SUPPORTING ONLINE MATERIAL FOR

Poly(ADP-ribose) Binding to Heterogeneous Nuclear Ribonucleoproteins Modulates Alternative Splicing Activity

Yingbiao Ji and Alexei V. Tulin

This PDF file includes:

Figures 1 to 8.

Figure 1. The expression level of Squid/hrp40 is strongly reduced in the *Squidhrp40* mutant, indicating the specificity of the Squid/hrp40 antibody (8D2). **A.** 10 μg total proteins from the third-instar larvae of the homozygous S*quid/hrp40* mutant (sqd^{j6E3}) and the wild-type (y,w) fly was immunoblotted with the Squid/hrp40 antibody (8D2). The residual Squid/hrp40 present in the S*quid/hrp40* mutant came from the maternal expression of the S*quid/hrp40* gene. **B.** The same blot as in (**A**) was stripped and probed with mouse anti-alpha-tubulin (Sigma) to show the equal loading.

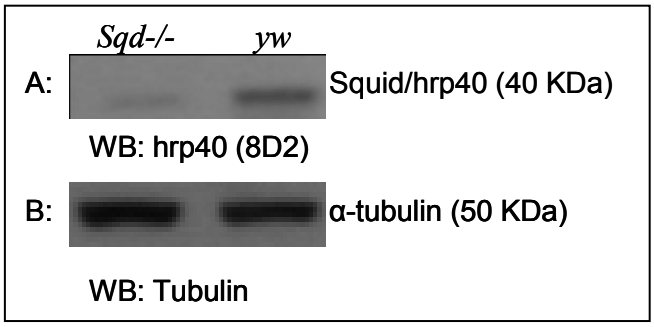
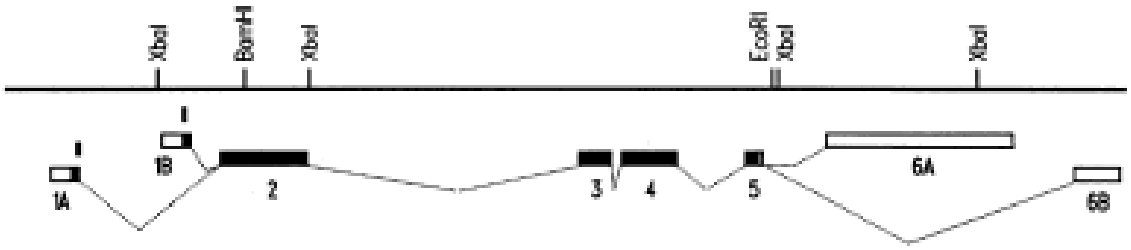
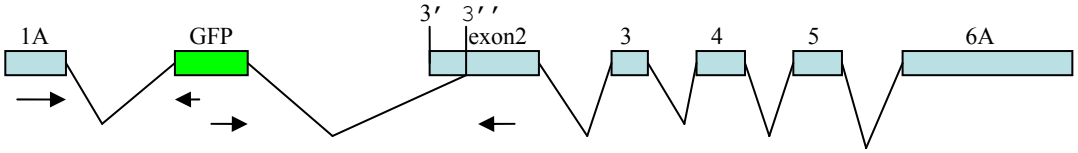


Figure 2. The transcript structure and expression of the hrp38:GFP fusion gene.

A. The transcript structure of the *Hrb98DE/hrp38* gene.



B. Hrb98DE/hrp38:GFP fusion gene.



GFP was inserted between the exon1A and exon2 of the *Hrb98DE/hrp38* gene. Sequencing the RT-PCR products using indicated primers (arrow) showed that GFP was also spliced in-frame to exon1A and exon2 using the 3'-most (3'') splice site, producing the Hrb98DE-PE:GFP

C. The amino acid sequence of Hrp38-PE:GFP fusion protein:

MGGHDNWNNGQNEEQDIHHHHHHGVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFI
CTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDT
LVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAHYQQNTPIGD
GPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSTLEDSITEPEHMRKLFIGGLDY
RTTDENLKAHFEKWGNIVDVVVMKDPRTKRSRGFGFITYSHSSMIDEAQKSRPHKIDGRVVEPKRAVPRQD
IDSPNAGATVKKLFVGALKDDHDEQSIRDYFQHFGNIVDINIVIDKETGKKRGFAFVEFDDYDPVDKVVLQ
KQHQLNGKMVDVKKALPKQNDQQGGGGGRGGPGGRAGGNRGNMGGGNYGNQNGGGNWNNGGNNWGNNRGGN
DNWGNNSFGGGGGGGGGYGGGNNSWGNNNPWDNGNGGGNFGGGGNNWNNGGNDFGGYQQNYGGGPQRGGGN
FNNNRMQPYQGGGGFKAGGGNQGNYGGNNQGFNNGGNNRRY.

The amino acid sequence of hrp38-PE:GFP fusion protein was derived from the RT-PCR sequences obtained in Figure B and the hrp38-PE sequence in the flybase. The 6X His-GFP insertion is highlighted in green. The pADPr-binding motif is highlighted in yellow. Based on our sequences, it appears that the pPGC construct (ATC-6XHis-GFP-TCGACACTCGAG) (Morin *et al*., 2001) was inserted into the intron between exon 1A and exon 2 of the *Hrb98DE/hrp38* gene in the *ZCL588* strain.

D. The expression of hrp38:GFP fusion protein. Upper panel: 50 μg total proteins from the third-instar larvae of the indicated genotypes was subjected to immunoblotting analysis using anti-GFP antibody, showing that about 55 kDa hrp38:GFP fusion protein was detected from the *ZCL588* (hrp38:GFP) and *PARP1-DsRed; hrp38:GFP* fly strains, respectively, but not from the wild-type strain (*yw*). Lower panel: The same blot as shown in the upper panel was stripped and probed with the mouse actin antibody (Millipore) as the loading control. It appears that the *ZCL588* (hrp38:GFP) strain had an expression level equal to the hrp38:GFP fusion protein with the *PARP1-DsRed; hrp38:GFP* strain after normalization with the loading control.

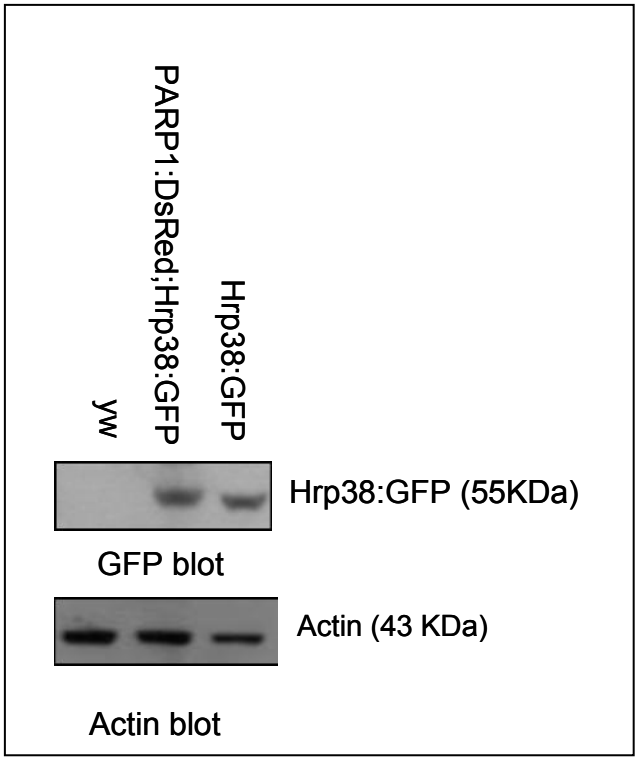
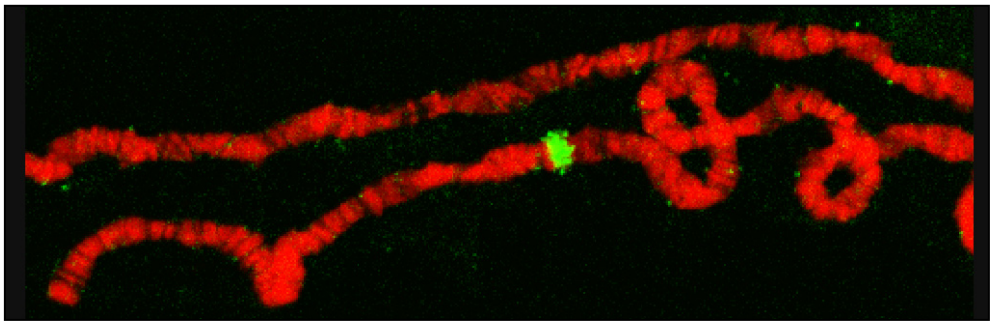


Figure 3.

A. The accumulation of hrp38:GFP in the 93D puffs after heat-shock treatment. *Drosophila* polytene chromosomes of the hrp38:GFP line were immunostained with the anti-GFP antibody: Hrp38:GFP, green; DNA, red.



B. There is no significant difference in the expression levels of hrp38:GFP and Squid between the hrp38:GFP and *Parg;* hrp38:GFP lines after heat-shock treatment. After one hour of heat-shock treatment, 30 µg total proteins from the third-instar larvae of the hrp38: GFP and *Parg;* hrp38:GFP lines was immunoblotted with anti-GFP and anti-Squid antibody (8D2), respectively. The blot was stripped and probed with mouse anti-actin antibody for loading control. HS: heat-shock treatment.

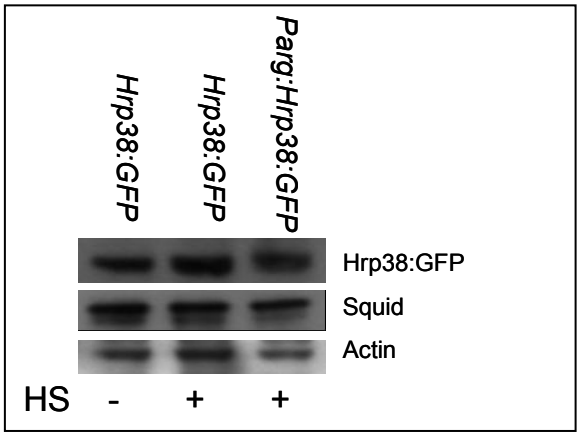
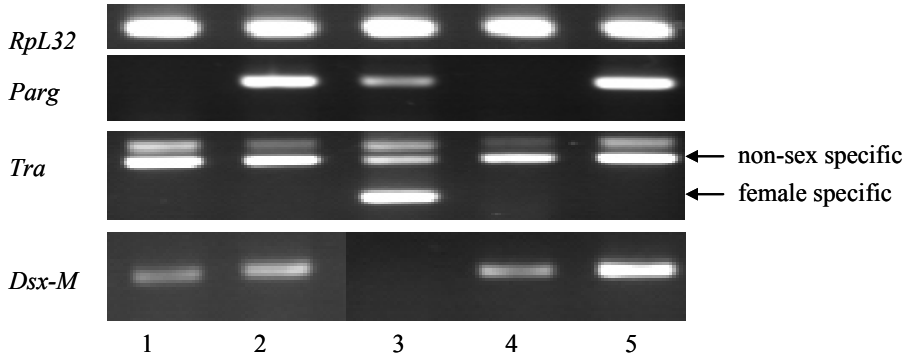


Figure 4.

A. RT-PCR confirmed the genotypes for the real-time RT-PCR assay in Figure 8B in the main text and Figure 4B in the Supplemental Materials. Lane 1. *Parg* (-/-), the third-instar larvae male; 2. *yw,* the third-instar larvae male (control); 3. *Parg* (+/-), the third-instar female; 4. *Parg* (-/-),pharate male; 5. *yw* pharate male (control).



B. There is no significant difference in the *RpL32* expression level relative to the wild type among the indicated genotypes and treatment in Figure 7C in the main text. The *RpL32* expression level as an internal control in Figure 8B was measured by real-time RT-PCR. The error bar represents the standard deviation from two independent experiments. Lane1. *Parg* (-/-), the third-instar larvae male; 2. *yw,* the third-instar larvae male (control); 3. *Parg* (+/-), the third-instar female; 4. *Parg* (-/-), pharate male; 5. *yw* pharate male (control).

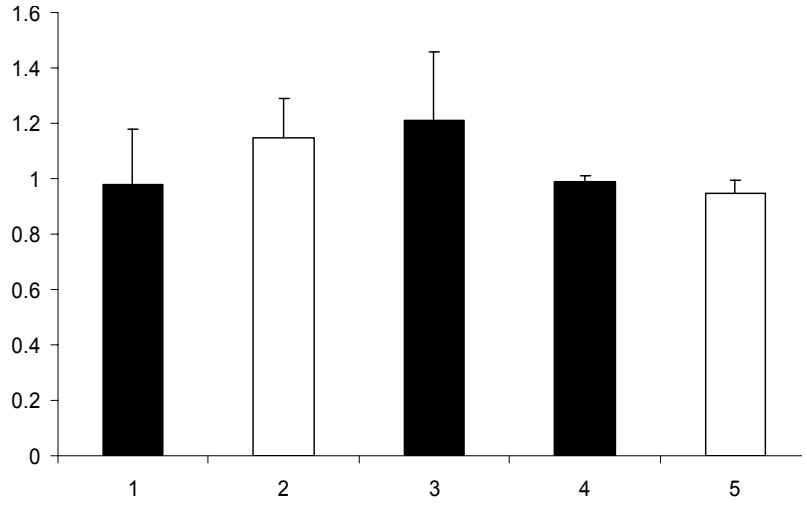
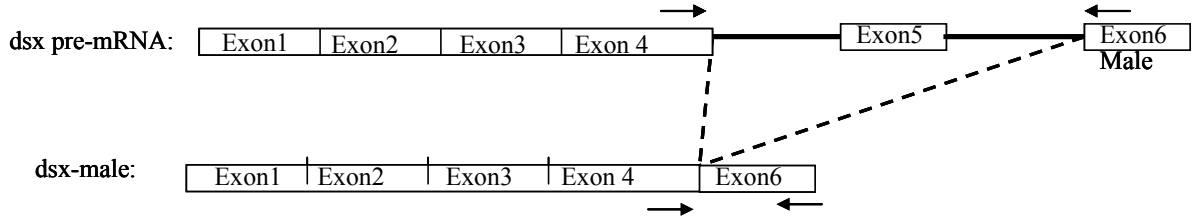


Figure 5. Inhibition of alternative splicing of the *dsx* gene by PARG loss-of-function. **A.** The structures of dsx pre-mRNA (unspliced) and dsx-male (spliced) transcripts in the *doublesex* (dsx) locus. The primers (arrows) specific for the dsx-male transcript were used for dsx-male quantification. **B**. The *dsx-male* expression level relative to *RpL32* in the indicated genotypes measured by real-time RT-PCR. The error bar represents the standard deviation from two independent experiments. Lane1. *Parg* (-/-), the third-instar larvae male; 2. *yw,* the third- instar larvae male (control); 3. *Parg* (+/-), the third-instar female; 4. *Parg* (-/-), pharate male; 5. *yw* pharate male (control). **: P<0.01.

A.



B.

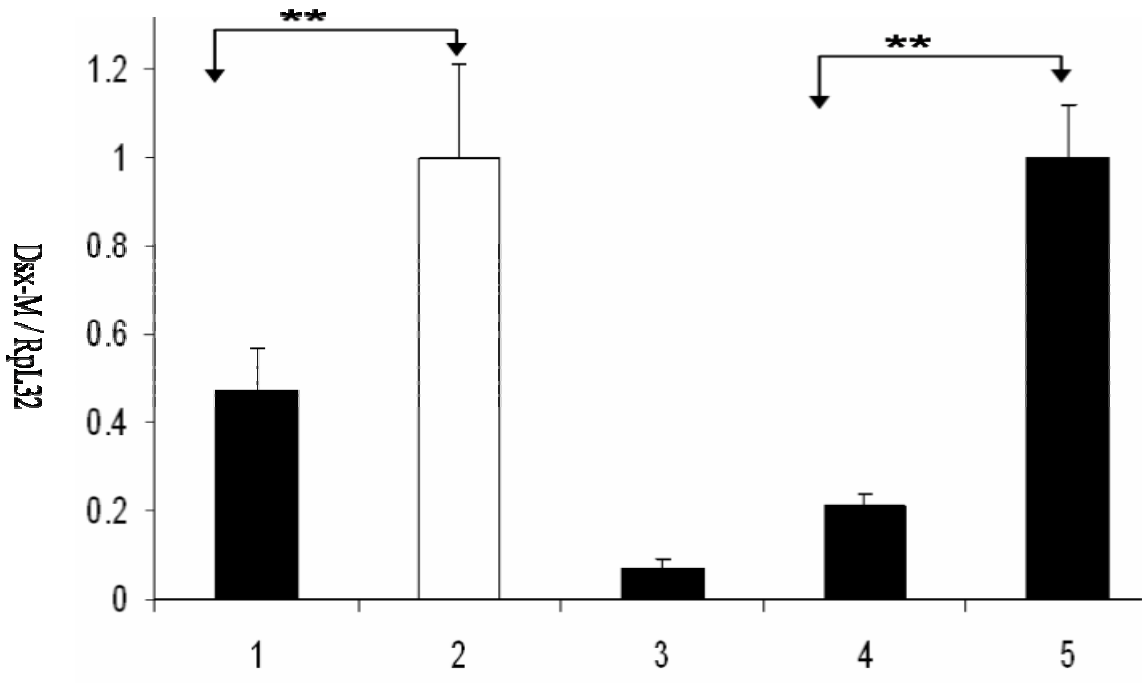


Figure 6. There is no significant difference in the *RpL32* expression level relative to the wild type among the indicated genotypes and treatment in Figure 8D in the main text. The error bar represents the standard deviation from two independent experiments. The expression level of the *RpL32* expression level as an internal control was measured by real-time RT-PCR.

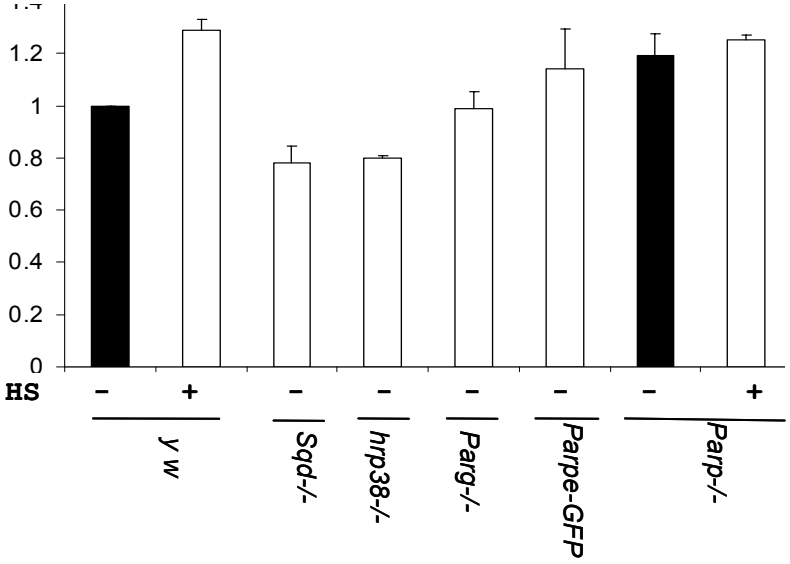


Figure 7. The structure of Hrsr-Omega-RC. Introns in lower case are marked in blue; G triple and quarters in exon 2 are marked in red.

```
   1 ACTCTCAAAT GAAAAGTGTT CAAGTGCATT CAAAGTGAAG CTGAAAAAAT AACCAGTTAA
  61 AAATAGTACA AAGAAATTTT CTTTCTTGCA ATTTGCAAGC AGTAGCTACA ACCAAAAATG
 121 GAAAAGTGTA AAAATCGTGT CCCAGCAGAC GAGCAGCAGC AGTACGAGTA TTGCAAAATG
 181 CAGGGGCAAG GGCCCACGTA GTATTTTTCC ACGTCGGGCA TTTAATGCTC TCGAGTTGGA
 241 AACAATGAAA CCATACGCAA ACCCCCTGGA AAAGATGTGA TTAGTCATCG ATTTTGCTGG
 301 TCAGCGTCGG GGAATTTCGC AATGCAGCAG GCAGTTTTCT CAAATTTGGC TAGAAAGTGA
 361 CCCACTAGGC AGTCTGAGGC AGTTATCCAG GATGTAAGGA TGTGTGCAGT ACTCTCTGTG
 421 TCGAATGGCG ATGGCCCTGA CCTGCGGATT TTGAAAGATT GAAATAGGAA GCCAGgtata
 481 catacacagc aacgaatgca gcgtgttcta tattcttaat tcgtaagcca aacctatgtt
 541 taagacgtag tttatataac cgtacatatg tatgtagcga aacacgtttt caaacgtcgc
 601 atcgaagcca tttcttctgc agccattgca atttcatcgt gttgcgtaat cgcaaattgt
 661 aaatgtgtcc ctactacttc acatttgtgt gtatccaagt cgaaagttct cgaactattc
 721 tcgtgagcaa cccccggatc gaaaggccac caactggctc gttctctctc tccagaaaca
 781 gcttactctt acgtttacaa ttgactctct acctatcggg tatacacatt tttatatatg
 841 tatacactca gagacacccc aatcccccac catacatatg gcgagatgtg tgaatgtatc
 901 gcagttggac gaaaacggca gctgcgcaga aaacgctgcg gcgtacatac aaacgagctc
 961 tttgtttgcc tttccccagt tttgcttccc catacgtata actggtgttt atcagcggta
1021 atctcgccag cctgactttg tccctgtcgc ttctattgac attctaaact agattgtaga
1081 cgttttaaca tttacctagc tgttaatctt atttgtaaac atctctatat gcaacctgcc
1141 ataccactta accaagcaat atgtatttct ttctctaaac tttatagTTG GGCGTTGAAA
1201 GTTGATATCG ATATCGATCC GTGAAAAGTC GATACCCTGC GCAAGCATGG GGCGGCATAT
1261 GGGTGCTGAA AACGCACTCG GCCCGATCCC GATTGCAGCG TTATTCGAAA GCTGTGTCTG
1321 CGACCGTGAC TGAGATCATA TGCGTACATA TATCTAATGT CCGGGGTCGT AGGCCAGCCA
1381 GGGTGCTCGA TTCTGTCAGA TTGATTGTGC GGATTGTGTT ATAGGAACAC TGGTGTATCG
1441 ACTTCTCTGC TCCACTATGG GTGAAGGATA CCCTACCGAA AAGGCCTTCT GTCGCTTACT
1501 ATCATCGAAC AAGTTCCGTA AAGGGCAGAC ATACGTACAC GTGGCAGCAT ATTACGTTCA
1561 ATGACACATC GTCTCTGGAT TAGTAGTTGA ACCAACGAGC TCGAATATAC CCTGCCAGTT
1621 GGTTGTAAAT ATTAATTTTG TTCTTATATT CAGTTGTAAA GTCAATACGG AAGACATTTC
1681 CACACCAACC ATGTGTCACT TATGTTCTTA AATACCAGAA ACTGTTTAAA TTTTTGTTCT
1741 TATACACTTC ACCAATTAAG AATTACCTGT ACTGATGTAA GTGTACCTGT TGAATCACAA
1801 CAAAAATAGA AAAAATGAAT AACAAAAGAG CTCTATAACA GAAAAGCCAC AGAAAAAGTG
1861 AATAAAAATT CAAAAATTCA AAAAATAAAT TTCGAAACAT TT
```

Figure 8. The structure of the *Ddc*(*dopa decarboxylase*) gene. Introns in lower case are marked in blue; G triple and quarters in intron 1 and 2 are marked in red.

```
   1 TCAGTTAAGA GGAGAACGCC AAGCGCACAG CAATCAGCAC CGAAATATCA GCATCGAAAT
  61 ATCAGCAAAT AAATATTAGC TGTTCTAAAC CAGGAGGGCA AACTGAACTT GGAGCAAAGA
 121 TTTAGTTCGG AACGGAAGTA AAGCTCGGCA ACAAGTGCAA ACAATTAAAA GCAGTTAACT
 181 AAAGTGCAAC Ggtgagagac gaaagtgtgg ctcctcaaca gcctcagctg cctgaagtgc
 241 ttggccaaca taatgagtgc atgtgcatgc gaaagattca tttcggggct aacgctgcgt
 301 atacgtaatg tgtatctaaa actgggcata tactatagcc ttgcttcggt tcaatttgat
 361 agttcgggcc ccgaattcta tagtgcttaa gcttttctcg gctttcggta tctgcatgct
 421 tttgtgtatc tattaaaata agattttagc tggcaacaag tcgtcgtctc aatgccaact
 481 tgtttacgtt gttaaaattg gaatttagaa aaaaaaaaaa aataaagcag tcttgattaa
 541 tgcaagaatg cattaaacat tctaattacc atactaattc acagcctata cttaagcagc
 601 gcactcgatg ggaaaacgct ttaaactatt aataccttaa taccttatta ttataactat
 661 tatcatcatc gttttgccta tcaagtaatt agtattcaat gtcgttcatt tgtcgtgttt
 721 gcagcgatag catttttgt ttgagctgct gcactgatta gcactatctt caaaaacgca
 781 cttctattaa taacactttc aataatcgca cattctttca tattagCTCT AACCATTCGA
 841 GTTCATATCA TTGCAAAAGT CAAACGAAAA GTAAATCTCT GAAATGAGCC ACATACCCAT
 901 TAGTAACACA ATTCCAACAA AACAAACTGA TGGTAATGGT AAAGCTAACA TTTCGCCGGA
 961 TAAGCTGGAT CCCAAGGTTT CGgtatgtct attgggttta ggtatagagc caacaattat
1021 gcacgtctga taaccaaata cttttgcatc cacatcaagA TCGACATGGA GGCGCCGGAG
1081 TTCAAGGATT TTGCCAAGAC AATGGTCGAC TTTATAGCCG AATATCTGGA GAATATACGC
1141 GAAAGgtgag ccagattaag actccctact caattagctt gaattaaact taatttagcg
1201 tatgaatttc atttatatgg tatcagaatc agtcgcttga cctcagcatt ttacgttcga
1261 atcgaaagtt cgttctgctc gattcgaatc cccgggcaag tgaatgacat ttcgcacacg
1321 ttttgagatt agtcacggga aagtcgcacc gatcggacat ttccattgct atatatatct
1381 atatatatat atcattttgt ttaggggttt gaggcgacct tcccattagc tcgagggcca
1441 agtactttcg ctgctcttgg gccgaaaact aattaattaa atggctttgt tgagttggcg
1501 tgtcaaggtc gtttttcatg tatacgagta tagatataat tgcactgcta acgccttggc
1561 caaaagcaat tcgggtattt cactattctt gggcaattct tctaacggct tcgtttccat
1621 taccttgaaa atcaaagtca gctaagtaaa caattttctt actacagctg ctgagtttgt
1681 ttgcccatcg acagtcgctg aaattaatgg ttaattgaaa atcaagctta agtagagcgt
1741 aatataataa ttcattttgc tttattaaag ttccttcgac attgaagttt caaaactatt
1801 ttcttagtta gataactttt taaacgaatc tttgttaatt gaagatacat atatatagag
1861 aaattatctt tttatttttct tttttcacct cttagtagta cttccttta attgaaagga
1921 tagaaaatcc caccatcatt atcagcattg cctctctatc tatattctgt tcccatagca
1981 atttgctaca tattcgtatt gattttcatg gcagtggcaa caagttgggg gtggttggat
2041 gggtgtcctt caaccccaat gattcctgat gcctttgttg gctaactgag tttcgcagcc
2101 aattagcaag gagcttttac tgaatgggcg ccaaaatgca atcagaagct aacgcaattt
2161 tcgcaattac agGCGCGTTC TGCCGGAAGT GAAGCCTGGC TACCTGAAGC CATTGATTCC
2221 GGATGCTGCG CCCGAGAAGC CGGAGAAGTG GCAGGATGTG ATGCAGGACA TCGAGCGAGT
2281 CATCATGCCG GGCGTGACAC ACTGGCACAG TCCCAAGTTT CATGCCTACT TCCCCACGGC
2341 CAACTCGTAT CCAGCGATCG TTGCGGACAT GCTGAGTGGA GCGATTGCCT GCATCGGATT
2401 CACGTGGATC GCCAGTCCCG CGTGCACGGA ACTCGAGGTG GTCATGATGG ATTGGCTGGG
2461 CAAGATGCTG GAGCTGCCGG CAGAGTTCCT GGCCTGTTCG GGCGGCAAGG GTGGCGGTGT
2521 CATCCAGGGC ACGGCCAGTG AGTCCACACT GGTGGCTCTG CTGGGAGCCA AGGCCAAGAA
2581 GTTGAAGGAG GTGAAGGAGC TCCATCCGGA GTGGGATGAG CACACCATCT TGGGCAAGTT
2641 GGTGGGCTAC TGCTCGGACC AGGCTCACTC ATCCGTGGAG CGGGCTGGTC TTCTGGGCGG
2701 AGTAAAGCTC CGTTCCGTGC AGTCCGAGAA TCACAGAATG CGTGGTGCTG CCCTGGAAAA
2761 GGCCATCGAA CAGGATGTGG CCGAGGGTTT GATTCCCTTC TACGCGGTGG TCACCCTGGG
2821 CACCACCAAC TCCTGCGCCT TCGACTACTT GGATGAGTGT GGACCGGTGG GAAACAAGCA
2881 CAATTTGTGG ATCCATGTGG ACGCTGCCTA TGCCGGATCC GCTTTCATTT GCCCCGAGTA
2941 TCGCCACCTG ATGAAGGGCA TCGAATCAGC AGACTCTTTC AATTTCAATC CACACAAATG
3001 GATGCTGGTG AACTTTGACT GCTCGGCCAT GTGGCTGAAG GATCCCAGTT GGGTGGTCAA
3061 CGCGTTCAAT GTGGACCCTC TTTACCTGAA GCACGACATG CAGGGATCAG CTCCGGACTA
3121 TCGTCACTGG CAAATCCCAC TTGGACGGCG ATTCAGGGCA CTGAAGCTCT GGTTCGTCCT
3181 CCGGCTGTAC GGTGTCGAGA ATCTCCAGGC CCACATCCGC AGACACTGCA ACTTTGCCAA
3241 GCAGTTCGGG GATCTCTGCG TGGCGGACTC CAGATTTGAA CTGGCCGCCG AGATCAATAT
3301 GGGATTGGTC TGCTTCCGGC TGAAGGGCAG CAACGAGCGG AACGAAGCTC TTCTCAAGCG
3361 AATCAATGGA CGCGGCCACA TCCACTTGGT TCCCGCCAAG ATCAAGGATG TCTACTTCCT
3421 GCGCATGGCC ATTTGCTCGC GATTCACCCA GTCCGAGGAC ATGGAGTACT CGTGGAAGGA
```

```
3481 GGTCAGCGCC GCTGCCGACG AGATGGAACA GGAGCAGTAA AGTGGTTGTG CAGGTCTGTT
3541 CCGTGTTTAG TATATAAATT AATATAGTAA ACTTAAATTG GACCAGTATG ATATATAATG
3601 CATTGTGACT TGGAACCCGG AACAGACCAT ACACTTTCCA CTTGCGACAT GTTTAGGGAA
3661 TTTACATCGC AACAAAAGAT GGTTCGTCCA TCGCTACATT ATATTTATAG TATCCTATCA
3721 TTGTATCATT GATGTTGTTC ATGATTTTTA TTGTTAACGT TATGCGCCTA ATTAAAACAA
3781 ATGTATTCTG CTTAAAAATA CAAACGAATT GTAACTATAA A
```