into the *bfp* reporter by overlap PCR. All primers and oligonucleotides used in the plasmid construction are listed in Supplementary Table 2.

Parasite transfection and genotyping

The procedures for parasite transfection and genotyping were carried out as previously described ^{34,73}. Briefly, the schizonts were isolated from infected mice using a 60% Nycodenz density gradient centrifugation. The parasites were then electroporated with 5 μg plasmid using a Nucleofector 2b Device (Lonza, Germany). The transfected schizonts were immediately intravenously injected into a naïve mouse, and pyrimethamine (Pyr) selection (6 mg/ml in drinking water) was applied the day following transfection. Pyr-resistant parasites were typically observed about 7 days after drug selection. Single clone of parasite was obtained by limiting dilution in mice, and genomic DNA was extracted from infected mouse blood for PCR genotyping using specific primers listed in Supplementary Table 2. PCR confirmation of correct 5′ and 3′ homologous recombination in each gene modification are presented in Supplementary Fig. 12.

Negative selection with 5-fluorocytosine

To remove the pYCm plasmids, we employed negative selection using 5-fluorocytosine. A mouse infected with the modified parasite clone was given drinking water containing 2 mg/ml of 5-fluorocytosine (Sigma-Aldrich, cat#F6627) in a dark bottle. After -3 days, most of the surviving parasites no longer carried pYCm plasmids and underwent limiting dilution cloning by injecting into mice via the tail vein. Seven days later, blood smears were used to identify the mice that were infected with parasites, and these parasites were genotyped again and used as the single cloned parasite.

Gametocyte induction in mice

The ICR mice were treated with phenylhydrazine ($80\,\mu\text{g/g}$ body weight; Sangon Biotech, China, cat#A600705-0025) to induce hyperreticulocytosis. Three days post-treatment, the mice were infected with 4×10^6 asexual stage parasites via tail vein injection. The peak of gametocytaemia usually occurred on day three post-infection. Male and female gametocytes were counted using Giemsa-stained thin blood films, and gametocytaemia was calculated as a percentage of the number of male or female gametocytes over the number of parasitized erythrocytes.

Gametocyte purification

The procedures for gametocyte purification were carried out according to previously described methods⁴⁸. Briefly, ICR mice were intraperitoneally treated with phenylhydrazine 3 days prior to parasite infection. Starting from 2 days post-infection, the mice were orally administered 0.12 mg/d of sulfadiazine (Sigma, cat#S8626) for 2 days to eliminate asexual stage parasites. Approximately 1 ml of mouse blood containing gametocytes was collected from the orbital sinus and then suspended in 6 ml of gametocyte maintenance buffer (GMB). GMB comprises 137 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 20 mM glucose, 20 mM HEPES, 4 mM NaHCO₃, 0.1% BSA, and has a pH of 7.2. The 7 ml

parasite sample was layered on top of a 2 ml 48% Nycodenz/GMB cushion in a 15 ml centrifugation tube. The cushion consisted of 27.6% w/v Nycodenz in 5 mM Tris-HCl (pH 7.2), 3 mM KCl, and 0.3 mM EDTA. After centrifugation at $1900\,g$ for 20 min, the gametocytes were collected from the interphase and washed twice with GMB for further use.

Exflagellation assay of male gametocytes

 $2.5\,\mu$ l of mouse tail blood with gametocytes was mixed with $100\,\mu$ l of exflagellation medium. The exflagellation medium was composed of RPMI 1640 supplemented with $100\,\mu$ M xanthurenic acid (XA, Sigma, cat#D120804), 2 unit/ml heparin, and pH 7.4. The mixture was incubated at 22 °C for 10 min. The number of parasite exflagellation centers (ECs) and total red blood cells were counted within a 1 × 1-mm square area of a hemocytometer under a light microscope. The exflagellation rate was calculated as the number of ECs per 100 male gametocytes.

In vitro ookinete culture

Mouse blood with the gametocytes was collected in the heparincontaining tubes and immediately mixed with the ookinete culture medium. This medium consisted of RPMI 1640 supplemented with 25 mM HEPES, 10% fetal calf serum, 100 μ M XA, and had a pH of 8.0. The blood/medium volume ratio was 1:10. The parasite samples were incubated at 22 °C for 16 h and analyzed using Giemsa-stained thin blood films. The number of ookinetes (including normal and abnormal ookinete in morphology) per 100 female gametocytes was calculated as the ookinete conversion rate.

Parasite genetic cross

ICR mice were treated intraperitoneally with phenylhydrazine for gametocyte induction. Three days post-treatment, an equal number (3×10^6) of asexual stage parasites from two different gene knockout lines were mixed and injected via the tail vein into the phenylhydrazine pre-treated mice. After 3 days, mouse blood with mixed gametocytes from two different parasite lines was collected from the mice and subjected for the in vitro gametocyte-gamete-zygote-ookinete development analysis using the in vitro ookinete culture described above.

Mosquito transmission of the parasite

Approximately 100 female *Anopheles stephensi* mosquitoes were allowed to feed on an anesthetized mouse with 4–6% gametocytaemia for 30 min. To evaluate midgut infection of parasite, mosquito guts $(n \sim 30)$ were dissected and stained with 0.1% mercurochrome 7 days post-feeding, and oocysts were tallied under a microscope. For quantifying salivary gland sporozoites, mosquito salivary glands $(n \sim 30)$ were dissected 14 days after feeding, with the sporozoites counted using a hemocytometer. Transmission efficacy was assessed by allowing ~ 30 infected mosquitoes to feed on a naïve mouse for 30 min at day 14 post-feeding. Parasite transmission from mosquito to mouse was monitored 5 days later via Giemsa-stained thin blood films. These procedures were performed in triplicate.

Flow cytometry analysis and sorting of male and female gametocytes

To analyze DNA content of male gametocytes, parasites containing gametocytes from the *DFsc7* or *DFsc7*;Δ*Rbpm1* lines were divided into two equal parts. One part was promptly fixed with 4% paraformaldehyde in PBS, while the other was exposed to exflagellation medium at 22 °C for 8 min to initiate gametogenesis before fixation. After staining with 4 μM Hoechst 33342 (Thermo Fisher Scientific, cat# 62249) for 10 min at room temperature and subsequent PBS washes, the samples were analyzed via flow cytometry on a BD LSRFortessa device (BD Biosciences, San Jose, CA, USA). Based on cell size and granularity, forward and side scatter signals were used to distinguish red blood cells from debris, doublets and white blood cells. Male gametocytes were identified by GFP fluorescence and analyzed for Hoechst 33342

fluorescence. For sorting gametocytes, parasites containing gametocytes were kept in GMB at 4 °C and sorted on a BD FACS AriallI based on GFP and mCherry fluorescence for male and female gametocytes, respectively. Sorted gametocyte purity was verified by re-analysis of a sample fraction.

Bulk RNA sequencing (RNA-seq)

Total RNA from 2 × 10⁷ purified gametocytes was isolated using TRIzol (Thermo Fisher Scientific, cat#15596026) according to the manufacturer's instructions. RNA integrity was confirmed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). mRNA was isolated with Oligo (dT) beads, fragmented, and reverse-transcribed to cDNA using random primers. Using DNA polymerase I, RNase H, dNTPs, and buffer, a second cDNA strand was synthesized. The resulting cDNA fragments were purified with the QIAQuick PCR Purification Kit (Qiagen, cat#28104), end-repaired, A-tailed, and ligated to Illumina sequencing adapters. The ligation products were size-selected using agarose gel electrophoresis, PCR amplified, and sequenced using the Illumina NovaSeq 6000 by Genedenovo Biotechnology Co., Ltd (Guangzhou, China).

Differential expression analysis of RNA-seq data

Illumina-generated paired-end FASTQ files were trimmed using Trim Galore (v0.6.10)75 (trim_galore --illumina -q 20 --paired --stringency 3 --length 25 -e 0.1 --fastqc --gzip) to remove the sequencing adapters and low quality reads. To refine the dataset, rRNA and tRNA were removed via a genome alignment program HISAT2 (v2.2.1)76 (hisat2 -p 12 -q --unconc-gz). The cleaned reads, around 40 million per sample, were aligned to the *Plasmodium yoelii* 17X reference genome (PlasmoDB-62 release) using HISAT2 (hisat2 -p 12 -q). The resulting BAM files were sorted by position and indexed with SAMtools (v1.16.1)⁷⁷ (samtools -sort | samtools index). Mapped reads were summarized using featureCounts (v2.0.3)⁷⁸. Gene expression analysis were performed in R (v4.2.1). Gene expression levels were normalized using transcripts per million (TPM) with the R package t-arae/ngscmdr (v0.1.0.181203)⁷⁹. Differential expressed genes (DEGs, fold change > 2, and false discovery rate < 0.05) were assessed by the R package edgeR (v3.40.2)80. The volcano plot of DEGs were generated by the ggplot2 (v3.4.2)81.

Bioinformatic analysis of global intron retention

A GFF file containing genomic intron information was crafted using a perl script from agat package^{§2} and an in-house bash script, then converted into a BED file with BEDOPS convert2bed (v2.4.41)^{§3}. Deep-Tools bamCoverage (v3.5.1)^{§4} was used to generate the bigWig files for peak visualization in Integrative Genomics Viewer (IGV, v2.16.1)³⁸, and calculate the peak score of each exon and intron regions. Low expressed genes (TPM below 30) were excluded. To exclude potential false hits, introns with peak scores exceeding 50% of adjacent exons were discarded in parental parasites. In mutant parasites, introns with peak scores under 50% of neighboring exons were also omitted. Before differential intron retention analysis, the introns were normalized based on the gene expression level:

Normalized intron counts = $\frac{Intron region counts \times 1000}{Corresponding gene counts}$

Differentially retained introns (fold change > 2 and false discovery rate <0.05) were assessed with the R package edgeR (v3.40.2)⁸⁰. These introns were further validated by IGV visualization and RT-PCR.

Bioinformatic analysis of RBP in the *P. falciparum* **and** *P. berghei* 189 putative RBPs had been predicted in silico in the *P. falciparum*³². Among them, 179 RBPs have homologous proteins in *P. yoelii* and *P. berghei*. Differential expression analysis of the 179 RBPs between male and female gametocytes of *P. berghei* was based on the public dataset

by Yeoh, L.M., 2017³¹. The RNA-seq FASTQ files from NCBI SRA database (Accession: PRJNA374918) were processed using Trim Galore (v0.6.10) and HISAT2 (v2.2.1) for quality trimming and rRNA/tRNA removal, respectively. The cleaned reads were mapped to the P. berghei ANKA strain genome (PlasmoDB-62 release) using HISAT2 (v2.2.1), and the resulting BAM files were sorted and indexed with SAMtools (v1.16.1). Mapped reads were summarized using featureCounts (v2.0.3), and the differential expression analysis of RBPs was performed by the R package edgeR (v3.40.2). Differential expression analysis of the 189 RBPs between male and female gametocytes of P. falciparum is based on the public dataset from Lasonder E. 2016³⁰. The RNA-seq FASTQ files from NCBI SRA (Accession: PRJNA305391) were processed similarly as above. The cleaned reads were mapped to the Plasmodium falciparum 3D7 reference genome. Given the absence of biological replicates in this dataset, differential expression analysis was performed by Cufflinks (v2.2.1)85 (cuffdiff -p 8 -- dispersion-method blind --library-norm-method geometric --library-type ff-firststrand). RBPs with fold change > 2 and false discovery rate < 0.05 were considered differentially expressed. The volcano plot of differentially expressed RBPs were generated by the ggplot2 (v3.4.2).

Antibodies and antiserum

The following primary antibodies were utilized: rabbit anti-HA (Cell Signaling Technology, cat#3724 S; IFA, 1:1000 dilution; IB, 1:1000 dilution), rabbit anti-mCherry (Abcam, cat# ab167453; IFA, 1:1000 dilution), rabbit anti-histone H3 antibody (Abcam, cat#ab1791; IFA, 1:1000 dilution), rabbit anti-Myc (Cell Signaling Technology, cat#2272 S; IFA, 1:1000 dilution; IB, 1:1000 dilution), mouse anti-α-Tubulin (Sigma-Aldrich, cat#T6199; IFA, 1:1000 dilution; IB, 1:1000 dilution; U-ExM, 1:500 dilution), mouse anti-β-Tubulin (Sigma-Aldrich, cat#T5201; IB, 1:1000 dilution) and mouse anti-HA (Santa Cruz Biotechnology, cat#sc-57592; IFA, 1:200 dilution). The secondary antibodies included: Alexa Fluor 555 goat anti-rabbit IgG (Thermo Fisher Scientific, cat#A-21428; IFA, 1:1000 dilution), Alexa Fluor 488 goat antirabbit IgG (Thermo Fisher Scientific, cat#A-31566; IFA, 1:1000 dilution), Alexa Fluor 555 goat anti-mouse IgG (Thermo Fisher Scientific, cat# A-21422; IFA, 1:1000 dilution; U-ExM, 1:500 dilution), Alexa Fluor 488 goat anti-mouse IgG (Thermo Fisher Scientific, cat#A-11001; IFA, 1:1000 dilution), Alexa Fluor 488 goat anti-mouse TER-119 (BioLegend, cat#116215; IFA, 1:500 dilution), Alexa Fluor 488 conjugated streptavidin (Invitrogen, cat# S32354; IFA, 1:1000 dilution), HRP-conjugated goat anti-rabbit IgG (Abcam, cat#ab6721; IB, 1:5000 dilution) and HRPconjugated goat anti-mouse IgG (Abcam, cat#ab6789; IB, 1:5000 dilution). The antiserum, including rabbit anti-BiP (IB, 1:1000 dilution) and rabbit anti-P28 (IFA, 1:1000), were previously in-house prepared in the laboratory86.

Immunofluorescence assay

Parasites fixed in 4% paraformaldehyde in PBS were placed on poly-L-lysine-coated coverslips in a 24-well plate and centrifuged at 550 g for 5 min. They were then permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature, blocked with 5% BSA/PBS at 4 °C overnight, and incubated with primary antibodies in 5% BSA/PBS for 1 h at room temperature. After three PBS washes, the samples were incubated with fluorescently labeled secondary antibodies in 5% BSA/PBS for 1 h at room temperature. Hoechst 33342 at a 1:5000 dilution in PBS was applied for 15 min at room temperature. Finally, the coverslips were washed, mounted in 90% glycerol, and sealed with nail varnish. Imaging was performed with a Zeiss LSM 780 confocal microscope at 100 × magnification.

Ultrastructure expansion microscopy (U-ExM)

According to the method described in 87, gametocytes were fixed in 4% paraformaldehyde in PBS, then transferred to poly-D-lysine-coated coverslips in a 24-well plate and centrifuged. They were incubated in a

1.4% formaldehyde (Sigma-Aldrich, cat#F8775) and 2% acrylamide (Sigma-Aldrich, cat# A4058) mixture in PBS overnight at 37 °C. Afterward, the coverslips were gelled in a monomer solution containing 23% sodium acrylate (Sigma-Aldrich, cat#408220), 10% acrylamide, and 0.1% N,N'-methylenbisacrylamide (Sigma-Aldrich, cat#M1533) in PBS with tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) at 37 °C for 1h of polymerization. After polymerization, the coverslips were moved to a 6-well plate with denaturation buffer (200 mM SDS, 200 mM NaCl, 50 mM Tris-HCl, and pH 8.8) for 15 min at room temperature to detach the gels. The gels were denatured in 1.5 ml Eppendorf tubes with denaturation buffer at 95 °C for 30 min, incubated with ddH₂O at room temperature overnight in a 10 cm dish for the first round of expansion. The expanded gels were incubated with mouse anti-α-Tubulin antibody diluted in 2% BSA/PBS at room temperature for 3 h, washed 3 times with PBS, and incubated with antimouse Alexa 555 diluted in 2% BSA/PBS at room temperature for 3 h. After 3 washes in PBS, the gels were transferred into 10 cm dishes and incubated with ddH₂O at room temperature for the second round of expansion. Subsequently, gel blocks of ~5 mm × 5 mm were excised from the expanded gels and placed in the cavity well of cavity well microscope slides, covered with a coverslip, and imaged using a Zeiss LSM 980 confocal microscope.

Protein extraction and immunoblot

Asexual blood parasites, gametocytes or ookinetes were lysed in RIPA buffer (Solaribio, cat#R0010) containing a protease inhibitor cocktail (MedChemExpress, cat#HY-K0010). After ultrasonication, the lysate was centrifuged at 14,000 g at 4 °C for 10 min. The resulting supernatant was mixed with SDS-PAGE loading buffer and heated at 95 °C for 5 min. Following SDS-PAGE separation, samples were transferred to a PVDF membrane (Millipore, cat#IPVH00010) and blocked with 5% milk in 1 × TBST (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween20) at 4 °C overnight. PVDF membranes were then incubated with primary antibodies at room temperature for 1h. After washing with 1×TBST, the membranes were incubated with an HRP-conjugated secondary antibody and then washed again with 1 × TBST. Finally, the membranes were visualized using a high-sensitivity ECL chemiluminescence detection kit (Vazyme, cat#E412-01), and the light emission was recorded either by X-ray film or by Azure Biosystems C280 (Azure Biosystems, USA).

Isolation of nuclear and cytoplasmic fractions

The procedures were performed with modifications according to the previous study88. Nycodenz-purified gametocytes were first released from red blood cells by incubating them with 0.15% saponin/PBS on ice for 5 min and then washed twice with ice-cold PBS. The parasite pellet was resuspended in ice-cold lysis buffer (20 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM MgCl₂, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 0.65% Nonidet P-40) supplemented with protease inhibitor cocktail. The lysate was transferred to a 1ml Dounce tissue grinder and homogenized gently for 80 strokes on ice. Nuclei were pelleted at 9000 g at 4 °C for 10 min, and the resulting supernatant represented cytoplasmic fractions. The nuclear pellet was washed twice with ice-cold lysis buffer before resuspension in one pellet volume of high salt buffer (20 Mm HEPES pH 7.8, 1M KCl, 1mM EDTA, 1mM EGTA, and 1mM DTT) supplemented with a protease inhibitor cocktail. After vigorous shaking at 4 °C for 30 min, the extract was centrifuged at 14,000 g at 4 °C for 10 min, and the resulting supernatant represented nuclear fractions. Immunoblotting was performed to analyze the proteins in each fraction.

Protein immunoprecipitation

Nycodenz-purified gametocytes containing 3×10^7 male gametocytes were lysed in 1 ml lysis buffer (0.01% SDS, 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM DTT) supplemented with protease inhibitor

cocktail. The lysate was transferred to a 1 ml Dounce tissue grinder and homogenized gently for 100 strokes on ice. The homogenate was transferred to an Eppendorf tube and incubated on ice for 10 min before centrifugation at 14,000 g at 4 °C for 10 min. The resulting supernatant was divided into two equal portions, with one portion mixed with 20 µl pre-balanced anti-GFP nanobody agarose beads (KT HEALTH, cat#KTSM1301) and the other portion mixed with anti-Myc nanobody agarose beads (KT HEALTH, cat#KTSM1306). Both portions were incubated at 4 °C for 2 h with rotation. The beads were then washed three times with lysis buffer before elution with SDS-PAGE loading buffer, followed by incubation at 95 °C for 5 min. Immuno-blotting was performed on equal volumes of the supernatant samples.

Transmission electron microscopy

Nycodenz-purified gametocytes were fixed at 8 mpa and 15 mpa in 2.5% glutaraldehyde in 0.1M phosphate buffer at 4 °C overnight, as previously described⁸⁹. Then, the samples were post-fixed in 1% osmium tetroxide at 4 °C for 2 h, treated *en bloc* with uranyl acetate, dehydrated, and embedded in Spurr's resin. Thin sections were sliced, stained with uranyl acetate and lead citrate, and examined in an HT-7800 electron microscope (Hitachi, Japan).

TurboID-based proximity-labeling and biotinylated protein pull-down

Nycodenz-purified gametocytes containing 1×10^8 male gametocytes from either the *Rbpm1::TurbolD* or *Rbpm1::T2A::TurbolD* line were incubated with 50 μ M biotin (Sigma-Aldrich, cat#B4639) at 37 °C for 20 min. After biotinylation, the parasites were pelleted, washed thrice with 1 ml ice-cold PBS to remove excess biotin, and then lysed with RIPA buffer containing a protease inhibitor cocktail via ultrasonication. The lysate was incubated on ice for 10 min before centrifugation at 14,000 g at 4 °C for 10 min. The supernatant was then mixed with 50 μ l pre-balanced streptavidin sepharose (Thermal Scientific, cat#SA10004) at 4 °C overnight. The beads were washed five times with 1 ml ice-cold RIPA buffer and then washed five times with 1 ml ice-cold PBS. The washed beads were resuspended in 200 μ l 100 mM Tris-HCl pH 8.5 followed by digestion with 1 μ g trypsin at 37 °C overnight.

Peptide desalting and mass spectrometry

Trifluoroacetic acid (TFA; Sigma-Aldrich, cat#T6508) was added to the trypsin-digested sample to a final concentration of 1%, and the precipitation of sodium deoxycholate was removed by centrifugation. The resulting supernatant was desalted using in-house-made StageTips that were packed with SDB-RPS (3 M EMPORE, cat#2241) and conditioned with 50 µl of 100% acetonitrile (ACN; Sigma-Aldrich, cat# 34851). After loading the supernatant onto the StageTips, centrifugation was performed at 3000 g for 5 min. The StageTips were then washed twice with 50 µl of 1% TFA/isopropyl alcohol (Sigma-Aldrich, cat# 19030) followed by a wash with 50 µl of 0.2% TFA. The peptides were eluted in glass vials (CNW Technologies, cat# A3511040) using 80% ACN/5% NH₄OH and dried at 45°C using a vacuum centrifuge (Eppendorf, Hamburg, Germany, cat#5305). The peptide samples were resolved in 2% ACN/0.1FA for LC-MS analysis. Liquid chromatography was performed on a high-pressure nano-flow chromatography system (Elute UHPLC, Bruker Daltonics). Peptides were separated on a reversed-phase column (40 cm × 75 µm i.d.) at 50 °C packed with 1.8 μm 120 Å C18 material (Welch, Shanghai, China) with a pulled emitter tip. A solution is 0.1% FA in H₂O, and B solution is 0.1% FA in ACN. The gradient time is 60 min and the total run time is 75 min including washes and equilibration. Peptides were separated with a linear gradient from 0 to 5% B within 5 min, followed by an increase to 30% B within 55 min and further to 35% B within 5 min, followed by a washing step at 95% B and re-equilibration. LC was coupled online to a hybrid TIMS quadrupole time-of-flight mass spectrometer (Bruker timsTOF Pro) via a CaptiveSpray nano-electrospray ion source. We performed data-dependent data acquisition in PASEF mode with 10 PASEF scans per topN acquisition cycle. Singly charged precursors were excluded by their position in the m/z-ion mobility plane and precursors that reached a 'target value' of 20,000 a.u. were dynamically excluded for 0.4 min. We used 100 ms to accumulate and elute ions in the TIMS tunnel. The MS1 m/z-range was acquired from 100 to 1700, and the ion mobility range from 1.5 to 0.7 V cm⁻². For dataindependent acquisition, we adopted the isolation scheme of 25 Da × 32 windows to cover 400-1200 mz. DIA files (raw) files were input to DIA-NN (v1.8.1)90 FASTA files downloaded from https://www.uniprot. org (UP000072874) were added. "FASTA digest for library-free search" and "Deep learning-based spectra, RTs, and IMs prediction" were enabled. "Generate spectral library" was also enabled. "Protein inference" was set to "gene". Other parameters were kept at their default settings. The protein groups and precursor lists were filtered at 1% FDR, using global q-values for protein groups and both global and runspecific q-values for precursors.

RNA isolation, RT-PCR and RT-qPCR

Total RNA was extracted from parasites using TRIzol reagent. cDNA was synthesized with the HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, cat#R212-02), using provided random hexamers, and utilized for PCR or qPCR analysis. qPCR was performed using 2×RealStar Green Fast Mixture (GenStar, cat#A301-101) with the following cycling program: a single incubation at 95 °C for 30 s, followed by 40 cycles (95 °C for 5 s, 60 °C for 40 s) on a CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). The housekeeping gene *gapdh* (PY17X_1330200) was used as a reference gene in the RT-qPCR. The relative expression was calculated using the 2^{ΔΔCT} method. The primers used for RT-PCRs and RT-qPCRs are listed in Supplementary Table 2.

UV crosslinking RNA immunoprecipitation (UV-RIP)

The Nycodenz-purified gametocytes, containing 6×10^7 male gametocytes in 6 ml ice-cold PBS, were placed in 10 cm dishes. Subsequently, they were irradiated using an HL-2000 HybriLinker (UVP, Upland, CA, USA) with 254 nm UV light at intensities of 400 mJ/cm² and 200 ml/cm². The gametocytes were then collected, centrifuged, and resuspended in 1 ml lysis buffer (1% TritonX-100, 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA) supplemented with 400 U/ml RNaseOUT (Thermo Fisher Scientific, cat#10777019) and a protease inhibitor cocktail. The lysate was transferred to a 1 ml Dounce tissue grinder and gently homogenized for 100 strokes on ice. The homogenate was then transferred to a tube and incubated at 4 °C for 25 min with rotation, followed by treatment with 30 U TURBO DNase (Thermo Fisher Scientific, cat#AM2238) at 37 °C for 15 min. The lysates were centrifuged at 14,000 g and 4 °C for 10 min. The supernatant was divided into two equal parts. One part was mixed with 20 µl of anti-GFP nanobody agarose beads (KT HEALTH, cat#KTSM1301), and the other part was mixed with 20 µl of anti-HA nanobody agarose beads (KT HEALTH, cat#KTSM1305). The mixtures were incubated with rotation at 4 °C for 2 h. The beads were washed six times with 500 µl RIP wash buffer (Millipore, cat#CS203177) at 4 °C and then incubated with 117 ul RIP wash buffer, 15 µl 10% SDS and 18 µl 10 mg/ml proteinase K (Millipore, cat#CS203218) at 55 °C for 30 min. RNA was isolated using phenol-chloroform extraction, and the purified RNA was reverse transcribed with random hexamer primers and determined by RT-qPCR.

In vitro RNA transcription (IVT)

To prepare biotinylated probes for Fig. 6O, P, IVT templates with T7 RNA polymerase promoter were obtained by PCR using the *P. yoelii* genome as a template. For Fig. 7H, IVT templates with T7 RNA polymerase promoter were obtained by PCR using the plasmid used in the *bfp* reporter assay as a template. Supplementary Table 2 provides a list of primers used to obtain the IVT templates. Subsequently,

Biotinylated RNA was produced using a MEGAscript kit (Thermo Fisher Scientific, cat#AM1334) and a biotin RNA labeling mix (Roche, cat#11685597910). To create a 20 μ l reaction volume, 1 μ g of PCR-amplified IVT templates were incubated at 37 °C for 2 h with 2 μ l of 10× reaction buffer, 2 μ l of T7 RNA polymerase enzyme mix, 2 μ l of biotin RNA labeling mix, and RNase-free water. The DNA templates were then removed from the RNA using TURBO DNase, and the biotinylated RNA was purified using the RNAclean Kit (TIANGEN, cat#4992728). In this process, the *kinesin8b* 14 probe, *kinesin8b* 11 probe, *PF16* E1 probe, and *PF16* II probe all have a length of 500 nt. Additionally, the *kinesin8b* 14 probe, *kinesin8b* I1 probe span the corresponding intron sequences.

RNA pull-down

Biotinylated RNA pull-down was performed using an RNA pull-down Kit (BersinBio, cat# Bes5102) following the manufacturer's protocol. Briefly, 1 µg of biotinylated RNA was denatured at 90 °C for 2 min and immediately cooled on ice for 2 min. The denatured RNA was then incubated with RNA structure buffer and RNase-free water at room temperature for 20 mi to facilitate RNA secondary structure formation. For cell lysate preparation, Nycodenz-purified gametocytes containing 3×10⁷ male gametocytes were lysed by RIP buffer, and the resulting lysate was centrifuged at 14,000 g at 4 °C for 10 min. The supernatant was then incubated with DNase I and agarose beads to remove the chromosomes, followed by incubation with folded RNAs, streptavidincoupled beads, and RNase inhibitor at room temperature for 2 h. The beads were subsequently washed five times with NT2 buffer at 4 °C, and proteins were retrieved from the beads by rinsing them with protein elution buffer. The retrieved proteins were then subjected to immunoblot assay.

bfp reporter assay

The Nycodenz-purified gametocytes from either *DFsc7* or *DFsc7;*Δ*Rbpm1* lines, which contain a *bfp* expression cassette in the *p230p* locus, were suspended in 200 μl of GMB. The samples were then transferred to a 15 mm glass bottom cell culture dish and imaged using a Zeiss LSM 780 confocal microscope at room temperature with 100× magnification. The laser illumination was set at 561 nm (mCherry), 491 nm (GFP), and 405 nm (BFP). BFP-positive parasites indicated that the intron in the *bfp* expression cassette had been spliced.

Other bioinformatic analysis and tools

The genomic sequences of target genes were downloaded from the PlasmoDB database (http://plasmodb.org/plasmo/). The sgRNAs of target gene were designed using EuPaGDT (http://grna.ctegd.uga.edu/). The analysis of flow cytometry data was performed using the FlowJo software (Tree Star, Ashland, OR, USA). The Gene Ontology (GO) enrichment analysis was performed using PlasmoDB. Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) with either a two-tailed Student's *t*-test or Mann-Whitney test as appropriate. Error bars represent the standard error of the mean (SEM) for triplicate experiments. *p* values were indicated in the figures above the two groups being compared, with a value <0.05 considered significant. The protein signal on the blotting membrane was quantified using ImageJ software (NIH, Bethesda, MD, USA), and the background was subtracted from each signal. Each signal was then normalized to the Bip signal.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All relevant data in this study are submitted as supplementary source files. Source data are provided with this paper. The RNA-seq data for the *P. yoelii* male- and female gametocyte transcriptome have been deposited in the Gene Expression Omnibus database under the accession number GSE222860. The RNA-seq data for male gametocyte transcriptome of the *P. yoelii Rbpm1* knockout parasite line is available under accession number GSE223170. The mass spectrometry proteomic data have been deposited in the ProteomeXchange with identifier PXD044094 (https://www.iprox.cn//page/project.html?id=IPX0006804000). Source data are provided with this paper.

Code availability

All code and supporting files for transcriptome and intron retention analysis in this study were available in Zenodo (https://doi.org/10.5281/zenodo.10979262).

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Author contributions

J.G. and J.Y. designed the study. J.G., P.W., X.Z.(Xiaoming Zhang), W.L., and X.Z. (Xiaolong Zhang) generated the modified parasites. J.G. performed phenotype analysis, protein analysis, electron microscopy, RNA analysis, and reporter assays. P.W. conducted mass spectrometry and protein analysis. X.M. performed the bioinformatics analysis. L.J., J.L., H.C., and J.Y. supervised the work. J.G., X.M., and J.Y. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Lubin Jiang, Jian Li, Huiting Cui or Jing Yuan.

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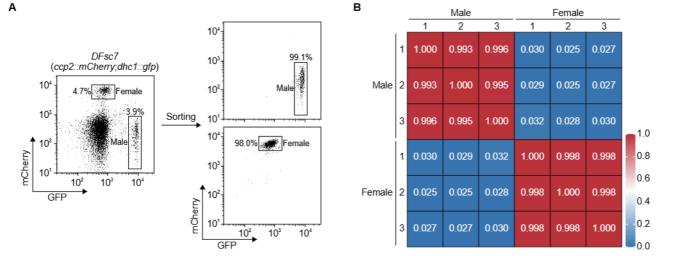
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Supplemental Information

An axonemal intron splicing program sustains *Plasmodium* male development

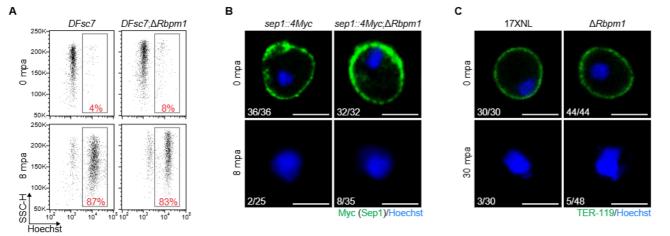
Jiepeng Guan^{1,#}, Peijia Wu^{1,#}, Xiaoli Mo^{1,#}, Xiaolong Zhang^{3,#}, Wenqi Liang¹, Xiaoming Zhang¹, Lubin Jiang^{3,*}, Jian Li^{1,*}, Huiting Cui^{1,*} and Jing Yuan^{1,2,*}

- 1. Supplementary Figures 1-13 and figure legends
- 2. Supplementary Table 1. List of genetically modified parasite strains used in this study
- 3. Supplementary Table 2. Oligonucleotides and primers used in this study



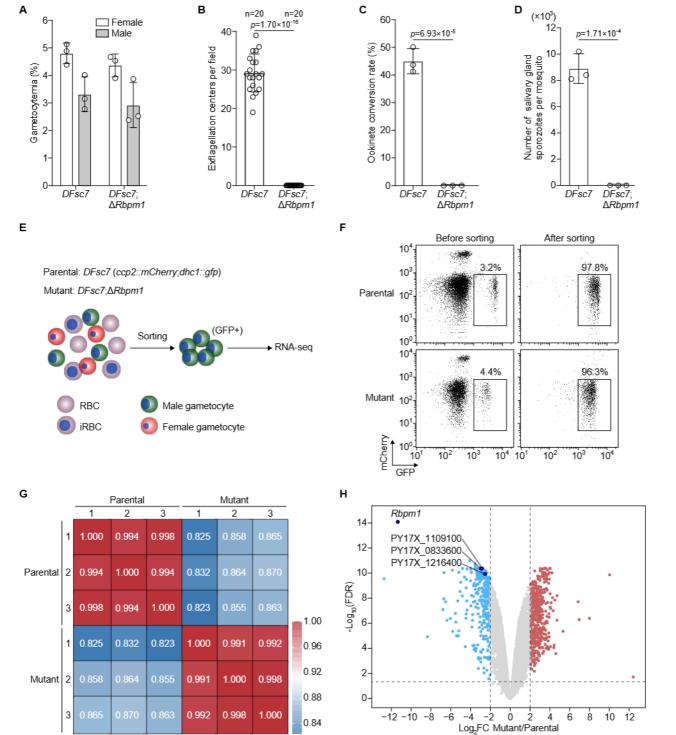
Supplementary Figure 1. Purification of male and female gametocytes of the *P. yoelii* parasite for transcriptome analysis

- **A.** Purification of male (GFP+) and female (mCherry+) gametocytes from a *P. yoelii* parasite reporter line *DFsc7* using flow cytometry sorting. The purity of gametocytes was shown. Representative from three independent experiments.
- **B.** Pearson's correlation coefficient analysis of global gene expression between male and female gametocytes based on RNA-seq data with three biological replicates.



Supplementary Figure 2. Normal ability of genome replication and erythrocyte rupture for the RBPm1-null parasite during male gametogenesis

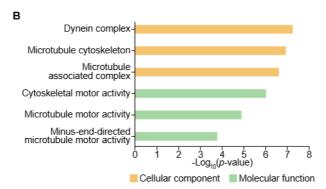
- **A.** Flow cytometry analysis of genomic DNA content in male gametocytes during gametogenesis. The DFsc7; $\Delta Rbpm1$ is a DFsc7-derived RBPm1-null parasite line. Male gametocytes (GFP+) were gated, and genomic DNA content was measured based on the Hoechst 33342 fluorescence intensity. Representative for three independent experiments.
- **B.** IFA detection of the parasitophorous vacuole membrane (PVM) rupture. SEP1 protein is a marker for PVM. Male gametocytes from the sep1::4Myc parasite and the derived RBPm1-null parasite $sep1::4Myc;\Delta Rbpm1$ were analyzed. Representative for three independent experiments. Scale bars: 5 µm.
- C. IFA detection of the erythrocyte plasma membrane (EM) rupture. TER-119 protein is a marker of mouse EM. Male gametocytes from the 17XNL and $\Delta Rbpm1$ parasites were stained with anti-TER-119 antibody. Representative for three independent experiments. Scale bars: 5 μ m.



Supplementary Figure 3. Generation, purification and transcriptome analysis of male gametocytes with RBPm1 deficiency

- **A-D.** Phenotype analysis of DFsc7 (parental) and DFsc7; $\Delta Rbpm1$ (mutant) parasite lines, including gametocyte formation (**A**), male gamete formation (**B**), ookinete formation $in\ vitro\ (\mathbf{C})$, and salivary gland sporozoite in mosquitoes (**D**). Data are means \pm SEM of three independent experiments, two-sided t-test.
- **E.** Flowchart showing the purification of male gametocytes (green, GFP+) from both DFsc7 and DFsc7; $\triangle Rbpm1$ parasites for transcriptome analysis via RNA-seq.
- **F.** Flow cytometry detection of male gametocytes (GFP+) before and after sorting, with indicated purity. Representative results from three independent experiments.
- **G**. A heatmap showing the Pearson correlation coefficient between DFsc7 (parental) and DFsc7; $\Delta Rbpm1$ (mutant) male gametocyte RNA-seq data.
- **H.** A volcano plot showing the differentially expressed genes in male gametocytes between the DFsc7 (parental) and DFsc7; $\Delta Rbpm1$ (mutant) lines. The threshold for the log_2 fold change (log_2FC) and false discovery rate (FDR) are ± 2 and 0.05, respectively. There are 481 genes up-regulated and 295 genes down-regulated in male gametocytes after loss of RBPm1. Three down-regulated genes (PY17X_1109100, PY17X_0833600, and PY17X_1216400), which exhibited intron retention in further study, are highlighted.

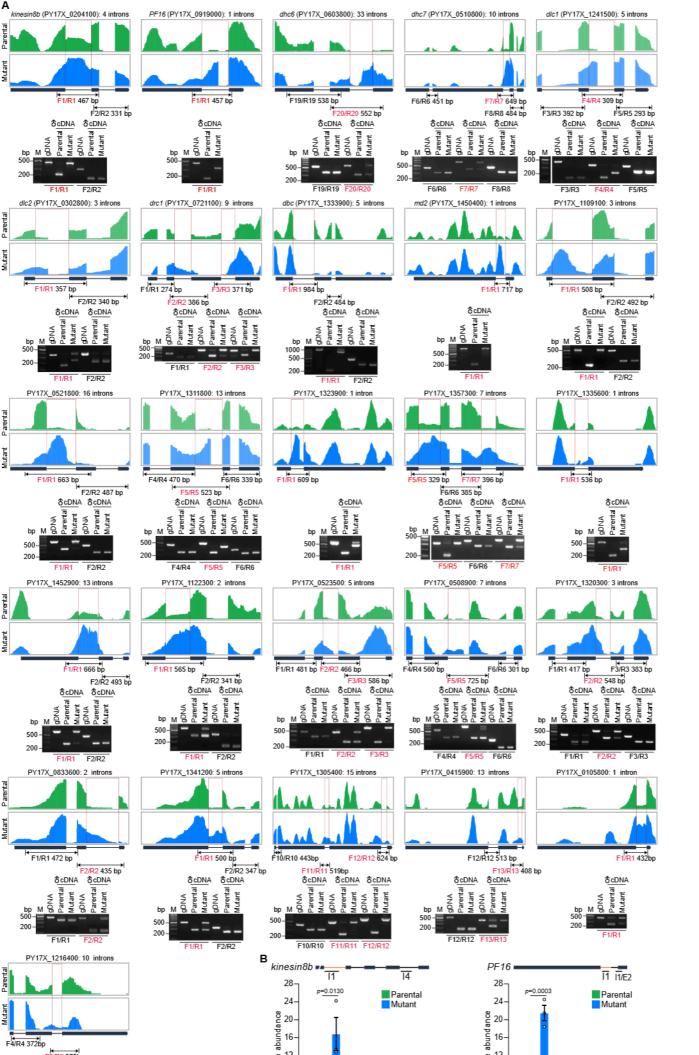
| Molecular function | Gene ID | Total number of introns in gene | # of the retained intron | Length of the retained intron (bp) | Male transcription (normalized counts) | Female transcription (normalized counts) | Ratio (Male/Female) |
|-------------------------------|---------------------------|---------------------------------------|--------------------------|------------------------------------|--|--|------------------------|
| Basal body & axoneme assembly | kinesin8b (PY17X_0204100) | 4 | 1 | 239 | 5148 | 2 | 3217 |
| | PF16 (PY17X_0919000) | 1 | 1 | 276 | 580 | 38 | 15 |
| Axoneme motility | dhc6 (PY17X_0603800) | 33 | 20 | 241 | 842 | 5 | 160 |
| | dhc7 (PY17X_0510800) | 10 | 7 | 235 | 1070 | 3 | 318 |
| | dlc1 (PY17X_1241500) | 5 | 4 | 193 | 296 | 68 | 4 |
| | dlc2 (PY17X_0302800) | 3 | 1 | 195 | 503 | 19 | 27 |
| | drc1 (PY17X_0721100) | 9 | 2, 3 | 179, 150 | 597 | 93 | 6 |
| | dbc (PY17X_1333900) | 5 | 1 | 772 | 2059 | 40 | 51 |
| Function unknown | md2 (PY17X_1450400) | 1 | 1 | 505 | 1534 | 53 | 29 |
| | PY17X_1109100 | 3 | 1 | 353 | 1662 | 55 | 30 |
| | PY17X_0521800 | 16 | 1 | 278 | 777 | 38 | 20 |
| | PY17X_1311800 | 13 | 5 | 248 | 1342 | 3 | 446 |
| | PY17X_1323900 | 1 | 1 | 322 | 710 | 103 | 7 |
| | PY17X_1357300 | 7 | 5, 7 | 212, 143 | 2644 | 4 | 630 |
| | PY17X_1335600 | 1 | 1 | 338 | 1320 | 3 | 417 |
| | PY17X_1452900 | 13 | 1 | 367 | 1686 | 6 | 295 |
| | PY17X_1122300 | 2 | 1 | 240 | 161 | 10 | 15 |
| | PY17X_0523500 | 5 | 2, 3 | 192, 318 | 348 | 1 | 533 |
| | PY17X_0508900 | 7 | 5 | 380 | 485 | 10 | 49 |
| | PY17X_1320300 | 3 | 2 | 183 | 195 | 24 | 8 |
| | PY17X_0833600 | 2 | 2 | 302 | 526 | 12 | 46 |
| | PY17X_1341200 | 5 | 1 | 208 | 731 | 1 | 803 |
| | PY17X_1305400 | 15 | 11, 12 | 261, 330 | 1583 | 14 | 116 |
| | PY17X_0415900 | 13 | 13 | 171 | 431 | 2 | 201 |
| | PY17X_0105800 | 1 | 1 | 193 | 107 | 3 | 33 |
| | PY17X_1216400 | 10 | 5 | 134 | 712 | 1 | 596 |



Supplementary Figure 4. Expression and function information of 26 intronretained genes identified in the RBPm1-null male gametocytes

A. List of 26 genes with intron retention detected in the RBPm1-null male gametocytes. Among them, 22 genes had one retained intron while 4 genes (*drc1*, PY17X_1357300, PY17X_0523500, and PY17X_1305400) possessed two retained introns after loss of RBPm1. Information including the protein function, gene ID, exon-intron structure, retained intron, and gender transcription, is provided. Gender transcription (TMM normalized counts) were from the gametocyte transcriptome data in this study.

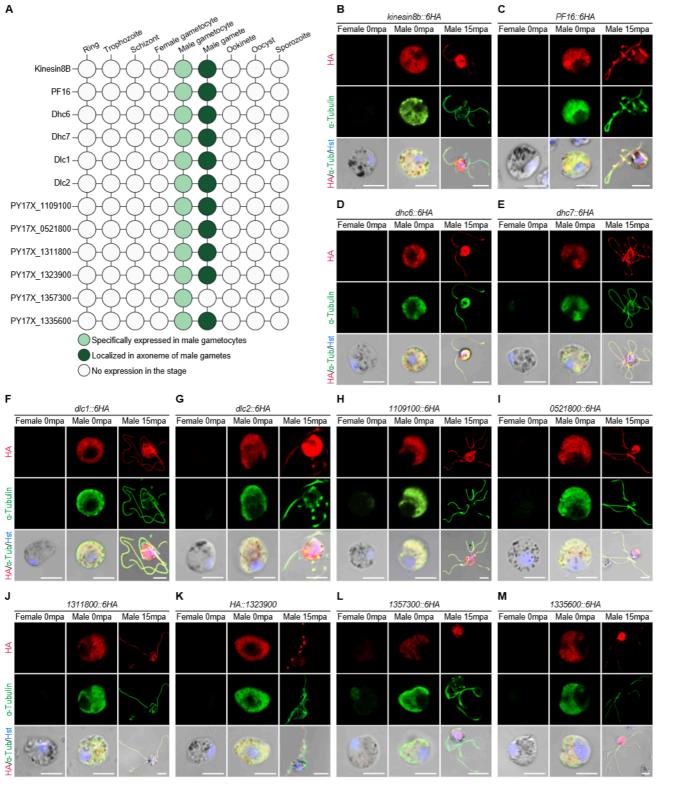
B. Gene ontology enrichment analysis of the 26 genes indicates male-specific or preferential biological processes. Hypergeometric test was applied.



Supplementary Figure 5. Verification of intron retention for 30 introns at 26 genes in the RBPm1-null male gametocytes

A. RT-PCR confirmation of intron retention in the 26 genes after loss of RBPm1. For each gene, the mapped views of the RNA-seq results (*DFsc7* in green and *DFsc7*;Δ*Rbpm1* in blue, representative for three biological replicates) and the exonintron structure (black) are shown in the upper panels. Intron retention is highlighted with red boxes. The primers (F or R) designed for detecting the intron via RT-PCR and the expected PCR products are shown. RT-PCR analysis using the genomic DNA (gDNA) from 17XNL parasite, complementary DNA (cDNA) from male gametocytes of parental and mutant parasites showed the intron retention (red) and intron splicing (black).

B. RT-qPCR confirmation of intron retention in the *kinesin8b* and *PF16* genes after loss of RBPm1. The top schematic of gene exon-intron structure shows the positions of the retained intron (orange line) and the RT-qPCR amplicon. RT-qPCR analysis using cDNA from male gametocytes of parental and mutant parasites showed the retention of *kinesin8b* intron1 and *PF16* intron1. Data are means \pm SEM from three independent experiments, two-sided *t*-test.



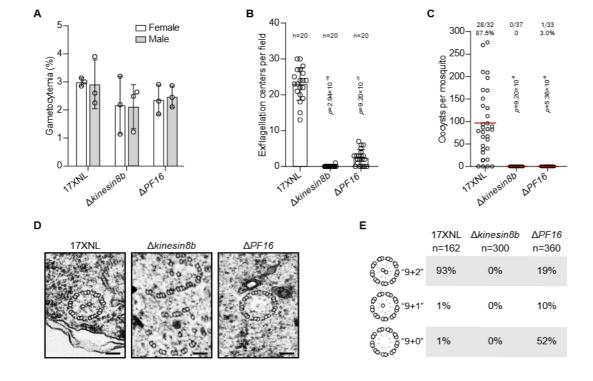
Supplementary Figure 6. RBPm1-regulating genes encode axoneme-associated proteins

A. Summary of protein stage expression and localization of 12 selected intron-retained genes in the *P. yoelii*. These genes include 6 annotated genes (*kinesin8b*, *PF16*, *dhc6*, *dhc7*, *dlc1*, *dlc2*) and 6 unannotated genes (PY17X_1109100, PY17X_0521800, PY17X_1311800, PY17X_1323900, PY17X_1357300, PY17X_1335600). Each gene was endogenously tagged at the N- or C-terminus with a 6HA in the 17XNL, generating the HA-tagged lines for protein expression and localization analysis. Due to the space limit, only the results of protein expression at the gametocytes were shown in **B-M**. **B-M**. Protein expression and localization analysis of the 12 intron-retaining genes in gametocytes. IFA of the HA-tagged target protein and α-Tubulin in female gametocytes (0 mpa) and male gametocytes (0 and 15 mpa). Representative results from two independent experiments. Scale bars: 5 μm.

| kinesin8b (PY17X_0204100) | PF16 (PY17X_0919000) |
|--|---|
| exon 1 intron 1 spliced exon 2 17XNL ··· CTAAAGgttggggctagttaga······agGAAAC··· K | exon 1 intron 1 spliced exon 2 17XNL···TTGCAAgtaaaataaacaaaaa······agGAAATA··· E I |
| ΔRbpm1····CTAAAGgttggggctagttaga······agGAAAAC··· | ΔRbpm1···TTGCAAgtaaaataaaacaaaa······agGAAATA··· |
| dhc6 (PY17X_0603800) | dhc7 (PY17X_0510800) |
| exon 20 intron 20 spliced exon 21 17XNL ··· TTATAT G g t ······ g a g c a t t a a ····· a g ATT CAC ··· \[\begin{array}{c} \text{exon 20} \\ \text{TXNL ··· TTATAT G} \\ \text{E } \\ \text{T } \\ \text{S} \end{array} | exon 7 intron 7 spliced exon 8 17XNL ··· CCACAACgt ······ a a a g c t t a g ····· agTTAAT ··· |
| $\Delta Rbpm1 \cdots \underbrace{\frac{1}{L} \underbrace{\frac{1}{Y} \underbrace{G}}_{H} \cdots \underbrace{\frac{1}{K} \underbrace{\frac{1}{K}}_{H} \underbrace{\frac{1}{K}}_{H}}_{h} \cdots \underbrace{\frac{1}{K} \underbrace{\frac{1}{K}}_{H} \underbrace{\frac{1}{K}}_{H}}_{h} \cdots \underbrace{\frac{1}{K} \underbrace{\frac{1}{K}}_{H} \underbrace{\frac{1}{K}}_{H}}_{h} \cdots \underbrace{\frac{1}{K}}_{H} \underbrace$ | $ \frac{ \text{intron 7 retained} }{ P Q R} \cdots \frac{ \text{a a a g c t t a g}}{ K A *} \cdots \frac{ \text{ag}}{ \text{TTAAT}} $ |
| dlc1 (PY17X 1241500) | dlc2 (PY17X 0302800) |
| exon 4 intron 4 spliced exon 5 17XNL ··· TACATTAg taacatattaataa ······ ag TAAGTT ··· Y I S | exon 1 intron 1 spliced exon 2 17XNL ··· ATAAGAg t a t a a a g a a c a a t g ······ a g AAATCT ··· R |
| ΔRbpm1····TACATTAgtaacatattaatataa······agTAAGTT···· | intron 1 retained ΔRbpm1···ΔΤΑΔΘΑ gtataaagaacaatg······ag AAATCT··· |
| drc1 (PY17X 0721100) | dbc (PY17X 1333900) |
| exon 2 intron 2 spliced exon 3 | exon 1 intron 1 spliced exon 2 |
| 17XNL ··· TTGAAAgtattgagaaaataa ······ ag CTGAAA··· intron 2 retained | 17XNL ··· AAAAAT g t ······ g t g g t g t a g ······ a g ATTTGT ··· I intron 1 retained |
| ∆Rbpm1··· TTGAAAgtattgagaaaataa ····· agCTGAAA··· | $ \Delta Rbpm1 \cdots \underbrace{AAA}_{K} \underbrace{AAT}_{N} \underbrace{gt \cdots gt g}_{V} \underbrace{gt g}_{V} \underbrace{tag}_{*} \cdots ag \underbrace{ATTTGT}_{*} \cdots $ |
| md2 (PY17X_1450400) | PY17X_1109100 |
| exon 1 intron 1 spliced exon 2 17XNL ··· AGAAAT Gg t ······· t c t a g c t a a ······ a g AT T T C ··· intron 1 retained | exon 1 intron 1 spliced exon 2 17XNL ··· GCGGGGg taatttaatattata ······ agTTCTAAG··· intron 1 retained |
| $\frac{\Delta Rbpm1 \cdots \underline{AGAAAT}}{R} \frac{Ggt}{N} \frac{\cdots \cdots \underline{tct}}{S} \frac{\underline{agctaa}}{S} \frac{\cdots \cdot \underline{ag}}{\star}$ | intron 1 retained ΔRbpm1····GCGGGGgtaatttaatattata······agTTCTAAG···· |
| PY17X_0521800 | PY17X_1311800 |
| exon 1 intron 1 spliced exon 1 17XNL ···ATAAGg t ······ t g t t t a t a a ······ a g C GCAGTA ··· I S | exon 5 intron 5 spliced exon 6 17XNL ··· GAAGACgtcagtatataaaaa ······ agATTAAG··· E D I K |
| $ \Delta Rbpm1 \cdots \underline{ATA} \underline{AGgt} \cdots \cdots \underline{tgt} \underbrace{tta}_{k} \underbrace{taa}_{k} \cdots \cdots \underbrace{agCGCAGTA}_{k} \cdots $ | intron 5 retained ΔRbpm1 GAAGACgtcagtatatataaaaaagATTAAG |
| PY17X 1323900 | PY17X 1357300 |
| exon 1 intron 1 spliced exon 2 17XNL AATATT Ggt g c a a a a t a a a g AAAGA E R | exon 5 intron 5 spliced exon 6 17XNL TGTACAgttaaaaaatagagGGAATT |
| $\Delta Rbpm1 \cdots \underbrace{\frac{AAT}{N} \frac{ATT}{I} \frac{Ggt}{G} \cdots \frac{gca}{A} \frac{aaa}{K} \frac{taa}{k} \cdots agAAAGA \cdots}_{\text{intron 1 retained}}$ | intron 5 retained ΔRbpm1··· TGTACAGttaaaaaatag ····· agGGAATT··· |
| PY17X_1335600 | PY17X 1452900 |
| exon 1 intron 1 spliced exon 2 17XNL ··· TATTTA Gg t ······ a a a t t a t g a ······ a g GGACT ··· | exon 1 intron 1 spliced exon 2 17XNL ··· CAACTGg t ······ g a g a a g t a g ····· ag GTTCCT ··· |
| $\Delta Rbpm1 \cdots \underbrace{TATTTA}_{Y} \underbrace{Ggt}_{G} \cdots \underbrace{K}_{K} \underbrace{tga}_{M} \cdots \underbrace{ag}_{M} GGACT \cdots$ | Q L intron 1 retained ΔRbpm1CAACTGg t g a g a a g t a g agGTTCCT |
| PY17X 1122300 | PY17X 0523500 |
| exon 1 intron 1 spliced exon 2 17XNL ··· TTAAATAgtaagtaaaataaaagCAAAC | exon 2 intron 2 spliced exon 3 17XNL ··· ACCAGgt ······ c a a a a g t a g ······ a g CCAACGA··· |
| L N intron 1 retained ΔRbpm1··· TTA AAT Agtaagtaaataaa·····agCAAAC··· | T S intron 2 retained ΔRbpm1ΔCCAGgtcaaaagtagagCCAACGA |
| | |
| PY17X_0508900 exon 5 intron 5 spliced exon 6 17XNL ··· ATTCAGgtatgcaatttttaa····· ag AAATTC··· | PY17X_1320300 exon 2 intron 2 spliced exon 3 17XNL···AAAAATgtatgatgcatatttaa······agATGATA··· |
| I Q intron 5 retained ΔRbpm1ΔTTCAGgtatgcaatttttaaag AAATTC | K N intron 2 retained ΔRbpm1···ΔΑΔΑΑΤgtatgatgcatatttaa······agATGATA··· |
| T Q V C N F * | K N V * |
| PY17X_0833600 | PY17X_1341200 |
| exon 1 intron 2 spliced exon 2 17XNL ··· ATT AAG g t a a t g t a a a a a g ······ a g GAA AAT ··· K intro 2 outsined E N | exon 1 intron 1 spliced exon 2 17XNL ··· GAA GAAg t ······ a t g a t t t a a ······ a g CGAAAC ··· R N |
| ΔRbpm1···ATT AAG gta atgtaaaaag······agGAAAAT··· | ΔRbpm1···· GAA GAA g t ······ a t g a t t t a a ······ a g C GAAAC ··· E E ···· M I ··· * |
| PY17X 1305400 | PY17X 0415900 |
| exon 11 intron 11 spliced exon 12 17XNL ··· TTAAGAgt ······ a a g a a a t a a ····· a g GTGAAA ··· | exon 13 intron 13 spliced exon 14 17XNL···AAAAAAGg t ······ t t t t t g t a a ······ a g ATAAGG ··· |
| L R V K intron 11 retained ΔRbpm1··· TTAAGAgt ····· aagaaataa ····· agGTGAAA··· L R ··· K K ** | ΔRbpm1···AAAAAGgt.·····tttttgtaa······agATAAGG··· K K ···· F L * |
| PY17X 0105800 | PY17X 1216400 |
| | <u> </u> |

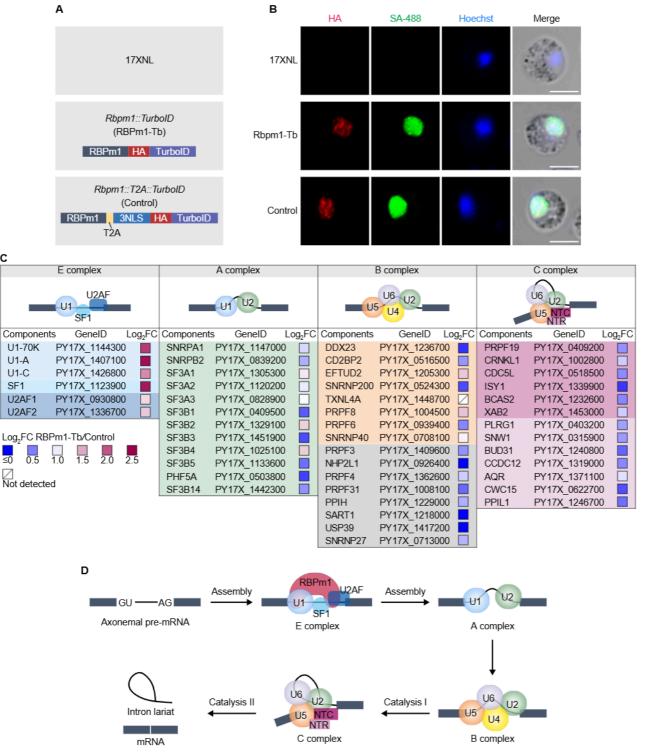
Supplementary Figure 7. Intron retention causes premature stop codons in RBPm1 target transcripts

Amino acid and nucleotide sequence analysis of the retained introns in the 26 genes. For each gene, intron retention (highlighted in orange lowercase letter) creates at least one premature stop codon (red asterisk) at the transcripts in the RBPm1-null parasites.



Supplementary Figure 8. Depletion of *kinesin8b* or *PF16* phenocopies RBPm1 deficiency

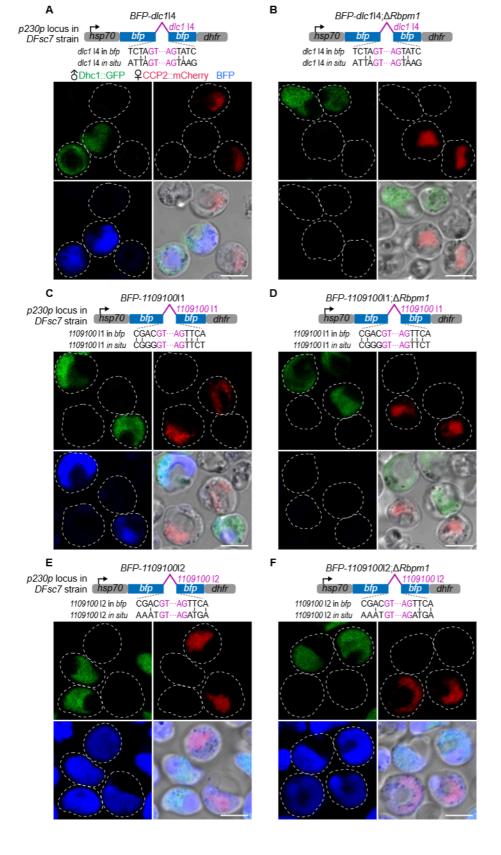
- **A.** Female and male gametocyte formation in mice. Data are means \pm SEM from three independent experiments.
- **B.** Exflagellation center (EC) formation of male gametocytes at 10 mpa. Data are means \pm SEM from three independent experiments, two-sided *t*-test.
- C. Midgut oocyst formation in mosquitoes at 7 days after blood feeding. x/y at the top represents the number of mosquitoes containing oocysts/the number of dissected mosquitoes, and the percentage represents the infection prevalence of mosquitoes. Red lines show the mean value of oocyst numbers, two-sided Mann-Whitney U test. Representative results from two independent experiments.
- **D.** Transmission electron microscopy of axoneme architecture in male gametocytes at 8 mpa. Scale bars: 100 nm.
- **E.** Quantification of axoneme formation in the mutant parasites in **D**. n is the total number of the intact and defective axoneme structures observed in each group. Representative for three independent experiments.



С

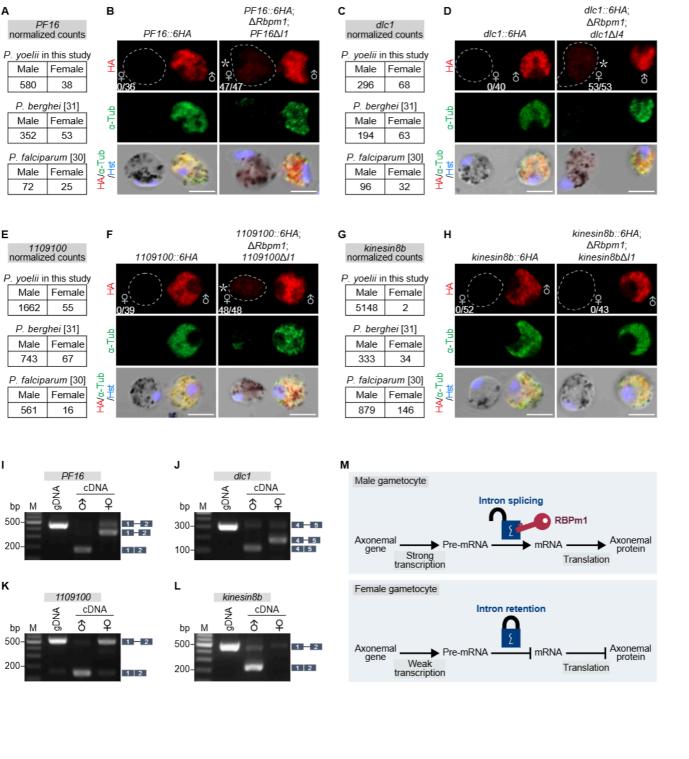
Supplementary Figure 9. RBPm1 interacts with spliceosome E complex

- **A.** A schematic of two modified parasite lines generated for detecting the RBPm1-interacting proteins in gametocytes by TurboID ligase-based proximity labeling. Endogenous RBPm1 was C-terminally tagged with an HA::TurboID motif by CRISPR-Cas9 in 17XNL, generating the *Rbpm1::TurboID* line. A control line *Rbpm1::T2A::TurboID* was generated, in which a "ribosome skip" T2A was inserted between RBPm1 and 3NLS::HA::TurboID for separated nuclear expression of RBPm1 and TurboID.
- **B.** Co-staining of HA-tagged TurboID ligase (red) and biotinylated proteins (SA-488, green) in male gametocytes of the *Rbpm1::TurboID* and *Rbpm1::T2A::TurboID* lines. Gametocytes incubated with 50 μM biotin at 37°C for 20 minutes were co-stained with the fluorescent-conjugated streptavidin (SA-488) and anti-HA antibody. Scale bars: 5 μm. Representative for three independent experiments.
- C. Protein interaction analysis between RBPm1 and spliceosome at different stages of assembly based on the data from TurboID-based proximity labeling and mass spectrometry. The upper panels show the spliceosome complexes from early to later stages during assembly, including E, A, B, and C complexes. The lower panels show the enrichment level (log₂FC value) of protein components in different spliceosome complexes.
- **D.** Proposed model showing RBPm1 interaction with the early spliceosome E complex for intron splicing of axonemal genes.



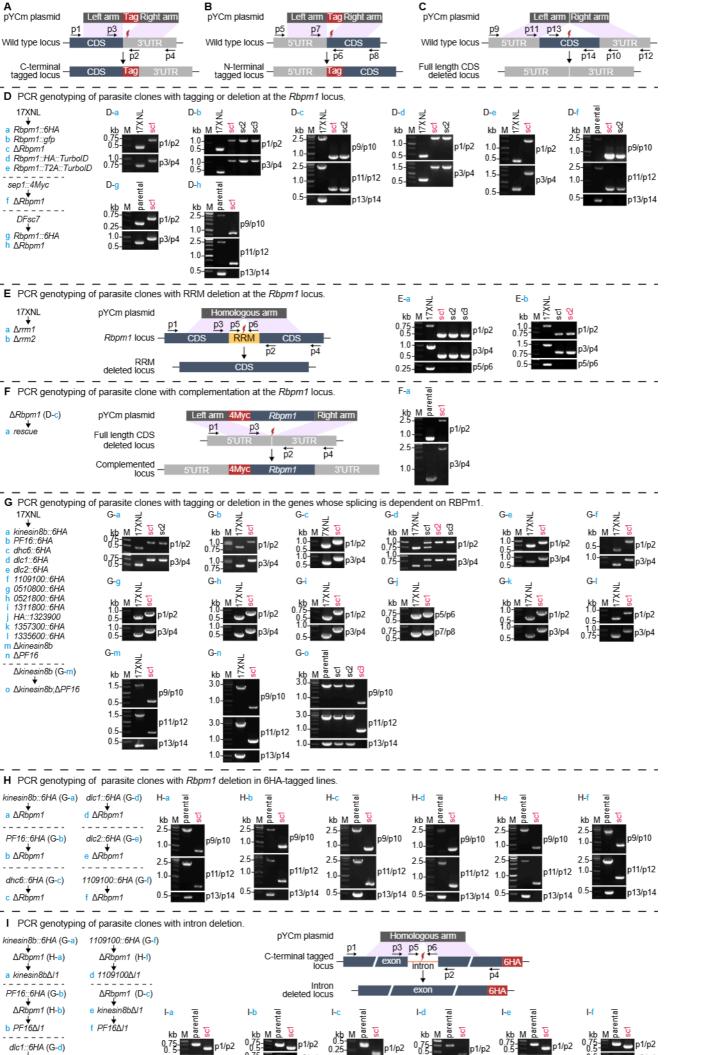
Supplementary Figure 10. RBPm1-dependent splicing of axonemal introns inserted in the reporter gene. Related to Figure 7

- **A.** A transgenic line *BFP-dlc1*I4 with a *dlc1* intron4 (*dlc1*I4, purple line)-inserted *bfp* cassette integrated at the *p230p* locus of the *DFsc7* line. Similar analysis as in **Figure 5B**. *dlc1* intron4 was inserted into the *bfp* gene at the nucleotides 455-456 to mimic the splice site (vertical lines) of *in situ dlc1*I4. BFP expression was detected specifically in male gametocytes of the *BFP-dlc1*I4 parasites. Representative for three independent experiments. Scale bars: 5 μm.
- **B.** A *BFP-dlc1*I4 derived RBPm1 mutant line, *BFP-dlc1*I4; $\Delta Rbpm1$, showed no BFP expression in male gametocytes. Representative for three independent experiments. Scale bars: 5 μ m.
- C. Effect of the *PY17X_1109100* intron1 (*BFP-1109100*I1) insertion on the gametocyte expression of BFP. Similar analysis as in A. *PY17X_1109100* intron1 was inserted into the *bfp* gene at the nucleotides 390-391 to mimic the splice site (vertical lines) of *in situ PY17X_1109100* intron1. BFP expression was detected specifically in male gametocytes of the *BFP-1109100*I1 parasites.
- **D.** A *BFP-1109100*I1 derived RBPm1 mutant line, *BFP-1109100*I1; $\triangle Rbpm1$, showed no BFP expression in male gametocytes. Similar analysis as in **B**.
- **E.** Effect of the *PY17X_1109100* intron2 (*BFP-1109100*I2) insertion on the gametocyte expression of BFP. Similar analysis as in **A**. *PY17X_1109100* intron2 was inserted into the *bfp* gene at the nucleotides 384-385. BFP expression was detected in both male and female gametocytes of the *BFP-1109100*I2 parasites.
- **F.** A *BFP-1109100*I2 derived RBPm1 mutant line *BFP-1109100*I2; $\Delta Rbpm1$, showed BFP expression in both male and female gametocytes. Similar analysis as in **B**.



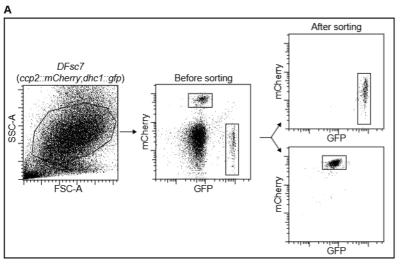
Supplementary Figure 11. Intron retention prevents expression of axonemal proteins in female gametocytes

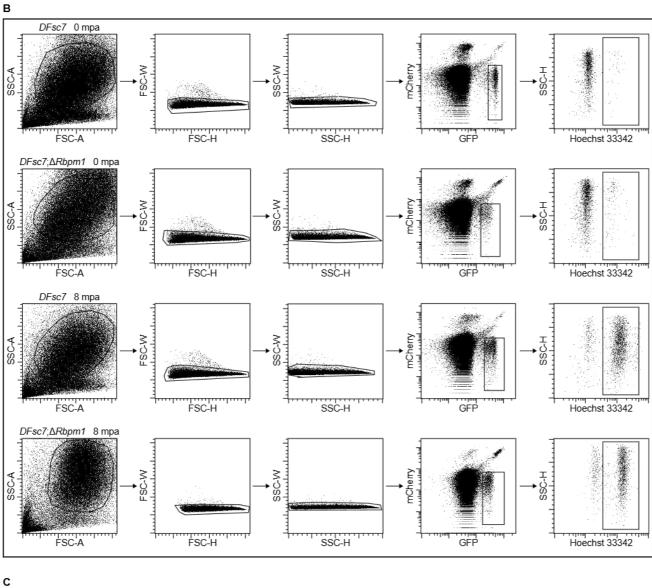
- **A.** Transcription level of the *PF16* gene in male and female gametocytes. TMM normalized counts are from the gametocyte transcriptomes in this study and the published dataset contributed by Yeoh, L.M. and Lasonder, E.
- **B.** IFA of 6HA-tagged PF16 in female and male gametocytes of the PF16::6HA and $PF16::6HA;\Delta Rbpm1;PF16\Delta I1$ (PF16 intron1-deleted line described in the **Figure 5D**) parasites. In each image, one male and one female gametocytes were shown. The HA-positive female gametocyte was highlighted with an asterisk. x/y represents the number of HA-positive female gametocytes/the total number of female gametocytes tested. Representative for three independent experiments. Scale bars: 5 μ m.
- C and D. Low-level expression of the Dlc1 protein detected in female gametocytes after deletion of the *dlc1* intron4. Similar illustration as in A and B. The *dlc1* intron4-deleted line was described in the **Figure 5G**.
- **E** and **F**. Low-level expression of the PY17X_1109100 protein detected in female gametocytes after deletion of the *PY17X_1109100* intron1. Similar illustration as in **A** and **B**. The *PY17X_1109100* intron1-deleted line was described in the **Figure 5J**.
- **G and H.** Undetectable expression of the Kinesin8B protein in female gametocytes after deletion of the *kinesin8b* $\Delta intron1$. Similar illustration as in **A** and **B**. Note that the *kinesin8b* transcripts are almost undetectable in female gametocytes of *P. yoelii*.
- **I, J, K, and L.** RT-PCR confirmation of intron retention at the transcripts of 4 genes (*PF16*, *dlc1*, *PY17X_1109100*, and *kinesin8b*) in female gametocytes. Genomic DNA (gDNA) from 17XNL parasite, complementary DNA (cDNA) from the purified male and female gametocytes of *DFsc7* were analyzed. Exons are indicated by boxes and introns by lines. Representative for three independent experiments.
- **M.** Proposed different roles of RBPm1-target introns in axonemal gene expression at male and female gametocytes respectively. In male gametocytes, RBPm1 (as a key)-directed splicing of axonemal intron (as a lock) allows protein expression of the axonemal genes. In female gametocytes, dual blockage via weak transcription and IR prevents protein expression of the axonemal genes.

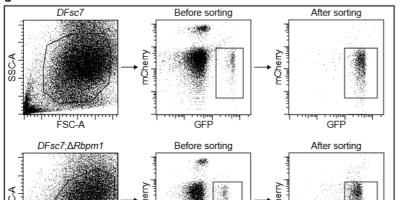


Supplementary Figure 12. Genotyping of genetically modified parasite lines

- **A-C.** Schematic diagrams showing CRISPR/Cas9-mediated gene tagging at C-terminus (**A**), gene tagging at N-terminus (**B**), and gene deletion (**C**) via double cross-over homologous recombination. 'p' represents the primers for PCR, and the red lightning bolt represents the sites for sgRNA recognition.
- **D-L.** Genotyping PCR results showing correct 5' and 3' homologous recombination in the modified parasite lines in this study. For each modification, usually 1-3 parasite single clones (sc) were obtained via limiting dilution. The red-colored sc was selected for further analysis. Oligo sequences used for plasmid construction and primers used for genotyping PCR are listed in Supplementary Table 2.







Supplementary Figure 13. Flow cytometry gating strategies

- **A.** Gating strategy for sorting male and female gametocytes from a *P. yoelii* parasite reporter line *DFsc7* presented in Supplementary Fig. 1A. Forward and side scatter signals were used to distinguish red blood cells from debris, doublets, and white blood cells. Male and female gametocytes were sorted based on GFP fluorescence and mCherry fluorescence. After sorting, gametocyte purity was assessed by re-analysis of a sample fraction.
- **B.** Gating strategy for analyzing DNA content of male gametocytes presented in Supplementary Fig. 2A. Forward and side scatter signals were used to distinguish red blood cells from debris, doublets, and white blood cells. Male gametocytes were identified by GFP fluorescence and analyzed for Hoechst 33342 fluorescence.
- C. Gating strategy for sorting male gametocytes from the DFsc7 and DFsc7; $\Delta Rbpm1$ lines presented in Supplementary Fig. 3F. Forward and side scatter signals were used to distinguish red blood cells from debris, doublets, and white blood cells. Male gametocytes were sorted based on GFP fluorescence. After sorting, male gametocyte purity was assessed by re-analysis of a sample fraction.

Table S1. List of genetically modified parasite strains in this study

| Presente with gree tagging | Table S1. List of ge | netically mod | ified parasite strains in this study | |
|---|--------------------------------------|---------------|--|--|
| ### Annual Processor | | | | Resource |
| DPCP | 17XNL | 1 | Plasmodium yoelii 17XNL strain | NIH |
| Grant April | Parasites with gene tagging | | | |
| Riginary 19th 177944, | DFsc7 | 17XNL | | Liu et al. 2018 |
| Page 1779-11. Report Cheminally tagged with BPA Fig. 512 | sep1::4Myc | 17XNL | sep1 C-terminally tagged with 4Myc | Jiang et al. 2020 |
| Page 17 Page | Rbpm1::6HA | 17XNL | Rbpm1 C-terminally tagged with 6HA | Fig S12 |
| Rigent 1747-174000 1775N. Rigent Cheminally tagged with 914-174000 Fig. 912 | | | | |
| Page 1778 | | | | |
| Page 175.0 PAR 1779N. | <u> </u> | | | - |
| ## 1701L | | | | |
| ## 9.512 ## 17.00 ## 17. | | | | |
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| 1709/100_08-874 1779/NL | dlc1::6HA | 17XNL | | |
| | dlc2::6HA | 17XNL | dlc2 C-terminally tagged with 6HA | Fig S12 |
| 1715/00 6944 17794. 1716/00 C-terminally tagged with 694A Fig. 512 | 1109100::6HA | 17XNL | 1109100 C-terminally tagged with 6HA | Fig S12 |
| 17.79.11 | 0521800::6HA | 17XNL | 0521800 C-terminally tagged with 6HA | Fig S12 |
| 1927/200 c944 | 1311800::6HA | 17XNL | 1311800 C-terminally tagged with 6HA | Fig S12 |
| 1335000 C-learninally tagged with 644. Fig. 512 | | | 1323900 N-terminally tagged with HA | Fig S12 |
| Report 64 AL UT-70K - Mayor Report 64 AL UT-AL - Mayor Per 512 Parasites with gene knockout 1770 ML Deletion of the whole coding sequences of rReport Per 512 Author 4 1770 ML Deletion of the whole coding sequences of report Jiang et al. 20 sept - Mayor 2 1770 ML Deletion of the whole coding sequences of rReport Per 512 Deletion of the whole coding sequences of rReport Fig. 512 Per 512 Deletion of the whole coding sequences of Report Fig. 512 Per 512 Almeanido Deletion of the whole coding sequences of Report Fig. 512 Per 512 Almeanido Deletion of the whole coding sequences of Report Fig. 512 Per 512 Almeanido Deletion of the whole coding sequences of Report Fig. 512 Per 512 | | | | |
| Export - SHA, U1-A. 4Mpc Report - SHA U1-A. C-terminally tagged with 4Mpc Fig. S12 Report - SHA, U1-C. 4Mpc Report - SHA, U1-C. 4Mpc Report - SHA U1-C. C-terminally tagged with 4Mpc Fig. S12 Amount - Amount - State - | | | | |
| Report - SHA - UP-C - Adaptic Report - SHA UP-C - C-terminally tagged with - Adaptic Pig S12 | | | | |
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| Sep1:4Myc DRibpint Sep1:4Myc Deletion of the whole coding sequences of Ribpint Fig. S12 | | | | Jiang et al. 2020 |
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| Deletion of the whole coding sequences of PF16 Fig. S12 | ∆kinesin8b | 17XNL | Deletion of the N-terminal 1184 bp coding sequence of kinesin8b | Fig S12 |
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| Deletion of the whole coding sequences of gep1 Jiang et al. 20 | | | | +- |
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| Deletion of the 1018-1519 bp coding sequence of Rbpm1 Fig S12 | | locus | | |
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| BFP-Kin8b 12;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-Kin8b 12 expression cassette Fig S12 BFP-PF1611 DFsc7 Coding region of p230p gene was replaced with the BFP-PF1611 expression cassette Fig S12 BFP-PF1611;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-PF1611 expression cassette Fig S12 BFP-Qlc114 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-Qlc114;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-110910011;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;ARbpm1 DFsc7;∆Rbpm1 | BFP-Kin8b11;∆Rbpm1 | DFsc7;∆Rbpm1 | Coding region of p230p gene was replaced with the BFP-Kin8b11 expression cassette | Fig S12 |
| BFP-PF1611 DFsc7 Coding region of p230p gene was replaced with the BFP-PF1611 expression cassette Fig S12 BFP-PF1611;\(\Delta\Phi\)PF1611 DFsc7;\(\Delta\Phi\)PF1611 Coding region of p230p gene was replaced with the BFP-PF1611 expression cassette Fig S12 BFP-PI6114 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-I10910011 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012;\(\Delta\Phi\Phi\Phi\Phi\Phi\Phi\Phi\Phi\Phi\Phi | BFP-Kin8b12 | DFsc7 | Coding region of p230p gene was replaced with the BFP-Kin8b12 expression cassette | Fig S12 |
| BFP-Pf1611;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-Pf1611 expression cassette Fig S12 BFP-dlc114 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-dlc114;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910011;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-Kin8b11 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | - |
| BFP-dlc114 DFsc7 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-dlc114;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-dlc114 expression cassette Fig S12 BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910011;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-Kin8b11 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | |
| BFP-IIc114:∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-IIc114 expression cassette Fig S12 BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910011;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-P71611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | - |
| BFP-110910011 DFsc7 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910011;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus Fig S12 Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-F1611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | |
| BFP-110910011:∆Rbpm1 DFsc7:∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910011 expression cassette Fig S12 BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-P71611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | |
| BFP-110910012 DFsc7 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 BFP-110910012;∆Rbpm1 DFsc7;∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-P71611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | - |
| BFP-110910012:∆Rbpm1 DFsc7:∆Rbpm1 Coding region of p230p gene was replaced with the BFP-110910012 expression cassette Fig S12 Parasites with axonemal intron inserted at the gep1 locus gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-PF1611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | |
| Parasites with axonemal intron inserted at the gep1 locus gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-PF1611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | | _ |
| gep1-Kin8b11 17XNL Insertion of Kinesin8b intron 1 into exon 3 of gep1 Fig S12 gep1-PF1611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | vosang rogion or proup gono was repraced with the Dr F-110010012 expression cassette | 1 1g 3 12 |
| gep1-PF1611 17XNL Insertion of PF16 intron 1 into exon 1 of gep1 Fig S12 | | | Insertion of Kinesin8b intron 1 into exon 3 of aep1 | Fig S12 |
| | | | | - |
| | nt: not tested | | · · | |

Table S2. Oligonucleotides and primers used in this study

| Oligo sequences for constr | ucting gene tagging plasmids | | | | | | | |
|--|--|--|--|---|--|--|--|--|
| Gene name | Gene ID | Тад | Left homo | logous arm Reverse primer | | ologous arm | | of agRNA |
| Rbpm1 | PY17X 0716700 | C-terminal 6HA | CCCAAGCTTGCCGAACCTA | CATGCCATGGATTGTTATAA | Forward primer CCGCTCGAGTTTATGTCATT | Reverse primer CCGGAATTCGTTCCAATGAA | | Reverse oligo AAACTTTATGGGTCCTTTC |
| · | PY17X_0716700 | C-terminal GFP | AGAAGCCAAA CCCAAGCTTGCCGAACCTA | CATGCCATGGATTGTTATAA | TTTTGAGGT CCGCTCGAGTTTATGTCATT | GACAAACAA CCGGAATTCGTTCCAATGAA | TATTGTAAGGGAAAGGACC | CTTAC AAACTTTATGGGTCCTTTC |
| Rbpm1 | | | AGAAGCCAAA CCCAAGCTTGCCGAACCTA | TCCGGTTGTTGC CATGCCATGGATTGTTATAA | TTTTGAGGT CCGCTCGAGTTTATGTCATT | GACAAACAA CCGGAATTCGTTCCAATGAA | CATAAA TATTGTAAGGGAAAGGACC | CTTAC AAACTTTATGGGTCCTTTC |
| Rbpm1 | PY17X_0716700 | C-terminal HA-TurbolD | AGAAGCCAAA | TCCGGTTGTTGC CATGCCATGGATTGTTATAA | TTTTGAGGT | GACAAACAA | CATAAA TATTGTAAGGGAAAGGACC | CTTAC AAACTTTATGGGTCCTTTC |
| Rbpm1 | PY17X_0716700 | C-terminal 3NLS-HA-TurbolD | AGAAGCCAAA | TCCGGTTGTTGC | TTTTGAGGT | GACAAACAA | CATAAA | CTTAC |
| kinesin8b | PY17X_0204100 | C-terminal 6HA | CCCAAGCTTGATGACCAAAT GAAGAGCTT | CATGCCATGGAGTTTTATTT TTTGTGATGCTAG | CCGCTCGAGAAATGTTTGA ACTTTACCATTC | GGGCTTAAGGAAAAAGGCA ATGATGCCAT | TATTGTGCGTCAGTTAATTT ATGA | AAACTCATAAATTAACTGA GCAC |
| PF16 | PY17X_0919000 | C-terminal 6HA | CCCAAGCTTACATCCTGTAT TTCTAAAATC | CATGCCATGGTTCTTCGGTA TTTTCAACTTC | CCGCTCGAGAACAAGGAAA TAATAACAAGT | GGGCTTAAGCTCGCAATTC CTCCTTTTGC | TATTGTGTGTACATACTTCA | AAACCACATGAAGTATGTA |
| ihcō | PY17X_0603800 | C-terminal 6HA | CCCAAGCTTGTGTGTGCAA | CATGCCATGGATTATATATA | CCGCTCGAGAAAAAAATACG | CCGGAATTCACGATTATATT | TATTGGAAGTAGAAATGCC | AAACACAACGGCATTTCT/ |
| dhc7 | PY17X_0510800 | C-terminal 6HA | ACGCAAATAT CGGGGTACCTGATGATAAT | AATAAATAGA CATGCCATGGTAGTAGCAT | ATATACAAAT CCGCTCGAGTTTTTCGTTTA | ATACCAGTA CCCCTTAAGCATCACATTCT | | AAACCCTTCAGATCCTAGT |
| 0/c1 | PY17X_1241500 | C-terminal 6HA | GAATTTATTT CCCAAGCTTCTCGAAAATG | ATGAACTTTAA CATGCCATGGAAGATGAGC | ATTTTTTAA CCGCTCGAGATTGAAAAGG | TTCATTTTA CCGGAATTCCAAAATTATAG | GAAGG TATTGTTACTGAAAAGTACG | TCCAC AAACTATAACGTACTTTTC |
| | - | | CTTCCTCTCAA CGGGGTACCCCTTATCATA | TACGGATCCATC CATGCCATGGACACTTATAT | CAACATGCTCA CCGCTCGAGAATATGATAAT | GAAATTGCAA GGGCTTAAGGGGGTGAAAA | TTATA TATTGCTATATGATGTATGC | GTAAC AAACTATAGCATACATCAT |
| dic2 | PY17X_0302800 | C-terminal 6HA | ATCAGAATAA | ACAACAATTG CGGGGTACCGGATTGTCGT | TAAATGTGG CCGCTCGAGGGGGTCCATA | AATAATTTGG CCGGAATTCTGGGTTTAAC | TATA TATTGTTGCTGCTTGGCTTG | TAGC AAACAGCAACAAGCCAAG |
| 1109100 | PY17X_1109100 | C-terminal 6HA | CCCAAGCTTGTACCACAAA CAATGGTGGA CGGGGTACCCAATAAATAG TGGAAACAAA | CATTATTGTAAGTG | TAAGATGGAA | CTTGTACACC CCCCTTAAGGGGAAACAAT | TTGCT | AGCAAC AAACACTTGGTCATATAGA |
| 0521800 | PY17X_0521800 | C-terminal 6HA | | CATGCCATGGCAGTTTATCC CCTAATAAAT | AATAATCTC | ATATACGAAT | TATTGTGAAGTCTATATGAC CAAGT | TTCAC |
| 1311800 | PY17X_1311800 | C-terminal 6HA | CGGGGTACCCCCCGAGGA CCAATCTTTTG | CATGCCATGGTTGTCCAGTT AAATAATACA | CCGCTCGAGAAAAAAAGAG AAATAAAGAA | CCCCTTAAGGTGTGCCTAA AAGTATGTTC | TATTAATCAGAGGATGAAGT | AAACTGCAACTTCATCCTC |
| 1323000 | PY17X_1323900 | N-terminal HA | CCCAAGCTTGGCATTACTGT CGAAGGACT | CATGCCATGGATTAATTACA | CCGCTCGAGATGGAACAAA AAAAAAATAG | CCCCTTAAGACCTGTTTATC AAAATGCGA | TATTGTAAATATGAAGGGAG | AAACTCCTCCTCCCTTCA |
| 1357300 | PY17X_1357300 | C-terminal 6HA | CGGGGTACCACCCCCGATC | AGGAAAGGTA CATGCCATGGATCTATTTCA | ICCGCTCGAGCATACAAATA | CCCCTTAAGTATGCCAGCTT | TATTGCATATGCACATTATC | AAACCTTTTGATAATGTGG |
| 1335600 | | C-terminal 6HA | TTAATACAAC CCCAAGCTTCGAAATAAATG | CATGCCATGGCTTTTTTTA | AATTAAAAAG CCGCTCGAGAATTTTTATAA | GTATGCCAG CCGGAATTCCTAATGATCTG | TATTGCCCTTTATGGCTACC | TATGC AAACTAATAGGTAGCCAT |
| | PY17X_1335600 | | GAGATAAA CCCAAGCTTGGAAAAGGTC | TATTTGTCTTTAAAAG CATGCCATGGTTCATACCCT | TGAACTTAGC CCGCTCGAGAAGTTTTTCGT | TTAATTGC CCGGAATTCGTAACTTGCTT | TATTA TATTGACAGAGATAGAGGC | AGGGC AAACTCTCTGCCTCTATC |
| U1-70K | PY17X_1144300 | C-terminal 4Myc | CTTCTAGGGG | TCATTATGATC | AAAAATATCATAAT | AAAATGACA | AGAGA | TGTC |
| J1-A | PY17X_1407100 | C-terminal 4Myc | CCCAAGCTTCAGGATTTGTT GAAGCCAGA CCCAAGCTTCACATAGTTCA | CATGCCATGGTCGTTTCGC ATATGATATTTTTAAC | CCGCTCGAGCCTTTTTTTTT TTTTTGGAATTATT | GGGCTTAAGGCATATCTGT GTGCTTTGTA GGGCTTAAGGAGTATATAC | TATTGTATGTCCATATTCCC | AAACAAATAGGGAATATG CATAC AAACGGGGGGAACATAGC |
| J1-C | PY17X_1426800 | C-terminal 4Myc | CCCAAGCTTCACATAGTTCA CCAGTAGGT | CATGCCATGGCTCATTATTT TTATTTACAAAATTTGC | GGAAGTATAT | GGGCTTAAGGAGTATATAC ACGTTCGAAA | TATTAGAGCAGGCTATGTTC | AAACGGGGGAACATAGC GCTCT |
| Diagnosite PCR primers for | C-terminal tagging | | | | | | | |
| Gene name | Gene ID | Tag | P1 | P2 | P3 | P4 | | |
| Rbpm1 | PY17X_0716700 | C-terminal 6HA | | | TGAAAATATGCAACAACCG | TGATTCTACCTGTACACCAG | | |
| | | | G GGATAAAATGGTTGAAGTTA | GGTTAAAAGCTAAAAAGGC | G TGAAAATATGCAACAACCG | TGATTCTACCTGTACACCAG | | |
| Rbpm1 | PY17X_0716700 | C-terminal GFP | G GGATAAAATGGTTGAAGTTA | C GGTTAAAAGCTAAAAAGGC | G TGAAAATATGCAACAACCG | | | |
| Rbpm1 | PY17X_0716700 | C-terminal HA-TurbolD | G | c | G | TGATTCTACCTGTACACCAG | | |
| Rbpm1 | PY17X_0716700 | C-terminal 3NLS-HA-TurbolD | GGATAAAATGGTTGAAGTTA G | C | TGAAAATATGCAACAACCG G | TGATTCTACCTGTACACCAG | | |
| Rbpm1 | PY17X_0716700 | C-terminal 6HA | GGATAAAATGGTTGAAGTTA G | GGTTAAAAGCTAAAAAGGC C | TGAAAATATGCAACAACCG G | TGATTCTACCTGTACACCAG | | |
| unesināb | PY17X_0204100 | C-terminal 6HA | TTAATAAACAAACTAGTTGC | GAATGGTAAAGTTCAAACAT | CGCACCAAAGATCTCAAAC | CCCACTTAGATATTTCGAAA | | |
| PF16 | PY17X_0919000 | C-terminal 6HA | | TATAGTAAGAAATAGCAGAC | CACACCTGGATATTCTGAAA | CCTTGAAGTAATCTTATCAC | | |
| | | | | | CAAATCTGAAAAACATAAAT | | | |
| shc6 | PY17X_0603800 | C-terminal 6HA | CGTTAAGATATGCAACTGTC | TACAAAAATAGCAAACGGTT | CC GCCATGCATCCATAGCAAA | GTCTCGAACAAATTTAAATG | | |
| shc7 | PY17X_0510800 | C-terminal 6HA | GACGATGAATCAAATTCGAA | ACAGGTTAAAAAATTAAACG | A | GTTTTGAATTTCTTCTAACTC | | |
| Nc1 | PY17X_1241500 | C-terminal 6HA | GGTCCAACATAATTTGTGAA G | GAAAATTTAGAAAACATATG | AGATGGATCCGTAGCTCAT C | GACCTCTTACTATGTGTAAC | | |
| Nc2 | PY17X_0302800 | C-terminal 6HA | GGCATAAGTTGAATAAGCAT | CATATGAGTGTTTTCATCCC | GCATTATTTTGTAGGAGGAG | ATCATTCCCTATATTTCCTG AATT | | |
| 1109100 | PY17X_1109100 | C-terminal 6HA | TCTACAAAAGAGGAAGCTTC | TTCCATCTTATATGGACCCC | GACACTTACAATAATGACGA | CCGAAGCACAATTAGCAAT | | |
| 0521800 | PY17X_0521800 | C-terminal 6HA | CAAGGTAATAATATGTACAT | AACTTCCATGATATTTTGAG | ATTTATTAGGGGATAAACTG | TGTGTATATTGACAACATCC | | |
| | | | G | | CAGAGGATGAAGTTGCATG | GGCGTAAGCCAAAGAATGA | | |
| 1311800 | PY17X_1311800 | C-terminal 6HA | ATAGGAAAAACTGGATTTCC | TCCCTTTGTTTTTTTCGTTG | G | AC | | |
| 1357300 | PY17X_1357300 | C-terminal 6HA | AAAACTGCAGGAGAAAATG C | CGGGGGAAGGGGTATATTT G | GAACTTCCGATAATTCAATA G | ACAATATGCCACGTGCACA A | | |
| 1335600 | PY17X_1335600 | C-terminal 6HA | CTGATGATATTTTTGAAACC | TAGGAAATATGAATTAGGAG | AAAATCGAGAGATGCAAAA C | ATGATTATGGGGATATAGTC | | |
| U1-70K | PY17X_1144300 | C-terminal 4Myc | TACCTAGAAGATTAGGAGG | TATCATTTGGAATATCAAAG | GCCAGGAAAATGGAGAATA | CCCTATATATGTGGTTATAT | | |
| U1-A | PY17X_1407100 | C-terminal 4Myc | GAAAATGTTAATACTGAAGC | CTACATATATTGTGCGTTTT | AGTTAAAAATATCATATGCG | TAACGGATGGTTAGATAAGA | | |
| U1-C | PY17X_1426800 | C-terminal 4Myc | GTGAATATTGCGATATATAT | | CAAATGAATAAAGAAAATGC | CGGAATAACTGCTCATTTAT | | |
| Diagnosite PCR primers for | | - 12 | С | [G | | | | |
| | | | | I | | | I | |
| Gene name | Gene ID | Tag | P5 | P6 | P7 | P8 | | |
| 1323900 | PY17X_1323900 | N-terminal HA | GGGGGGAATGATGGAAAAA A | C ACTAMATGAAGGCGACTCA | GTTCTTTTACCTTTCCTTGT | GGAAAGGTTTTGTGTAGCTA | | |
| kinesin8b | PY17X_0204100 | N-terminal 6HA | GTTTACTCTCTTGTCCACAT | GATCCTGCACATTTTTAAGA | GTGGATCTACTTGATATACT | CATCATAATATTGAGTTTTA | | |
| PF16 | PY17X_0919000 | N-terminal 6HA | GCAAAAATGTAGACAATAAT | CGACCTATTATAATCGTCAA | AAAAAATTTCACAAAAAAGG | GGTATGATACCTTTATCAAC | | |
| Oligo sequences for constr | ucting gene knockout plasmid: | | ,00 | | | | | |
| | | 3 | | | | | | |
| Gene name | ucting gene knockout plasmids | | Left homo | logous arm | Right home | ologoue arm | Target site | of agRNA |
| | Gene ID | Gene size (bp) / deleted gene size (bp) | Forward primer | logous arm Reverse primer | Forward primer | ologous arm Reverse primer | Target alte Forward oligo | Reverse oligo |
| Rbpm1 | T | Gene size (bp) / deleted | Forward primer CGGGGTACCGCATACACGA GGAAATACTA | Reverse primer CATGCCATGGTTTGTGTATT TTATTTTTCGTCG | Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA | Forward oligo TATTGTAGGGGGGTTAAGC TACTAT | Reverse oligo AAACATAGTAGCTTAACC CCTAC |
| | Gene ID | Gene size (bp) / deleted gene size (bp) | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCCGGTTGTGC | Reverse primer CATGCCATGGTTTGTGTATT TTATTTTTCGTCG CATGCCATGGATTTTGATCT | Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT CCGCTCGAGACAGACAAC | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA CCGGAATTCCTTTCTCTCA | Forward oligo TATTGTAGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC | Reverse oligo AAACATAGTAGCTTAACC CCTAC AAACATTTTGTATACTGAC |
| kinesināb | Gene ID PY17X_0716700 | Gene size (bp) / deleted gene size (bp) 1904/1904 | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCCGGTTGTGC ATTTATTATT CCCAAGCTTACGGTTTCTAA | Reverse primer CATGCCATGGTTTGTGTATT TTATTTTCGTCG CATGCCATGGATTTGATCT TTACTATATT CATGCCATGGTTTTTTTTTT | Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGGACAG CCGCTCGAGAACAAGGAAA | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA CCGGAATTCCTTTCTCTCA TTCATTTCTTG GGGCTTAAGCTCGCAATTC | Forward oligo TATTGTAGGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAT TATTAGTGCATGTGTTTGGA | Reverse oligo AAACATAGTAGCTTAACCI CCTAC AAACATTTTGTATACTGAG GTAAC AAACACAGTCCAAACACA |
| rinesin8b PF16 | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 | Gene size (bp) / deleted gene size (bp) 1904/1904 5137/1184 | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCCGGTTGTGC ATTTATTATT CCCAAGCTTACGGTTTCTAA | Reverse primer CATGCCATGGTTTGTGTATT TTATTTTCGTCG CATGCCATGGATTTTGATCT TTACTATATT | Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGGACAG | Reverse primer CCGGAATTCGTTCCAATGAA GACAACAA CCGGAATTCCTTTCTCTTCA TTCATTTCTTG | Forward oligo TATTGTAGGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAT | Reverse oligo AAACATAGTAGCTTAACC CCTAC AAACATTTTGTATACTGAG GTAAC |
| inesinab PF18 Diagnostic PCR primera foi | Gene IID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout | Gene size (bp) / deleted gene size (bp) 1904/1904 5137/1184 1809/1809 | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCCGGTTGTGC ATTTATTATT CCCAAGCTTACGGTTTCTAA AGTAATAATATCAC | Reverse primer CATGCCATGCTITGTGTATT TIATHTITGTCG CATGCCATGCATTTTACTATATT CATGCCATGC | Forward primer CCGCTCGAGTTTATGCATT TTTTGAGGT CCGCTCGAGACCAGAACAAC TTAATGGACAG CCGCTCGAGAACAAGGAAA TAATAACAAGT | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA CCGGAATTCCTTTCTCTCA TTCATTTCTTG GGGCTTAAGCTCGCAATTC CTCCTTTTGC | Forward oilgo TATTGTAGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAT TATTAGTGCATGTGTTTGGA CTGT | Reverse oligo AAACATAGTAGCTTAACC CCTAC AAACATTTTGTATACTGAC GTAAC AAACACAGTCCAAACACA |
| cinesin8b PF16 Diagnostic PCR primers for Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID | Gene elze (bp) / deleted gene elze (bp) 1904/1904 5137/1184 1809/1809 | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCGGTTGTGC ATTTATTATT CCCAAGCTTACGGTTTCTAA AGTAATAATATCAC P10 | Reverse primer CATGCCATGGTTTGTGTATT TTATTTTCGTCG CATGCCATGC | Forward primer CCGCTCSAGATTTATGCATT TTTTGAGGT CCGCTCSAGACAACAAC TTAATGGACAG CCGCTCSAGAACAACAAC TAATAACAAGT P12 | Reverse primer CCGGANTICGTTCCAATGAA GACAAACAA CCGGAATTICCTTTCATTCATTTCTTCATTTCTTCATTTCTTCATTCCTTCATTCATTTCTTC | Forward oilgo TATTGTAGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAAT TATTAGTGCATGTGTTTGGA CTGT P14 | Reverse oligo AAACATAGTAGCTTAACC CCTAC AAACATTTTGTATACTGAC GTAAC AAACACAGTCCAAACACA |
| kinesinäb PF16 Diagnostic PCR primers for Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 | Gene size (bp) / deleted gene size (bp) | Forward primer CGGGCTACCGCATACACGA GGAATACTA CGGGGTACCCGGTTGTGC ATTTATTATT CCCAAGCTTACCGTTTCTA AGTAATATATCAC P10 GGTTAAAAGCTAAAAAAGGC C | Reverse primer CATGCCATGCTTTGTTGTTGTTTTTTTTGTTCS CATGCCATGCATTTTACTTATTT CATGCATGCTTTTTTTTTT | Forward primer CCGCTCGAGTTATGTCATT TTTTGAGGT CCGCTCGAGAACAACAACAAC TTAATGGACAG CCGCTCGAGAACAACAACAAACAAACAAACAAACAAACAA | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA CCGGGAATTCCTTTCTCTTCA TTCATTTCTTG GGGCTTAAGCTGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTG G | Forward oligo TACTGATAGGGGGGTTAAGC TACTGAT TATTGATAGGCATGATAGAAAAAT TATTGATGCATGTGTTTGGA CTGT P14 GGTTACAAAGGCAAACCCT C | Reverse oligo AAACATAGTAGCTTAACCI CCTAC AAACATTTTGTATACTGAG GTAAC AAACACAGTCCAAACACA |
| kinesin8b PF16 Diagnostic PCR primers for Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID | Gene elze (bp) / deleted gene elze (bp) 1904/1904 5137/1184 1809/1809 | Forward primer CGGGGTACCGCATACACGA GGAAATACTA CGGGGTACCCGGTTGTGC ATTTATTATT CCCAAGCTTACGGTTTCTAA AGTAATAATATCAC P10 | Reverse primer CATGCOATGCTTTGGTATT THATTITTGGTCG CATGCOATGCATTTGATCT THACTATATT CATGCOATGCATTTTTACTATATT CATGCOATGCTTTTTTTTTACTATATT THATTAACAGTCA P11 CGACGAAAAAATAAAAATAACAC | Forward primer CCGCTCGAGTTTATGTCATT TITTIGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG CCGCTCGAGAACAACAAC TAATAACAAGT P12 CGTAATTTCAAAAATGAAGA TATGATATTTTTTTTTTT | Reverse primer COGAATICOTTCCATGAA GACAACAA COGGAATICCTTTCTCTCA TTCATTICTTG GGGCTTAAGCTCGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTG CGGATGCAAACTATAACATCT CGGATGCAAACTATAACATCT | Forward oilgo TATTGTAGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAAT TATTAGTGCATGTGTTTGGA CTGT P14 | Reverse oligo AAACATAGTAGCTTAACCC CCTAC AAACATTTTGTATACTGAG GTAAC AAACACAGTCCAAACACA |
| cinesināb PF1d Diagnoetic PCR primers for Gene name Rbpm1 | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 | Gene size (bp) / deleted gene size (bp) | Forward primer CGGGCTACCGCATACACGA GGAATACTA CGGGGTACCCGGTTGTGC ATTTATTATT CCCAAGCTTACCGTTTCTA AGTAATATATCAC P10 GGTTAAAAGCTAAAAAAGGC C | Reverse primer CATGCOATGCTTTGTGTATT TTATHTHTCGTCG CATGCCATGCATTTGATCT TTACTATATT CATGCATGCTTTTTTTTTATTAACAGTCA P11 CAACGAAAAAATAAAATACAC CAACGAAAAAATAAAAT | Forward primer CCGCTCGAGTTATGTCATT TTTTGAGGT CCGCTCGAGAACAACAACAAC TTAATGGACAG CCGCTCGAGAACAACAACAAACAAACAAACAAACAAACAA | Reverse primer CCGGAATTCGTTCCAATGAA GACAAACAA CCGGGAATTCCTTTCTCTTCA TTCATTTCTTG GGGCTTAAGCTGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTG G | Forward oligo TACTGATAGGGGGGTTAAGC TACTGAT TATTGATAGGCATGATAGAAAAAT TATTGATGCATGTGTTTGGA CTGT P14 GGTTACAAAGGCAAACCCT C | Reverse oligo AAACATAGTAGCTTAACCC CCTAC AAACATTTTGTATACTGAG GTAAC AAACACAGTCCAAACACA |
| cinesināb PF1d Diagnostic PCR primers for Gene name Rbpm1 kinesināb | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0204100 | Gene size (bp) / deleted gene size (bp) | Forward primer CGGGGTACCAGGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATI COCCAAGCTTACCGGTTCTA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC C CTGTCCAATTAGTTGTTCTG | Reverse primer CATGCOATGCTTTGTGTATT TTATHTHTCGTCG CATGCCATGCATTTGATCT TTACTATATT CATGCATGCTTTTTTTTTATTAACAGTCA P11 CAACGAAAAAATAAAATACAC CAACGAAAAAATAAAAT | Forward primer CCGCTCGAGTTTATGTCATT TITTIGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG CCGCTCGAGAACAACAAC TAATAACAAGT P12 CGTAATTTCAAAAATGAAGA TATGATATTTTTTTTTTT | Reverse primer COGAATICOTTCCATGAA GACAACAA COGGAATICCTTTCTCTCA TTCATTICTTG GGGCTTAAGCTCGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTG CGGATGCAAACTATAACATCT CGGATGCAAACTATAACATCT | Forward oilgo TACTAT TATTGATGAGGGGTTAAGC TACTAT TATTGATGACCTCAGTATAC AAAAT TATTGATGCATGTGTTTGGA CTGT P14 GGTTACAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T GATTCTGTATGTTGAGGGG | Reverse oligo AAACATAGTAGCTTAACCC CCTAC AAACATTTTGTATACTGAG GTAAC AAACACAGTCCAAACACA |
| Rbpm1 kinesin8b PF10 Oligo sequences for constr | Gene IID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0204100 PY17X_0919000 ucting RRM deletion plasmide | Gene size (bp) / deleted gene size (bp) | Forward primer CGGGTACCGCATACACGA GGAATACTA GGGGGTACCCGGGTTGTGC ATTTATTATT CCCCAAGCTTACAGGTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC C CTGTCCATTAAGTTGTTCTG T AAAGCACAATAATGTATTGG | Reverse primer CATGCCATCGCTTGGTGATT TTATITITICGTCG CATGCCATCGCATTTGTATCT TTACTATATT CATGCCATCGCTTTTTTTTTATACAGTCA P11 CGACGAAAAATAAAATACAC AAA CCTATATATGTGGATCTACTT G CCTCCCCACTTTTAGTACTG Mutation Mutation | Forward primer COGCTICAGE TOTAL THE TRANSPIT TO THE | Reverse primer COGAATICOTTOCATISAA CACAACAA CACAACAA COGGAATICOTTICTCTTCA TICATTICTTC GGGCTITAASCTCGCAATIC CTCCTTTTGC P13 GCGGACACTCTAAGTACTC GCAACAGTAATACCTAGCGA A | Forward oligo TATTGTAGGGGGGTTAAGC TACTAT TATTGTTACCCTCAGTATAC AAAAT TATTGTTACCCTCAGTATAC AAAAT TATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T AAGCTTCAAGATCCGTAACT | Reverse oligo AACATAGTAGCTTAACCC CCTAC AACATTTGTATACTGAG GTAAC AACATTTGTATACTGAG AACACAGTCCAAACACA CACT s of sgrNA |
| cinesināb PF1d Diagnostic PCR primers for Gene name Rbpm1 kinesināb | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0204100 PY17X_0919000 ucting RRM deletion plasmids Homolo Forward primer | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 5137/1184 1809/1809 P9 AAGCGAAATAAATGAAACG GTTTACTCTCTTGTCCACAT CAGATTATTGTCATATCATC | Forward primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTITATIT CCCCAAGCTTACCGTTTTGC CCCAAGCTTACCGTTTCTAA AGTAATAATACAC P10 GGTTAAAAGCTAAAAAGGC C CTGTCCATTAAGTTGTTCTG T AAAGCACAATAATGTATTGG | Reverse primer CATGCCATGCTTGTGTATT TTATITITICGTCG CATGCCATGCATTGTATT TTACTATATT CATGCCATGC | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG CCGCTCGAGAAACAAGAAA TAATAACAAGT P12 CGTAATTTCAAAAATGAAGA TATGATATTTTTTTTTTT | Reverse primer COGAATICATION COGAATICATICATICAT CAGACAA CAGAACAA COGGAATICATICATICATICAT CAGCTIAAGCTCCAATICATICATICATICATICAT P13 CCGGACACACTCTAAGTACTC CCAACAGTAATACTAGCAA Primer | Forward oilgo TATTGTAGGGGGGTTAAGC TACTGTAT TATTGTTACCCTCAGTATAC AAAAT TATTGTACCCTCAGTATAC AAAAT TATTGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TAGGTTCTGTATGTTGAGGGG AAGCCTTCAAGATCCGTAACT Target sib Forward oilgo | Reverse oligo AAACATAGTAGCTTAACCC CCTAC CCTAC AAACATTTGTATACTGAG GTAAC CACT of sgRNA Reverse oligo |
| cinesinão Diagnostic PCR primers for Gene name Ròpmi cinesinão PF10 Strain | Gene IID | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809/1809 1909/1809/1809/1809/1809/1809/1809/1809/ | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATT CCCAAGCTTACAGGTTCTAA AGTAATAATATACAC P10 GGTTAAAAGCTAAAAAGGC C TGTGCCAITAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC STAACTCXAAAAAATATAAATCACC | Reverse primer CATGCCATGCTTGTGTATT TTATITITICGTCG CATGCCATGCATTGTATT TTACTATATT CATGCCATGC | Forward primer COGCTICAGE TOTAL THE TRANSPIT TO THE | Reverse primer COGAATICATION COGAATICATICATICAT CAGACAA CAGAACAA COGGAATICATICATICATICAT CAGCTIAAGCTCCAATICATICATICATICATICAT P13 CCGGACACACTCTAAGTACTC CCAACAGTAATACTAGCAA Primer | FORWARD GIBO TATTOTAGGGGGGTTAAGC TACTAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC ATATTAGTAGAAAAGGCAAAACCCT GATTCTGTATGTTGAGGGG TATCAGTAGTTGAGGGGGT AAGCTTCAAGATCCGTAACT Target sib FORWARD GIBO TATTATTAGAAAAATAAAAAT TGT | Reverse oligo AAACATATTAACCI GTAAC AACATTTGATACTGAC GTAAC AACATTTGATACTGAC CACT s of sgRNA Reverse oligo AAACACATTTTATTTTCAAC AAT |
| cinesin80 Diagnostic PCR primers for Gene name Rhpm1 sinesin80 PF16 Strain | Gene ID | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 13137/1184 1809/1809 PB AGCGAAATAAATGAAACG GTTTACTCTCTTGTCCACAT CAGATTATCTCATC GGGCTTAGGGACATATAC ATATTCTCCT (GGGCTTAGGGACATATACC) (GGGCTTAGGTAGTATATATACC) (GGGCTTAGGTAGTATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) (GGGCTTAGGTATATATATACC) | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATT CCCAAGCTTACAGGTTCTAA AGTAATAATATACAC P10 GGTTAAAAGCTAAAAAGGC C TGTGCCAITAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC STAACTCXAAAAAATATAAATCACC | Reverse primer CATGCATGCTTTGTGTATT TTAINTITCGTCG CATGCATGCATTTTGATCT TTACTATATT CATGCATGCTTTTTTTTTT | Forward primer COGCTICAGE TO TATTITIS ACAG TOCOGTICAGE ACAGAACAAC TITATIS ACAG TATTIS ACAG P12 CGTAATITICAAAAAATGAAGA TATGATAATTITIS CGTCTC GACATGACTIGTACAGGT primers Reverei ATCTGAGGGGTGCCTATTITIS | Reverse primer COGAMITICATION GACAACAA COGGAMITICATITCTCA TICATITETTS GGCTIANGCTCGCAMITIC CTCCTTITEC P13 GCGGACACTCTAAGTACTG GCGACACTCTAAGTACTG GCAACAGTAATACCATCT GCAACAGTAATACCATCG S primer GTTCCTCAAATGGTTCATTTA | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oligo AAACATATIATORIA or sgriia or sgriia everse oligo AAACAATITTATATICTATACATAAACACAATITTATATATA |
| idnesin80 PF16 Diagnostic PCR primera for Gene name Ropm1 dinesin80 PF16 Strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0204100 PY17X_0919000 ucting RRM deletion plasmids Homolo Forward primer CCGCTCGACCAGAACGAA CTTCACACAC | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809/1809 1909/1809/1809/1809/1809/1809/1809/1809/ | FORWARD primer CGGGGTACCACGA GGAATACTA CGGGGTACCCCGATTCACCGA GGAGATACTCA CGGGGTACCCCGGTTGTCC ATTIATIATI CCCAAGCTTACCGGTTCTCA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC CTCTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTCG FORWARC GTAACTCAAAAAAATTAAATGACG | Reverse primer CATGCATGCTTTGTGTATT TTAINTITCGTCG CATGCATGCATTTTGATCT TTACTATATT CATGCATGCTTTTTTTTTT | Forward primer COGCTCGAGTATTATCATT TITTIGAGGT COCCTCGAGACAGAACAAC TTAATGACAG P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTTTTTT | Reverse primer COGAMITICATION GACAACAA COGGAMITICATITCTCA TICATITETTS GGCTIANGCTCGCAMITIC CTCCTTITEC P13 GCGGACACTCTAAGTACTG GCGACACTCTAAGTACTG GCAACAGTAATACCATCT GCAACAGTAATACCATCG S primer GTTCCTCAAATGGTTCATTTA | FORWARD GIBO TATTOTAGGGGGGTTAAGC TACTAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC ATATTAGTAGAAAAGGCAAAACCCT GATTCTGTATGTTGAGGGG TATCAGTAGTTGAGGGGGT AAGCTTCAAGATCCGTAACT Target sib FORWARD GIBO TATTATTAGAAAAATAAAAAT TGT | Reverse oligo AAACATATACATAACAAAACATATACATAACAAAAACAATATAACAAAAAA |
| idnesin80 PF16 Diagnostic PCR primers for Gene name Ropm1 dinesin80 PF16 Strain Arm11 Arm11 Diagnostic PCR primers for | Gene IID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 ucting RRM deletion plasmids Forward primer CCGCTCGACCAGAACGAA GTTTATCTAA CCGCTCGACCATGAGAACGAA RRM deletion | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909/1909 1909/1909/1909/1909/1909/1909/1909/1909 | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCCAAGCTTACCGGTTTGTC CCCAAGCTTACCGGTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC CTGTCCATTAAGTTGTTCTG TAAAGCACAATAATGTATTGG FORWARC GTAACTCAAAAAAATAATTAAATG ACG CGACACCCCTCAGATATTCAAA | Reverse primer CATGCATGCTTTGTGTATT TTATITTTTGTCG CATGCATGCATTTTGATTT TTATATATT CATGCATGCTTTTTTTTTT | Forward primer COGCTCAGAGACAGAACAAC TITTIGAGGT COGCTCAGAGACAGAACAAC TITATGAGCAG COCCTCAGAGACACAACAAC TAATGACAAG P12 CGTAATTTCAAAAATGAAGA TATGATATTTTTTTTTTT | Reverse primer COGRATICOTTCCATGAA CACAACAA COGRATICCTITCCTCA TICATTICTTC GOGCTIANGCTCGCAATTC CTCCTTITGC P13 CCGGACACTCTAAGTACTC GCAACAGTAATACTAGCGA A sprimer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTGAG | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oilgo AAACATAGTAGCTTAACC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC CACT or sgRNA Reverse oilgo AAACAATTTTATTTTT AAT AAACAAACTTTATGGATCCTTTTAAACAACTTTTTGATTTTTTAAACAACAATTTTTTTAAACAACAACTTTTTTT |
| idnesin80 PF16 Diagnostic PCR primera for Gene name Ropm1 dinesin80 PF16 Strain Arrm1 Arrm2 Diagnostic PCR primera for Strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0204100 PY17X_0919000 ucling RRM deletion plasmide Forward primer CGG-TICGA/SCGA/GAA/GAA GTGT/TICGA/SCGA/GAA/GAA RRM deletion P1 | Gene size (bp) / deleted gene size / | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCAAGCTTACAGGTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC CTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC GTAACTCAAAAAATTAAATCAC CGACACCCTCAGATATTCAA | Reverse primer CATGCATGCTTTGTGTATT TTATITTTTGTCG CATGCATGCATTTTGTTTTTTTTTTTTTTTT | Forward primer CGGCTCAGAGACAGAACAAC TITTIGAGGT CCGCTCAGAGACAGAACAAC TITATGAGACAG CCGCTCAGAGACACAACAAC TAATGACAAG P12 CGTAATTTCAAAAAATGAAGA TATGATATTTTTTTTTT | Reverse primer GCGGAATICCTTCAATGAA GACAACAA COGGAATICCTTTCTCTCA TICATTICTTG GGGCTTAAGCTCGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTC GCAACAGTAATACTAGCGA A Primer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTGGG P6 | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oilgo AAACATAGTAGCTTAACC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC CACT or sgRNA Reverse oilgo AAACAATTTTATTTTT AAT AAACAAACTTTATGGATCCTTTTAAACAACTTTTTGATTTTTTAAACAACAATTTTTTTAAACAACAACTTTTTTT |
| inesin8b PF16 Gene name Ropm1 Inesin8b Nigo sequences for constr Strain wwm1 strain Strain Strain | Gene ID | Gene size (bp)/ deleted gene eize (bp) 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG G G G G G G G G G G G G G G G G G | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC CCGAGGTTACCGGTTTGTGC CCCAAGGTTACGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC C CTGTCCATTAAGTTGTTCTG T AAAGCACAATAATGTATTGG FORWAR GTAACTCAAAAAAATTAAATG ACG CGACACCCTCAGATATTCAA | Reverse primer CATGCCATGCTTGTGTATT TTATTTTTTGTCG CATGCCATGC | Forward primer COGCTICAGAGAACAAC TITTTGACAT TITTGAGAGT COCCTICAGAGAACAACAAC TITATTGACAAG P12 CGTAATTTCAAAAAATGAAGA TATTAACAAGT P13 CGTAATTTCAAAAAATGAAGA TATGATATTTTTGCGTCTC CGACATGACTTGTACAGGT primers ATCTGAGGGGTGTCTTTTTTTTTTTTTTTTTTTTTTTTT | Reverse primer GCGGAATCGTTCCAATGAA GACAACAA COGGAATCCTTTCTCTCA TCATTICTTG GGGCTTAAGCTCCCAATTC P13 GCGGACACTCTAAGTACTC CGATGCAAACTATAACATCT GCAACAGTAATACTAGCGA Primer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTAGG P6 ATTTATATGAGAATGACATGG A | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oilgo AAACATAGTAGCTTAACC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC CACT or sgRNA Reverse oilgo AAACAATTTTATTTTT AAT AAACAAACTTTATGGATCCTTTTAAACAACTTTTTGATTTTTTAAACAACAATTTTTTTAAACAACAACTTTTTTT |
| idnesin80 PF16 Diagnostic PCR primera for Gene name Ropm1 idnesin80 PF16 Diagnostic PCR primera for Strain Arrm1 Strain Strain Arrm1 | Gene ID | Gene size (bp) / deleted gene size / | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCAAGCTTACAGGTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC CTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC GTAACTCAAAAAATTAAATCAC CGACACCCTCAGATATTCAA | Reverse primer CATGCATGCTTTGTGTATT TTATITTTTGTCG CATGCATGCATTTTGTTTTTTTTTTTTTTTT | Forward primer CGGCTCAGAGACAGAACAAC TITTIGAGGT CCGCTCAGAGACAGAACAAC TITATGAGACAG CCGCTCAGAGACACAACAAC TAATGACAAG P12 CGTAATTTCAAAAAATGAAGA TATGATATTTTTTTTTT | Reverse primer GCGGAATICCTTCAATGAA GACAACAA COGGAATICCTTTCTCTCA TICATTICTTG GGGCTTAAGCTCGCAATTC CTCCTTTTGC P13 GCGGACACTCTAAGTACTC GCAACAGTAATACTAGCGA A Primer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTGGG P6 | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oilgo AAACATAGTAGCTTAACC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC CACT or sgRNA Reverse oilgo AAACAATTTTATTTTT AAT AAACAAACTTTATGGATCCTTTTAAACAACTTTTTGATTTTTTAAACAACAATTTTTTTAAACAACAACTTTTTTT |
| idnesināb PF16 Gene name Rāppm1 dinesināb PF16 Strain Arrm1 Arrm2 Strain | Gene ID | Gene size (bp)/ deleted gene size (bp)/ size (bp) 1904/1904 1904/1904 1809/1809 P8 AAGCGAAATAAATGAAACG G GTTACTCTCTTGTCCACAT CAGATTATCTCTCTTGTCCACAT CAGATTATCATCTCTCTGTCCACATATACTCTCTCTGGGCTTAAGGGACATATAC ATATCTCCT GGGTTAAGTGATATACC GGTTGTTGC P2 AGGGTCTGATATTACTCCT GGTTGTTGCTAAGTGTTATATCC GGTTGTTGC ATATCTCCT ATATCTCTCT ATATCTCCT ATATCTCCT ATATCTCCT ATATCTCCT ATATCTCTCT ATATCTCCT ATATCTCTTTTCCT ATATCTCTTTTCCT ATAGGGATTATACTCACACACACACACACACACACACACA | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTGC CCGAGGTTACCGGTTTGTGC CCCAAGGTTACGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAGGC C CTGTCCATTAAGTTGTTCTG T AAAGCACAATAATGTATTGG FORWAR GTAACTCAAAAAAATTAAATG ACG CGACACCCTCAGATATTCAA | Reverse primer CATGCCATGCTTGTGTATT TTATTTTTTGTCG CATGCCATGC | Forward primer COGCTICAGAGAACAAC TITTTGACAT TITTGAGAGT COCCTICAGAGAACAACAAC TITATTGACAAG P12 CGTAATTTCAAAAAATGAAGA TATTAACAAGT P13 CGTAATTTCAAAAAATGAAGA TATGATATTTTTGCGTCTC CGACATGACTTGTACAGGT primers ATCTGAGGGGTGTCTTTTTTTTTTTTTTTTTTTTTTTTT | Reverse primer GCGGAATCGTTCCAATGAA GACAACAA COGGAATCCTTTCTCTCA TCATTICTTG GGGCTTAAGCTCCCAATTC P13 GCGGACACTCTAAGTACTC CGATGCAAACTATAACATCT GCAACAGTAATACTAGCGA Primer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTAGG P6 ATTTATATGAGAATGACATGG A | Forward oilgo TATTOTAGSGGSTTAMSC TATTOTAGSGGSTTAMSC TATTOTATCCCTCAGTATAC AMANT TATTOTACCCTCAGTATAC ATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT CATTCTTAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAACTTCAAAAACCTTAAAAACTTAAAAAA | Reverse oilgo AAACATAGTAGCTTAACC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC CACT or sgRNA Reverse oilgo AAACAATTTTATTTTT AAT AAACAAACTTTATGGATCCTTTTAAACAACTTTTTGATTTTTTAAACAACAATTTTTTTAAACAACAACTTTTTTT |
| idnesināb PF16 Gene name Rāppm1 dinesināb PF16 Strain Arrm1 Arrm2 Strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 ucting RRM deletion plasmids Homolo FY17X_0716706 HOMOlo FY17X_0716700 HOMOlo | Gene size (bp)/ deleted gene size (bp)/ size (bp) 1904/1904 5137/1184 1809/1809 P8 AAGCGAAATAAATGAAACG GETTACTCTCTTGTCCACAT CAGATTATCTCTCTTGTCCACAT CAGATTATCATCTCCTGGCTTAAGGGACATATAC ATATTCTCCT GGGCTTAAGTGATATACC GGTTGTTGC | FORWARD primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATT CCCCAAGCTTACCGGTTCCA AGTAATAATACAC P10 GGTTAAAAGCTAAAAAGGC CCCCCACTACAAAAAGCC AAACAAAAAATAATGTATTGG FORWARC GTAACTCAAAAAAATAATGAATGACC CGACACCCCTCAGAATATTCAAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACCAGTG C Left homol | Reverse primer CATGCATGCTTTGTGTATT TTATITTTTGTCG CATGCATGCATTTGTTTTTTTTTTTTTTTTT | Forward primer COGCTCGAGTTTTTGTCATT TTTTGAGGT COCCTGAGACAGAACAAC TTAATGAGACAG COCCTGAGACAGAACAAC TTAATGACAAG P12 CGTAATTTCAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGTACAGGT ATGATATTTTACAGGT TAT TTGGCTTCTTAGGTTCGCT TAT TTGGCTTCTTAGGTTCGCT Reverse ATCTGAGGGTGTCGTATTGTT TAT TTGGCTTCTTAGGTTCGGCT P5 TTTCATTGAGGAGAATTCCAC GGGGGTTAAGCTACTATTG G | Reverse primer COGRATICOTTOCATGAA CAAACAA COGRATICOTTOCATGAA CAACAACAA COGRATICOTTOCTOCA TICATTICTTG GOCTITAGACCTCCCAATTCC CTCCTTTTGC P13 CCGACACCTCTAAACTACCC CGATGCAAACTATAACATCT GCAACAGTAATACTAGCGA A Primer GTTCCTCAAATGGTTCATTTA TTCTTTGAATATCTGAG ATTTATTGAATATCTGAG ATTTATTGAATATCTGAG CTAACTTCAACCATTTTATC P6 ATTTATATGAGATGACATGG CTAACTTCAACCATTTTATC | Forward oilgo TATTGTAGGGGGGTTAAGC TATTGTAGGGGGGTTAGC TATTGTTACCCTCAGTATAC AAAAT TATTGTTACCCTCAGTATAC AAAAT TATTGTAGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACAGAGTCCGTAACT Target sib TATTGTTAAAGAAAAATAAAAAT TGT TATTGTAAAGGAAAAGGACC CATAAA Target sib | Reverse oilgo AAACATATACCAACACACACACACACACACACACACACA |
| idesinable PET of Gene name Report idesinable PET of Gene name Report idesinable PET of Strain Strain Strain Comment Strain Comment Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 ucting RRM deletion plasmids Homolo PY17X_0919000 ucting RRM deletion plasmids Homolo COG_TTGGACCATGGAGAACGAA GITTATCTAA COG_TTGGACCATTGGAGGA ATTCGACAAAA ATTCGACAAAA ATTCGACAAAA GITTATGTAA COG_TTGGACATTGGAGGA GITTGGAACATTCTGAAACT ucting gene in eitur complemen Gene ID | Gene size (bp)/ deleted gene size (bp)/ deleted gene size (bp) 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG G. G. GETTACTCTCTTGTCCACAT CAGATTATCATCTCTTGTCCACAT CAGATTATCATCTCTTGTCCACATATCATCTCTTAAGGGACATATACATATCTCCT GGGCTTAAGGGACATATACCATATCTCCT GGGTTGTGC P2 AGGGTTGTGCTAAGTGTTATATCC GGTTGTTGC ATATCTCT GCTTGTTGCTAAGTGTTATATCC GGTTGTTGC Taggattatagggtatatatatatatatatatatatatat | FORWARD primer CGGGGTACCAGGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCCAAGGTTACAGGTTCTAA GGTAAAAGCTAAAAAAGGC CGTTAAAGCTAAAAAAGGC CTGTCCAATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARG CGAACACCCTCAGAATATTCAA P3 CTTCAACAGGAATTATTAAA ATTGAATGGGAAACGAGTG G Left homol Forward primer | Reverse primer CATGCOATGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | Forward primer CGGCTGAGATTTATGTCATT TTTTGAGGT CGGCTGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TAATAATATTTTGGCTCTC CGACATGACTTGTACAGGT G Primere Reversa ACTGAGAGGTGTCGTATTGTT TAT TTGGCTTCTTAGGTTCGGCT P5 TTTCATTGGAGAGAATTCCAC GGGGGTTAAGCTACTATTG G Right home Forward primer | Reverse primer COGAATICOTTCCAATGAA CAACAA CAACAA CAACAA CAACAA CAACAA CAACAA | FORWARD GIIGO TACTAT TATTOTAGGGGGTTAAGC TATTOTATAGCATCAGTATAC TATTOTACCATCAGTATAC TATTOTACCATCAGTATAC P14 GGTTACAAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T AAGCTTCAAGATCCGTAACT Target all FORWARD GAAAAAAT TGT TATTGTAAGGAAAAATAAAAAT TGT TATTGTAAGGGAAAGGACC CATAAAA Target all FORWARD GIIGO TATTATTAGAAAAATAAAAAT TGT TGT TGT TATTGTAAGGGAAAGGAACCC CATAAAA Target all | Reverse oilgo AAACATAGTAGCTTAACC CCTAC CCTAC GTAC GTA |
| idestination Gene name Ropert Gene name Ropert direstination Fit of Strain www. strain www. Strain www. Strain www. Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0919000 PY17X_0919000 pv17X_0919000 pv17X_09190000 pv17X_09190000000000000000000000000000000000 | Gene size (bp)/ deleted gene size (bp)/ 1904/1904 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG GGTTAACTCTCTTGTCCACAT CAGATTATTGTCATATCATC GGGCTTAAGGGACATATAC ATATTCTCCT GGGCTTAAGTGTTATATCC GGGTTTAAGTGTTATATCC P2 AGGGCTAAGTGTTATATCC ATATTCTCCT ATGGCCCATATACATATATCC GGGTTTAGTGTTATATTCC T ATGGCCCATATACATATATATCC ATGGCCCATATACATATATATCC T ATGGCCCATATACATACACAC hatton plasmids Tag N-terminal 4Myc | Forward primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTTGCC ATTIATIATT CCCAAGCTTACCGGTTCCA GTAAAAAATACAC P10 GGTTAAAAAGCTAAAAAAGCC CTGTCCATTAAGTTGTTCTG TAAAGCACAATAATGTATTGG FORWART GTAACTCAAAAAAATAATTAATG CGACACCCCTCAGATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGAGTG G FORWART GTAACTCAAAAAAATTAAATG ACG CGACACCCCTCAGAATATTCAAA LETT homol FORWART GGGGTACCCGATACACGA CGGGGTACCCCGATACACGA CGGGGTATACCCGATACACCGA CGGGGTATACCCGATACACCACCATACACCATACACCACACCACACCACACACCAC | Reverse primer CATGCATGCTTTGTGTATT TTATITTTTGTCG CATGCATGCATTTGTTTTTTTTTTTTTTTTT | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGATACAGGT ATCTGAGGGTGTCTC CGACATGACTTGTACAGGT TAT TTGGCTTCTTAGGTTCGCTT TAT TTGGCTTCTTAGGTTCGCT Right home Forward primer CCGCTCGAGTTTTACTATTT CCGCTCGAGTTTTACTTTTTTTTTT | Reverse primer COGAATICOTTCCAATGAA CAACAA CAACAA CAACAA CAACAA CAACAA CAACAA | FORWARD GIBD TATTGTAGGGGGGTTAAGC TACTAT TATTGTAGCCTCAGTATAC AAAAT TATTGTACCCTCAGTATAC ACTGT P14 GGTTACAAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACTGTATGTTGAGGGG TACTGTATGTTGAGGGG TATTGTTAAGGAAAAAAT TGT TATTGTTAAGGAAAAAAAA | Reverse oligo AAACATAGTAGCTTAACC CCTAC CCTAC AAACATTTTGTATACTCAI GTAAC AAACATTTTGTATACTCAI GTAAC CACT or sgrna Reverse oligo AAACACAATTTTATTTTATAC or sgrna Reverse oligo Reverse oligo AAACACAATTTTATTTTACTTAC or sgrna Reverse oligo |
| idestination Gene name Ropert Gene name Ropert direstination Fit of Strain www. strain www. Strain www. Strain www. Gene name | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 ucting RRM deletion plasmids Homolo PY17X_0919000 ucting RRM deletion plasmids Homolo COG_TTGGACCATGGAGAACGAA GITTATCTAA COG_TTGGACCATTGGAGGA ATTCGACAAAA ATTCGACAAAA ATTCGACAAAA GITTATGTAA COG_TTGGACATTGGAGGA GITTGGAACATTCTGAAACT ucting gene in eitur complemen Gene ID | Gene size (bp)/ deleted gene size (bp)/ 1904/1904 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG GGTTAACTCTCTTGTCCACAT CAGATTATTGTCATATCATC GGGCTTAAGGGACATATAC ATATTCTCCT GGGCTTAAGTGTTATATCC GGGTTTAAGTGTTATATCC P2 AGGGCTAAGTGTTATATCC ATATTCTCCT ATGGCCCATATACATATATCC GGGTTTAGTGTTATATTCC T ATGGCCCATATACATATATATCC ATGGCCCATATACATATATATCC T ATGGCCCATATACATACACAC hatton plasmids Tag N-terminal 4Myc | Forward primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTTGCC ATTIATIATT CCCAAGCTTACCGGTTCCA GTAAAAAATACAC P10 GGTTAAAAAGCTAAAAAAGCC CTGTCCATTAAGTTGTTCTG TAAAGCACAATAATGTATTGG FORWART GTAACTCAAAAAAATAATTAATG CGACACCCCTCAGATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGAGTG G FORWART GTAACTCAAAAAAATTAAATG ACG CGACACCCCTCAGAATATTCAAA ATTGAATGGGAAACGAGTG CTTCAACAGGAAATTATTGAA Left homol FORWART GTAACTGAACACGCATTACACGA CGGGGTACACCGATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTACCACCGATACACCGA CGGGGTATACACGA CGGGGTACCACCGATACACCGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACCGA CGGGGTACCACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTACCCGATACACCGA CGGGGTACACCGATACACCGA CGGGGTACCCCGATACACCGA CGGGGTACCCCGATACACCGA CGGGGTACCCCCATACACCGA CGGGGTACCCCCATACACCGA CGGCGCACCCCCCATACACCGA CGCGCTACCCCCACCACCCCCA CGGGGTACCCCCCATACACCGA CGCCCCCCCCCC | Reverse primer CATGCCATGCTTTGTGTATT TTATITTTTTTTTTTTTTTTTTTTTTTTT | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGATACAGGT ATCTGAGGGTGTCTC CGACATGACTTGTACAGGT TAT TTGGCTTCTTAGGTTCGCTT TAT TTGGCTTCTTAGGTTCGCT Right home Forward primer CCGCTCGAGTTTTACTATTT CCGCTCGAGTTTTACTTTTTTTTTT | Reverse primer CCGAATTCATTCATCA ACAAACAA CCGGAATTCCTTTCATCA CCGAATTCATTCTTCA CTCATTCTTC CTCCTTTTCC P13 CCGACACACTCTAACTCTC CAACCACTCAACTCATCATCATCA CGACACACTCAAACTCTCACCACACACACACACTCAACTCATCA | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TATTOTAGGGGGGTTAGC TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC GGTTACAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACTGTATGTTGAGGGG TATTGTAAGGATCCGTAACT Target sib TOTTAGGAAAAAAAAAAT TGT TATTGTAAAGGAAAAAAAA | Reverse oligo AAACATATATATATATA of agrina of agrina Reverse oligo AAACATITTATATATATATATA Reverse oligo AAACAATITTATATATATATATA AACATTATATATATATATATATAT |
| inesin80 Gene name REPORT Gene name REPORT REPORT Strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0919000 PY17X_0919000 pv17X_0919000 pv17X_09190000 pv17X_09190000000000000000000000000000000000 | Gene size (bp)/ deleted gene size (bp)/ 1904/1904 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG GGTTAACTCTCTTGTCCACAT CAGATTATTGTCATATCATC GGGCTTAAGGGACATATAC ATATTCTCCT GGGCTTAAGTGTTATATCC GGGTTTAAGTGTTATATCC P2 AGGGCTAAGTGTTATATCC ATATTCTCCT ATGGCCCATATACATATATCC GGGTTTAGTGTTATATTCC T ATGGCCCATATACATATATATCC ATGGCCCATATACATATATATCC T ATGGCCCATATACATACACAC hatton plasmids Tag N-terminal 4Myc | Forward primer CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCACGATACACGA GGAATACTA CGGGGTACCCCGGTTTGCC ATTIATIATT CCCAAGCTTACCGGTTCCA GTAAAAAATACAC P10 GGTTAAAAAGCTAAAAAAGCC CTGTCCATTAAGTTGTTCTG TAAAGCACAATAATGTATTGG FORWART GTAACTCAAAAAAATAATTAATG CGACACCCCTCAGATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGAGTG G FORWART GTAACTCAAAAAAATTAAATG ACG CGACACCCCTCAGAATATTCAAA ATTGAATGGGAAACGAGTG CTTCAACAGGAAATTATTGAA Left homol FORWART GTAACTGAACACGCATTACACGA CGGGGTACACCGATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTACCACCGATACACCGA CGGGGTATACACGA CGGGGTACCACCGATACACCGA CGGGGTATACACGA CGGGGTATACACGA CGGGGTATACACCGA CGGGGTACCACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTATACACCGATACACCGA CGGGGTACCCGATACACCGA CGGGGTACACCGATACACCGA CGGGGTACCCCGATACACCGA CGGGGTACCCCGATACACCGA CGGGGTACCCCCATACACCGA CGGGGTACCCCCATACACCGA CGGCGCACCCCCCATACACCGA CGCGCTACCCCCACCACCCCCA CGGGGTACCCCCCATACACCGA CGCCCCCCCCCC | Reverse primer CATGCCATGCTTTGTGTATT TTATITTTTTTTTTTTTTTTTTTTTTTTT | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGATACAGGT ATCTGAGGGTGTCTC CGACATGACTTGTACAGGT TAT TTGGCTTCTTAGGTTCGCTT TAT TTGGCTTCTTAGGTTCGCT Right home Forward primer CCGCTCGAGTTTTACTATTT CCGCTCGAGTTTTACTTTTTTTTTT | Reverse primer CCGAATTCATTCATCA ACAAACAA CCGGAATTCCTTTCATCA CCGAATTCATTCTTCA CTCATTCTTC CTCCTTTTCC P13 CCGACACACTCTAACTCTC CAACCACTCAACTCATCATCATCA CGACACACTCAAACTCTCACCACACACACACACTCAACTCATCA | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TATTOTAGGGGGGTTAGC TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC GGTTACAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACTGTATGTTGAGGGG TATTGTAAGGATCCGTAACT Target sib TOTTAGGAAAAAAAAAAT TGT TATTGTAAAGGAAAAAAAA | Reverse oilgo AAACATATATATTATATA of sgRNA Reverse oilgo AAACATITTATTATATATTATATATATATATATATATATA |
| inesin8b Performance Gene name Report Inesin8b Performance Strain Arma Strain Arma Strain Arma Arma Gene name Commance Strain Arma | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 ucting RRM deletion plasmids Homolo Forward primer CGGTCGACCAGAGAGAAA GTITATCTAA CCGGTCGACCATTGGAGGA ATTCCACAAA RRM deletion P1 CAACAATTGTGAAAACAGAG GTTGGCAACATTCTGAACT ucting gene in situl complemen Gene ID PY17X_0716700 in situl complementation gene in situl complementation gene | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909/1909 1909/1909/1909/1909/1909/1909/1909/1909 | FORWARD primer CGGGGTACCACGA GGAATACTA CGGGGTACCACGA GGAATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCCAAGCTTACCGGTTTCTAA AGTAATAATACAC P10 GGTTAAAAGCTAAAAAAGC CCTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC GTAACTCAAAAAAATATAAATG ACG CGACACCCTCAGATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGGATAGGGGAAACGAGTG G FORWARD FORWARD FORWARD FORWARD FORWARD CGGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGGGGTACCACCA GGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGAATACCTA GGGGGTACCGCATACACCA GGAATACCTA GGGGGTACCGCATACACCA GGGGGTACCGCATACACCA GGAATACCTA CGGGGGTACCGCATACACCA GGAATACCTA CTCTTCTTTCTTTCTTTTTTTTTT | Reverse primer CATGCCATGCTATT TATHITITICGTCG CATGCATGCATTITITITITITITITITITITITITITITITITITIT | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGATACAGGT ATCTGAGGGTGTCTC CGACATGACTTGTACAGGT TAT TTGGCTTCTTAGGTTCGCTT TAT TTGGCTTCTTAGGTTCGCT Right home Forward primer CCGCTCGAGTTTTACTATTT CCGCTCGAGTTTTACTTTTTTTTTT | Reverse primer CCGAATTCATTCATCA ACAAACAA CCGGAATTCCTTTCATCA CCGAATTCATTCTTCA CTCATTCTTC CTCCTTTTCC P13 CCGACACACTCTAACTCTC CAACCACTCAACTCATCATCATCA CGACACACTCAAACTCTCACCACACACACACACTCAACTCATCA | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TATTOTAGGGGGGTTAGC TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC GGTTACAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACTGTATGTTGAGGGG TATTGTAAGGATCCGTAACT Target sib TOTTAGGAAAAAAAAAAT TGT TATTGTAAAGGAAAAAAAA | Reverse oilgo AAACATTATGGTAACC CATAC GTAAC AACATTTTGTATACTGAG GTAAC CACT of sgRNA Reverse oilgo AAACAATTTTATGGTCTTTAC of sgRNA Reverse oilgo AAACAATTTATGGTCCTTTAC of sgRNA Reverse oilgo AAACAATTTATGGTCCTTTAC |
| inesin80 PF10 Gene name Ropm1 Idean80 PF10 Rome name Ropm1 Idean80 PF10 Strain Virm1 Virm1 Virm2 Diagnostic PCR primers for strain Gene name Ropm1 Idean80 Strain Strain Virm1 Gene name Ropm1 Idean80 | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 Loting RRM deletion plasmids Homolo FORWARD PRIMER ALCAGAGA GITLATICTAA COGGTCGAGCAGAACGAA ATTCCACAAA RRM deletion P1 CAACAATTGTGAAAACAGAG GTTGGCAACATACTGAACT Loting gene in situ complement Gene ID PY17X_0716700 In situ complementation gene P1 AAGCGAAATGAAACG | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909/1909 1909/1909/1909/1909/1909/1909/1909/1909 | FORWARD primer CGGGGTACCACGA GGAATACTA CGGGGTACCACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATI CCCAAGGTTACCGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAAGCTAAAAAAGGC CCCTCTCCAATTAAGTTGTTCTG AAAGCACAATAATGATTGTGT FORWART GTAACTCAAAAAAATTAAATG ACG CGACACCCTCAGAATAATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGACTG G FORWART GTAACTCAAAAAATTATAATG ACG CGACACCCTCAGAATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGACTG G GGAAATACTA P3 | Reverse primer CATGCCATGCTATT TTATITITITITITITITITITITITITITITITI | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATAATTTTTGGCTCTC CGACATGACTTGATACAGGT ATCTGAGGGTGTCTC CGACATGACTTGTACAGGT TAT TTGGCTTCTTAGGTTCGCTT TAT TTGGCTTCTTAGGTTCGCT Right home Forward primer CCGCTCGAGTTTTACTATTT CCGCTCGAGTTTTACTTTTTTTTTT | Reverse primer CCGAATTCATTCATCA ACAAACAA CCGGAATTCCTTTCATCA CCGAATTCATTCTTCA CTCATTCTTC CTCCTTTTCC P13 CCGACACACTCTAACTCTC CAACCACTCAACTCATCATCATCA CGACACACTCAAACTCTCACCACACACACACACTCAACTCATCA | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TATTOTAGGGGGGTTAGC TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCCTCAGTATAC AAAAT TATTOTAGCATCAGTATAC GGTTACAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TACTGTATGTTGAGGGG TATTGTAAGGATCCGTAACT Target sib TOTTAGGAAAAAAAAAAT TGT TATTGTAAAGGAAAAAAAA | Reverse oilgo AAACATTATGGTAACC CATAC GTAAC AACATTTTGTATACTGAG GTAAC CACT of sgRNA Reverse oilgo AAACAATTTTATGGTCTTTAC of sgRNA Reverse oilgo AAACAATTTATGGTCCTTTAC of sgRNA Reverse oilgo AAACAATTTATGGTCCTTTAC |
| inesin80 PF16 Gene name Rippm1 Inesin80 PF16 Strain Strain Arrim1 Arrim2 Rippm2 Gene name Rippm1 Strain Strain Arrim1 Arrim2 Rippm3 Rip | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 Gene ID PY17X_0716700 PY17X_0716700 PY17X_0716700 PY17X_0919000 Loting RRM deletion plasmids Homolo Forward primer CGGTCTGAACCATGGAGGA ATTCCACAAA ATTCACAAA RRM deletion P1 CAACCATTGTGAAAACAGAG GTTGGCAACATATCTGAACT Loting gene in situ complement Gene ID PY17X_0716700 In situ complementation gene P1 AAGGGAAATAAATGAAACG GGGCGAAAACGAACGAACGAACGAACGAACGA | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1904/1904 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809 1909/1809/1809 1909/1809/1809/1809/1809/1809/1809/1809/ | FORWARD primer CGGGGTACCACGA GGAATACTA CGGGGTACCACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATI CCCAAGGTTACCGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAAGCTAAAAAAGGC CCCTCTCCAATTAAGTTGTTCTG AAAGCACAATAATGATTGTGT FORWART GTAACTCAAAAAAATTAAATG ACG CGACACCCTCAGAATAATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGACTG G FORWART GTAACTCAAAAAATTATAATG ACG CGACACCCTCAGAATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGACTG G GGAAATACTA P3 | Reverse primer CATGCCATGCTATTTTATTTTTTTTTTTTTTTTTTTTTT | Forward primer CCGCTCGAGTTTATCTCATT TTTTGAGGT CCGCTCGAGACAGAACAAC TTAATGAGACAG CCGCTCGAGACAGAACAAC TTAATGACAAG P12 CGTAATTTCAAAAATGAAGA TATGATAATTTTTTGGCTCTC CGACATGACTTGTACAGGT ATGATATTTTTTTTTACAGTTCTTTTTTTACAGTTCAGGTTTACAGGTTTATTTTTTTT | Reverse primer CCGAATTCATTCATCA ACAAACAA CCGGAATTCCTTTCATCA CCGAATTCATTCTTCA CTCATTCTTC CTCCTTTTCC P13 CCGACACACTCTAACTCTC CAACCACTCAACTCATCATCATCA CGACACACTCAAACTCTCACCACACACACACACTCAACTCATCA | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TACTAT TATTOTAGCCTCAGTATAC AAAAT TATTOTACCCTCAGTATAC AAAAT TATTOTACCCTCAGTATAC TATTAGTGCATGTGTTTGGA CTGT P14 GGTTACAAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T AAGCTTCAAGATCCGTAACT Target sib FORWARD GIBDO TATTATAGAAAAATAAAAAT TATT TATTGTAAAGGAAAAGAACC CATAAA Target sib FORWARD GIBDO TATTGTAAGGGAAACCCATAAA Target sib FORWARD GIBDO TATTGCGGACGCTAATCGTA GCTA | Reverse oligo AAACATATTATGATACATTAC of sgRNA Reverse oligo AAACATTTATTTTATATTATTATTATTATTATTATTATTA |
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| conesinão PF16 Cane name Raport Cane name Raport Strain Arma Arm | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0716700 PY17X_0716700 PY17X_0919000 pv17X_0919000 pv1 complementation Gene ID PY17X_0716700 pv17X_0716700 pv17X_0716700 pv1 complementation gene pv1 AAGCGAAATAAATGAAACG Gutting information gene pv1 AAGCGAAATAATGAAACG gutting information gene pv1 AAGCGAAATAAATGAAACG gutting information gene pv1 AAGCGAAATGATGAAGG gutting information gene pv2 AAGCGAAGGAGGAGGAAGAACG gutting information gene pv2 AAGCGAAGGAGGAGGAAGAACG gutting information gene pv2 AAGCGGAAGGAGGAAGAAGAACG gutting information gene pv2 AAGCGGAAGGAGGAAGAACG gutting information gene pv2 AAGCGGAAGGAGGAACG gutting information gene pv2 AAGCGGAAGGAGGAACG gutting information gene pv2 AAGCGGAAGGAGGAACGAACG gutting information gene pv2 AAGCGGAAGGAGGAACG gutting information gene pv2 AAGCGGAAGGAGGAACGAACG gutting information gene pv2 AAGCGGAACGGAGGAGGAACG gutting information gene pv2 AAGCGGAACGGAGGAGGAACG gutting information gene pv2 AAGCGAAGGAGGAGGAACGAACG AAGCGAAGGAGGAACG | Gene size (bp)/ deleted gene size (bp)/ deleted gene size (bp)/ 1904/1904 5137/1184 1809/1809 AAGCGAAATAAATGAAACG GETTAACTCCTCTTGTCACACT CAGATTATTGTCATATCATC GOGGTTAAGGGACATATAC ATATTCTCCT GGGGTTAAGTGTTATATCC P2 AGGGTTAAGTGTTATATATCC GGTTGTGC ATGACCCAATACATACACAC hatton plasmids Tag N-terminal 4Myc P2 GGTTAAAAGCTAAAAAGGC C GGGGTTAAAAAGCTAAAAAAGGC C Begous arm Reverse primer CCCCTCGAGCATGTTTAGG | FORWARD primer CGGGGTACCACGA GAMATACTA CGGGGTACCACGA GAMATACTA CGGGGTACCCCGGTTGTGC ATTIATIATT CCCCAMCCTTACGGTTTCTAA AGTAATAATATCAC P10 GGTAAAAGCTAAAAAGGC CTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARG GTAACTCAAAAAATATAAATG ACG CGACACCCTCAGATATTCAA P3 CTTCAACAGGAATTATTGAA ATTGAATGGGTACCGATACACCA GGAAATACTA P3 CGGCGTACCGCATACACCA GGAAAAAATAAAATACAC AAA CGGCGAAAAAAATAAAAT | Reverse primer CATGCCATGCTATTGTTATTT TTATITTTGTCG CATGCCATGCATTTTTTTTTTTTTTTTTTTTT | Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT CCGCTCGAGTAGAGACACAC TTTATTGAGCAG P12 CGTAATTTCAAAAAATGAAGA TAATAACAAGT P12 CGTAATTTCAAAAAATGAAGA TATGATATTTTTGGCTCTC CGACATGACTTGTACAGGT G primers Reveres ATCTGAGGGGTTATGTCGTTTTATTTTATTTCATTTTTATTCATTTTTTGGGTTCATTGTGGGTTTATGTGGGTTTATGTCAGGTTGAGGGTTAAGGTTATGTGGGTTTATGTGGTTCAGGTTTATGTGGTTCAGGTTTAGGTTCAGGTTAGGTTTAGGTTCAGGTTAGGTTAGGTTAGGTTTAGGTTCAGGTTAG | Reverse primer COGANTICOTTCAATGAA CAAACAA COGGAATICCTTTCATTCA COGGAATICCTTTCTCA COGGAATICCTTTCTCA TCATTTCTTC GCGCTTAAGCTCOCAATTC P13 GCGGACACTCTAAGTACTC GCGACACTCTAAGTACTC GCAACAGTAATACATCT GCAACAGTAATACATCT GCAACAGTAATACATCT GCAACAGTAATACATCT GCAACAGTAATACATCT CAACATCAAATGGTTCATTTA TTCTTTGAATATCTGAG ATTTATTATCAGAATGACATGG CTAACTTCAACCATTTATC NOGOUS STM REVERSE PITMER CCGGAAATCGTTCCAATGAA GACAAACAA S Primer | FORWARD GIIGO TATTOTAGGGGGGTTAACC TATTOTAGGGGTATAC TATTOTAGGGATGGGTTAGA P14 GGTTACAAAGGCAAACCCT CATTOTAGGGATGGGTTGGA GGTTACAAAGGCAAACCCT GATTCTGTATGTTGAGGGG TATGTATGTTAAGGAAAACTGTAACT Target allb FORWARD GIIGO TATTGTAAGGAAAAAAAAAAT TGT TATTGTAAGGAAAAAAAA | Reverse oligo AAACATAGACTAACACAC GTAAC GTAAC AACATATTGATACTGAC GTAAC AACATATTGATACTGAC GTAAC CACT of sgRNA Reverse oligo AAACATACTACGACTTACC CCGC of sgRNA Reverse oligo AAACTACCTACGATTAGC CCGC of sgRNA Reverse oligo AAACTACCTACGATTAGC CCGC |
| conesin80 PF10 Gene name Rapom Conesin80 PF10 Gene name Rapom Strain Arrm1 Arrm2 Diagnostic PCR primers for constrain Arrm2 Diagnostic PCR primers for constrain Arrm1 Arrm2 Diagnostic PCR primers for constrain Strain Arrm2 Diagnostic PCR primers for constrain Strain Strain Arrm2 Cone name Rapom 1 Diagnostic PCR primers for constrain Strain Strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 Gene ID PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PV17X_0919000 PV17X_0919000 PV17X_0919000 PV17X_0919000 PV17X_0919000 PV17X_0919000 PV17X_0716700 In siw complementation gene P1 AAGCGAAATAAATGAAACG GGGGTGAAAGAAAAAAAAAA | Gene size (bp)/ deleted gene size (bp) 1904/1904 5137/1184 1809/1809 PB | FORWARD primer CGGGGTACCACGA GGAATACTA CGGGGTACCACGA GGAATACTA CGGGGTACCCCGGTTGTCC ATTIATIATI CCCAMGGTTACCGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAGCTAAAAAAGCC CTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWAR GTAACTCAAAAAATAAATTAAAT ACG CGACACCCTCAGAATATTCAA P3 CTTCAACAGGAATATTGAA ATTGAATGGGAAATATTGAA ATTGAATGGGAAATATTAAA ATTGAATGGGAAATATTAAA CGGGCTACCGCATACACGA GGAAATACTA P3 CGACACCACCACAAAAAAAAAAAAAAAAAAAAAAAAAA | Reverse primer CATGCATGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | Forward primer COGCICGAGGAGAACAAC COGCICGAGGACACAACAAC TITATGAGACAG COCCICGAGAACAACAACAAC TAATGACAAG P12 CGTAATITCAAAAAATGAAGA TAATAACAAGT P12 CGTAATITCAAAAAATGAAGA TATGATATITTTGGCGTCTC CGACATGACTTGTACAGGT ACTGAAGGTTCTTACGGTT TTGGCTTCTTAGGTTCGGCTT TAT TTGGCTTCTTAGGTTCGACT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTTGAGGT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT Right home Forward primer CCGCTCGAGTTTATGTCATT TTTTGAGGT Right home FORWARD Right home FORW | Reverse primer CCGAATTCCTTCAATCAA CAAAACAA CCGGAATTCCTTTCTCA CCGGAATTCCTTTCTTCA TCAATTCTTC CCCTTTTCC P13 CCGGACACTCTAAGTACTC CGACACACTCTAAGTACTC CGACACACTCTAAGTACTC CGACACACTCTAAGTACTC CGACACTCTAAGTACTC CGACACACTCTAACTCTC CCGACTCTAACTCTCAACTCTCTCAACTCTCAACTCTCAACTCTCTCAACTCTCTCTCAACTCTCCAACTCTCCAACTCTCAACTCTCCAACTCTCCAACTCTCCAACTCTCAACTCTCAACTCTCCAACTCTCTCAACTCTCAACTCTCAACTCTCAACTCTCAACTCTCAACTCTCAACTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCAACTCTCTCTCAACTCTCTCTCTCAACT | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TATTOTAGGGGGTTAGGA P14 GGTTACAAAAGGCAAACCCT CATTOTAGGGAAACCCT GATTCTTAGTGAAAACCCT GATTCTGTATGTTAAGGGGAAACCCT Target all FORWARD GAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | Reverse oligo AAACATAGTACATTTACT of sgRNA Reverse oligo AAACATTTATTATTATTATTATTATTATATATATATATA |
| Consisted to the control of the cont | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 Gene ID PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 Loting RRM deletion plasmide Homolog Forward primer COGTCGAGCAGAGAGAA ATTIATCTAA COGGTCGAGCAGAACAA RRM deletion P1 CAACAATTGTGAAAACAGAG GTTGGCAACATTCTGAACT Loting gene in situ complement Ucting gene in situ complement P1 AAGCGAAATAATGAAACG P1 AAGCGAAATAATGAAACG P1 COMPLETE COMPLETE COMPLETE P1 AAGCGAAATAATGAAACG P1 COMPLETE COMPLETE P1 CAACACG COMPLETE P1 CAGCGAGAGAGAAAAAAAAAAACG P1 CATGCCATGCTGATAAAGA AAAAACAACG COGGGGTACCCAGCTATAT TATGTCTTGG COGGGGTACCCAGCTATAT TATGTCTTGG COGGGGTACCCAGCTATAT TATGTCTTGG COGGGGTACCCAGCCATATT TATGTCTTGG COGGGGTACCCAGCCTATAT | Gene size (bp) / deleted gene size (bp) 1904/1904 5137/1184 1809/1809 PB | FORWARD PRIMER GGGGGTACCACGA GGAATACTA GGGGGTACCACGA GGAATACTA COCAAGCTTACCACGA CGGGGTACCCCGGTTGTCC ATTIATIATI P10 GGTTAAAAAGCTAAAAAGCC CTCTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARD GGTAACTCAAAAAAATAATGATTGA FORWARD P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGAGTG P3 CTTCAACAGGAATTATTGAA ATTGAATGGGAAACGAGTG P3 CGGGGTACCGCATACACGA GGAAATACTA P3 CGACGCAAAAAATAAATACAC AAA FORWARD FORWARD FORWARD CGGGGTACCGCATACACGA GAAATACTA ATTCAACTAAAAGAAAAATACAC AAA FORWART CCGGGGTACCGCATACACGA FORWART CCGACGAAAAAATAAAATACAC AAA ACTCAACTAAAAGGAAAACATA TCAGGGTGCTTAAAATATTTC CAGGGTGCTTAAAAAATTTTC CAGGGTGCTTAAAAAAATTTTC CAGGGTGCTTAAAAAATTTTC CAGGGTGCTTAAAAATTTTC CAGGGTGCTTAAAAATTTTC CAGGGTGCTTAAAAATTTTC CAGGGTCCTAAAATTTTC CAGGGTCCTAAAAATTTC CAGGGTCCTAAAATTTC CAGGGTCCTAAAATTTC CAGGGTCCTAAAATTATTTC CAGGGTCAAAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAATTTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAATTTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAATTTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAATTTC CAGGGTCCTAAAATTATTC CAGGTCAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAAATTATTC CAGGGTCCTAAATTTC CAGGTCCTAAATTTC CAGGTCCTAAAATTATTC CAGGTCCTAAAATTATTC CAGGTCCTAAATTATTC CAGGTCCTAAATTATTC CAGGTCCTAAATTATTC CAGGTCAAATTATTC CAGGTCCTAAATTATTC CAGGTCCTAAATTATTC CAGGTCCTAAATTAT | Reverse primer CATGCCATGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | FORWARD primer COGCICGAGACAGAACAAC TITITIGAGGT COCCICGAGACAGAACAAC TITITIGAGACAG COCCICGAGACAGAACAAC TAATGACAAG P12 CGTAATITCAAAAATGAAGA TATGATAATITTIGGCGTCTC CGACATGACTTGTACAGGT A primers Reverse TITICATTGGAGAGACATCATTGT RIght home Forward primer CGGGGGTTAACCAGTTTTGT RIght home Forward primer CCGCICGAGTTTATGTCATT TITITIGAGTT RIght home Forward primer CCGCICGAGTTTATGTCATT TITITIGAGGT ANATACAATGGGGATAAAAAT AAATACAATGGGGATAAAAAAT AAATACAATGGGGATAAAAAAT AAATACAATGGGGATAAAAAAT AAATACAATGGGGATAAAAAAT AAATACAATGGGGATAAAAAA | Reverse primer COGAMITICATICAMITA GACAAACAA P13 GCGGATICCTITCTCTCA TCATTICTTG P13 GCGGACACTCTAAGTACTG CGACACTCTAAGTACTG CGACACACTCTAAGTACTG CGACACACTCTAAGTACTG CGACACACTCTAAGTACTG CAACAGTAATACTATCAGCA A PTIMER PTIMER PG ATTITATATGAGATGACTGT CTAACTTCAACCATTTTATC PG ATTITATATGAGATGACTATTAC PG ATTITATATGAGATGACATGACACACATGACTCAACCTCAACCTTCAACCATTTTATC PG ATTITATATCAGACATTTTATC PG ATTITATATCAGACATTTTATC PG ATTITATATCAACCATTTTATC PG ATTITATATCAACCATTTTATC ROGGUSS REVERSE PITMER CCGGAATTCCATCCAATGAA GACAAACAA S Primer AGTGTATTTGCGTTT TTACTTGCAAATATTT TTACTTGCAATTT TTACTTGCAAATATT TTACTTGCAAATATT TTACTTGCAAATATTT TTACTTGC | FORWARD GIBDO TATTOTAGGGGGGTTAAGC TACTAT TATTOTAGGGGGGTTAAGC TACTAT TATTOTAGCCTCAGTATAC AAAAT P14 GGTTACAAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T AAGCTTCAAGATCCGTAACT Target sib FORWARD GIBDO TATTATAGGAAAAAAAAAT TATT TATTATAAGGAAAAAAAA | Reverse oligo AAACATAGTACTAACC COTAC COTAC AAACATTTTGTATACTGAC GTAAC AAACATTTTGTATACTGAC GTAAC AAACACTTTTGTATACTGAC AAACACACACT of sgRNA Reverse oligo AAACTACATTTTTATTGTATACC AAACACACATACTTACC of sgRNA Reverse oligo AAACTACGTACGATTACC CCGC of sgRNA Reverse oligo AAACTACGTACGATTACC CCGC aaaCCACACTACTTTGTACAC AAACACCACATACTTTGTAAACACACAACACA |
| resin8b F16 Gene name bppm1 inesin8b F16 Iligo eequences for constr strain rrm1 rrm2 Iligo eequences for constr gene name bppm1 inesin8b F16 Iligo eequences for constr strain rrm2 Iligo eequences for constr Gene name bppm1 Iligo eequences for constr strain strain strain strain strain | Gene ID PY17X_0716700 PY17X_0204100 PY17X_0919000 gene knockout Gene ID PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_0919000 ucting RRM deletion plasmids Homolo FY17X_0716700 PY17X_0819000 ucting RRM deletion plasmids Homolo FORWARD primer CCGCTCGAGCATTGGAGGA ATTCGACAAA ATTCGACAAA ATTCGACAAA TRM deletion P1 CAACAATTGTGAAAACAGAG GTTGGAACATATCTGAACT ucting gene in situ complement Gene ID PY17X_0716700 In situ complementation gene P1 AAGCGAAATAAATGAAACG GUILing intron deletion plasmids P1 HOMOlo Forward primer CATGCCAGGATGAAGA CGGGGAAATAAAGAAGG CGGGGAAATAAAGGAAGGAAGAAAAAAGCAAGAGGAAGAAGAAGAAGAA | Gene size (bp) / deleted gene size (bp) 1904/1904 1904/1904 1904/1904 1904/1904 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909 1909/1909/1909/1909/1909/1909/1909/1909 | FORWARD primer CGGGGTACCAGGA GGAAATACTA CGGGGTACCAGGA GGAAATACTA CGGGGTACCCCGGTTGTCC AGGGGTACCCCGGTTGTCC AGGGGTACCCCGGTTTCTAA AGTAATAATATCAC P10 GGTTAAAAAGCTAAAAAAGCC CTGTCCATTAAGTTGTTCTG AAAGCACAATAATGTATTGG FORWARC GGACACCCTCAGATTATTCAA P3 CTTCAACAGGAAATAATTATAGA ATTGAATTGGAAACCACTG GGGGTACCGCATACACGA GGAAATAATTAAATCAC AAA P3 CGACGCAAAAAATAAAATACAC AAA FORWARC FORWAR | Reverse primer CATGCCATGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | Forward primer COGCICGAGGAGAACAAC TITITGAGGT COCCICGAGGACAGAACAAC TITITGAGACAG P12 CGTAATITCAAAAAATGAAGA TAATAACAAGT P12 CGTAATITCAAAAAATGAAGA TATGATAATITTTGGCGTCTC CGACATGACTTGTACAGGT A primers Reverse TITICATTGGGTTCATTGTT TAT Right home Forward primer CGGGGGTTAAGCTACTTTG Right home Forward primer CCGCTCGAGTTTATGTCATT TITTGAGGT P5 TITICATTGGGTTCATTTTTTTTTGAGGTT RIGHT HOME FORWARD AGGTT RIGHT HOME FORWARD THE PRIMER RIGHT HOME FORWARD THE PRIME | Reverse primer COGAMITICATICAMITA GACAAACAA P13 GCGGATICCTITCTCTCA TCATTICTTG P13 GCGGACACTCTAAGTACTG CGACACTCTAAGTACTG CGACACACTCTAAGTACTG CGACACACTCTAAGTACTG CGACACACTCTAAGTACTG CAACAGTAATACTATCAGCA A PTIMER PTIMER PG ATTITATATGAGATGACTGT CTAACTTCAACCATTTTATC PG ATTITATATGAGATGACTATTAC PG ATTITATATGAGATGACATGACACACATGACTCAACCTCAACCTTCAACCATTTTATC PG ATTITATATCAGACATTTTATC PG ATTITATATCAGACATTTTATC PG ATTITATATCAACCATTTTATC PG ATTITATATCAACCATTTTATC ROGGUSS REVERSE PITMER CCGGAATTCCATCCAATGAA GACAAACAA S Primer AGTGTATTTGCGTTT TTACTTGCAAATATTT TTACTTGCAATTT TTACTTGCAAATATT TTACTTGCAAATATT TTACTTGCAAATATTT TTACTTGC | Forward oilgo TATTGTAGGGGGGTTAGCT TATTGTAGGGGGGTTAGCT TATTGTTACCCTCAGTATAC AAAAT TATTGTACCAGTGGTTTGGA CTGT P14 GGTTACAAAGGCAAACCCT C GATTCTGTATGTTGAGGGG T AAGCTTCAAGATCCGTAACT Target sib Forward oilgo TATTGTTAAAGGAAAAAAAAAT TGT TATTGTAAAGGAAAAGGACC CATAAA Target sib Forward oilgo TATTGTAAAGGAAAAGGACC CATAAA Target sib Forward oilgo TATTGTGAGGGGCTAACCGTAGCTAGCTAGACTAGAAAAATAAAAT GGTG TATTGTGAGCGTTGACTAACGTAGCTAGCTAGATG TATTGTGACGCTTGACTAAACTAA | Reverse oligo AAACATACTTAGGATAGC CACT of agrina of agrina Reverse oligo AAACATITTATTAGGATAGC CTAC of agrina Reverse oligo AAACAATITTATGGGTCCTT anacatitataggatagatagatagatagatagatagatagata |

| | 011100110111 | CCGGAATTCGTGGGAAAAA ATAAGTATTAC | GTTGAGCGCGGGTTCTAAGC | CCCTTAGAAATATGGAA | AAGGGCTTAGAACCCGCGCT | TCAACAAGAGTGACAAT | TATTGGAAAATGAGAACAAA AAAA | TCC TTTTTTTGTTCTCATTT |
|--|--|--|--|--|--|---|--|--|
| Diagnostic PCR primers for in | tron deletion | | | | I | | | |
| Strain | P1 | P2 | P3 | P4 CGCTCATTTAAAACCTGTTT | P5 | P6 | | |
| | CTGAAATAGAATGCTTAAAA GAAC GTACGTGCAGGTGCTTTAC | GCATIGICITIAICIGITAI | AATTTGGGTTCTATAACCCC | AC ACTION TO ACCORDING TO ACCOR | C C | CGGATAATGAACCATCGATT | | |
| PF16∆I1 | C C | | CATTTATTAAATCAGGGTGC | GCACAATAATGTATTGGAAA | CCTAGTTGTAATTTCTCAAA | ACGATTTGGTTATATATAAT | | |
| | GCACACGTAATCAAATTTAA | TCTTTAAAATGTAGCAACTT A | GCTTCCTCTCAAATACATTA | CCATCTGATACTTTCAATGC | TTCAAAAAGTAGAAGTATGC | CTGAAAAGTACGTTATAAGG | | |
| 1109100 AI1 | CATATGCAAATAATATTTGC AC | TATTTCTAAGGGCTTAGAAC | ATTGTCACTCTTGTTGAGCG | TATTCATCTAAGCAGAAAAA T | CGGTAGCAGTATATGTAGTT | CTGATTATAAAAGGAAAGAC | | |
| Oligo sequences for construct | ting <i>bfp</i> reproter assay plass | nide | | | | | | |
| Expression element | Forward primer | Reverse primer | Reporter gene | Forward primer | Reverse primer | Expression element | Forward primer | Reverse primer |
| The 5'-UTR of hsp70 | CGGGGTACCGTAAAGAGGA TGATGTATGT | TTTTAATTGCAA | qua | CATGCCATGGGGATGGTGA GCAAGGGCGAGGAGCT | CCGCTCGAGTTACTTGTACA GCTCGTCCATGC | | CCGCTCGAGTGTTCATTTTT CTTATTTAT | AACAGTCCGA |
| Reporter gene | Upstream bi Forward primer | fp sequence Reverse primer | Int Forward primer | ron Reverse primer | Downstream Forward primer | bfp sequence Reverse primer | Overla Forward primer | p PCR Reverse primer |
| | CATGCCATGGGGATGGTGA | TAGCCCCAACCTTGAAGTC | CGACTTCAAGGTTGGGGCT | TGCCGTCCTCCTACAAATAA | TTATTTGTAGGAGGACGGC | CCGCTCGAGTTACTTGTACA | CATGCCATGGGGATGGTGA | CCGCTCGAGTTACTTGTAC |
| Mar Internation | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | GACGCCCTTCA CTGAATATACTTGTGGCCGT | ACGCCACAAGTATATTCA | ACAAAATAAC CACGCTGAACCTGTTTACAT | AACATCCTGGG ATGTAAACAGGTTCAGCGT | GCTCGTCCATGC CCGCTCGAGTTACTTGTACA | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | GCTCGTCCATGC CCGCTCGAGTTACTTGTAC |
| | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | TTACGTCGCC TTTATTTTACTTGAAGTTCAC | TGAACTTCAAGTAAAATAAA | ATATTAAAAA GTGGCGGATCCTAAATAAA | GAGGGGCGAGG TTTTATTTAGGATCCGCCAC | CCGCTCGAGTTACTTGTACA | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | GCTCGTCCATGC CCGCTCGAGTTACTTGTAC |
| hfn-dic114 | | | ACAAAACAAA ACAACATCTAGTAACATATT | AGAGAAAAGCC GGCCATGATACTGAAAAGT | AACGTGGAGG TACTTTTCAGTATCATGGCC | GCTCGTCCATGC CCGCTCGAGTTACTTGTACA | CATGCCATGGGGATGGTGA | GCTCGTCCATGC CCGCTCGAGTTACTTGTAC |
| | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | | AATATAATTT GGGCGTCGACGTAATTTAA | ACGTTATAAGG CCTCCTTGAACTGATTATAA | TTATAATCAGTTCAAGGAGG | GCTCGTCCATGC CCGCTCGAGTTACTTGTACA | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | CCCCTCGAGTTACTTGTAC |
| hfn 440040012 | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | TTCAGCTCGA TAAGTATTACGTCGACGCC | TATTATAATAA GGGCGTCGACGTAATACTT | AAGGAAAGAC CCTCCTTGAACTAAGCAGAA | ACGGCAACAT TTCTGCTTAGTTCAAGGAGG | GCTCGTCCATGC CCGCTCGAGTTACTTGTACA | GCAAGGGCGAGGA CATGCCATGGGGATGGTGA | GCTCGTCCATGC CCGCTCGAGTTACTTGTAC |
| Diagnostic PCR primers for ex | GCAAGGGCGAGGA xpression cassette integrated | d Into the p230p locus | ATTTTTTCCCA | AAATTTTAAA | ACGGCAACAT | GCTCGTCCATGC | GCAAGGGCGAGGA | GCTCGTCCATGC |
| P1 | P2 | P3 | P4 | P5 | PG | | | |
| GGAAAAGTATGATAACGAT (| | | | | TGCTGAGTCAGTGGTGTTC | | | |
| G (| С | TOGGACTGTTCTTCTTCAGG | AGATGATATOGCTATATATO | GATGCATCTATAACTCCAGA | lc | | | |
| Oligo sequences for construct | | | Int | ron | Downetream g | and saguance | Overla | n DCP |
| Strain | Forward primer | Pp1 sequence Reverse primer | Forward primer | Reverse primer | Forward primer | Reverse primer | Forward primer | Reverse primer |
| gep 1-Annoul I | GAAGTTTGGA | CTGATATAAG | AGTTAGATCGT | TACATCTATCCTACAAATAA ACAAAATAAC | TTATTTTTGT | CATGCCATGGCTTTGAGGA GAGAGTGTGGG | | GAGAGTGTGGG |
| gep1-PF1611 | ATAAAAGATG | TTTATTTTACTTGCAATAAAA ATGGAGCCG | TTTATTGCAAGTAAAATAAA ACAAAACAAA | CATTAATCTCCTAAATAAAA GAGAAAAGCC | TTTTATTTAGGAGATTAATG GAGGAAGATT | GGGCTTAAGGTAGACTTAC CAACTTCATT | CGGGGTACCATGTGTTGAA ATAAAAGATG | GGGCTTAAGGTAGACTTAC CAACTTCATT |
| Strain | Target site Forward oligo | e of agRNA | | | | | | |
| | TATTGTATTGATTCCGAGTC | Reverse oligo AAACTTTCAGACTCGGAATC | | | | | | |
| nen1-DF1611 | | AAACCCATTAATCTCTTGCA | | | | | | |
| Diagnostic PCR primers for ax | AATGG | ATAAC | | | | | | |
| Strain | D1 | P2 | PS | P4 | | | | |
| | TATTGAGCTACTGTCAGCAG | | GCTTATATCAGCATCAAAAA | CTCATTTTTCGGCTTATCAC | | | | |
| | | | G CGGCTCCATTTTTATTGCAA | GAAAGCAATGATGTCTCATC | | | | |
| Primers for RT-PCR | GIGATGTACGAAAAATTCGA | GGCAAGIGIIIAIAGAAIIG | CGGCTCCATTTTATTGCAA | c | | | | |
| | Potentia de la constanta de la | Bd======= | Batana a sanana | | Bd | B-1 | Bd | Balance and an arrange |
| Primer name | Primer sequence | Primer name | Primer sequence | | Primer name | Primer sequence GGAATTTGCAGAAAGTACC | Primer name | Primer sequence |
| | CAAAGTTAATCTTCAAGAGT | kinesin8b R1 | AGTTCATCTTGCAATCCTTC | | PY17X_1357300 F6 | C GGAGAAGCCACTCAACTTA | PY17X_1357300 R6 | CGATTTTTTTTCCGATTTTT |
| | GAAAACATAACAGATAAAGA | | CTCCTCAAGCATCTTAATAT | | PY17X_1357300 F7 | T CAATGAAGAAGGAAATTCC | PY17X_1357300 R7 | GTATCATTAAACATGGAATG GAACTATCCGAGCAACTCT |
| | CATTTATTAAATCAGGGTGC | | TCGGTATTTTCAACTTCATC | | PY17X_1335600 F1 | G | PY17X_1335600 R1 | С |
| dhcő F19 | CAAGAAAATGATGCATTAAA | dhcő R19 | GTAGCGTTGATTTTCCACTA | | PY17X_1452900 F1 | CAGCCTGAATATAAACCTAA | | ATATGCTGTTTAAACTCCAC |
| | TTTCTACAACAAGAGAGAAT GAGGCAATTATGATGACGT | dhcō R20 | GGAGTCATATAATTGATCTT | | PY17X_1452900 F2 | GTGGAGTTTAAACAGCATAT | PY17X_1452900 R2 | ATCAGCAAAACTTATGTGG |
| ahc7 F6 | G G | dhc7 R6 | CTTCAAAACATGATTTAACA | | PY17X_1122300 F1 | GCCTTTTTAGCTATTTTTGT G | PY17X_1122300 R1 | GTAGAATCCGAAGTAATTC |
| dhc7 F7 | CCTACTAATCTTATAACATT | dhc7 R7 | TTTGTATCTCAATAAGCTTC | | PY17X_1122300 F2 | CGAATTACTTCGGATTCTAC | PY17X_1122300 R2 | TTCCATTTATGATAATCGTC |
| dhc7 F8 | ATCTGATCCAAGAATACAAC | dhc7 R8 | GCTCACGAATATTAGTTCCC | | PY17X_0523500 F1 | TAACACCATATAACAATAAC | PY17X_0523500 R1 | ATAATGTGAAAAGATCTTC |
| | ACGTTTTAAAGAACAAAATG | dic1 R3 | TAATGTATTTGAGAGGAAGC | | PY17X_0523500 F2 | CACCACATCTATCTTACCAG | PY17X_0523500 R2 | GGGATCCAGAATTGCAAGC T |
| | TGGTCCAACATAATTTGTGA AG | dic1 R4 | TGAATAGAAGTCTCTGCATT CG | | PY17X_0523500 F3 | GACAATATTCCAGACACTAA | PY17X_0523500 R3 | AATTTGTGGAAATGTCCAG |
| dic1 F5 | GCTACATTTTAAAGAATTCG | dic1 R5 | GCACAATATATATGAGCATG | | PY17X_0508900 F4 | CAAATTCCGATTTTTACGAT | PY17X_0508900 R4 | CCTTCTTTTTACTCATTAAT |
| dic2 F1 | ATGAGTTCCGAAAATTTTTC | dic2 R1 | TTCAACTTGTATAGCATTTG | | PY17X_0508900 F5 | AGACAGAGGACTTAATTCA G | PY17X_0508900 R5 | GTGTGTCATCAATTAGTTG |
| alc2 F2 | AAATCTAATATTCCTTATCA | dic2 R2 | CTTTGCTGAGATGTTAAACG | | PY17X_0508900 F6 | GCTTTCTTATGATGTTAACA | PY17X_0508900 R6 | CGCATTTGCTTTCTTCGCT |
| drc1 F1 | ATGCTACAAAACAATCTTAA | drc1 R1 | TTTCAAATTTCTTAACAGAC | | | GGGAAGATAACATTGAGGA | PY17X_1320300 R1 | |
| drc1 F2 | | | | | PY17X_1320300 F1 | G | | TATGTACTAGTTTTTTCTGC |
| drc1 F3 | GTCCAACCACTTTAAGATGG | drc1 R2 | CTATTAACACATTTACACTG | | PY17X_1320300 F1 PY17X_1320300 F2 | CTCATGAAAATTTGGAAGAG | PY17X_1320300 R2 | |
| | GTCCAACCACTTTAAGATGG ATTTTGAAGATGATCAAGAG | | | | | · | PY17X_1320300 R2 PY17X_1320300 R3 | CTCGTCATGTGATTGCTTA |
| abc F1 | ATTTTGAAGATGATCAAGAG CGCAGCGGATAATATCCAA | drc1 R3 dbc R1 | CTATTAACACATTTACACTG C | | PY17X_1320300 F2 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATGGC | | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA |
| abc F1 | ATTTTGAAGATGATCAAGAG | drc1 R3 dbc R1 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTTCCT | | PY17X_1320300 F2 PY17X_1320300 F3 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGAAAGC | PY17X_1320300 R3 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TTTAAAGGGTCGGGGTTCT G |
| abc F1 | ATTTTGAAGATGATCAAGAG CGCAGCGGATAATATCCAA | drc1 R3 dbc R1 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTTCCT TTTCAAAATTTACTTCCTCC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATGGC ACAGAACCCCGACCCTTTAA A | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TTTAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTC |
| dbc F1 dbc F2 md2 F1 | ATTTTGAAGATGATCAAGAG CGCAGCGGATAATATCCAA C GAGTTATCAAAAACACTGGA T CTAGCTAGAGCTATGCGTA A | drc1 R3 dbc R1 dbc R2 md2 R1 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTTCCT TTTCAAAATTTACTTCCTCC CGTACTTTAATTTACGTATC C | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATGGC A CAGAACCCCGACCCTTTAA A GCAGTAATGACAATAGAAGT | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TTTAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTTATCC |
| dbc F1 dbc F2 ma2 F1 PY17X_1109100 F1 | ATTITIGAAGATGATCAAGAG CGCAGCGGATAATATCCAA C GAGTTATCAAAAACACTGGA T CTAGCTAGAGCTATGCGTA A TTGTCACTCTTGTTGAGCGC | drc1 R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTTCCT TTTCAAAATTTACTTCCTCC CGTACTTTAATTTACGTATC C TCAATAGGAACTTGTGAATC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC A CAGAACCCCGACCCTTAAA A GCAGTAATGACAATAGAAGT AGGATAAAAACTCGACATCC | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TITAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTATCC TACAAATGATACTTTTTGCG |
| dbc F1 dbc F2 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA C GAGTTATCAAAAAACACTGGA T CTAGCTAGAGGCTATGCGTA A TTGTCACTCTTGTTGAGCGC | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 | CTATTAACACATTTACACTG C CGTTITTATCATTTTCTTCCT TTTCAAAATTTACTTCCTCC CGTACTTTAACTTACCTACTC CTAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGTC TTCGAAAAGTTCTTCGACAC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATGGC A CAGAACCCCGACCCTTTAA A GCAGTAATGACAATAGAAGT | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 | CTCGTCATGTGATTGCTTA CATTCATGAGTTCTTTTGA TTTAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTATCC TACAAATGATACTTTTTGCG CCTGACTCCATATTATATCT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGTT GAGTT CTAGAGAGACAGTGGA T TGTGCACTGTTGAGAGGG GGGTGTGTTAGAAAAGTTTT CGAAACTGATGTTAAAAAATA | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 | CTATTAACACATTTACACTG C CGTTITATCATTTTCTTCCT TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC C TCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGTC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC ACAAACCCCGACCCTTTAA ACCAGTAATGACAATAGAAGT AGGATAAAAACTCGACATCC GAGATTTGTACGAATGTCGA CACAAAGAAGGCAATCATGT | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT GTATTTAATGCCTCAATTTTC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTTGCC CCTGACTCCATATTATATCT GTCATTGATAGATGATACTTTT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA. GAGTTATCAAAAACACTGGAT TCTAGCTAGAGCTATGCGTA ATTGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAGTTTT CGAAACTGATGATTAAAAATA CGCAGTAAATATAGAGTTAG | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 PY17X_0521800 R2 | CTATTAACACATTTACACTG C COTTITATCATTTTCTTCCT TITCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC C TCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 | CTCATGAMAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC ACAACCCCGACCCTTTAA ACCAGTAATGACAATAGAAGT AGGATAAAAACTCGACATCC GAGATTTGTACGAATGTCGA CACAAAGAAGGCAATCATG GATTCTGAGGAATTTGATCA | PY17X_130300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTTGCC CCTGACTCCATATTATATCT GTCATTGATAGTTCTTTGCAACATTTAGTTCATAGTTCATTGCACATTTAGTTCATCATTTAGTTCATTGCAACATTTAGTTCATCATTTAGTTCATCATTTAGTTCATCATTTAGTTCATCATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACATTTAGTTGCAACAACATTAGTTGCAACATTAGTTGCAACATTAGTTGCAACATTAGTTGCAACATTAGTTGCAACAACATTAGTTGCAACAACATTAGTTGCAACATTAGTTGCAACATTAGTTGCAACATTAGTTGCAACAACATTAGTTGCAACAACAACAACAACAACAACAACAACAACAACAACAA |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGTTATCAAAAACACTGGA TCTAGCTAGAGCTATGCGTA ATTGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAGTTTT CGAAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATAGTGTTTTACCTAAATC | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_1311800 R4 | CTATTAACACATTTACACTG C COTTITATCATTTTCTTCCT TITCAAAATTTACTTCCTCC CGTACTTTAATTTACGTATC C CAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTATTTTCCCACTCT | | PY17X_130300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_015900 F13 PY17X_015900 F12 | CTCATGAMAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC ACAGACCCCGACCCTTTAA A GCAGTAATGACAATAGAAGT AGGATATGACAATAGAAGT GAGATTTGTACGAATGTCGA CACAAAGAAGGCAATCATG GATTCTGAGGAATTGTAC CGTTGAAAATGTTTCCTCAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0415900 R12 | CTCGTCATGTGATTGCTTAL CATTCATGAGTTTCTTTGA TITTAAAGGGTCGGGGTTCT GTATTTAATGCCTCAATTTTCC GGATGTCGAGTTTTTATCC CCTGACTCCATATTATATCT GTCATTGATAGTTCTTTGCAA TCTTATGCATTTTTAGTATCT CATCATTTAGTTCTTTGCAA TCTTATGCATTTTTTTTCCAA |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGTTATCAAAAACACTGGA TCTAGCTAGAGCTATGCGTA ATTIGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAATA CGCAGTAAATATAGAGTTAG ATAGTGTTTTATCCTAAAATC GATATTACCCATAAGGTGTG | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTTCCT TTTCAAAATTTACTTCCTCC CGTACTTTAATTTACGTATC C CAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGTC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTTTTTCCACTCT CGTAGCTAAATTATCATGTAG | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F12 PY17X_0415900 F13 | CTCATGAMAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC A CAGAACCCCGACCCTTTAA A GCAGTAATGACAATAGAAGT AGGATATGACAATAGAAGT CAGATTTGTACGAATGTCGA CACAAGAGAGGCAATCATG GATTCTGAGGAATTTGATCA CGTTGAAAATGTTTCCTCAA GCCACATTTTTACGAAAATG GCCACATTTTTACGAAAATG | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0415900 R12 PY17X_0415900 R13 | CTCGTCATGTGATTGCTTAL CATTCATGAGTTCTTTGA TITTAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTTCC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTTGCC CCTGACTCCATATTATATC GTCATTGATAGTTCTTTGCAA TCTTATGATTAGTTCTTCCAATTATTATCTCC AGTATTGATTTTTTCTCC |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F6 | ATTITIGANGATGATCAAGAG CGCAGCGGATAATATCCAA GAGITATCAAAAACACTGGA TCTAGCTAGAGCTATGCGTA ATTIGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAGTTTC CGAAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATTAGTGTTTTATCCTAAATC GATATTACCCATAAGGTGTG ATTAGAGTTGTTTCCTTCC | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R5 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTCCT TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC C CGTACTTTAATTTACGTATC C CGTAGGAACTTGTGAATC GCTGTGTATATGTACGAGGC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCATTTCTCCACTCT CGTAGCTAAATTATCATGTAG ATATCTATATTCATAGACCC | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0105800 F1 | CTCATGAAAATTTGGAAGGA AAAATATTAAAATTGGAAGCCCGAGCCCTTTAA AGCAGTAAGAACCCCGACCCCTTTAA AGCAGTAATGACAATAGAACT AGGATAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCCAC AAAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAGGGTCGGGGTTCT TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTTGCC CCTGACTCAATATATATCT GTCATTGATAAGTTGATTGTCA CATCATTTAGTCTTTTGCA ACGTCATCAATATATTCTCC AGTATTTAGTCTTTTTGCAA CCTTATGCAATTTTTTTCTCC AGTATTTTTTTCTCC AGTATTTTTTTTCTCC AGTATTTTTTTTCTCC AGTATTTTTTTTCTCC AGTATTTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTCC AGTATTTTATCTC AGTATTTTATCTCC AGTATTTTATCTC AGTATTTTATCTC AGTATTTTATCTC AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTATCT AGTATTTTTATCT AGTATTTTTATCT AGTATTTTTATCT AGTATTTTTATCT AGTATTTTTATCT AGTATTTTTTT AGTATTTTTTT AGTATTTT AGTATTT AGTATT AGTATTT AGTATT AGTAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F6 PY17X_1313800 F6 PY17X_1323900 F1 | ATTITGAAGATGATCAAGAG CGCAGGGGATAATATCCAA CGAGGTATATATCAAA CATAGCTAGAGGATAATATCAAAACAACATGGAA TIGTCACTCTGTTGAGAGCA GGGGTGTTAGAAAAACAATTAGAGTTAAAAATAATAATAATAATAATAATAATAAT | orct R3 obc R1 obc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R4 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_131300 R6 PY17X_1323900 R1 | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TITCAAAATTTACTTCCTC COTTACTTTACATTTACTTCCTC COTTACTTTACT | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F12 PY17X_105400 F12 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0105800 F1 PY17X_1216400 F4 | CTCATGAAAATTTGAAAGG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATTGGC CAGAACCCCGACCCTTTAA AGCATAATAAAAATCGAAATCGAAACCCCGACATTGAAAAAAATCCGACATCC GAGATTTGTACGAAATTGAACT CACCAAGAAGGCAATCATC CATTCTGAGGAATTTGATCA CATTCTGAGGAATTTCCTCAA GCCACATTTTTACCAAAATT GCTACCACATTTTATCC GAGAGGAAACATAAATTTGTC CAGAGGGAAACATAAATTTGTC GAGAGGAAACATAAATTTGTC | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R1 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R12 PY17X_0415900 R12 PY17X_0415900 R13 PY17X_0105800 R1 PY17X_1215400 R4 | CTCGTCATGTGATTGCTTAL CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT TATTAATGCCTCAATTTTC GGATGTCGAGTTTTTATCC CTCGACTCCATATTATCC CCTGACTCCATATTATTCT CATCATTATATATCT CATCATTATATCTCC CATCATTATAGTCTTTGCAA TCTTATGCATTTTTACCC AGTATGTCTCTGGCCCTTA ACGTCCTCAAACTTGAGCT CTTTTGACTCATAAGTTTG CTTTTTGACTCATAAGTTTG CTTTTTGACTCATAAGTTT CTTTTGACTCATAAGTTT CTTTTTGACTCATAAGTTT CTTTTTGACTCATAAGTTT CATCATATACTCATAAGTTT CATCATATACTCATAAGTTT CATCATATATCTCATAAGTTT CATCATATACTCATAAGTTT CATCATATATCTCATAAGTTT CATCATATATCT CATCATATATCT CATCATATATCT CATCATATATCT CATCATATATCT CATCATATATAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0821800 F1 PY17X_0821800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F6 PY17X_1357300 F5 | ATTITGAAGATGATCAAGAG CGCAGGGGATAATATCCAA CGAGGTATATATCAAA CATAGCTAGAGGATAATATCAGAT CTAGCTAGAGGATATGAGGTA ATTIGTCACTCTTGTTGAGAGCG GGGTGTTAGAAAAGATTAGAGTTAGA ATAGTGATTTAAAAATA CGCAGTAAAATATAGAGTTAG ATAGTGTTTTATCCTAAAAC GATATTACCCATAAGGTGTG ATTAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R6 PY17X_1323900 R1 | CTATTAACACATTTACACTG C CGTTTTATCATTTTCTCCT TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC C CGTACTTTAATTTACGTATC C CGTAGGAACTTGTGAATC GCTGTGTATATGTACGAGGC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCATTTCTCCACTCT CGTAGCTAAATTATCATGTAG ATATCTATATTCATAGACCC | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0105800 F1 | CTCATGAAAATTTGGAAGGA AAAATATTAAAATTGGAAGCCCGAGCCCTTTAA AGCAGTAAGAACCCCGACCCCTTTAA AGCAGTAATGACAATAGAACT AGGATAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCC AAAAAAAAACTCGACATCCAC AAAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 | CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGTTCT TATTAATGCCTCAGATTTC GGATGTCGAGTTTTATCCT TACAAATGATACTTTTTGCG CCTGACTCCATATTATATCT GTCATTGATAAGTTGATCTC ACTCATTATAGTTCTTGCAA TCTTATGCATTTTTGCAA ACGTCCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTT |
| dbc F1 dbc F2 ma2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F2 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_131300 F5 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR | ATTITGANGATGATCANGAG CGCAGGGGATAATATCCAA CGCAGGGGATAATATCCAA CGCAGTAGAGAGAAAAAAAAAA | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_131300 R5 PY17X_1323900 R1 PY17X_1357300 R5 | CTATTAACACATTTACACTS C COTTITATCATTTTCTCCT TITCAAAATTTACTTCCTC CGTACTTTAACTTACCACTC CGTACTTTAATTTACGTATC CCTGTGTATATCTACGAGTC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAAACTTCTTCGACAC GCTCTCTACTATTCCTCACG GTCTTCTATTTCCTACTC CGTACCTAAAATATCATGTAG ATATCTATATTCATAGACCC GTCTTTCCAGTTTATACAC GGATCGTTTTATACAC GGATCGTTTTATACAC | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F2 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_1216400 F4 PY17X_1216400 F5 | CTCATGAAAATTTGGAAGGA AAAATATTAAAATTGGAAGGC CAGATGGAGTATTAATTGGC CAGAACCCCGACCCTTTAA AGCATGAATAAAAACTCGACATCC AGGATAATGACAATAGAACT AGGATAAAAAACTCGACATCC ACACAGAGAAGGCAATCATG CACAGAGGAATTGATCA CGTTGAAGAATTGTCCCA CGTTGAAGAATTTGTCCCA GCCACATTTTTACCAAAAATG GCTACCCTGTCTTATTTTATC GAGAGAAACATAAATTTGT CGCAGAACATTAAAAAAGGG G | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_015900 R12 PY17X_015900 R13 PY17X_015900 R13 PY17X_015900 R13 PY17X_015900 R13 PY17X_015900 R13 PY17X_015900 R13 | CTCGTCATGTGATTGCTTA- CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTCT TAITTAATGCCTCAATTTTC GGATGTCGAGTTTTATCCT TACAAATGATACTTTTTGCC CCTGACTCAATATATCT GTCATTGATAAGTTGATTATCT GTCATTGATAAGTTGATTGCA ACGTCATATATATCTC AGTATTATGCTTTTTGCAA TCTTATGCATTTTTTGCAA CCTTATGCAATTTTTACTCC AGTATGTCTTTGGACT CTTTTTCAACTCATAAGTTT G ATCGTTTCAATCATAAGTTT G ATCGTTTCAATCATAAGTTT G ATCGTTTCAATCATAAGTTT G ATCGTTTCATTCAGTTTCAG |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_131300 F1 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR Location | ATTITGAAGATGATCAAGAG CGCAGGGGATAATATCCAA CGAGGTATATATCAAA CATAGCTAGAGGATAATATCAGAT CTAGCTAGAGGATATGAGGTA ATTIGTCACTCTTGTTGAGAGCG GGGTGTTAGAAAAGATTAGAGTTAGA ATAGTGATTTAAAAATA CGCAGTAAAATATAGAGTTAG ATAGTGTTTTATCCTAAAAC GATATTACCCATAAGGTGTG ATTAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1323900 R1 PY17X_1323900 R1 PY17X_1357300 R5 | CTATTAACACATTTACACTG C CGTTITATCATTTTCTTCCT TTTCAAAATTTACTTCCTC CGTACTTTACATTTACCTACC CGTACTTTACATTTACCTACC CTCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC CTCGTCAGAATTAAAGTTT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCCACTC CGTAGCTAAATATCATGTGAA TATCTTATTTCTATGACAC CGTCTTTCTATTTCTATGACAC CGTCTTTCTATTTCTATGACAC CGTCTTTCTATTTCTATGACAC CGTCTTTCTATTTCTATAGACACC GTCTTTCCAGGTTTTATACACC GGATCGTTTTATGTACACAC GGATCGTTTTATTCTATACACC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F12 PY17X_0415900 F13 PY17X_0415900 F1 | CTCATGAAAATTTGAAAGG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATTGGC CAGAACCCCGACCCTTTAA AGCATAATAAAAATCGAAATCGAAACCCCGACATTGAAAAAAATCCGACATCC GAGATTTGTACGAAATTGAACT CACCAAGAAGGCAATCATC CATTCTGAGGAATTTGATCA CATTCTGAGGAATTTCCTCAA GCCACATTTTTACCAAAATT GCTACCACATTTTATCC GAGAGGAAACATAAATTTGTC CAGAGGGAAACATAAATTTGTC GAGAGGAAACATAAATTTGTC | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0105800 R1 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT TATTAAAGGGTCGAGTTTTATCC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTGCC CCTGACTCCATATTATATCT GTCATTGATAAGTTGATCC CATCATTTAGTTCTTTGCAA TCTTTAGTCATTATATCT CATCATTTAGTTCTTTCCAA ACGTCCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTT G Reverse primer |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGATTATCAAAAACACTGGAT TGTAACATGATGATA TTGTCACTCTTGTTGAGCGC GGGTGTTTAGAAAAATTAC CGCAACTGATGTTAAAAATTAC GCAACTGATGTTAAAATTAGAGTTTG ATTAAGATTTACCTAAAATC GATATTACCCATAAGGTGTG ATTAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG AATATAAGAGTGTGT AATATAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAAATG AATATAAGACTCTACCAG Gene ID PY17X_1330200 | drcf R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_131300 R1 PY17X_1337300 R5 PY17X_1357300 R5 Forward primer GAGGAGGTAGATCAGCTTT GT | CTATTAACACATTTACACTG C CGTTITATCATTTTCTCCT TTTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTGATATGTACAGATC TCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC CTCGTCAGAATTAAAGTTT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCACTCT CGTAGCTAAAATATCATGTAG ATACTATATTCATAGACCC GTCTTTCAGTTTATTCAACACC GTCTTTCAGTTTATTCAACACC GTCTTTATTTCAAGACCC GTCTTTCAGTTTATTAACACC GTCTTTCAGTTTATTAACACAC GAACGTTTTATTTATATCAAA | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F10 PY17X_1305400 F13 PY17X_0415900 F12 PY17X_0415900 F13 PY17X_0415900 F14 PY17X_0415900 F15 | CTCATGAAAATTTGAAAGG AAAATATTAAAATTGAAAGC CAGATGGAGTAATTAATGGC CAGAACCCCGAGCCCTTTAA AGCAGTAATGACAATAGAAGT AGGATAAAAAACTCGACATCG AGAATTGTACGAAATTGTAC CACCAGAAGAAGGCAATCTGT GATTCTGAGGAATTTGATCA CCTGAGAAATTTGTACCAAAATT GCTGAGAAATTTTCATCA CCCACATTTTTACCAAAATT GCTACCTGTCTTATTTTATC CAGAGGGAAACATAAATTTGT CGCAGACATTAAAAAAGGG Gene ID PY17X_1215400 | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT G TATTTAATGCCTCAATTTCC GGATGTCGAGTTTTTATCC TACAAATGATACTTTTTGCC CCTGACTCCAATTATATCT GTCATTGATAAGTTGATCC CATCATTTAGTTCTTTTATCCC AATAGTCTCTTTGACC CTTTTCTAACTCATAAGTTG |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH | ATTITGAAGATGATCAAGAG CGCAGCGGGATAATATCCAA CGCAGCGGGATAATATCCAA GAGTTATCAAAAACACTGGAT TIGTCAACTGATGATGAGAGCTATGAGAGCTATGAGAGAGTTAGAAAATTAAAATAAC CGCAGTAAATATAAGAGTTTG ATTAAGAATTAAAATC ATTAAGAATTGATTAAAATC ATTAAGATTGATTACTAAATC GTATTTGCTCGACAAAAATG ATTATACCATAAGGTTTG ATTAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG ATATAAGCATTACACAG Gene ID PY17X_1330200 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R8 PY17X_131300 R1 PY17X_1323900 R1 PY17X_1357300 R5 Forward primer GAGCAGGTAGATCAGCTTT GT GT GAGCAGGTAGATCAGCTTAT | CTATTAACACATTTACACTG C CGTTITATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTGATATGTACAGATC TCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCACTCT CGTACCTAAAATATCATGTAG ATATCTATATTCATACACC GTCTTCCAGTTTATACACC GGCTTTCAGTTTATCACA GGATCGTTTATGTACAAACCCAGTTTATCAAAACACACCAGTTAAAGGCAAACACCAGTTACAAACTCAAACTCAACTTAAAAGCAAACACCAGTTACAAACTCAACTGAGCAAACACCAGTTACAAACTCAACTGAGCAAACACCAGTTACAAACTCAACTGAGCAAATTCAAACTGAGCAAACACCAGTTACAAACTCAAACTCAAACTCAACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAACTTGATCACTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCAACTGAGCAACACCAGTTACAAACTCAACTTGATCAACTGAGCAACACCAGTTACAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAAACTCAACTTACAAAAACTCAACTTACAAAACTCAACTCAAAAACACAACTACT | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F12 PY17X_0415900 F13 PY17X_0415900 F1 PY17X_0415900 F1 PY17X_0415900 F1 PY17X_0415900 F1 PY17X_0415900 F1 PY17X_0415900 F1 Location | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTTATTAATGGC CAGAACCCCGAGCCCTTTAA AGCATGAAGAACTCGACATCC GAGATTGTACGAACTCGACATCC GAGATTTGTACCGAACTCGACATTCGACCATCT T GATTCTGAGGAATTTGATCA GCTGAGGAATTTCCTCAA GCCACATTTTTACCAAAATT GCTACCACATTTTACCAAAATTG GCTACCACATTTTACCAAAATTG GCTACCACATTTTACCAAAATTG CAGAGGAAAACATAAATTGT CGCGAGAACATTAAAAAGAGG G Gene ID | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTAL CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT TATTAAATGCCTCAAGTTTTCCC GGATGTCGAGTTTTTACCC TACAAATGATACTTTTCCG GCCTGACTCCAATTATATCT GTCATTGATAAGTTCATCCC CATCATTAGTTCTTTCCAA TCTTACAAATTGATCTC CATTCATTAGTCTCTCCAAAATTGACCC CTTTTCTAACTCAAAATTGAGCT CTTTTCTAACTCATAAGTTT G Reverse primer CCCGTTATCTTCTTCATCACCA CA TGGCCCTTAACTCATACACT CA TGGCCCTTAACTCATCACACACACA TGGCCCTTAACTCATCACACACACACA TGGCCCTTAACTCATCACACACACACACACACACACACAC |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_131800 F6 PY17X_1357300 F5 Primers for RT-qPCR Location GAFDH o-tubuiln 2 | ATTITGAAGATGATCAAGAG CGCAGCGGGATAATATCCAA CGCAGCGGGATAATATCCAA GAGTTATCAAAAACACTGGAT TIGTCAACTGATGATGAGAGCTATGAGAGCTATGAGAGAGTTAGAAAATTAAAATAAC CGCAGTAAATATAAGAGTTTG ATTAAGAATTAAAATC ATTAAGAATTGATTAAAATC ATTAAGATTGATTACTAAATC GTATTTGCTCGACAAAAATG ATTATACCATAAGGTTTG ATTAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG ATATAAGCATTACACAG Gene ID PY17X_1330200 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_131300 R5 PY17X_1323900 R1 PY17X_1357300 R5 Forward primer GAGCAGGTAGATCAGCTTT GT ATOTTGGTCAAGCAGGTAT CC GGGAGAAGGTATGGACGAA | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTACTTTAATTTACGTATC CTCAATAGGAACTTGTGAATC GCTGTGATATGTACAGACC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGACAC GTCTTCTATTTCTCACTCT CGTACCTAAAATTCATAGACCC GTCTTCCAGTTTAATACAC GGATCGTTTATTACACA GGATCGTTTATTATCACA Reverse primer ACTCTAAAGGCAACACCAG TTA CAACTTAAAGGCAACACCAG TTA CAACTTGATCACTGGGCATT CAACTGCACAGCAGCAGCTTCATAC CTCAAGGCAGTGGGCATCTG CTCAGGCAGTGGGCATCTG CTCAGGCAGTGGGCATCTG CTCAGGCAGTGGGCATCTCC CTTCAGGCAGTGGCATCTCCTCACTCTCCTCCTCCTCCTCCTCCTCCTCCTCC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F10 PY17X_1305400 F13 PY17X_0415900 F12 PY17X_0415900 F13 PY17X_0415900 F14 PY17X_0415900 F15 | CTCATGAAAATTTGAAAGG AAAATATTAAAATTGAAAGC CAGATGGAGTAATTAATGGC CAGAACCCCGAGCCCTTTAA AGCAGTAATGACAATAGAAGT AGGATAAAAAACTCGACATCG AGAATTGTACGAAATTGTAC CACCAGAAGAAGGCAATCTGT GATTCTGAGGAATTTGATCA CCTGAGAAATTTGTACCAAAATT GCTGAGAAATTTTCATCA CCCACATTTTTACCAAAATT GCTACCTGTCTTATTTTATC CAGAGGGAAACATAAATTTGT CGCAGACATTAAAAAAGGG Gene ID PY17X_1215400 | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_0415900 R19 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTAL CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT TATTAAATGCCTCAACTTTTCC GGATGTCGAGTTTTTATCC CCTCACTCCAATTATATCT GTCATTGATAACTCTTTCCAC AGTATGTCATAACTCTTTCACAC AGTATGTCAAAATTGACTCTTCCAAAATTGACTCTTTCAAAATTGACTCTTTCAAAATTGACTCTTTTCTAACTCAAAATTGACTCTTTTCTAACTCAAAATTGACTCTTTCTAAACTTTTCAACTCTTTCTAAACTTTTCAACTCTTTCTAACTCATAAGTTTTCAACTCTTTCTT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F6 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH o-tubulin 2 β-tubulin | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA CAGATTATCAAAAAACACTGGA TTAGCATAGAGCTATGCGTA ATTIGCACTCTTGTTGAGCGC GGGTGTTAGAAAAATTACAGTTAG CGAAACTGATGTTAAAAAAT CGCAGATAAATATAGAGTTAG ATTAGACATTATACCAAAATTACCAATTATACCATAAGGTTGTAAAATTACCATAAGGTTGAAATTAGAATTGTATTCCTTACATC GTATTTGCTCGACAAAAATG AATATAAGCATCTATCACAG Gene ID PY17X_1330200 PY17X_0524100 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_109100 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_131800 R5 PY17X_131800 R5 PY17X_131500 R5 COUNTY REPORT | CTATTAACACATTTACACTG C CGTTITATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTGATATGTACAGATC TCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCACTCT CGTACCTAAAATATCATGTAG ATATCTATATTCATACACC GTCTTCCAGTTTATACACC GGCTTTCAGTTTATCACA GGATCGTTTATGTACAAACCCAGTTTATCAAAACACACCAGTTAAAGGCAAACACCAGTTACAAACTCAAACTCAACTTAAAAGCAAACACCAGTTACAAACTCAACTGAGCAAACACCAGTTACAAACTCAACTGAGCAAACACCAGTTACAAACTCAACTGAGCAAATTCAAACTGAGCAAACACCAGTTACAAACTCAAACTCAAACTCAACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAACTTGATCACTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCACTGAGCAACACCAGTTACAAACTCAACTTGATCAACTGAGCAACACCAGTTACAAACTCAACTTGATCAACTGAGCAACACCAGTTACAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAACTCAACTTACAAAACTCAACTTACAAAAACTCAACTTACAAAACTCAACTCAAAAACACAACTACT | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F10 PY17X_1405400 F12 PY17X_0415900 F12 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 W17X_0415900 F13 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC CAGAACCCCGAGCCCTTTAA AGCATAAAAAACTCGACATCC GAGATTGTACGAAATTGTACAATTGAAATTGTACAATTGTACAATTGTACAATTGTACAAATTGTACAAATTGTACAAATTTTTTACGAAAATTGTACAAATTGTACAAATGCACAAATTAAATTTGTCAAAAATGAAAATTGTACAAATGCAAAATTAAATTGTACAAATGCAAAATTAAAATTGTACAAAATGCAAAATTGATCAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R15 PY17X_0415900 R15 PY17X_0415900 R16 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTAA GAGGGTCGGGGTTC1 GATTTAAATGCCTCAATTTTC GGATGTCGAGTTTTTACC CCTGACTCCATATTTTCC CATCATTTTACTCC AGTATGTCATTTTTCC CATCATTTTACTCC AGTATGTCTTTGCAC CTTTTCTAACTCATAAGTTACTC CTTTCTAACTCATAAGTTC CTTTCTAACTCATAAGTTC ATCGTTTCTTACTCATAAGTTC CTTTTCTAACTCATAAGTTC CTTTTCTAACTCATAAGTTC CATCGTTTCTTCATCACCA CA CACCGTTTATCTTCTTCTCATCACCA CA GAGGGTTAAACAATGAGGAG GAGGGTTAAACAATGAGGAG CCAATGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTTGCACAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCACACCAC CCAATGTGTAAAATATTGCACACACCACATGTGTAAAATATTGCACACTACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACTACACATGAGGAAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGGAGTTAAAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATAATAATATATTCACACATGAGTATAATAATTGCACATGAGTATAATAATTATTCACACATGAGTATAATATTGCACATGAGTATAATAATTATTCACACATGAGATAATAATAATAATAATAATAATAATAATAATAAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1331800 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH G-bubulin 2 β-bubulin | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA. GAGATTATCAAAAAACACTGGAT TTGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAATTCCAAC CGCACTAAAAAATACACTGAA ATTGTCACTCTTGTTGAGAAAATTTC CGAAATAAAAATACAGTTAG ATTAAGATTTTTATCCTAAATC GATATTACCCATAAGGTGTG AATTAAGATTGTTTCCTCC GTATTTTCTCGACAAAAAATG AATTAAAGATTGTATCACAG Gene ID PY17X_1330200 PY17X_0524100 PY17X_1210100 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1313800 R1 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD PRIMER GAGCAGGTAGATCAGCTTT GT ATGTTGGTCAAGCAGGTAT CG GGGACAAGATCACGAGCA A TGTCACCTCTTGTTGAGCGC | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTACTTTAATTTACGTATC CTCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC TTCGAAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCACTCT CGTAGCTAAAATATCATGTAG ATACTATATTCATATGACC GTCTTCTATTTCAATTCAA | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_0833600 F2 PY17X_1341200 F2 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_105400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_015800 F1 PY17X_1216400 F5 Location 1216400 kinesin8b I1 kinesin8b I1 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAATGGC CAGATGGAGTATTAATGGC CAGAAACCCCGAGCCCTTTAA AGCAGTAAGAAACTCGACATCC GAGATTTGTACGAAATGGAATTGTACA CACAAAGAAGAAGTCAAATTGTACA GCTTGAAGAATTTTACCAAAATTTTTCAGAAAATTTTTACGAAAATTTTTTCAGAAAATTTTTTCAGAAGAGAACATTAAATTTGTCAGAAGAGAAAAAATTTTTTCAGAAGAAAAAAATTTGTCAGAAAAATTTAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R15 PY17X_0415900 R15 PY17X_0415900 R16 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTAA GAGGGTCGGGGTTC1 GATTTAAATGCCTCAATTTTC GGATGTCGAGTTTTTACC CCTGACTCCATATTTTCC CATCATTTTACTCC AGTATGTCATTTTTCC CATCATTTTACTCC AGTATGTCTTTGCAC CTTTTCTAACTCATAAGTTACTC CTTTCTAACTCATAAGTTC CTTTCTAACTCATAAGTTC ATCGTTTCTTACTCATAAGTTC CTTTTCTAACTCATAAGTTC CTTTTCTAACTCATAAGTTC CATCGTTTCTTCATCACCA CA CACCGTTTATCTTCTTCTCATCACCA CA GAGGGTTAAACAATGAGGAG GAGGGTTAAACAATGAGGAG CCAATGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTTGCACAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCACACCAC CCAATGTGTAAAATATTGCACACACCACATGTGTAAAATATTGCACACTACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACTACACATGAGGAAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGGAGTTAAAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATAATAATATATTCACACATGAGTATAATAATTGCACATGAGTATAATAATTATTCACACATGAGTATAATATTGCACATGAGTATAATAATTATTCACACATGAGATAATAATAATAATAATAATAATAATAATAATAAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F4 PY17X_131800 F5 PY17X_1337300 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH G-bubulin RDpm1 1100100 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA CAGATTATCAAAAAACACTGGAT CTAGCTAGAGAGCTATGCGTA TIGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAACTTT CGAAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATTAGCCATAAATATCCAAATC GATATTACCCATAAGGTGTG ATTAGAGATTGTTACCAAAAAGTAG ATTAGAGATTGTATCCTCAAATC GATATTACCCATAAGGTGTG AATTAAGACTGTTCCTCC GATATTACCCATAAGGTGTAATAAGACTGTATTCCTCACAG PTTTX_1330200 PY1TX_1330200 PY1TX_1210100 PY1TX_17210100 PY1TX_0716700 | orct R3 obc R1 obc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R4 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD PRIMER GAGCAGGTAGATCAGCTTT GT ATCHTGGTCAAGCAGGTAT CCGGAGAAGGTATGACCGAA A TTGTCAGCTCTTGTTGAGCCGC A TTGTCAGCTCTTGTTGAGCCGC | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CCTGTACTTTAATTTACGTATC CTCAATAGGAACTTGTGAATC GCTGTGTATATGTACGAGC TTCGAAAAAGTTCTTCGACAC CTCGTCAGAATTAAAGTATT TATAAAAACTTCTTCGTCAG GTCTTCTATTTCTCACTCT CGTAGCTAAAATATCATGTAG ATACTATATTCATATGACC GTCTTCTATTTCAATTCAA | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_015800 F1 PY17X_1216400 F5 Location 1216400 kinesin8b 11 kinesin8b 14 PF16 I1 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAAATTGGC CAGAACCCCGACCCTTTAA GCAGTAATGACAATTCGAATGCC GAGAATTGTACCAATGCCA CACAAAGAAGCGCAATTCAAC CACAAAGAAGGCAATTCACAC GACAATTTTACCGAAATTCCAC GCTTGAAAATTTTACTCAA GCCACATTTTTACTCAAAATG GCTACCTGTCTTATTTTATC GAGAGGAAACATTAAAATTGT C GCGCAGACATTAAAAAAGAGG G GCCACATTTTAAAAAAGAGG CACACTTCAAAAAATTGT C C GCCAGACATTAAAAAAGAGG C GCCACACATTAAAAAAGAGG C C C C C C C C C C C C C C C | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R19 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CAACCCTGAAGAGATACCT CAAA GGCTAGATTAGAT | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTAA GAGGGTCGGGGTTC1 GATTTAAATGCCTCAATTTTC GGATGTCGAGTTTTTACC CCTGACTCCATATTTTCC CATCATTTTACTCC AGTATGTCATTTTTCC CATCATTTTACTCC AGTATGTCTTTGCAC CTTTTCTAACTCATAAGTTACTC CTTTCTAACTCATAAGTTC CTTTCTAACTCATAAGTTC ATCGTTTCTTACTCATAAGTTC CTTTTCTAACTCATAAGTTC CTTTTCTAACTCATAAGTTC CATCGTTTCTTCATCACCA CA CACCGTTTATCTTCTTCTCATCACCA CA GAGGGTTAAACAATGAGGAG GAGGGTTAAACAATGAGGAG CCAATGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CACCCATAGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTTGCACAC CCAATGTGTAAAATATTGCACAC CCAATGTGTAAAATATTGCACACCAC CCAATGTGTAAAATATTGCACACACCACATGTGTAAAATATTGCACACTACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACACTACACATGAGGAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACTACACATGAGGAAGTTAAACAATGAGGAAG CCAATGTGTAAAATATTGCACACACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGGAGTTAAAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATATTGCACATGAGTATAATAATTGCACATGAGTATAATAATAATATATTCACACATGAGTATAATAATTGCACATGAGTATAATAATTATTCACACATGAGTATAATATTGCACATGAGTATAATAATTATTCACACATGAGATAATAATAATAATAATAATAATAATAATAATAAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F4 PY17X_131800 F5 PY17X_1337300 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAPDH G-bubulin RDpm1 1100100 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGTTATCAAAAACACTGGAT CTAGCTAGAGAGCTATGCGTA TIGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAATTACACTGAT TCGCAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATTAGTGTTTATCCTAAATC GATATTACCCATAAGGTTGG ATTAGACCATAAGATTGCTCC GTATTTGCTCGACAAAAATG AATATAAGCATCTATCACAG Gene ID PY1TX_1330200 PY1TX_0524100 PY1TX_1210100 PY1TX_0716700 PY1TX_1109100 | orct R3 obc R1 obc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R4 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD PRIMER GAGCAGGTAGATCAGCTTT GT ATCHTGGTCAAGCAGGTAT CCGGAGAAGGTATGACCGAA A TTGTCAGCTCTTGTTGAGCCGC A TTGTCAGCTCTTGTTGAGCCGC | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TITCAAAATTTACTTCCTC CGTACTTTAACATTTACCTAC C CTACTAGAAACTTTACTTCCTC CCTCCCCACACACTCCACCACCACCACCACCACCACCACC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_015800 F1 PY17X_1216400 F5 Location 1216400 kinesin8b 11 kinesin8b 14 PF16 I1 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAAATTGGC CAGAACCCCGACCCTTTAA GCAGTAATGACAATTCGAATGCC GAGAATTGTACCAATGCCA CACAAAGAAGCGCAATTCAAC CACAAAGAAGGCAATTCACAC GACAATTTTACCGAAATTCCAC GCTTGAAAATTTTACTCAA GCCACATTTTTACTCAAAATG GCTACCTGTCTTATTTTATC GAGAGGAAACATTAAAATTGT C GCGCAGACATTAAAAAAGAGG G GCCACATTTTAAAAAAGAGG CACACTTCAAAAAATTGT C C GCCAGACATTAAAAAAGAGG C GCCACACATTAAAAAAGAGG C C C C C C C C C C C C C C C | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R19 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CAACCCTGAAGAGATACCT CAAA GGCTAGATTAGAT | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTAA GAGGGTCGGGGTTCT AGAGGTCGAGTTTTTAA GGATGTCGAGTTTTTAC CATCAAATGATACTTTTGCC CATCATTATACTC CATCATTTACTCTC AGATGTCATAGTTTTACTCC AGATGTCATGAGTTTTACTCC AGTATGATCTCTGGCCCTTA ACGTCCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTA ACGTCCTCAAACTTCAAGTTT ACGTCTTCAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTTCATCATAAGTT ACGTTTCTTTCATCATCAACA ATCGTTTCTTCTTCTTCATCA ACGAGGTTAAACAATGAGCAG ACAAGGTTAAACAATGAGCAG ACAAGGTTAAACAATGAGCAG CCAATGTGTAAAATATTGCA |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0S21800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_131800 F5 PY17X_133500 F1 PY17X_133500 F1 PY17X_13500 F5 PY17X_13500 F5 PTIME for RT-qPCR Location GAPDH G-bubuin 2 P-bubuin 1 Ropm1 1109100 0833000 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA GAGTTATCAAAAACACTGGAT CTAGCTAGAGAGCTATGCGTA TIGTCACTCTTGTTGAGCGC GGGTGTGTTAGAAAAATTACACTGAT TCGCAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATTAGTGTTTATCCTAAATC GATATTACCCATAAGGTTGG ATTAGACCATAAGATTGCTCC GTATTTGCTCGACAAAAATG AATATAAGCATCTATCACAG Gene ID PY1TX_1330200 PY1TX_0524100 PY1TX_1210100 PY1TX_0716700 PY1TX_1109100 | orct R3 obc R1 obc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R4 PY17X_1311800 R4 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_131300 R7 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD PRIMER GAGCAGGTAGATCAGCTTT GT ATCHTGGTCAAGCAGGTAT CCGGAGAAGGTATGACCGAA A TTGTCAGCTCTTGTTGAGCCGC A TTGTCAGCTCTTGTTGAGCCGC | CTATTAACACATTTACACTG C COTTITATCATTTTCTCCT TITCAAAATTTACTTCCTC CGTACTTTAACATTTACCTAC C CTACTAGAAACTTTACTTCCTC CCTCCCCACACACTCCACCACCACCACCACCACCACCACC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_015800 F1 PY17X_1216400 F5 Location 1216400 kinesin8b 11 kinesin8b 14 PF16 I1 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAAATTGGC CAGAACCCCGACCCTTTAA GCAGTAATGACAATTCGAATGCC GAGAATTGTACCAATGCCA CACAAAGAAGCGCAATTCAAC CACAAAGAAGGCAATTCACAC GACAATTTTACCGAAATTCCAC GCTTGAAAATTTTACTCAA GCCACATTTTTACTCAAAATG GCTACCTGTCTTATTTTATC GAGAGGAAACATTAAAATTGT C GCGCAGACATTAAAAAAGAGG G GCCACATTTTAAAAAAGAGG CACACTTCAAAAAATTGT C C GCCAGACATTAAAAAAGAGG C GCCACACATTAAAAAAGAGG C C C C C C C C C C C C C C C | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R19 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CAACCCTGAAGAGATACCT CAAA GGCTAGATTAGAT | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTAA GAGGGTCGGGGTTCT AGAGGTCGAGTTTTTAA GGATGTCGAGTTTTTAC CATCAAATGATACTTTTGCC CATCATTATACTC CATCATTTACTCTC AGATGTCATAGTTTTACTCC AGATGTCATGAGTTTTACTCC AGTATGATCTCTGGCCCTTA ACGTCCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTA ACGTCCTCAAACTTCAAGTTT ACGTCTTCAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTAACTCATAAGTTT ACGTTTCTTTCATCATAAGTT ACGTTTCTTTCATCATCAACA ATCGTTTCTTCTTCTTCATCA ACGAGGTTAAACAATGAGCAG ACAAGGTTAAACAATGAGCAG ACAAGGTTAAACAATGAGCAG CCAATGTGTAAAATATTGCA |
| dbc F1 dbc F2 md2 F1 pv17X_1109100 F1 pv17X_1109100 F2 pv17X_0821800 F2 pv17X_0821800 F2 pv17X_1311800 F4 pv17X_1311800 F6 pv17X_1311800 F6 pv17X_1331800 F6 pv17X_1323900 F1 pv17X_1357300 F5 pv17X_ | ATTITGAAGATGATCAAGAG CGCAGGGGATAATATCCAA CGAGTGATATCAAAAACACAGGAT CTAACTAGAGAGATAATACCGTA TIGTCACTCTTGTTGAGAAATATC CGAGATAAAAAATAATAC CGCAGTAAATATAGAGTTTA AATATACCCATAAGGTGTA ATTAGAGTTTAAAAATA CGAAACTAAATATAGAGTTGA ATTAGTGTTTTATCCTAAATC GATATTACCCATAAGGTGTG ATTAGAGATTGATTACCTACAG Gene ID PY17X_1330200 PY17X_1210100 PY17X_016700 PY17X_1109100 PY17X_0833600 | ### doc R1 ### doc R1 ### doc R2 ### doc R1 ### doc R2 ### doc R1 ### do | CTATTAACACATTTACACTS C CTTTTATCATTTTCTCCT TITCAAAATTTACTTCCTC TITCAAAATTTACTTCCTC CGTACTTTAATTTACCTATC C CTCCTCACAAATTTACTACACTC CTCGTAAAACTTCTTCGACAC CTCCTAGAAATTAAAGAACTTCTTCGACAC CTCCTAGAAATTAAAACTTCTTCGACAC GTCTTCTATTTCTCACACTC CGTAGCTAAAACTTCATCAGACC GTCTTCTATTTCTCACACTC CGTAGCTAAAATATCATGTAG ATACTATATTCATAGACCC GTCTTCCAGTTTTATACAC GGATCGTTTTATGTATCAAA Reverse primer ACTCTAAAGGCAACACCAG TTA CAACTTCATCACTCTC CAACTTCATCACTCTC ATCACTCGTTTCCCACTTC ACTCCACTCGTTTCCCACTTC ACTCCACTCGTTTCCCACTTC ACTCCACTCGTTTCCCACTTC ACTCCACTCGTTTCCCACTTC ACTCCACTCGTTTTCCCACTC GCTGTTTATATGTACCAGGTC GCGGTTTTCATGACT GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTATTCATACACTC GCTGTTTTATTCATACACTC GCTGTTTTATTATTCATACACTC GCTGTTTTATTTATTCATACACTC GCTGTTTTATTATTATCACACTC GCTGTTTTCATTCATACACTC GCTGTTTTATTATTATCACACTC GCTGTTTTCATTCATCACTC GCTGTTTTCATTCATCACTC GCTTTTTATTTATTTATCACACTC GCTGTTTTCATTCATCACTC GCTTTTCATCACTCC GCTTTTCATCACTCC GCTTTTCATTCATCACTC GCTTTTCATCACTC GCTTTCATCACTC GCTTTCATCACTC GCTTTCATCACTC GCTTTCATCACTC GCTTTCATCACTC GCTTTCATCACTC GCTTTCACCTC GCTT | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F1 PY17X_1305400 F12 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_105400 F13 PY17X_0415900 F13 P | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAAATTGGC CAGAAGCCCCGACCCTTTAA AGCAGTAATGACAATAGACATAGACATAGACATAGAAATTGTACAACAATAGAAATTGTCAACAAAGAAGAGCAATCATG CATTCTGAGGAAATTTGATCA CGTTGAAAAATGTTTCCTCAA GCCACATTTTTACGAAAATTGCAACCTGTCTTATTTTATC GAGAGGAAACATAAATTGT CGCAGACATTAAAAAAGAGC Gene ID PY17X_1218400 PY17X_0204100 PY17X_0919000 PY17X_0919000 | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_015800 R12 PY17X_015800 R12 PY17X_015800 R1 PY17X_015800 R1 PY17X_015800 R1 PY17X_015800 R1 PY17X_015800 R1 PY17X_015800 R1 ACCCTGAAGAGATACCT CAACCTGAAGAGATACCT CAACCTGAAGAGATACCT CAACCTGAAGGAAATGCAACG AGCAAGGAAAGGA | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TITAAAGGGTCGGGGTTCI A TATTTAATGCCTCAATTTTC GGATGTCGAGTTTTTTACC CTCAACTCCATATTTTCC CATCATTTTTGCA CATCATTTTACTCC AGTATGTCTTTGGACCTTAACTCATACTCATACTCATACTCATACTCATACTCATACTCATACTCATACTCTTCT |
| dbc F1 dbc F2 md2 F1 pV17X_1109100 F1 pV17X_1109100 F2 pV17X_0821800 F2 pV17X_0821800 F2 pV17X_0821800 F2 pV17X_1311800 F6 pV17X_1311800 F6 pV17X_131300 F5 pV17X_1323900 F1 pV17X_1357300 F5 Primers for RT-qPCR Location GAPDH 10-tubulin p-tubulin photology primers for RT-qPCR Location GR3900 F1 pV17X_13000 F1 p | ATTITGAAGATGATCAAGAG CGCAGGGGATAATATCCAA CGCAGGGGATAATATCCAA CAGATTATCAAAAACACTGGAT TTGTCACTCTTGTTGAGGCC GGGTGTGTTAGAAAAATAT CGCAGATAAATATAGAGTTTA CGCAGTAAATATAGAGTTTAAAAATA CGCAGTAAATATAGAGTTGG ATTAGCCATAAGAGTGTG ATTAGCCATAAGGTGTG ATTAAGATTGATTCCTTCC GTATTTGCTCGACAAAAATA AATATAAGCATCTACACAG Gene ID PY17X_1330200 PY17X_1210100 PY17X_016700 PY17X_016700 PY17X_01633600 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_131300 R1 PY17X_1323900 R1 POFWARD PRIME GGGCACAGGAGACAGGTAT ACTIGGTCAGGAGACAGATACCGTTC TGGGAGAACAGGTATCACGAGACA ATGCGCCCATGGGTGTGGACAGACACACGGGACACACGGACACACGAGACACCACGGACACACGGACACACGGGACACACGGACACACGGACACACGGACACACGGACACACGGACACACGACACACACGAC | CTATTAACACATTTACACTS C CTTTTATCATTTTCTCCT TITCAAAATTTACTTCCTC TITCAAAATTTACTTCCTC CGTACTTTAATTTACGTATC CGTGTGTATATGTACACACTC TCGAATAGGAACTTGTGAATC TCGAAAAAGTTCTTCGACAC CTCGTCAGAATTAAAATATT ATAAAAAACTTCTTCGACAC GTCTTCTATTTCCACACTC CGTAGCTAAAATATCATGTAG ATACTATATTCATAGACCC GTCTTCTATTTATACAC GGATCGTTTTATACAC GGATCGTTTTATACAC CGACTGTTATATGTACCAACTTACACACTC TCCACCTCCACTTTACACCCACTTACACTCCACTCTCACACTTACACTCACTCCACTTTACACCCACTTACACTCCACTCTCACACTTACACCAC | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F1 PY17X_1305400 F12 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAAATGGC CAGATGGAGTATTAATGGC CAGATACCCCGACCCTTTAA AGCATAATAACAACTCGACATCC GAGATATGACAATAGAATTGACA CACAAAGAAGGCAATCATG CATTCTGAGGAATTGACA CGTTGAAAAATGTTCCCAA GCCACAATTTTACCAAAATTGTCCA GCCACAATTTTACCAAAATTGTCCAC GCCACAATTTTACCAAAAATTGTCCCAC GCCACAATTTTACCAAAAATTGTCCCACACACTCATCCACACACA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_1216400 R4 PY17X_1216400 R5 | CTCGTCATGTGATTGCTTA CATTCATGAGTTCTTTTGA TTTAAGGGTCGGGGTTCT ACTTCATGAGTTCTTATCTC GGATGTCGAGTTTTTACTC CCTGACTCCATATTATCT GTCATTGATAGTTCTTGCAT ACGTCCTCATATATATCT ACGTCCTCATAGTTTTTACTC AGATGTCTCTAGACTCTTTACATC AGATGTCTCTAGACTCTTACACTC AGATGTCTCTAGACTCTTACACTC AGATGTCTCTAGACTCTTACACTCATCAACTTCTCTTCT |
| dbc F1 dbc F2 md2 F1 pV17X_1109100 F1 pV17X_1109100 F2 pV17X_0521800 F1 pV17X_0521800 F2 pV17X_0521800 F2 pV17X_1311800 F5 pV17X_1311800 F5 pV17X_1323900 F1 pV17X_1323900 F1 pV17X_1323900 F1 pV17X_1357300 F5 primers for RT-qPCR Location GAPDH c-bubulin Rippm1 1100100 0833000 primers for RIP-qPCR Location Kinesin8b I1 kinesin8b I1 | ATTITGAAGATGATCAAGAG CGCAGCGGGATAATATCCAA CGCAGCGGGATAATATCCAA GAGTTATCAAAAACACTGGAT TTGTCAACTGTGTTAGAGAGGC GGGTGTTTAGAAAAATTAGAGTTTA CGCAGATAAATATAGAGTTTA ATAGTGTTTAAAATAGAGTTTG ATTAGAAATTGAATTGAATAGAGTTTG ATTAGAATTGATTACCAAAATG GGRE ID PY17X_130200 PY17X_0524100 PY17X_0524100 PY17X_0833600 GGRE ID PY17X_0833600 PY17X_0833600 PY17X_0804100 PY17X_0804100 PY17X_0804100 PY17X_0204100 | drct R3 dbc R1 dbc R2 md2 R1 py17X_1109100 R1 py17X_1109100 R2 py17X_0521800 R2 py17X_0521800 R2 py17X_1311800 R4 py17X_1311800 R5 py17X_1311800 R5 py17X_1313800 R5 py17X_1313800 R5 py17X_1313800 R5 py17X_1323900 R1 py17X_1357300 R5 Forward primer GAGCAGGTAGATCAGCTTT GT ATGTTGGTCAAGCAGGTAT CGCACAAGATCACGAGACA ATG CGCACAAGATCACGAGACA ATG GGGTAGTTGGACGAC ATGGGTGTGGAAAAAAGTC CAGACAAGATCACGAGACA ATGAGAGAGAAATGCACGAC ATGGGTGTGGAAAAAAGTC CAGACAAGATCACGAGACACAC CTTC GGAGAAAGATCACGAGACACACCTC GGAGAAAGATCACGACACACCTC GGAGAAAGAAAAAGTC CCCCCCCCCC | CTATTAACACATTTACACTG C CTTITTACATTTTCTCCT TITCAAAATTTACTTCCTC TITCAAAATTTACTTCCTC CGTACTTTAACTATTCCTC CGTACTTTAACTATTCCCTC CGTACTTAATTACGTATC CCTGTGTATATGTACAGATC TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTCATCTCAG GTCTTCTATTTCTCACTCT CGTAGCATAATATCATCTAG ATACTATATTCATAGACCC GTCTTTCCAGTTTTATCACAC GGATCGTTTTATTCATCAAC Reverse primer ACTCTAAAAGGCACACCAGTTC CACTTCACACTCTCACACACACTCC GCTCTTCACACTCACACACACTCC GCTGTGTATCATCACACACTCCACTC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F2 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGC CAGATGGAGTATTAAATTGGA CAGAACCCCGACCCTTTAA AGCAGTAATGACAATAGAACT AGGATAAAAAACTCGACATCC GAGATTTGTACGAACTCGACATTGTCACACAGAACTCGACAATTGAACA GCTGAGAATTGAACAACACACACACACACACACACACACA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CAACCETGAAGAGATACCT CAAA AGCAAGGAAAATACTCATGTG FORWARD PRIMER | CTCGTCATGTGATTGCTTA CATTCATGAGTTCTTTTG TTTAAGGGTCGGGGTTCT GGATGTCGAGTTTTATCC TACATAGTCGAGTTTTATCC TACATAGTATATTTCC CCTGATCCATATATATCC GTCATTGATAGTTTTTGCATCATCATCATCATCATCATTAGTTCTTTTCACCATCATTAGTTCTTCACCATCATTTAACTCTCCACATCATTTAACTCTCTCACATCAT |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F5 PY17X_131300 F5 PY17X_1357300 F5 PIMER for RT-qPCR Location GAPDH | ATTITGAAGATGATCAAGAG CGCAGCGGGATAATATCCAA. GAGATTATCAAAAACACTGGAT TTGTCACTCTTGTTGAGCGC GGGTGTTTAGAAAAATTC CGAAACTGATGTTAGAAAAATTAC CGAAACTGATGTTAAAAATAAC GCAGTAAATATAGAGTTTG ATTAGCATAATATAGAGTTTG ATTAGAATTTTAAAAC GATATTACCCATAAGGTTGT ATTAGAATTGTATTCCTTCC GTATTTGCTCGACAAAAATG ATATAAGATTGTATTCCTTC GTATTTGCTCGACAAAAATG PY17X_1330200 PY17X_1210100 PY17X_1210100 PY17X_1109100 PY17X_0833600 Gene ID PY17X_0833600 PY17X_0804100 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_0521800 R1 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD PRIMARY GAGCAGGAGGAGCA ATTGGCACAGGAGCA ATTGGCACAGGAGCA ATTGGCACAGGAGCA ATTGGCACAGGAGCAC ATTGGCACAGGAGCAC ATGGCACAGAGACACACCCCCATGGGTGTGGACAAAAAGCCACGAGGACACACCCCCCCAGGAGACACACCCCCC | CTATTAACACATTTACACTG C CTTITTATCATTTTCTCCT TITCAAAATTTACTTCCTC TITCAAAATTTACTTCCTC CGTACTTTATCATTTACCTAC CCTGTGCTATATTACGATC CCTGTGTATATGTACGAGTC TICGAAAAGTTCTTCGACAC CTCGTCAGAAATTAAAGTATT TATAAAAACTTCTTCGACAG GTCTTCTATTTCTCCACTCT CGTAGCTAAATATCATCAGAGAG ATATCTATATTCATAGACAC GTCTTCCAGTTTTATACAC GGATCGTTTATTCACACAC TCATCACACACACACACACACACACA | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F2 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 Location 1216400 Location | CTCATGAAAATTTGAAAGAG AAAATATTAAAATTGAAAGC CAGATGGAGTATTAATGGC CAGAACCCCGACCCTTTAA AGCAGTAATGACAATAGAAAT AGGATAAAAAACTCGACATCG AGAATTGTACGAATTGGAATTGTCA CACCAGAAGGCAATTGATCA GATTCTGAGGAATTTGATCA GCTTGAAAAATTTCTCTCAA GCCACATTTTTACCGAAAATG GCTACCAGAATTTTATCCTCAA GCCACATTTTTACCGAAAATG GCTACCTGTCTTATTTTATC CAGAGGAAACATAAATTTGT CGCAGACATTAAAAAGAGG PY17X_1216400 PY17X_0019000 PY17X_0919000 PY17X_0919000 PY17X_0919000 PY17X_1241500 PY17X_1241500 PY17X_1241500 PY17X_13032800 | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0105800 R1 PY17X_015800 R12 PY17X_015800 R1 FORWARD PRIMER CAACCCTGAAGAAGAATCCT CAACCCTGAAGAAGAATCCT CAACCCTGAAGAAGAATACCT CATCTAGTTGAAATTCTC GTGTGTACATACTTCATGTG FORWARD PRIMER TCAAAAAGTAAGATGAAGTATGC GTGCAATATCCTACACGGTT GAAACAATAATAACCGAAAT CG | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAGGGTCGGGGTTCT GGATGTCGAGTTTTTAGC TACTTAGTCGATTTTTGC CATCATAGTAGTTTTTGCC CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTTGCA CATCATTAGTCTTTTTGCA CATCATTAGTCTTTTTGCA CATCATTAGTCTTTTTGCA CATCATTAGTCTTTTCAGCT CTTTCTAACTCATAAGTTT GAGCTTTACTCTTCTTCATCAC CA CACCATGTCTTGATAACATCAGCA CACCATGTGATAAACATCAGCA CACATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CCAATGTGTAAAATATTGCAC CGATGTGTGTGAAATATTCCAC CGGTGATTCGTGGAATCTTC C GGTGATACACACATTACTA CGGGGTTAACACATTACTA CGGGGTTAACACACATTACTA CGGGGTTAACACACATTACTA CGGGGTTAACACACATTACTA CGGGGTTAACACACATTACTA CGGGGTTAACACACATTACTA CCACATACATACACACTTTTTTAA |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F5 PY17X_131300 F5 PY17X_1357300 F5 PIMER for RT-qPCR Location GAPDH | ATTITGAAGATGATCAAGAG CGCAGCGGGATAATATCCAA. GAGATTATCAAAAACACTGGAT TTGTCACTCTTGTTGAGCGC GGGTGTTTAGAAAAATTC CGAAACTGATGTTAGAAAAATA CGCAGTAAATATAGAGTTTG ATTAGAAATATAGAGTTTG ATTAGATATTAGAATTAG ATTAGATTTTAAAATC GATATTACCCATAAGGTTGT ATTAGAATTGTATTCCTTCC GTATTTGCTCGACAAAAATG ATATAGAGTTGTATCCTTC GTATTTGCTCGACAAAAATG PY17X_1330200 PY17X_1210100 PY17X_1210100 PY17X_1109100 PY17X_0833600 Gene ID PY17X_0833600 PY17X_0804100 | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD primer GAGCAGGATAGACCATCTTG ATGATGGTCAAGGATACAGGTAT ATGATGGTCAAGGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGAGAGAAAAAGCAAGATACAGAGAGAAAAAAGCAAGATACAGAGAGAAAAAAGCAAGATACAGAGAGAAAAAGCAAGATACAGAGAGAAAAAGCAAGATACAGAGAAAAAGCAAGATACACACCACCAGAGAAAAAGCAAGAAAAAGCAAGAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAACACCAC | CTATTAACACATTTACACTG C CTTITTACATTTACACTG C CTTITTACATTTTCTCCT TTCAAAATTTACTTCCTC TTCAAAATTTACTTCCTC CGTACTTTACTATTTACGTATC CCTGTGTATATGTACAGACT TTCGAAAAGTTCTTCGACAC CTCGTCAGAATTCAACACTTCTCACACTC CGTAGCTAATATCATCAGACAC GTCTTCTATTTCTCCACTCT CGTAGCTAATATCATCAGACAC GTCTTCTATTTCTCAACTC GTCTTCTATTTCTCAACTC GTCTTCAGATTTATACACAC GATCTTCAACACTCATTCAACACTC CACCACCACTCACTC | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F12 PY17X_015800 F1 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F5 Location 1214400 kinesin8b 11 kinesin8b 11 FF16 11/E2 Location dic1 14 dic1 13 dic2 11 dic2 13 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAATGGC CAGAACCCCGACCCTTTAA AGCAGTAATGACAATTGGAATTGAAAATTGAAAATTGAAAATTGAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R13 PY17X_1305400 R13 PY17X_0105800 R1 PY17X_015800 R1 PY17X_015800 R1 PY17X_0415900 R3 PY17X_0415900 R3 PY17X_0415900 R3 PY17X_075800 R1 POWART PIMER CAACCTGAAGAGAATACTCATGAGG FOWART PIMER TCCAAAAAGTAAAAGTAACG GTGAATATCTCACACGGTT GAAAACATAATAAGCGAAAT GGGTTAGAGCATACTTCATAGA GTTAGAGCATGCTTTATAGA | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT GGATGTCAGAGTTTTTCC GGATGTCAGAGTTTTTGCC CCTGACTCCAATTATATCT GTCATTGATAAGTTGATCT CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTGCA CATCATTAGTCTTTTTCCA ACGTCCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTTG ACGTCCTCAAACTTGAGCT CTTTCTAACTCATAAGTTC CATCATTTAACTCTCTCTCATCAC CACCATTCATCTCTCTC |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F6 PY17X_131800 F6 PY17X_1357300 F5 PImers for RT-qPCR Location GAPDH 0-tubulin 2 \$-tubulin 1 Rbpm1 | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA. GAGATTATCAAAAACACTGGAT CTAGCTAGAGAGCTATGCGTA TIGTCACTCTTGTTGAGAGGC GGGTGTTAGAAAAATTACAATTAC CGAAACTGATGTTAAAAATA CGCAGTAAATATAGAGTTAG ATTAGAATTATACAATTAGATTTACCTAAATC GATATTACCCATAAGGTGTG ATTAGAATTGTATTCCTTCC GTATTTGCTCGACAAAAATG AATATAAGATTGTATTCCTTCC GTATTTTGCTCGACAAAAATG AATATAAGATTGTATTCCTTCC GTATTTGCTCGACAAAAATG PY17X_1330200 PY17X_1210100 PY17X_016700 PY17X_016700 PY17X_016700 PY17X_016700 PY17X_0204100 PY17X_0204100 PY17X_0919000 PY17X_0919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 PY17X_01919000 | ### doc R1 ### doc R2 ### doc R1 ### doc R2 ### doc R1 ### do | CTATTAACACATTTACACTS C CTTTTATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAACATTTACTTCCTC CGTACTTTAACATTTACGTATC CCTCGTCAGAAATTTCTCACACC CTCGTAAAAATTCTTCCACACC CTCGTAAAAACTTCTTCGACAC GTCTCTATTTTCTCACACT CGTAGCTAAAACTTCTTCGACAC GTCTCTATTTTCTCACACT CGTAGCTAAAATATCATGTAG ATACTATATTCATAGACCC GTCTTCAGTTTATAACAC GGATCGTTTAATATACAC GGATCGTTTAATGTACCAACTTACACACACTTACACACTCACACTCACACTCACACTTACACACTCACACTTACACACTCA | | PY17X_1320300 F2 PY17X_0833600 F1 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F1 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F13 PY17X_0415900 F10 RY17X_0415900 F10 RY17X_14159100 F10 RY17X_141591 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAATTGGC CAGAATGGAGTATTAATTGGC CAGAATGGAGTATTAATTGGC CAGAATTGAAAAACTCGACATTCCGACATTGCAC CACAAAGAAGGCAATTCAATT | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R2 PY17X_1305400 R10 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_0415900 R12 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_1216400 R4 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CARCCTGAAGGATACCT CAAA GGCTAGATAGATACTTCATGTG TCAAAAAGTACATCTTCATGTG FORWARD PRIMER TCAAAAAGTACATCTTCATGTG GTGTGTACATACTTCATGTG GTGCATATCCTTACACGGTT GAAACCATAATAGCGAAAT CGTAGGACATAATAGCGAAAT CG GTAGGACATAATAGCGAAAT CG GTAGGACATAATAGCGAAAT CG GTTAGAGCAGTATTATAGA CGGTAGCAGGTATTATAGA CGGTAGCAGGTATTATAGAT CGGTAGCAGGTATTATAGAT CGGTAGCAGGATATATTAGATTTAGAT CGGTAGCAGTATATTAGATTTAGAT CGGTAGCAGTATATTAGATTTAGAT CGGTAGCAGTATATTAGATTTAGAT CGGTAGCAGTATATTAGATTTAGAT CGGTAGCAGGTATATCTTAGAT | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTTGA TITAAAGGGTCGGGGTTCT TATTTAATGCCTCACATTTTC GGATGTCGAGTTTTATCCT TACAAATGATACTTTTTGCG CCTGACTCCATATTTATCT CATCATTTTATCT CATCATTTTTATCT CATCATTTTTATCT CATCATTTTTATCT CATCATTTTTATCT CATCATTTTTATCT CATCATTTTATCT ACGATTTTAACTCATAAGTT ACGATTCTCTAGACTCTTTCACAC CTTTTCTAACTCATAAGTT ACGATTTCTTCTCATCAC CA CATCATTTCTACTCATAAGTT CACAATGTTCTTCTCATCAC CA CACATCTTCTTCTCATCAC CA CACATCTTCTTCTCATCAC CA CACATCTTCTTCTCTCTC |
| dbc F1 dbc F2 md2 F1 PY17X_1109100 F1 PY17X_1109100 F2 PY17X_0521800 F1 PY17X_0521800 F2 PY17X_1311800 F4 PY17X_1311800 F5 PY17X_1311800 F6 PY17X_1323900 F1 PY17X_1323900 F1 PY17X_1357300 F5 Primers for RT-qPCR Location GAFDH | ATTITGAAGATGATCAAGAG CGCAGCGGATAATATCCAA CGAGCGGATAATATCCAA CACATAGAGCATGAA TTAGCTAGAGCATAGCAT | drct R3 dbc R1 dbc R2 md2 R1 PY17X_1109100 R1 PY17X_1109100 R2 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_0521800 R2 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1311800 R5 PY17X_1323900 R1 PY17X_1357300 R5 FORWARD primer GAGCAGGATAGACCATCTTG ATGATGGTCAAGGATACAGGTAT ATGATGGTCAAGGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGATACAGAGAGAAAAAGCAAGATACAGAGAGAAAAAAGCAAGATACAGAGAGAAAAAAGCAAGATACAGAGAGAAAAAGCAAGATACAGAGAGAAAAAGCAAGATACAGAGAGAAAAAGCAAGATACACACCACCAGAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAGCAAGAAAAAA | CTATTAACACATTTACACTS C CTTTTATCATTTTCTCCT TTCAAAATTTACTTCCTC TTTCAAAATTTACTTCCTC CGTACTTTAACATTTACTTCCTC CGTACTTTAACATTTACGTATC CCTCGTCAGAAATTTCTCACACC CTCGTAAAAATTCTTCCACACC CTCGTAAAAACTTCTTCGACAC GTCTCTATTTTCTCACACT CGTAGCTAAAACTTCTTCGACAC GTCTCTATTTTCTCACACT CGTAGCTAAAATATCATGTAG ATACTATATTCATAGACCC GTCTTCAGTTTATAACAC GGATCGTTTAATATACAC GGATCGTTTAATGTACCAACTTACACACACTTACACACTCACACTCACACTCACACTTACACACTCACACTTACACACTCA | | PY17X_1320300 F2 PY17X_1320300 F3 PY17X_0833600 F1 PY17X_0833600 F2 PY17X_1341200 F1 PY17X_1341200 F2 PY17X_1305400 F10 PY17X_1305400 F12 PY17X_1305400 F12 PY17X_015800 F1 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F4 PY17X_1216400 F5 Location 1214400 kinesin8b 11 kinesin8b 11 FF16 11/E2 Location dic1 14 dic1 13 dic2 11 dic2 13 | CTCATGAAAATTTGGAAGAG AAAATATTAAAATTGGAAGAG CAGATGGAGTATTAATGGC CAGAACCCCGACCCTTTAA AGCAGTAATGACAATTGGAATTGAAAATTGAAAATTGAAAATTGAAAAAAAA | PY17X_1320300 R3 PY17X_0833600 R1 PY17X_0833600 R2 PY17X_1341200 R1 PY17X_1341200 R1 PY17X_1305400 R10 PY17X_1305400 R12 PY17X_1305400 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_0415900 R13 PY17X_1216400 R4 PY17X_1216400 R5 FORWARD PRIMER CAACCETGAAGAGATACCT CAAA ATTCCTAAGTTGAATTCTC GTGTGTACATACTTCATGTG FORWARD PRIMER FORWARD P | CTCGTCATGTGATTGCTTA CATTCATGAGTTTCTTTGA TITAAAGGGTCGGGGTTCT GGATGTCGAGTTTTATCC TACAAATGACTCAATTTTC GCATTGATAAATTATCT GTCATTGATAAATTATCT GTCATTGATAAATTATCT CATCATTTAGTCTTTTGCAA CCTCATCTAGACTATTATCTC AGATGCTCTCAAACTTTGCAA CCTCATCAAATTATCTCC AGATGCTCTCAAACTTGAGCT CTTTTCTAACTCATAAGTTT GAGTCTTCAAACTTCAACACATAAACTTCACCAC CCCATCATCATAAACTTCACCAC CCCATGTCTTAAACAATAACACACACAC CCCAATGTCTCTAAACACATAACAC CCCAATGTCTCTAAACACATACACAC CCCAATGTCTCTAAACACATACACAC CCCAATGTCTAAAACATAACACACACACACACACACACAC |

| Probe | Gene ID | Forward primer | Reverse primer | | Probe | Gene ID | Forward primer | Reverse primer |
|---|---------------|---|----------------------|--|--------------|---------|---|--------------------------|
| kinesin8b 14 probe | PY17X_0204100 | GTTAC | TAAGGTGAATGGTAAAGTTC | | ρψ | ľ | TAATACGACTCACTATAGGG AGAATGGTGAGCAAGGGCG AGGA | C |
| kinesin8b I1 probe | PY17X_0204100 | TAATACGACTCACTATAGGG AGAATGAAAAATTATTTATA GAC | | | bfp-Kin8b 1 | | TAATACGACTCACTATAGGG AGAATGGTGAGCAAGGGCG AGGA | C |
| PF16 E1 probe | PY17X_0919000 | TAATACGACTCACTATAGGG AGAAGATAAAGTTCCTATAG TTC | AAAATTTGATTAAATTTGGT | | bfp-PF16 I1 | 1 | TAATACGACTCACTATAGGG AGAATGGTGAGCAAGGGCG AGGA | CTTGTACAGCTCGTCCATG C |
| PF16 I1 probe | PY17X_0919000 | TAATACGACTCACTATAGGG AGACTAAAATCACCTTTAAA ACT | TCAGAATATCCAGGTGTGTA | | | | | |
| Note: The blue sequences are designed for the restriction enzyme digestion. The red sequences are T7 promoter sequence. | | | | | | | | |