Tribolium castaneum

All predicted exons (71,788) from the 'official gene set' of the recently sequenced genome of the red flour beetle, *Tribolium castaneum*, were used to calculate independent designs of long dsRNAs for the *Tribolium* genome (exons longer 560 nt were additionally splitted in two sequences, resulting in overall 80,671 input sequences). *Tribolium* has become an important model organism for developmental and evolutionary studies and is suitable for RNAi by injection of long dsRNAs. Information on predicted genes and sequences was obtained from BeetleBase that integrates sequence annotations for *Tribolium*.

NEXT-RNAi results

NEXT-RNAi HTML ouputs are available here

Overall 71,293 designs were obtained, covering 99.4% of the genome. 92.9% of all genes are covered by at least one design that does not show homology of 19 nt or longer to any other gene. 83.2% of all genes are additionally covered by at least one second, independent design.

Input files and settings used

Input FASTA file

beetlebase_exons_split.zip (6.6MB) containing exon target sequences as input file (-i input) (parsed from *Tribolium* GFF files).

```
>TC016216-RA-E3
GGCACGCACATCTCAGTCCTGCCAACGGAAATGATTTTGCACATTTTGCGGTGGGTGGTGAGTGCGGAG
CTGGATTTGAGGTCGCTGGAGATGTTTTCGATGGTGTGTCGGGGCTTTTATTTGTGCGCACGTGACCCG
GAGGTGTGGCGGTTGGCGTGCCTGAG
>TC008956-RA-E3_1
GCTGCACTTCCGACCGAAGACGCCGCCCCCCGACGAAGAGTCCCCGTGCAGTTTGCTCCCGTTCAGCGA
GAGTTATTTCGTCCCCGGTGCCAACATGTCGTTCTCAATCGAGGTCCAGTCGTTGGAAACGCCAAGTAC
ACTCCTGGACACTTCCGCTTTCTTAACCGATCCATTCACGTTTGATAATTTGGACAGTATAGAAGACGT
ACCGGTAACCGACACGCGGCAATCCAGTGATAAAATCGTGCAAACGGGCATTAACTTGAACGTTAATGT
CGGTCTCAATACGCTTACTCCTAGTGTTAACG
>TC008956-RA-E3_2
ATAATAGTAGTAGTGTTTTTTCAAATAGTAACGCGAACCTCACTGATAGTATCAAGCTCGAGGATGCGA
CGTCATCGTCGACGTCGGACGAAAACGACGAAAGGACTATCGGATGTTCGAGGTTTTACGTGTCGGATT
TGGGCGTCACGAAGAAAAATCAAACGTCCAAAACGCAAACCGTTACGAGTACCTTGACTAGTCCTAAGT
TTAATAGTGTCAACGCCGTGTCTGAGGGATGGAGTAGGAGTACAGCCACCGAAGGGCCCTTGACTAACA
CCAACGGACTGCTCTCGCAGTTTGGCCTGGATG
```

Targetgroup file (tab-delimited)

beetlebase_targetgroups.tab (732KB) defining which BeetleBase transcripts belong to the same gene (headers Target and TargetGroup) (TARGETGROUPS option)

```
Target        TargetGroup     TxnName         GeneName
TC004355-RA   TC004355        TC004355-RA     GLEAN_04355
TC004356-RA   TC004356        TC004356-RA     GLEAN_04356
TC004357-RA   TC004357        TC004357-RA     GLEAN_04357
TC004358-RA   TC004358        TC004358-RA     GLEAN_04358
TC004359-RA   TC004359        TC004359-RA     GLEAN_04359
TC004360-RA   TC004360        TC004360-RA     GLEAN_04360
```

Bowtie database/index for off-target evaluation

Bowtie database/index containing annotated BeetleBase transcripts for specificity calculations (-d input):
beetlebase_transcript.tar.gz (26MB)

Bowtie database/index for mapping of reagents

The Bowtie database/index (GENOMEBOWTIE option) for mapping of reagents to the *Tribolium* genome:
beetlebase_genome.tar.gz (148MB)
Bowtie indexes for genomes are also available through the Bowtie webpage.

FASTA file for homology evaluation

Transcriptome FASTA file to evaluate the homology of the designs using Blast (HOMOLOGY option):
beetlebase_transcript.zip (6.2MB)

Design criteria

Start of program

```
perl nextrnai.pl -i beetlebase_exons_split.fa -s 2500 -r d -d
beetlebase_transcript
-e NO -o options.txt -n Tcas_3.0
```

Descriptions for start parameters used are available here.

Options file

```
DESIGNWINDOW=80,250
DESIGNNUM=50
OUTPUTNUM=1
SIRNALENGTH=19
EFFICIENCY=SIR,0
REDESIGN=ON
BOWTIE=/usr/bin/
TARGETGROUPS=beetlebase_targetgroups.tab
PRIMER3=/usr/bin/
GENOMEBOWTIE=beetlebase_genome
GFF=GFF3
GBROWSEBASE=http://www.dkfz.de/signaling/cgi-bin/gbrowse_img/beetlebase/
GBROWSETRACK=GENE+TXN
AFF=YES
LOWCOMPEVAL=/usr/bin/
CANEVAL=6
HOMOLOGY=/usr/bin/,beetlebase_transcript.fa,1e-10
RANKD=SPEC
```

Descriptions for all options used are available here.