Supplemental Figure Legends

Figure S1. Solubilization of the trimming activity from crude mitochondrial fraction, Related to Figure 1.

(A) Schematic presentation of in vitro trimming assay.

(B) Crude mitochondrial fraction from BmN4 cells was treated with indicated detergents, and separated into the soluble (sup.) and insoluble (ppt.) fractions by centrifugation at 12,000 ×g for 15 min. In vitro trimming assay was performed using each fraction. Partial solubilization of the trimming activity was observed when treated with 0.5% CHAPS, 0.2% DMM, 0.5% OTG, or 0.2% TritonX-100. DC; sodium doxycholate, DMM; *n*-Dodecyl-β-D-maltoside, DM; *n*-Decyl-β-maltoside, OTG; *n*-Octyl-β-D-thioglucoside.

(C) BmN4 cells were transfected with dsRNAs for Trimmer candidates. Total RNAs were extracted and the mRNA levels for each target gene were analyzed by quantitative real time PCR. Mock indicates BmN4 cells transfected with dsRNAs for *Renilla* luciferase.

(D) BmN4 cells were transfected with dsRNAs for BmPARN and/or Trimmer and in vitro trimming assay was performed using 1,000 ×g pellet fractions. The mRNA levels for BmPARN were analyzed by quantitative real time PCR (left). BmPARN knockdown had no effect on the basal trimming activity (right). Mock indicates a control transfected with dsRNAs for *Renilla* luciferase.

Figure S2. BmPapi is the rate-limiting factor for trimming, Related to Figure 2.

(A) BmN4 cells were transfected with dsRNAs for BmPapi and the protein level of

BmPapi was analyzed by Western blotting (left). In vitro trimming assay was performed using 1,000 ×g pellet fraction from the BmPapi knockdown cells (right). BmPapi knockdown not only affected the length of the trimming products but also reduced the efficiency of the trimming reaction.

(B) BmPapi-FLAG stable cells were analyzed by Western blotting (left). In vitro trimming assay was performed using 1,000 ×g pellet fraction from BmPapi-FLAG stable cells (right). Increased expression of BmPapi facilitated the trimming reaction without affecting the expression level of Trimmer.

(C) Schematic presentation of BmPapi mutants and the summary of in vitro trimming assay using the 1,000 ×g pellet fraction from the cells overexpressing BmPapi mutants. BmPapi has three functional domains; N-terminal transmembrane (TM) domain predicted by SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/), two hnRNP K homology (KH) motifs, and a Tudor domain. All of three functional domains are required for BmPapi to promote the trimming reaction. Overexpression of BmPapi mutants possessing the Tudor domain show a dominant negative effect on the basal trimming activity.

(D–F) BmN4 cells were transfected with plasmids expressing C-terminally FLAG-tagged BmPapi wild type or mutants. The 1,000 ×g pellet (ppt.) and 17,000 ×g supernatant (sup.) fractions were analyzed by Western blotting with anti-FLAG antibody (D) or anti-BmPapi antibody (E). A part of BmPapi-ΔN mutant was detected in 1,000 ×g pellet fraction by anti-BmPapi antibody. In vitro trimming assay was performed using 1,000 ×g pellet fraction from the transfected cells (F). Except for BmPapi-ΔC3 mutant, all mutants failed to accelerate the trimming reaction. We note that the expression levels of the mutants vary substantially, allowing only qualitative analysis of their effects.

Mock indicates BmN4 cells transfected with the empty plasmid.

(G) BmN4 cells were transfected with plasmids expressing FLAG-tagged wild-type or BmPapi mutants, and the cell lysates were subjected to immunoprecipitation with anti-FLAG antibodies. The ΔTudor mutant was able to bind to Trimmer as well as the wild type but the interaction with Siwi and BmAgo3 was severely reduced. The KH motif mutant retained the interaction with both PIWI proteins and Trimmer.

(H, I) BmN4 cells were transfected with plasmids expressing FLAG-tagged wild-type or 5RK Siwi with sDMA site mutations, and the cell lysates were subjected to immunoprecipitation with anti-FLAG antibodies (H) or used for in vitro trimming assay (I). Siwi-5RK largely lost the interaction with BmPapi. While Siwi-5RK loaded 50-nt ssRNAs comparably to wild-type Siwi, the loaded RNAs were trimmed inefficiently. The Siwi-5RK mutant was substituted five arginines (R) to lysines (K) at amino acid positions 7, 9, 11, 13, 16.

(J) HEK293T cells were transfected with plasmids expressing Tdrkh-FLAG and/or MmPNLDC1. Mock is a negative control transfected with the empty plasmid. The whole cell lysate was prepared and analyzed by Western blotting with indicated antibodies. Asterisks indicate nonspecific signals. In vitro trimming assay for Mili-loaded ssRNAs was performed using the cell homogenates (without centrifugation). A trimming activity was detected only when Tdrkh and wild-type MmPNLDC1 were co-expressed.

Figure S3. Multiple sequence alignment of PNLDC1 and PARN proteins, Related to Figure 1.

(A, B) Multiple alignment of PNLDC1 and PARN amino acid sequences using Clustal W.

The asterisk indicates the glutamate mutated in the catalytic mutant. The red line shows the nuclease domain of BmPNLDC1. The region enclosed by the blue line indicates the putative transmembrane domain of PNLDC1 predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM/).

(C) An unrooted phylogenic tree of PNLDC1 and PARN proteins.

Figure S4. BmPapi and Trimmer depletion specifically elongates the 3′ end of piRNAs, Related to Figure 3.

(A) Schematic explanation for 5′- and 3′-end variation analysis.

(B) The length distribution of piRNAs from 25−45 nt libraries mapped to 1,811 transposable elements under knockdown of BmPapi or Trimmer. Reads were normalized to the total mapping reads. Knockdown of BmPapi or Trimmer alone extended the length of piRNAs.

(C) The 5′- and 3′-end variation analysis of piRNAs from 25−45 nt libraries mapped to 1,811 transposable elements under knockdown of BmPapi or Trimmer. The mode values of 5′- and 3′-ends at each piRNA locus were calculated and the percentage in each length are indicated (see Figure S3A). Knockdown of BmPapi or Trimmer extended the 3′ ends of piRNAs but not their 5′ ends.

(D, E) The effect of BmPapi and Trimmer knockdown on the relative abundance of 25–45-nt piRNAs mapped to silkworm transposable elements. piRNA reads were normalized by the total reads of 10 most abundant miRNAs (Liu et al., 2010). Each dot represents the abundance of piRNAs deriving from each transposable element, with the color reflecting the fold change compared to mock knockdown (D) or BmPapi knockdown (E). Overall, piRNA levels tended to decrease modestly by BmPapi single

knockdown (median 0.76 fold compared to mock knockdown, $p < 2.2 \times 10^{-16}$ by Wilcoxon signed rank test; D, left) and by Trimmer single knockdown (median 0.88 fold, $p < 2.2 \times 10^{-16}$; D, middle), and this decrease was enhanced by BmPapi + Trimmer double knockdown (median 0.53 fold, $p < 2.2 \times 10^{-16}$; D, right). The effect of Trimmer knockdown was more clearly observed when BmPapi single knockdown and BmPapi + Trimmer knockdown were compared (median 0.68 fold, $p < 2.2 \times 10^{-16}$; E).

A

pre-piRNA loading

5′ labeled 50-nt ssRNA
with 1st U

FLAG-Siwi
expressing
cell extract

FLAG-Siwi

FLAG-IP

pre-piRNA loaded Siwi

BmN4 cell
homogenate

e.g.
1,000 ×g

Pellet
(insoluble)

Sup.

Trimming &
2′-*O*-methylation

Me

Mature piRNA-RISC

Buffer

Pellet

β-elimination : – – +

50 nt

27 nt

B

Extraction from crude mito fraction

Buffer

Crude mito

0.3% Digitonin

0.5% CHAPS

0.2% DC

0.2% DMM

0.5% DM

0.5% OTG

0.2% TritonX-100

: Detergent

100%

33%

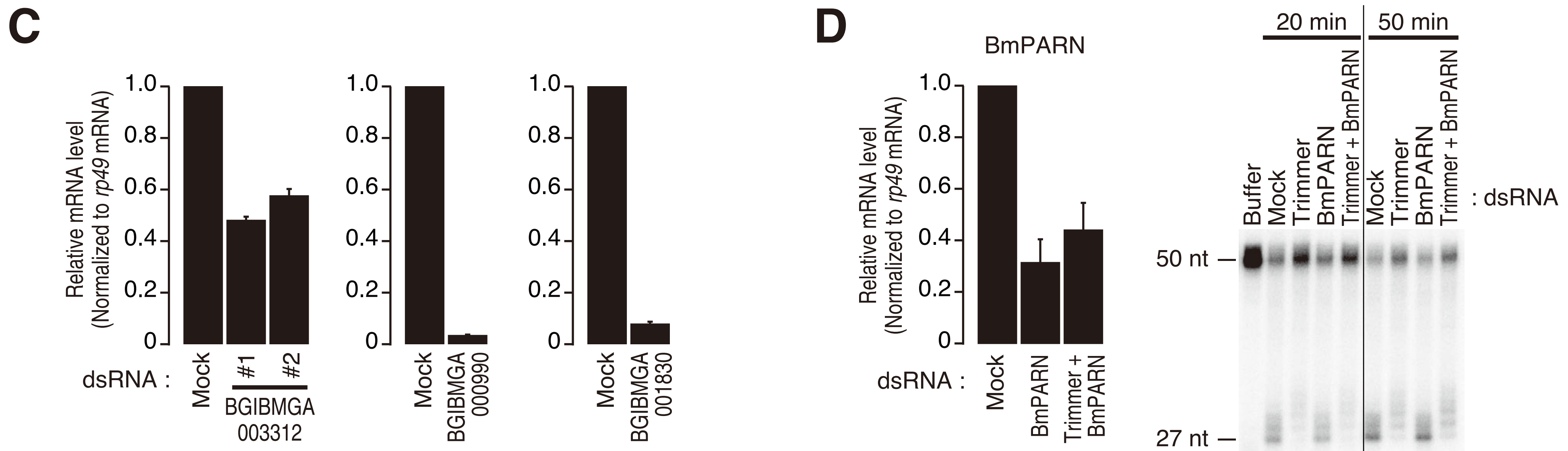
Sup.

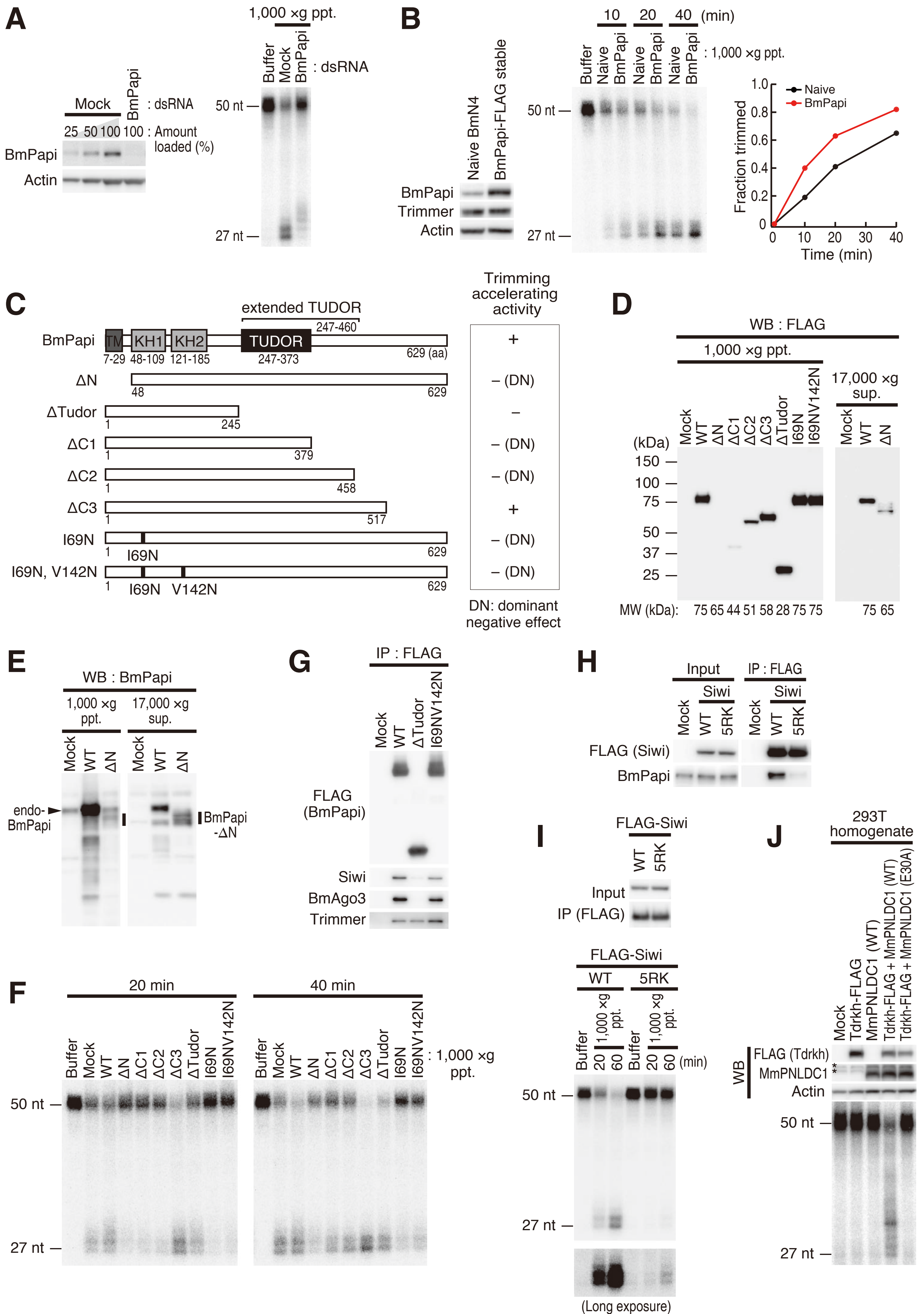
Ppt.

: Trimming
lysate

50 nt

27 nt




A
Mock
BmPapi
: dsRNA
25 50 100 100 : Amount loaded (%)
BmPapi
Actin
1,000 ×g ppt.
Buffer
Mock
BmPapi
: dsRNA
50 nt
27 nt
B
Naive BmN4
BmPapi-FLAG stable
BmPapi
Trimmer
Actin
10 20 40 (min)
Buffer
Naive
BmPapi
: 1,000 ×g ppt.
Naive
BmPapi
Fraction trimmed
Time (min)
C
extended TUDOR
247-460
BmPapi
TM
KH1
KH2
TUDOR
7-29 48-109 121-185
247-373
629 (aa)
ΔN
ΔTudor
ΔC1
ΔC2
ΔC3
I69N
I69N, V142N
V142N
Trimming accelerating activity
– (DN)
DN: dominant negative effect
D
WB : FLAG
1,000 ×g ppt.
17,000 ×g sup.
Mock
WT
ΔN
ΔC1
ΔC2
ΔC3
ΔTudor
I69N
I69NV142N
(kDa)
MW (kDa): 75 65 44 51 58 28 75 75
75 65
E
WB : BmPapi
1,000 ×g ppt.
17,000 ×g sup.
endo-BmPapi
BmPapi -ΔN
F
20 min
40 min
: 1,000 ×g ppt.
G
IP : FLAG
FLAG (BmPapi)
Siwi
BmAgo3
Trimmer
H
Input
IP : FLAG
Siwi
5RK
FLAG (Siwi)
I
FLAG-Siwi
Input
IP (FLAG)
WT
5RK
(Long exposure)
J
293T homogenate
Tdrkh-FLAG
MmPNLDC1 (WT)
Tdrkh-FLAG + MmPNLDC1 (WT)
Tdrkh-FLAG + MmPNLDC1 (E30A)
WB
FLAG (Tdrkh)
MmPNLDC1
Actin

Izumi *et al.* Supplemental Figure 3

A

Nuclease domain

```
                          10         20         30         40         50         60         70         80
BmPNLDC1_XP_004930511.1   ---------- ---------- ---------- -MDITKENYY EELDNIAKNI KDSCFISFDA EFSAILT--K ESFKYSLFDT
HsPNLDC1_NP_001258791.1   ---------- ---------- ---------- -MDVGADEFE ESLPLLQELV QEADFVGLDI EFTGLRSNLS GPQQISLFDL
MmPNLDC1_NP_001030038.1   ---------- ---------- ---------- -MDVGADEFE QSLPLLQELV AGADFVGLDI EFTGLRSNLS RPQQISLFDL
BmPARN_NP_001153677.1     ---------- ---------- ---------- -MEVIRKNFK EALPLVAASV KKADFLVIDT EFTGIINGRD VS----MFDT
HsPARN_NP_002573.1        ---------- ---------- ---------- -MEIIRSNFK SNLHKVYQAI EEADFFAIDG EFSGISDGPS VSALTNGFDT
MmPARN_NP_083037.1        ---------- ---------- ---------- -MEIIRSNFK INLHKVYQAI EEADFFAIDG EFSGISNGPS VTALTSGFDT
DrPARN_NP_957382.1        ---------- ---------- ---------- -MEVTRQNFK EVLPEVCNAV QEADFISIDG EFTGISDGPS VSALTNGLDT
CePARN-1_NP_506169.2      ---------- ---------- ---------- MVIVTDSNFL DAAGTLRKGL LYCDFVAIDF EFLGLDVS-- ---AISLHDT
CePARN-2_NP_496602.1      MSGDGDSSTT SSAGDGSTSK DKIPAFSEIR VIEVNRDNFA KIWPYLLVCI KSADFTAIDL ELSGLGGKGL RS------RD

                          90         100        110        120        130        140        150        160
BmPNLDC1_XP_004930511.1   NEERYNKYKT QISTMKMMQV GLTMFQYDRE LD-AYLATGY TFHLCPQLIG TINQS--LIF QASTLKFLCK HNFDFNKFIY
HsPNLDC1_NP_001258791.1   PSEWYLKTRQ SVQQFTVCQI GLSVFSAIEG EANKYIAHSC NFYLFPTTFG ILDS--EFSF QASSVQFLNQ YGFNYNKFLK
MmPNLDC1_NP_001030038.1   PSEWYLKTRQ SVQQFTICQI GLSMFSSIEG ESNKYVAHSC NFFLFPTTFG ILDS--EFSF QASSVQFLNQ YGFDYNKFLK
BmPARN_NP_001153677.1     PEEYYKCTQR GSSEFLLIQF GLCAFHWNHK EK-HYMNDAY NFYLFPR--G SPGPDRMFMC QSSSLDFLAS QGFDFNKLIK
HsPARN_NP_002573.1        PEERYQKLKK HSMDFLLFQF GLCTFKYDYT DS-KYITKSF NFYVFPKPFN RSSPDVKFVC QSSSIDFLAS QGFDFNKVFR
MmPARN_NP_083037.1        PEERYQKLKK HSMDFLLFQF GLCAFKYDHT DS-KHVTKSF NFYVFPKPFS RSSPDVKFVC QSSSIDFLAS QGFDFNKVFC
DrPARN_NP_957382.1        PEERYTKLRK HSMNFLLFQF GVCTFRYDQN QS-TYITKAF NFYIFPKPFS RTSPDIKFIC QSSSIDFLAS QGFDFNKVFR
CePARN-1_NP_506169.2      VESRYQILRD NVIKYRPCQL GLTLFKQKSN RA--YKADTY SVPLFQRFGD NDTS-----I SLPSMRFLVK NKFNLNQVFM
CePARN-2_NP_496602.1      IAERYQAIRD AAHTRSILSV GIATMKLTHK SE-KRRALRY ETQVFNILTF SEKP---FTI EPSALQFLAK HSFDFNRLIQ

                          170        180        190        200        210        220        230        240
BmPNLDC1_XP_004930511.1   EGLPYLSKSD EALLNRYRIE NN-------- ---------- -LFDYVSESL EFDEEKQINQ CSSEVSKWLS SSIEDTMYLD
HsPNLDC1_NP_001258791.1   NGIPYMNEEQ EKKIRHDILT ---------- ---------- ---GNWRVRS SPDKDQIKVV IDEVTRWLEL AKEGDWMTLP
MmPNLDC1_NP_001030038.1   NGIPYMNEEQ EKKIKHSILR ---------- ---------- ---GNWRVRS SLDKDQIKVV IDKVTQWLDL AEEGDQMTLP
BmPARN_NP_001153677.1     DGISYMTVPI ECKLRENLTE RQKTYS---- ---------- --KGKDSIKV PDEHKAYVED ICNQVHQFLE EGQLDEMEID
HsPARN_NP_002573.1        NGIPYLNQEE ERQLREQYDE KRSQANGAGA LS-----YVS PNTSKCPVTI PEDQKKFIDQ VVEKIEDLLQ SEENKNLDLE
MmPARN_NP_083037.1        SGIPYLNQEE ERQLREQFDE KRSQANGAGA L--------- ---AKCPVTI PEDQKKFIDQ VIEKIEDFLQ SEEKRSLELE
DrPARN_NP_957382.1        SGIPYLNQEE ECQLREQYEE RRGQMNGAGP VS-----YTP P-SGTGVCNV PEDQREFIRS VEEKVEALLK N-TDQTLDLE
CePARN-1_NP_506169.2      DGVEFCTRKE FKKFERALLA GTAASYLSRE VKSQIELLKV MVHEKCYQST SYHITHRTDE PMQKIPLKMK PNSSVSLRMP
CePARN-2_NP_496602.1      SGVRFQGANC P--------- ---------- ---------- ---LKTLFRE LLGSSATFCL HNGFIDLAFI YKQLYDIDLP

                          250        260        270        280        290        300        310        320
BmPNLDC1_XP_004930511.1   I-DNAICRYL LHLELRQRYP G-----ILTT DSLGNSKKIL IYRDKNVEGA KNAPIAILED NLIAYLLGFL HVIKLLETHK
HsPNLDC1_NP_001258791.1   G-ITGFQAFE VQLVLRQALP N---IWTVLK DEGVVVKKVS KQHRWYLQNT SCDRESCWKE NILLSARGFS VFFQMLVKAQ
MmPNLDC1_NP_001030038.1   G-IAGFQAFE VQLVLRQALP N---IWTVLK EEWVIVKKVS QPQRWYLEHA SCDQVSCWKE QILLSARGFS VFFQMLVKAQ
BmPARN_NP_001153677.1     K-CNSFVRLL LFQELRARFK DKILVETKIL ENKNRLLNVK RVKSQDDLKN HDALKKEREW EDFEDAVGFS KVARMISESE
HsPARN_NP_002573.1        P-CTGFQRKL IYQTLSWKYP KGIHVETLET EKKERYIVIS KVD-EEERKR REQQKHAKEQ EELNDAVGFS RVIHAIANSG
MmPARN_NP_083037.1        P-CTGFQRKL IYQTLSWKYP KGIHVETLET DKKERHIVIS KVD-EEERKR REQEKYTKEQ EELNDAVGFS RVIHAIANSG
DrPARN_NP_957382.1        P-CTGFQRKL IYQTLNSKYS KGLHVEALET EKKERFIQIS KVD-DEERRR REQQKQQREQ EELNDAVGFS RVIRAISKSG
CePARN-1_NP_506169.2      RNLSSVEKYM IIYELTKAFP Q----FLFTC DEKQQNLHVK NISDDYLKEK DNLERARARC S--ESVKGVS AILQVVHMTG
CePARN-2_NP_496602.1      DTLDGFVN-- ---NLSDMFP DN---YLPVA DSKYLAEYQT RMTASFLEYV FRRTQGDNEI ERQNNRYHIE ICFPSSEKSI

                          330        340        350        360        370        380        390        400
BmPNLDC1_XP_004930511.1   KPIVGHNMFL DMLFLHNQFI GPLPDSYTMF KKNINSVFPT IFDTKYISHA MSKKLTFSES WKSNALQDLY EFFAERKCKK
HsPNLDC1_NP_001258791.1   KPLVGHNMMM DLLHLHEKFF RPLPESYDQF KQNIHSLFPV LIDTKSVTKD IWKEMNFPRV SNLSEVYEVL NSDLNPTKNS
MmPNLDC1_NP_001030038.1   KPLVGHNMMM DLLHLHEKFF RPLPESYDQF KQNIHSLFPV LIDTKNVTKD IWKELRFPRV SNLLEVYEVL SSNLNPTKDS
BmPARN_NP_001153677.1     KLVVGHNMLL DVMHTLNHFF QPLPAEYTQF KEFTHCMFPH LLDTKYMSS- ---LPPFKDK VNSSILKHLL ATLSAAPFSL
HsPARN_NP_002573.1        KLVIGHNMLL DVMHTVHQFY CPLPADLSEF KEMTTCVFPR LLDTKLMAS- ---TQPFKDI INNTSLAELE KRLKETPFNP
MmPARN_NP_083037.1        KLVVGHNMLL DVMHTIHQFY CPLPADLNEF KEMAICVFPR LLDTKLMAS- ---TQPFKDI INNTSLAELE KRLKETPFDP
DrPARN_NP_957382.1        KLVVGHNMLL DVMHTIHQFC GPLPEELDDF KEVAMTVFPR LLDTKLMAS- ---TQPFKEI IHNTSLAELH KQLRQKPFRP
CePARN-1_NP_506169.2      KLVVGHSLLL DAMYMYHYFF SHLPANYQMF KDKFNALFPR IMDTKLLAQA LRFELPGVGD S-LENLGDYF GSDKSDKTVP
CePARN-2_NP_496602.1      KALKNATESV DVRLPEGFPD HAIPIDLHIH VCKYFAYHGF CHARNTEKG- ---CKLLHDV DTAIDLQRIK ENKKAFKRKR

                          410        420        430        440        450        460        470        480
BmPNLDC1_XP_004930511.1   LEYG--INIV RLTTPFDIKQ SYHEAGWDAY CSGYCFIRLG HWAACENRGK SHVIG--PRE KLAALAPYCN KVNVIR---G
HsPNLDC1_NP_001258791.1   GPE---IVHA SRCEKYVETK CPHEAAYDAF LCGSVLLKVA HLLLQKIYHI DPVPESSFPQ YLDVLAPYVN QVNLIR---A
MmPNLDC1_NP_001030038.1   GPV---IIHA RQCKKYAETK CPHEAAYDAF LCGSVLLKVA HLLLQRVHGN GAVHEPAFPQ YLDVLAPYVN QVNLIR---A
BmPARN_NP_001153677.1     PKV---VSDE GR-GYSQLHE KHHEAGYDAY VTGLCFLAMH SHLANMRGDD TTR---FLSI TSPLIKPFLN KVFLSRTAHQ
HsPARN_NP_002573.1        PKV---ESAE GFPSYDTASE QLHEAGYDAY ITGLCFISMA NYLGSFLSPP KI----HVSA RSKLIEPFFN KLFLMR--VM
MmPARN_NP_083037.1        PKV---ESAE GFPSYDTASE QLHEAGYDAY ITGLCFISMA NYLGSLLSPP KM----CVSA RSKLIEPFFN KLFLMR--VM
DrPARN_NP_957382.1        PTT---ECPE GLQSYDTSTE QLHEAGYDAF ITGLCFISMA NYLGSFLTPP KS----HISA RSKLLEPFYN KLFLMR--VI
CePARN-1_NP_506169.2      PELRGFIEPW MNPLEDESEN VYHNAGFDSY VTGEVFLKLA HIYINRRNNF KNEILDFDRI YQYLEAPILN RLPFQL---M
CePARN-2_NP_496602.1      RYNN-VIAES VAEKTGTDEE AWKNAKFEQN LLTRDFRELK RNVVTG---- ---------L HRAGVDAFMT AFAVIY----

                          490        500        510        520        530        540        550        560
BmPNLDC1_XP_004930511.1   GVSYMNFSEN DPPRNRPVLL HVKCLKDTV- IDVEKVASLL GSFGSIDIKP CGKRAALVAT GAQFMVDKIL KTYENNRDYR
HsPNLDC1_NP_001258791.1   GVPKINFSGP DYPSIRPPIL ILSV-KRWPG VSEQQVYHKF QNLCKFDVRR LTRSQFLLLT NKFKDARNIL KEYRDHPTLC
MmPNLDC1_NP_001030038.1   GVPKINFSGP DYPSIRPPIL ILTV-KRWPG VSEQQVYREF QNLCKFDVRR FTRSQFLLLT NKFKDARSVL KEYRNHPTLQ
BmPARN_NP_001153677.1     DSPFINLAGP EPLPPRDHVF HLAFPREWQR NEITQLFSPF GPITVQFIDD TSAFVALARR EQ---ATSVS KALAKHSKIT
HsPARN_NP_002573.1        DIPYLNLEGP DLQPKRDHVL HVTFPKEWKT SDLYQLFSAF GNIQISWIDD TSAFVSLSQP EQVKIAVNTS KYAESYRIQT
MmPARN_NP_083037.1        DIPYLNLEGP DLQPKRDHVL HVTFPKEWKT SDLYQLFSAF GNIQISWIDD TSAFVSLSQP EQVQIAVNTS KYAESYRIQT
DrPARN_NP_957382.1        DIPYLNMSGP DLQPKRDHVL YVTFPKEWKT SDLYQLFSAF GNIQVSWVDD TSAFVSLSQT EQVQIAMNTS RYAESYRIQT
CePARN-1_NP_506169.2      DVGCCYLTGD DSKGFRPDVI TIVR-RDRVA IEEDEFRYLE KALGTLMATY QFDIEWSKNK KELFLATNSP GSYAFLCEKF
CePARN-2_NP_496602.1      QQRRTLMADN SVDLKFLNRV PLSAKDKPLH LRHRPVPVPQ SEQIDAHLEA MVEINKQRDY IRMNAAATVI KSVQKEDTPC
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Putative TM region

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                          570        580        590        600        610        620        630        640
BmPNLDC1_XP_004930511.1   ISKYSVYKHS VAGR------ ---------- ---------- FAIWSGSIVT GGLALYLIHK KFKSILL--- ----------
HsPNLDC1_NP_001258791.1   ISLYRYWRHS PNVN------ ---------- ---------C LLQVCGIVTA WALLAFILGR SGT------- ----------
MmPNLDC1_NP_001030038.1   VSLYRSWRHS PNIT------ ---------- ---------C LLQVCSIVTT WAMIAFLLGR PMP------- ----------
BmPARN_NP_001153677.1     LTSYKYKKKI NDTPSSKTAT KTAEAANNDC KKTEKTPVRT SALQINATAT TPVNKVKIPE KVPRDLSNSV FPITPPRKRT
HsPARN_NP_002573.1        YAEYMGRKQE EKQIKRKWTE DSWKEADSKR LNPQCIP-YT LQNHYYRNNS FTAPSTVGKR NLSPSQ---E EAGLEDGVSG
MmPARN_NP_083037.1        YAEYVGKKQK GKQVKRKWTE DSWKEVDRKR P--------H MQGPCYHSNS FTAAGMLGKR TLSPDP---R EAALEDRESE
DrPARN_NP_957382.1        YAEYLQSRQK NTHSSRKWAS DGWADTSYPS VAMTTASGYS HTDNWHQAVK RSISPSLDEQ NHGADSSWTN YSVKKIKTEG
CePARN-1_NP_506169.2      SNDSSLAPLD ELDSGKKWTF E--------- ---------Q RQTAWRSFKN RGVGIGINAK RIRAQNQATK IQRAMEI---
CePARN-2_NP_496602.1      ED-------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                          650        660        670        680        690        700
BmPNLDC1_XP_004930511.1   ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
HsPNLDC1_NP_001258791.1   ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
MmPNLDC1_NP_001030038.1   ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
BmPARN_NP_001153677.1     FSTSSGGFEV NELESPTKKL KGASETKKRI DEKIIDR--- --FDSEAKPA CVTVFKDDDC WD
HsPARN_NP_002573.1        EISDTELEQT DSCAEPLSEG RKKAKKLKRM KKELSP---- --AGSISKNS PATLFEVPDT W-
MmPARN_NP_083037.1        EVSDSELEQT DSCTDPLSEG RKKSKKLKRM KKELSP---- --AGSVS-DS PAVLFEVPDT W-
DrPARN_NP_957382.1        SCTQTYADVA GSCDWPRLQA DEGGASVSPV AEEAELDEFS ANQSQASETT PPALFDVPQV W-
CePARN-1_NP_506169.2      ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
CePARN-2_NP_496602.1      ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
```
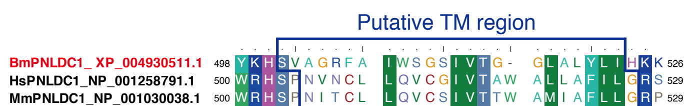
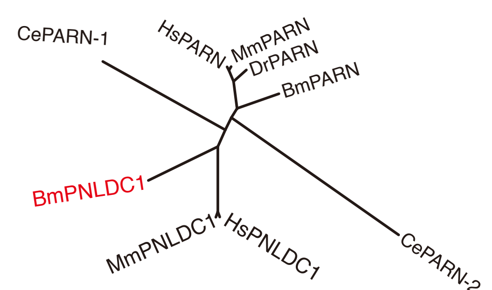

B



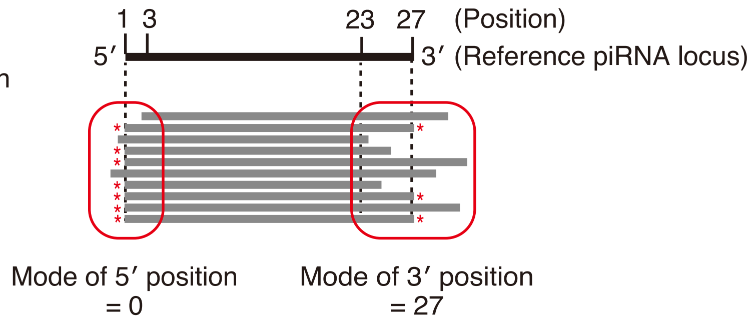
C



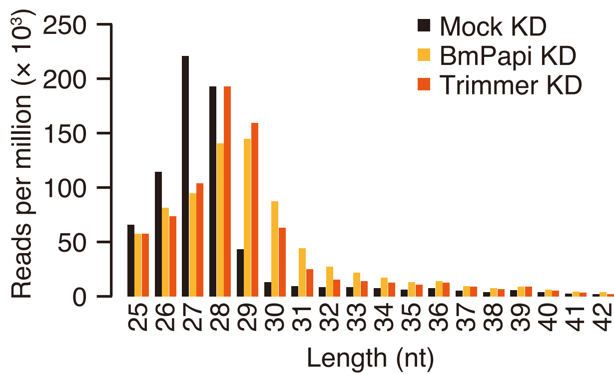
A

1. Collect the reads containing the same sequence in the 3–23 region to a piRNA locus

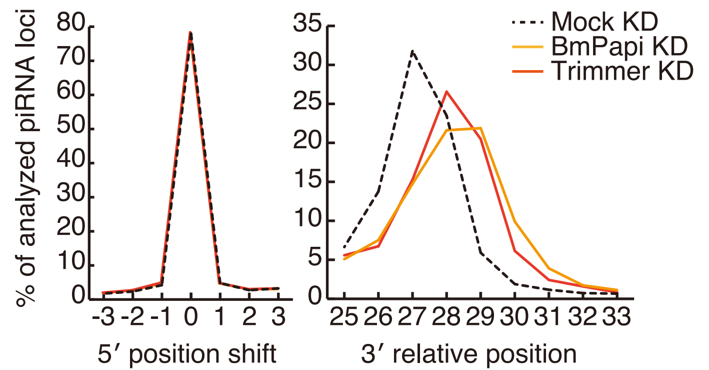
2. Calculate the mode value of 5′ and 3′ positions relative to the reference sequence in each piRNA locus



B



C



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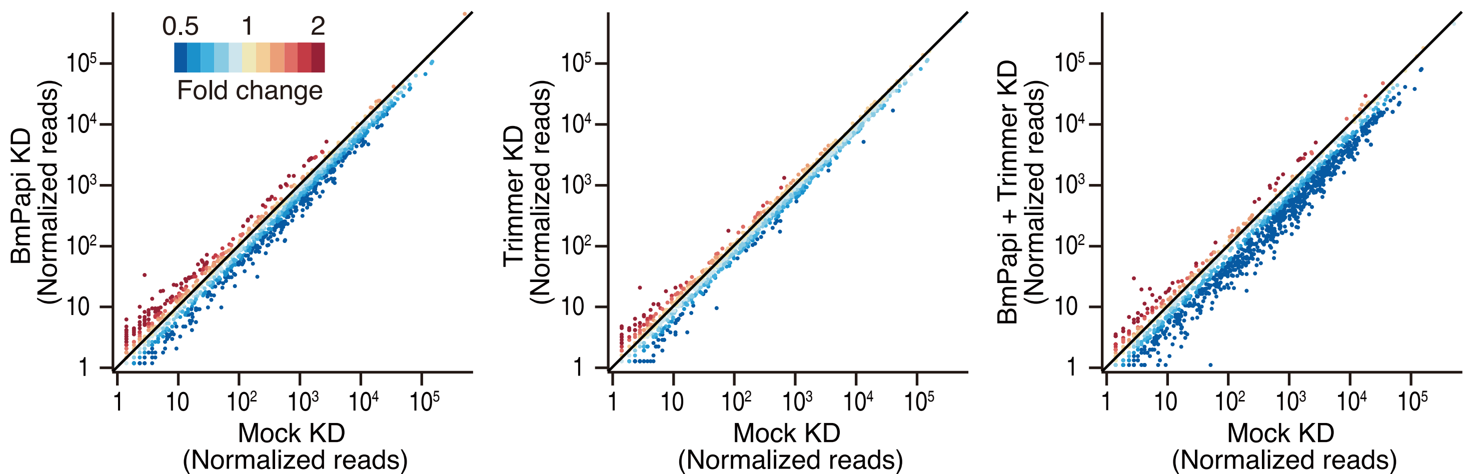

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