Supporting Information

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SI Materials and Methods

Plasmids. To generate a reporter gene, *Luciferase* was placed between the 1.8-kb intergenic region and 3′-UTR of the *RH4* gene. The 1.8-kb intergenic region was cloned into the restriction sites KpnI /XhoI in plasmid pVLH (1) and an 800-bp 3′-UTR from the *RH4* gene HindIII /PstI, respectively to generate another plasmid, pVLHRLucR. The entire fragment (5′-1.8-kb intergenic region / *Luciferase*/3′-UTR of *RH4*-3′) from pVLHRLucR was then subcloned into the restricted sites ApaI /SalI in plasmid pXL-BacII-DHFR (2) to generate another plasmid, pBRLucR.

Parasite Culture, Transfection, and Cloning of Transfected Parasite. To test the promoter activity of the 1.8-kb intergenic region, pBRLucR was transfected into Dd2 by electroporation using a Bio-Rad Gene Pulser II system (Bio-Rad); 50 μg of pBacRLucR and 50 μg of a helper plasmid pHTH that carries a piggyBac transposase were introduced into 2×10^8 ring stage-infected erythrocytes in 400 μL of electroporation reaction. The electroporation conditions and transfected parasites were cultured and selected as described previously (2). To remove any episomal plasmids, the transfected parasites that lost episomal plasmids were also cloned by limiting dilution as described previously (2). A single clone Dd2Luc was obtained. Dd2LucNM was derived from Dd2Luc by culturing Dd2Luc with neuraminidase-treat erythrocytes as described previously (3).

Southern Blot and PCR Analyses. To test if the foreign fragment was integrated into the chromosome of Dd2Luc, genomic DNA extracted from Dd2Luc was analyzed by Southern blot analysis. 2 μg of genomic DNA of Dd2Luc was digested with 5 units of ClaI (New England BioLabs) at 37 °C for 4 h and then separated on a 1% agarose gel. The DNA in the gel was processed for Southern blot assay as described previously (2). The hybrid probe used in Southern blot was PCR amplified using two primers (Table S1) and purified using the MicroSpinTM G-25 Columns (Amersham Biosciences). The transfection plasmid pBRLucR was used as a positive control and genomic DNA of the wild-type Dd2 was used as a negative control. To check if there were any episomal plasmids in Dd2Luc, genomic DNA from Dd2Luc was analyzed by PCR. Briefly, 1 ng of Dd2Luc genomic DNA was set up for two separate PCR reactions. One was processed using primer set F1/R1 (Table S1) that only amplifies a 1-kb fragment from episomal plasmids. Another was processed using primer set F2 /R2 (Table S1) that can amplify a 0.5-kb fragment from both episomal and integrated plasmids.

Inverse PCR. To investigate the integration site of the foreign fragment in the chromosome of Dd2Luc, inverse PCR assay was carried out as described previously (2). Primers used for PCR and sequencing are listed in Table S1.

Reverse Transcription and Real-Time PCR. To measure the amount of transcript for each gene, total RNA was extracted by TRIzol reagent (Invitrogen). Reverse transcription using the SuperScript First-Strand Synthesis System kit (Invitrogen) was carried out as described by the manufacturer. First strand cDNA synthesized from 50 ng of total RNA was amplified using the QuantiTect SYBR Green Supermix in the iCycler iQ real-time detection system (Bio-Rad) with the following conditions: 5 min at 94 °C for one cycle; 94 °C for 30 s, 50 °C for 30 s, 60 °C for 30 s for 40 cycles; and an extension cycle at 60 °C for 2 min; 0.5 μM of both forward and reverse primers were added in each reaction. Primer set for each tested gene was designed to generate a 100 ± 10-bp PCR fragment. The sequence of each primer is listed in Table S1. A housekeeping gene *60S ribosomal protein L18* (*60S L18*, MAL13P1.209) was used as a reference gene. The positive and negative control genes included *AMA1* (PlasmoDB ID: PF11_0344), a gene critical for parasite invasion and a silent *var* gene *Svar1* (PlasmoDB ID: PF07_0051), respectively.

5′-RACE. The TSSs of the *RH4* and *PEBL* genes were mapped using the SMART RACE cDNA Amplification Kit (BD Bioscience) as described in the product user manual. Briefly, 0.4 μg of total RNA extracted from schizont Dd2NM was used to synthesize the first-strand cDNA in 10 μL of reaction with 1× First-Strand buffer, 2 mM DTT, 1 mM dNTP Mix, and 1 μL of PowerScript Reverse Transcriptase (BD Bioscience). 5′-cDNA ends were amplified from 2.5 μl of 5′-RACE-Ready cDNA in 50 μl of 5′-RACE reaction with 1× Universal Primer A Mix, 0.2 μM gene specific primer (Table S1), and 1× Advantage 2 Polymerase Mix (BD Bioscience). The amplification conditions were: 94 °C for 30 s and 66 °C for 2 min for 5 cycles; 94 °C for 30 s, 64 °C for 30 s, and 62 °C for 2 min for 5 cycles; 94 °C for 30 s and 62 °C for 2 min for 20 cycles. Three different gene specific primers for each gene (Table S1) were used to generate the specific PCR products. The PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen) by following the manufacturer. At least 10 positive colonies were sequenced for mapping 5′-cDNA ends.

MNase Digestion. To generate nucleosomal DNA samples for nucleosomal mapping and ChIP, synchronized parasites were prepared as described previously (4); 1 mL of synchronized parasite-infected erythrocytes were saponin lysed in 10 mL of PBS, pH 7.4 (PBS, composition is 137 mM NaCl, 4.3 mM Na_2HPO4, 1.47 mM KH_2PO4; Invitrogen), with 0.15% saponin (Sigma) on ice for 10 min. Parasite were pelleted by centrifuging at $500 \times g$ for 5 min at 4 °C and resuspended in 20 mL of PBS with 1% paraformaldehyde (Electron Microscopy Sciences) and protease inhibitors (1 mM PMSF, Sigma, and complete protease inhibitor mixture, Roche) at room temperature for 10 min. The crosslink reaction was stopped by adding 2 mL of 1.25 M glycine (Sigma). Parasites were washed three times with PBS containing protease inhibitors (1 mM PMSF, Sigma, and complete protease inhibitor mixture, Roche). DNA of paraformaldehyde-crosslinked parasites were analyzed by MNase digestion using the Enzymatic Shearing kit (Active Motif) according to the product instruction manual. Briefly, 6×10^8 fixed parasites were thawed and resuspended in 200 μL of Lysis Buffer with protease inhibitor mixture (Active Motif) on ice for 30 min. For chromatin samples derived from young stage parasites (at 24 h post-invasion), 1 μL of Igepal CA-630 (Sigma) was added to the Lysis Buffer to aid in release of the nuclei. Swelled parasites were ejected through a syringe with a 30-gauge hypodermic needle for 10 strokes to release nuclei. Parasite nuclei were centrifuged at $12,000 \times g$ for 15 min at 4 °C and resuspended in 200 μL of Digestion Buffer (supplemented with 1× protease inhibitor mixture, Active Motif) with 2 Units of MNase (Active Motif) at 37 °C for 30 min. Enzymatic reaction was stopped by adding 4 μL of 0.5 M EDTA and stored at −80 °C before use. To determine if more than 80% of the chromatin samples were digested into mononucleosomal size, DNA was purified from the MNase digested chromatin samples. Briefly, 10 μL of MNase digested chromatin sample was treated in 100 mM NaCl at 65 °C for 4 h to reverse crosslink. Nuclear proteins were digested with

0.25 μg of Protease K (Active Motif) at 37 °C for 1 h. Nucleosomal DNA was purified using the QIAquick PCR purification kit (Qiagen) and eluted with 20 μL of H_2O. The purified nucleosomal DNA was analyzed by electrophoresis on 2% agarose gel and ready for Solexa mapping of nucleosome and nucleosome scanning by real-time PCR.

1. Deitsch KW, Calderwood MS, Wellems TE (2001) Malaria. Cooperative silencing elements in var genes. *Nature* 412:875–876.
2. Balu B, Shoue DA, Fraser MJ, Jr, Adams JH (2005) High-efficiency transformation of Plasmodium falciparum by the lepidopteran transposable element piggyBac. *Proc Natl Acad Sci USA* 102:16391–16396.
3. Dolan SA, Miller LH, Wellems TE (1990) Evidence for a switching mechanism in the invasion of erythrocytes by Plasmodium falciparum. *J Clin Invest* 86:618–624.
4. Gaur D, et al. (2006) Upregulation of expression of the reticulocyte homology gene 4 in the Plasmodium falciparum clone Dd2 is associated with a switch in the erythrocyte invasion pathway. *Mol Biochem Parasitol* 145:205–215.

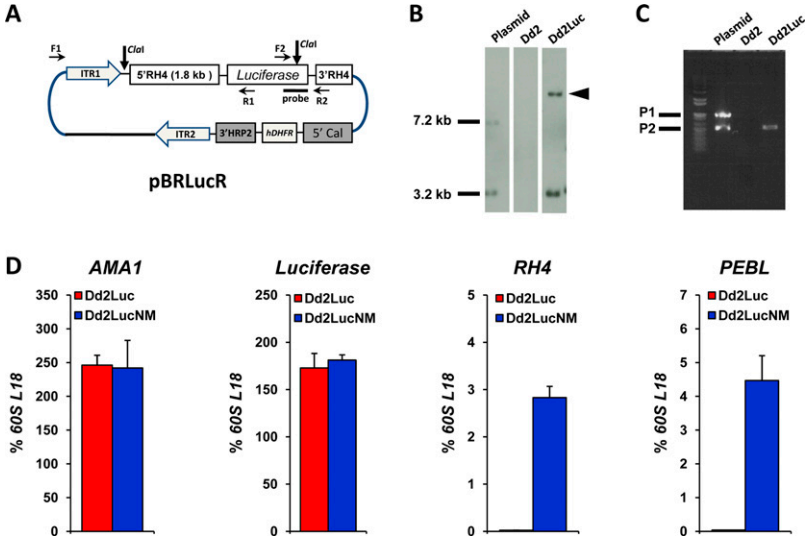

Fig. S1. The 1.8-kb intergenic region between the *RH4* and *PEBL* genes has promoter activity. (*A*) Schematic structures of plasmid pBRLucR. Transcription of the reporter gene *Luciferase* was controlled by two UTRs from the *RH4* gene, 1.8 kb intergenic region, and 3′-UTR of *RH4*. The primers used in PCR were indicated by the horizontal arrow. The hybridization probe was presented as a bar. The sites of the restricted enzyme ClaI were shown by the vertical arrow. Genomic DNA of Dd2Luc was analyzed by Southern blot (*B*) and PCR (*C*) analyses. The arrowhead indicates the integrated fragment containing the *Luciferase* gene in *B*. (*C*) The two PCR reactions for each sample were added to the same lane in a 1% agarose gel for DNA electrophoresis. P1 and P2 are shown as two different PCR products generated by primer sets F1/R1 and F2/R2, respectively. A DNA marker *1 kb plus* (M) (Invitrogen) was run on the left lane (*C*). (*D*) Total RNA samples from Dd2Luc and Dd2LucNM were collected at 44 h postinvasion for reverse transcription and real-time PCR analysis of transcription of *AMA1*, *Luciferase*, *RH4*, and *PEBL* genes. The signals for all of the genes from real-time PCR were shown as percentage of that of the *60S L18* gene as described in the legend of Fig. 1. Experiments were independently performed at least three times. The SE is shown as an error bar.

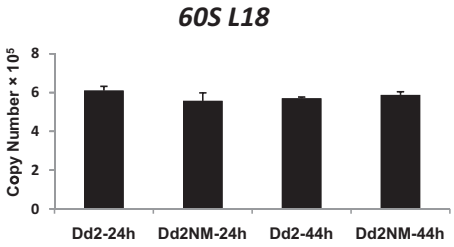

Fig. S2. Similar expression of the *60S ribosomal protein L18* gene at 24 and 44 h in Dd2 and Dd2NM. Expression level of the *60s L18* gene was shown as the copy number of cDNA × 10^5. Experiments were independently performed at least three times. The SE is shown as an error bar.

Table S1. Primers used in this study

Genes analyzed in 5′-RACE	Gene-specific primers	
RH4	5′-GATTCTGTGAGTTCTTAGAATCTGCTTCTGGATCG-3′	
	5′-GGTTTTGACTGTACGCCAAATGATGGCAACCCAAAGG-3′	
	5′-ATATGTGTAAATATATCCATATTG-3′	
PEBL	5′-AATGCCCTAGAAGAAAAGAGGAACCCAGAG-3′	
	5′-AGTTCTTATGTTATAGCATCAAAATCAGGG-3′	
	5′-TACAATATTAGCCCTTTTTGTTTCTTCC-3′	
Genes tested for transcription	Forward primer	Reverse primer
RH4	5′-GTTCTTTTGTAGTTTCTAAC-3′	5′-CGGAATCGAATCGTATTATG-3′
PEBL	5′-GCAAATGGTAGAGAAGATCC-3′	5′-GACATCTCTTCCAGAACTAC-3′
AMA1	5′-TAAGAACGCTAGTATGATCA-3′	5′-CCCTTACCATGACTTTTATA-3′
Luciferase	5′-TTGTGTTTGTGGACGAAGTAC-3′	5′-TTCTTGGCCTTTATGAGGATC-3′
60S L18	5′-AATATCACATGGCCAATCACC-3′	5′-CAATCTCTTATCATCTGTTATTGATCC-3′
Primers in ChIP		
3′RH4	5′-TTTTCAAAAGGAATTTAGTG-3′	5′-TCATCTTCAACTTCATCTTC-3′
M′RH4	5′-AGTCCAAAATAATGATGCTA-3′	5′-ATCGAACAAGATGTGATTAT-3′
5′RH4	5′-GATATGTACCAAGGAAATGAC-3′	5′-GTGTGTTTTATTTATATCATGTTG-3′
+3N-RH4	5′-TAAAAGATCTGGTTATATAAAAATC-3′	5′-ATTTTGTTTTTTCAAATGTTTGTTC-3′
+2N-RH4	5′-TGATAAATTATAGATTTTAGGTC-3′	5′-ACATATATACCTACATATTTATATG-3′
+1N-RH4	5′-AATACATTTATATAATAAAAGAAGGAAG-3′	5′-AACTTTGTAATTCCATAAAGATGAG-3′
−1N	5′-TTTTTTTTTTTATAATTATAGCATGTAC-3′	5′-TTTTATAAAAATAATATGTTCATTTTGTG-3′
3′PEBL	5′-CTTAATAATCCTATCCTATATGC-3′	5′-GCCTTTGAAGAAGCTAAT-3′
M'PEBL	5′-AGGTTCCTTTATTACATCATT-3′	5′-TATGTTTGATGGAAGACG-3′
5′PEBL	5′-TTCTCTGGAAGTTGTTCCT-3′	5′-TGATAAGTTGGCAAATGG-3′
+3N-PEBL	5′-AAAATATACACACACATAAATATATG-3′	5′-ATTAATATTATCAGGTTTCTACATG-3′
+2N-PEBL	5′-AATATTTTATTTATTAATTATTTTTTTC-3′	5′-AAAAGGAAAGGAGGATCTTAG-3′
+1N-PEBL	5′-AAATATTATTTTTAAAAATAAAAAAAAG-3′	5′-AAAAAAAAAATTAAGAACTTACC-3′
5′UTR-AMA1	5′-TAACACCTTGTTTTGAAACC-3′	5′-CGTTTAATAAGAGACTCTAACA-3′
5′UTR-Svar1	5′-CTATGTTGTATTATTCGATATTTTC-3′	5′-AGAATAGGAAAATACAAATTATAGC-3′
Primers for nucleosome scanning		
180(+)/288(-)	5′-TTTTTTTTATAGTTCTTATGTTATAGC-3′	5′-TTATAGATGTGGTTTATGTACATAC-3′
205(+)/319(-)	5′-GCATCAAAATCAGGGTTAAA-3′	5′-TGTTATAAATATTAAACAGGTTG-3′
245(+)/354(-)	5′-ATTTTTTTGATTTTTTATAGTATGTAC-3′	5′-AAGAAACAAAAAGGGCTAATATTG-3′
277(+)/388(-)	5′-CCACATCTATAAATAAATAACAAC-3′	5′-GATATTCCATTATGAACTTGTG-3′
300(+)/407(−)	5′-CCTGTTTAATATTTATAACATTTGTTC-3′	5′-ATTGCTTAATCATAATAATGATATTCC-3′
333(+)/446(-−)	5′-ATATTAGCCCTTTTTGTTTCTTC-3′	5′-ATATTTATGTGTGTGTATATTTTTG-3′
366(+)/456(−)	5′-TCACAAGTTCATAATGGAATATC-3′	5′-ATATATACATATATTTATGTGTGTG-3′
397(+)/507(−)	5′-GATTAAGCAATGAAAATAATATTTTC-3′	5′-CTACATGTAAAATAAATACATATTATG-3′
424(+)/525(−)	5′-AAAATATACACACACATAAATATATG-3′	5′-ATTAATATTATCAGGTTTCTACATG-3′
461(+)/557(−)	5′-ATATATATATATGTTTAATACATAATATG-3′	5′-CCTTGTGTAAGGTTTATATTTTAAA-3′
481(+)/584(−)	5′-CATAATATGTATTTATTTTACATGTAG-3′	5′-GATTATTTATCGAAATAATATTGCAC-3′
501(+)/583(−)	5′-CATGTAGAAACCTGATAATATTAA-3′	5′-ATTATTTATCGAAATAATATTGCAC-3′
529(+)/598(−)	5′-TATATTTAAAATATAAACCTTACAC-3′	5′-TATATATATATATAGATTATTTATCG-3′
554(+)/658(−)	5′-AAGGGGTGCAATATTATTTCG-3′	5′-AAAAAATAATTAATAAATAAAATATTAAC-3′
631(+)/726(−)	5′-AATATTTTATTTATTAATTATTTTTTTC-3′	5′-AAAAGGAAAGGAGGATCTTAG-3′
675(+)/775(−)	5′-ATATTATATATATATATATATGAAGGG-3′	5′-ATTTTTAAAAATAATATTTTATATTTATTTG-3′
702(+)/811(−)	5′-AATTCTAAGATCCTCCTTTCC-3′	5′-AATATTTTTAATATAAATAATTTTATTC-3′
721(+)/839(−)	5′-CCTTTTATATTAAAAAAAATAAAAC-3′	5′-AAAAAAAAAATTAAGAACTTACC-3′
757(+)/839(−)	5′-AAATATTATTTTTAAAAATAAAAAAAAG-3′	5′-AAAAAAAAAATTAAGAACTTACC-3′
798(+)/897(−)	5′-ATATTAAAAATATTATTAAGGTAAGTTC-3′	5′-CTTTTCTTTTCTTTTATTTCTCTATG-3′
833(+)/939(−)	5′-TTTTTTTTTTTTTTAATAATACACATC-3′	5′-TATATTTGTTCTTGTTTTTTCGTG-3′
856(+)/953(−)	5′-CATCTATATTAAGAATCATAGAG-3′	5′-TTTTTTTGTTCTCTTATATTTGTTC-3′
894(+)/1007(−)	5′-AAAGAAAAAAAAAAAAAAAAGGCAC-3′	5′-TATATGTATGAACAAGTACATG-3′
925(+)/1007(−)	5′-ACAAGAACAAATATAAGAGAAC-3′	5′-TATATGTATGAACAAGTACATG-3′
965(+)/1051(−)	5′-TTTTTTTTTTATAATTATAGCATGTAC-3′	5′-TTTTATAAAAATAATATGTTCATTTTGTG-3′
987(+)/1133(−)	5′-ATGTACTTGTTCATACATATAATTAT-3′	5′-GTTTTATTTTTATGGATTCTATAAGTAA-3′
1023(+)/1132(−)	5′-CACAAAATGAACATATTATTTTTATA-3′	5′-TTTTATTTTTATGGATTCTATAAGTA-3′
1052(+)/1132(−)	5′-AAAAAAAAAAAAAAAAAAAAAAAAAATG-3′	5′-TTTTATTTTTATGGATTCTATAAG-3′
1092(+)/1198(−)	5′-TATATAAAAATTTATTACTTATAGAATC-3′	5′-CTTTTATTATATAAATGTATTGCTTG-3′
1109(+)/1206(−)	5′-CTTATAGAATCCATAAAAATAAAAC-3′	5′-TCTTCCTTCTTTTATTATATAAATG-3′
1146(+)/1248(−)	5′-CAAAAAAAAAAACAAAAAAAAAACTAAC-3′	5′-TAATAACATAGTGTGGTTGTAG-3′

Table S1. Cont.

1178(+)/1277(–)	5′-AATACATTTATATAATAAAAGAAGGAAG-3′	5′-AACTTTGTAATTCCATAAAGATGAG-3′
1213(+)/1299(–)	5′-TTTAATAATATAATCTACAACCACAC-3′	5′-TTAAAGGTTCTTTATATATAATAACTTTG-3′
1231(+)/1315(–)	5′-AACCACACTATGTTATTACAAC-3′	5′-TATATATGTATCTATATTAAAGGTTC-3′
1266(+)/1373(–)	5′-AATTACAAAGTTATTATATATAAAGAAC-3′	5′-GGAAAGAAAAAAAAAAAAAAAAAAAAATATA-3′
1287(+)/1386(–)	5′-AAAGAACCTTTAATATAGATAC-3′	5′-ATGGCAACCCAAAGGAAAG-3′
1348(+)/1450(–)	5′-ATTTTTTTTTTTTTTTTTTCTTTCCTTTG-3′	5′-AAAAATGACCTAAAATCTATAATTTATC-3′
1362(+)/1459(–)	5′-TTTTTTCTTTCCTTTGGGTTG-3′	5′-ATTTTTTTTAAAAATGACCTAAAATC-3′
1396(+)/1494(–)	5′-TACAGTCAAAACCAAATCTT-3′	5′-ATATGTGTAAATATATCCATATTG-3′
1422(+)/1514(–)	5′-TGATAAATTATAGATTTTAGGTC-3′	5′-ACATATATACCTACATATTTATATG-3′
1464(+)/1565(–)	5′-ATATATACAATATGGATATATTTACAC-3′	5′-TTAGAACTCTACATAATAATCAAAC-3′
1489(+)/1581(–)	5′-ACATATAAATATGTAGGTATATATG-3′	5′-ATTTATGATGATTTTTTTAGAACTC-3′
1541(+)/1653(–)	5′-GTTTGATTATTATGTAGAGTTC-3′	5′-AGATCTTTTATAAGTTGAATGG-3′
1624(+)/1711(–)	5′-ATATATAACCATTCAACTTATAAAAG-3′	5′-TATTTATTTTGTTTTTTCAAATGTTTG-3′
1644 (+)/1706(–)	5′-TAAAAGATCTGGTTATATAAAAATC-3′	5′-ATTTTGTTTTTTCAAATGTTTGTTC-3′
1659(+)/1753(–)	5′-TATAAAAATCGAATTTTTGTTAAGAAC-3′	5′-AAATCAATAAAGAGAAAAATATAAACC-3′
1677(+)/1765(–)	5′-GTTAAGAACAAACATTTGAAAAAAC-3′	5′-AGAAAAAGAAAAAAATCAATAAAGAG-3′
1724(+)/1828(–)	5′-TAAGGTTTATATTTTTCTCTTTATTG-3′	5′-AAAAAAAAATAAACAGAGGAAAAAG-3′
1789(+)/1890(–)	5′-TTTTTTTCTTTTTTTCTTTTTCCTCTG-3′	5′-TTATCCACAATATATTCTTATTCATG-3′
1821(+)/1924(–)	5′-TTTTTTTTCTTCCATAAGGAATGAG-3′	5′-TTACCCAAGAGAAAAAATAAAAAATG-3′
1849(+)/1940(–)	5′-ACCCATTTAAAATAAACATGAATAAG-3′	5′-TATATATATTTTTTTTTTACCCAAGAG-3′
1874(+)/1976(–)	5′-GAATATATTGTGGATAACTTTTTTTC-3′	5′-TTATATGTGTGTCGTTTTACTTATG-3′
1896(+)/1989(–)	5′-TTTCATTTTTTATTTTTTCTCTTGGG-3′	5′-ATACATGTTTATATTATATGTGTGTC-3′
1930(+)/2050(–)	5′-AAATATATATAACATATATGATCATAAG-3′	5′-GAAGGATATATGTAAAAATAAATAAATA-3′
1962 (+)/2063(–)	5′-ACGACACACATATAATATAAACATG-3′	5′-TATATAAGTGTATGAAGGATATATG-3′
2032(+)/2121(–)	5′-ATTTTTACATATATCCTTCATACAC-3′	5′-TCTGGATCGTTTTTTTTTTCTTTTG-3′
2061(+)/2160(–)	5′-ATATAGATATGTACCAAGGAAATG-3′	5′-TTTATATCATGTTGATTCTGTGAG-3′
NR	5′-AAGAAGTTATTTATATGGAATATATC-3′	5′-ATAATAAGGAACAGGATAATATTC-3′
Primers in pBRLucR		
F1/R1	5′- AGTCAAAATGACGCATGATTATC -3′	5′- AAGAAGCTCAGAGATTGCATG -3′
F2/R2	5′-TGAACGTGAATTGCTCAACA-3′	5′-GCAACGCACTTTGAATTTTG-3′
5′-UTR-RH4	5′-CGGGGTACCGTCGACTACATTTAAATGTTCAAAATATAATATA-3′	5′-GCCGCTCGAGGTTTATTTTAAATGGGTGTACT-3′
3′UTR-RH4	5′-GCCCAAGCTTTTAATTTATATAAACATTGGAGAGTTACATTGG-3′	5′-AAAACTGCAGGGGCCCAGTAAAAAGTACCTTTTCGTC-3′
IPCR	5′-AGATGTCCTAAATGCACAGCGAC-3′	5′-GTCAATGCGGTAAGTGTCACTGA-3′