

proTRAC

version 2.1.2

- Documentation -

1. Scope and prerequisites

1.1 Introduction

proTRAC predicts and analyzes genomic piRNA clusters based on mapped piRNA sequence reads. proTRAC applies a sliding window approach to detect loci that exhibit high sequence read coverage. Subsequently, sequences mapped to these loci are analyzed with respect to typical piRNA and piRNA cluster characteristics to ensure high specificity. The latest proTRAC version can be found at <https://sourceforge.net/projects/protrac/files> and <http://www.smallRNAGroup-mainz.de/software>. Running proTRAC on your local machine requires the installation of a Perl interpreter. Perl is pre-installed on common Linux and Mac systems. For Windows you can download and install either StrawberryPerl (www.strawberryperl.com) or ActivePerl (www.activestate.com/activeperl/downloads). For piRNA cluster prediction proTRAC requires a genome file in FASTA format and a map file in so-called ELAND format that contains information about the mapped sequence reads.

1.2 Changes compared to previous versions

- 2.0.5 → 2.1 proTRAC now produces output files in a very nice html format. Some default values changed to produce best results when using mammalian piRNA datasets.
- 2.1 → 2.1.2 Bug fixed: Pseudogenes, lincRNAs and antisense transcripts in piRNA clusters were displayed as protein coding (when implementing Ensembl gene sets information).

2. Quick Start

You need a **genome file** (e.g. `genome.fasta`) and a file that contains your small RNA **sequence reads** (`seq.fasta`). Download and unzip [NGS TOOLBOX](#) (a collection of simple Perl scripts). Open a terminal/command prompt window and type the following commands:

```
perl TBr2_collapse.pl -i seq.fasta -o seq.collapsed
```

This will remove redundant sequences from `seq.fasta`

```
perl TBr2_duster.pl -i seq.collapsed
```

This will remove low-complexity reads from `seq.fasta.collapsed` and produce an output file named `seq.fasta.collapsed.no-dust`.

Download [sRNAmapper](#) and map your sequences to a genome using the following command:

```
perl sRNAmapper.pl -input seq.fasta.collapsed.no-dust -genome genome.fasta -alignments best
```

The map file will have the name `seq.fasta.collapsed.no-dust.map`.

Download [proTRAC](#) and start piRNA cluster prediction with the following command:

```
perl proTRAC_2.1.pl -map seq.fasta.collapsed.no-dust.map -genome genome.fasta
```

3. An exhaustive manual

3.1 Create a map file (mandatory)

There are two simple ways to create map files for proTRAC. You need a genome file in FASTA format and a file that contains your piRNA sequence reads in FASTA or FASTQ format. This file should not contain redundant sequences and information on read counts for each sequence should be provided in the header line as follows:

FASTA

```
>34
TGGCACGTGCGATGGCAGTTCAACGTTCA
>23
TAAATCCCGTAGGCTTTTAGCATCGACG
>2
TTATTTCGAAGCGCTTTACGACACGCGCGCA
```

or FASTQ

```
@34
TGGCACGTGCGATGGCAGTTCAACGTTCA
+
AAABBGHHHHHHHHHHHHKLLLLLLLLL
@23
TAAATCCCGTAGGCTTTTAGCATCGACG
+
@@19000ACCCCGHHHFFBBBB (6321
```

You can convert your FASTA/FASTQ file to the required format using the `collapse` tool (Perl script) from the NGS TOOLBOX which you can find in the software section of this page (or download NGS TOOLBOX from https://sourceforge.net/projects/NGS_TOOLBOX/files/). We recommend to apply an additional filtering step prior to genomic mapping to remove low complexity reads from your datasets. Otherwise your map file may become extraordinary large due to multiple mapping of low complexity reads to low complexity regions in the genome. For this purpose you can use the `duster` tool from the NGS TOOLBOX which you can find in the software section of this page (or download NGS TOOLBOX from https://sourceforge.net/projects/NGS_TOOLBOX/files/) or use a third party software like DUST (Morquolis et al. 2006, *J Comput Biol*). Alternatively you may use a genome with masked low complexity regions.

For mapping your piRNAs to the genome we recommend the Perl script `sRNAmapper.pl` (which displaced the older `piRmapper_1.0.pl`) which you can find in the software section of this page (or download from <https://sourceforge.net/projects/protrac/files/>). Start the mapping procedure from the command line or terminal with the following command:

```
perl sRNAmapper.pl -input piRNAs.fasta -genome genome.fasta -alignments best
```

With the above command `sRNAmapper` will create a map file that is named `piRNAs.fasta.map` (you can give it a different name using the option `-output othername.map`) and that will have the following format:

```
Chr1    238    TGTTACGGCTAGCTCAGTACGGC    23    TGTTACGGCTAGCTCAGTACGAA    2    +
Chr1    291    TACGCCAGCTCGACTCGCCTGTGCA    23    TACGCCAGCTCGACTCGCCTGTGCA    0    -
```

The columns refer to: Chromosome, starting coordinate of the mapped sequence, locus sequence, FASTA/FASTQ header of the mapped sequence, mapped sequence, mismatch to locus sequence, strand. You can alternatively use the `SeqMap` software (Jiang and Wong 2008, *Bioinformatics*) to map your piRNAs using the following command:

```
seqmap 0 piRNAs.fasta genome.fasta output.map /output_all_matches
```

The `0` in the above command refers to the number of allowed mismatches. You may adjust this value as desired and/or add the option `/allow_insdel:n` where `n` refers to the maximum number of allowed insertions and deletions. The output format will be the same as above.

3.2 Post process your map file (optionally, proTRAC 2.0.5 and later)

Typically a large proportion of sequences will produce more than one genomic hit. In practice this means that the real origin of a sequence with multiple genomic hits remains unknown. Therefore, when searching for genomic piRNA clusters, `proTRAC` by default apportions the read counts for the sequence in question equally to each hit locus. However, you have the option to process your map file in order to allocate read counts of multiple mapping sequences according to the transcription rate of the genomic region (calculated on the basis of uniquely mapping sequence reads). To this end, you may want to use the Perl script `reallocate` which you can find in the software section of this page (or download from <https://sourceforge.net/projects/protrac/files/>). Start the post processing procedure from the command line or terminal with the following exemplary command:

```
perl reallocate.pl mapfile.map 10000 1000 b 0
```

`10000` in the above command refers to the up- and downstream region that is considered for calculation of transcription rates. `1000` refers to the sliding window increment. `b` refers to the form of the function that is used to weight the sequence read counts according to the distance between the center of the window and the hit locus. `0` represents the threshold value for the minimum number of allocated reads per locus. A threshold of `0` will remove hits with no allocated read counts. Use higher values as desired. Use `-1` to keep all hits.

3.3 Run proTRAC

Once you have created a map file you can start proTRAC from the command line or terminal using the following command:

```
perl proTRAC_2.1.pl -map piRNAs.fasta.map -genome genome.fasta
```

3.4 Include RepeatMasker annotation (optionally)

You can optionally include RepeatMasker annotation in proTRAC image output files. Therefore you need a RepeatMasker annotation that can be obtained by running RepeatMasker on the genome you use for mapping. If you do not want to install the RepeatMasker software on your local machine you can find RepeatMasker outputs (assembly_name.out.gz) for selected model organisms on the RepeatMasker website (<http://www.repeatmasker.org/genomicDatasets/RMGenomicDatasets.html>). Make sure that the chromosome or scaffold names in the RepeatMasker output (query sequence column) correspond to the FASTA headers in the genome file. RepeatMasker output files should look like this:

SW score	perc div.	perc del.	perc ins.	query sequence	position begin	position end	in query (left)	matching repeat	repeat class/family	position begin	position end	in repeat (left)	repeat ID	
1741	7.8	5.5	14.0	Bf_V2_1	1	358	(7763997)	+	hAT-N3_BF	DNA/hAT-Tip100	410	735	(1077)	1
94	2.3	0.0	0.0	Bf_V2_1	848	936	(7763419)	+	(TA)n	Simple_repeat	1	89	(0)	2
1123	3.7	0.7	0.0	Bf_V2_1	1236	1369	(7762986)	C	CR1-7_BF	LINE/CR1	(1)	4088	3954	3
293	27.7	7.7	7.3	Bf_V2_1	2023	2295	(7762060)	+	hATw-2_BF	DNA/hAT-hATw	5524	5797	(408)	4

To include RepeatMasker annotation add the option:

```
-repeatmasker RepeatMasker_output.out
```

3.5 Include Ensembl Gene sets (optionally)

You can optionally include information from Ensembl Gene sets in proTRAC image output files. Gene sets can be downloaded from <http://www.ensembl.org/info/data/ftp/index.html>. Make sure that the chromosome or scaffold names in the Gene sets file (first column) correspond to the FASTA headers in the genome file. Gene Set files should look like this:

```
#!genome-build Zv9
#!genome-version Zv9
#!genome-date 2010-04
#!genome-build-accession NCBI:GCA_000002035.2
#!genebuild-last-updated 2014-02
7       ensembl gene    100958  101715  .       +       .       gene:id [...]
7       ensembl exon   100958  100975  .       +       .       gene:id [...]
7       ensembl CDS    100958  100975  .       +       0       gene:id [...]
```

To include Ensembl Gene Sets information add the option:

```
-geneset GeneSets.gtf
```

4. How proTRAC works

Initially proTRAC reads the provided map file and saves information about the total number of mapped reads and the number of reads and genomic hits for each sequence. For the prediction of a piRNA cluster proTRAC needs a threshold value for mapped reads per kb. There are three different ways to set this value:

1. Directly set a value with the option `-dens [read counts per kb]`
2. Use the option `-pdens [p-value]`. **This is the default and recommended method.** Applying this option proTRAC will scan the map file with a sliding window and save read counts per kb for each sliding window. Running proTRAC with the option `-pdens 0.01 (=default)` will set the threshold for mapped reads per kb to the value that corresponds to the top 1% of sliding windows in terms of read coverage. In other words. A maximum of 1% of the genome can be annotated as piRNA clusters. In mammals, piRNA clusters typically represent 0.1% to 0.3% of the whole genome. This option will ensure that the sensitivity/specificity of proTRAC will remain mostly equal irrespective of the total number of mapped sequence reads.
3. Use the option `-pdens [p-value]` in combination with the option `-est [integer]`. Instead of scanning the map file with a sliding window, proTRAC will create [integer] random sliding windows based on the data obtained from the map file and save read counts per kb. This approach may be faster especially for large map files.

Once a threshold for mapped reads per kb is defined proTRAC again reads the map file with a sliding window. The default sliding window size is 5kb and can be adjusted with the option `-swwsize [bp]`. The default sliding window increment is 1kb and can be adjusted with the option `-swincr [bp]`. If a genomic locus exhibits an appropriate sequence read coverage thus represents a putative piRNA cluster proTRAC starts to analyze the locus and the reads that map to this locus in detail and checks whether

1. the locus exhibits the minimum size of a typical piRNA cluster. The default value is 5kb and can be adjusted with the option `-clsize[bp]`.
2. a defined minimum fraction of mapped reads exhibits the typical size of piRNAs. The default minimum size is 24 nt (can be adjusted with the option `-pimin [nt]`), the default maximum is 30 nt (can be adjusted with the option `-pimax [nt]`). The default minimum fraction is 0.75 and can be adjusted with the option `-pisize [0..1]`. If you do not want proTRAC to consider length of mapped sequences as a criterion start proTRAC with the option `-pisize 0.0`.
3. a defined minimum fraction of mapped reads exhibits U at position 1 (1U) or A at position 10 (10A). The default minimum fraction is 0.75 and can be adjusted with the option `-1Tor10A [0..1]`. If you do not want proTRAC to consider positional nucleotide frequencies of mapped sequences as a criterion start proTRAC with the option `-1Tor10A 0.0`. The option `-1Tand10A` (note the 'and' instead of 'or') allows to bypass the option `-1Tor10A` if both the fraction of 1U and 10A reads is above a stated minimum. The default value for this option is 0.5.
4. a defined minimum fraction of reads maps to the main strand of the putative piRNA cluster. For mono-directional clusters the main strand is simply represented by the strand with the majority of mapped sequence reads. However, piRNA clusters may also be organized in a bi-directional manner. Hence, proTRAC splits each cluster between each pair of mapped reads to check whether the main strand switches. The default value for the minimum fraction of reads mapped to the main strand is 0.75 and can be adjusted with the option `-clstrand [0..1]`. In case of bi-directional piRNA clusters the user can set the minimum fraction of sequence reads that map to each arm of the cluster with the option `-clsplit [0..1]`. The default value for `-clsplit` is 0.1. If you do not want proTRAC to consider strand bias as a criterion start proTRAC with the option `-clstrand 0.5`.
5. the observed sequence read coverage is caused by a low number of sequences with an exceptionally high read count which would be atypical for genuine piRNA clusters. To this end proTRAC calculates the amount of mapped reads for the locus in question that is attributed to the top fraction of mapped sequences in terms of read counts. The parameters can be set with the option `-distr [int-int]` and the default is `-distr 1-90` which means that the top 1% of sequences mapped to this locus (sequences with highest read counts) should not account for more than 90% of all reads mapped to this locus. If you do not want proTRAC to consider how read counts distribute among different mapped sequences start proTRAC with the option `-distr 1-100`.
6. (NOT SET PER DEFAULT) the putative piRNA cluster exhibits a minimum number of hits at different sites. This number can be set with the option `-clhits [int]` and is 0 by default. Using this option may enhance specificity since genuine piRNA clusters usually exhibit a large number of sites corresponding to piRNAs.
7. (NOT SET PER DEFAULT) the putative piRNA cluster exhibits a minimum number of mapped sequence reads. This number can be set with the option `-clhitsn [int]` and is 0 by default. This option can be used if it is desired that smaller piRNA clusters should exhibit a higher amount of mapped reads per kb follows from the generally applied threshold.

5. Command line options

ftp = floating point number

int = integer

0..1 = floating point number between 0 and 1 (Type 1.0 for 1 and 0.0 for 0).

<code>-genome [file]</code>	Name of the file that contains the genomic sequence that was used for mapping the sequence reads.
<code>-map [file]</code>	Name of the file that contains mapped reads in ELAND3 format. Use SeqMap with option <code>/output_all_matches</code> or sRNAmapper to create an appropriate file.
<code>-help</code>	Shows this information.
<code>-h</code>	Shows this information.

`-repeatmasker [file]` Name of the file that contains the RepeatMasker annotation. Make sure that the names for the chromosomes/scaffolds are identical in your RepeatMasker and genome file.

`-geneset [file]` Name of the file that contains gene annotation (GTF-file from Ensembl database). Make sure that the names for the chromosomes/scaffolds are identical in your GTF- and genome file.

`-swsize [int]` Size of the sliding window (default=5000)

`-swincr [int]` Increment of the sliding window (default=1000)

`-nohc` Do not consider total number of genomic hits for the sequence in question for calculation of hit counts.

`-norc` Do not consider number of reads for the sequence in question for calculation of hit counts.

`-norpm` Do not normalize the hit count with the total number of mapped sequence reads (reads per million). Normalization will make the values comparable across different piRNA libraries and/or organisms.

`-dens [fpt]` Define an absolute minimum number of (normalized) read counts per kb.

`-pdens [0..1]` Define a p-value for minimum number of (normalized) read counts per kb. A p-value of 0.01 means that the (normalized) read counts in a sliding window must belong to the top 1% of all sliding windows.

`-est [int]` Use that option together with `-pdens`. Estimate the required minimum number of (normalized) read counts in a sliding windows based on n random 1kb sliding windows (faster). Without that option proTRAC will scan the map file and calculate the required minimum number of (normalized) read counts in a sliding window based on the observed distribution. We recommend not to use this option.

`-pisize [0..1]` Fraction of (normalized) read counts that have the typical piRNA size (default=0.75).

`-pimin [int]` Define the minimum length of a typical piRNA (default=24).

`-pimax [int]` Define the maximum length of a typical piRNA (default=30).

`-lTor10A [0..1]` Fraction of (normalized) read counts that have 1T (1U) or 10A (default=0.75).

`-lTand10A [0..1]` If the fraction of (normalized) read counts with 1T (1U) OR 10A is below the value defined by `-lTor10A`, accept the sliding window if BOTH the 1T (1U) and the 10A fraction reach this value (default=0.5).

`-distr [ftp-ftp]` To avoid false positive piRNA cluster annotation of loci with one or few mapped sequences represented by exceptionally many reads. You can e.g. type `-distr 10-75` which means that the TOP 10% of mapped sequences account for max. 75% of the piRNA clusters (normalized) read counts (default=1-90). Otherwise the locus is rejected.

`-clsize [int]` Set the minimum size for a piRNA cluster (default=5000).

`-clhits [int]` Minimum number of sequence hit loci per piRNA cluster (default=0).

`-clhitsn [ftp]` Minimum number of normalized sequence read counts per piRNA cluster (default=0).

`-clstrand [0..1]` Fraction of (normalized) read counts that map to the main strand (default=0.75).

`-clsplit [0..1]` Minimum fraction of (normalized) read counts on the smaller arm of a bi-directional piRNA cluster. Otherwise the cluster will be annotated as mono-directional (default=0.1).

`-image` Output .png image files for each piRNA cluster.

`-nohtml` Do not output .html files for each piRNA cluster.

`-notable` Do not output a summary table.

`-nofaspi` Do not output a FASTA file comprising mapped piRNAs for each cluster.

`-nofasc1` Do not output a FASTA file comprising all piRNA cluster sequences.

`-nomotif` Do not search for transcription factor binding sites.

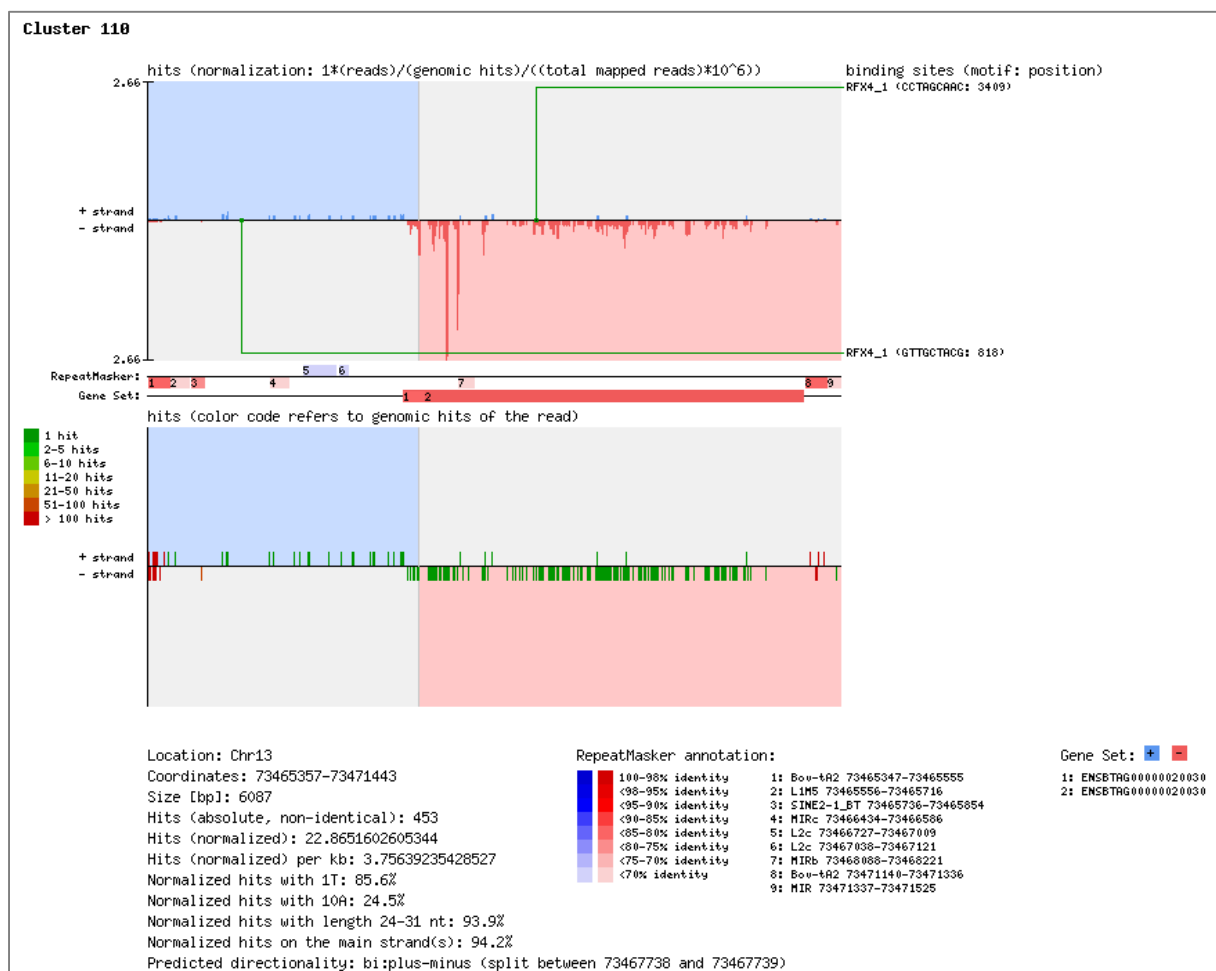
`-flank [int]` Include n bp of flanking sequence in output files.

`-pti` Output a file that contains information on mapped sequence reads in a tab-delimited table that comprises sequence, reads, genomic hits e.g:
TGGGCACGCAAATTCGAGTATCG 12 4

6. Output files

5.1 Image files

From proTRAC version 2.1 image files are replaced by HTML files. If you want proTRAC to output image files you have to use the option `-image`. If doing so, proTRAC will output one separate image file for each predicted piRNA cluster (1.png, 2.png ...). The image file is separated into different sections. At the top you will find the distribution of normalized hit counts (blue: hits on the plus strand, red: hits on the minus strand). The main strand is indicated by blue or red background shading. In case of bi-directional clusters (see above) the main strand switches inside the cluster. The position of identified transcription factor binding sites will be indicated with green lines. Additional information for identified binding sites is provided on the right side. Below you will find a second chart that shows the same locus. Each hit is displayed with a bar of equal size where the color refers to the number of genomic hits produced by the sequence in question. Dark green indicates a hit to a single copy locus whereas dark red indicates a hit to a multi-copy locus (e.g. a transposon). The color code legend is placed on the left side. If you included RepeatMasker and/or GeneSet annotation you will find according tracks between the two charts. Transposons are indicated with blue (plus strand) and red (minus strand) bars with color saturation that corresponds to its divergence from the consensus sequence. Each bar contains a number that refers to the list of transposon names in the RepeatMasker annotation section. This section also comprises the according color code legend. The track for GeneSet annotation comprises coding sequences (blue: plus strand, red: minus strand). As for the RepeatMasker track, numbers in bars refer to a list in the GenSet annotation section. Finally, you will find some cluster data (coordinates, normalized hit count etc) on the bottom of the image. This data is also included in the tabular output of proTRAC (results.table).



5.2 HTML files

From proTRAC version 2.1 image files are replaced by HTML files. In the cluster info section you can find some basic data on the predicted piRNA cluster. The plot on the right side shows parameter values (e.g. reads with 1U, reads with typical piRNA length etc.) that are important for piRNA cluster prediction. The lines indicate the

applied (default or user set) thresholds. The read coverage section starts with a topology plot where each hit is indicated by a bar. The color of the bar refers to the number of genomic hits produced by the sequence in question. Many adjacent red or yellow bars can indicate the presence of a multi copy locus such as tRNA genes or transposons. Below the topology plot you find the sequence read coverage plot (blue: reads on the plus strand, red: reads on the minus strand). The main strands are indicated by light blue or light red background color. You can get exact values of mapped reads by moving the mouse cursor over a desired position. Below the coverage plot you will find Gene Set and RepeatMasker annotation. You can include Ensembl GeneSets annotation files (.gtf) using the option `-geneset [file.gtf]`. You can include RepeatMasker annotation using the option `-repeatmasker [file.rm]`. A list of Gene Set and RepeatMasker elements for the predicted piRNA cluster can be found on the left side. Move the mouse cursor over a desired element to highlight it in the annotation bar. Transcription factor binding sites are listed below Gene Set and RepeatMasker annotation. Move the mouse cursor over putative binding site and you will see its position inside the piRNA cluster.



5.2 FASTA files with hit loci sequences (one for each piRNA cluster)

proTRAC will output one separate FASTA file for each predicted piRNA cluster (1.fasta, 2.fasta ...) that comprises mapped sequences in genome coordinates order (one sequence may occur several times in one file). The FASTA header comprises the following information: Chromosome, starting coordinate of the hit locus, read count of the mapped sequence, total number of genomic hits for the mapped sequence, strand. In case you used the option `-flank [value]` the FASTA header might end with 'FLANK' if the hit locus is not within the predicted piRNA cluster but within the flanking region.

```
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887259 11 1 +
TGATTTACAGCTGACTGCGCATGCAC
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887259 2 1 +
TGATTTACAGCTGACTGCGCATGCACA
```

```

>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887259 2 1 +
TGATTTCACAGCTGACTGCGCATGCACAG
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887263 2 1 +
TTCACAGCTGACTGCGCATGCACAG
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887281 1 1 +
TGCACAGCGATGAGAGAAACATTAAA
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887319 1 12 +
TAATAAACGACTCAGATAAA
>1 dna:chromosome chromosome:Zv9:1:1:60348388:1 REF 26887322 1 1 +

```

5.3 FASTA file with piRNA cluster sequences

proTRAC will output one FASTA file that comprises the sequences of all predicted piRNA clusters. The FASTA header comprises the following information: Chromosome, cluster coordinates, included flanking sequence ('+0 bp' if you do not use the -flank option), predicted directionality of the cluster.

```

>Chromosome_1 15411306-15416703 (+-0 bp) directionality: mono:plus
ATTTCTGTGTGGAGTTTTCAGGTTCTCCCATGTTCAAGTGTATGAGTGTAATGGGTGTTTCCAGTACTGGGTTCAGCTGGAA
GGGCATCCGCTGTGTAACATATGCTGGATAAGTTGGCGGTTTCATCTGCTGTGGCAACCCCTGATAAATAAGGGACTGAGCTG
AAAAAGAAATGAATGAATATATATATATTTCTGAATATGTAAACAATGTATATGAACTCAAAGTTAATCAAGTGTATGAAA
TTAATTTGATTAATATTTAATATAAGAGTATAATAACAACATTTGCTTTACAGTGTAGCTATAATATATATATATATATAT
ATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATATAT

```

5.4 Summary table of the proTRAC results

proTRAC will output one summary table that is divided into several sections. At the top you will find information on proTRAC version and the parameters set for the analysis. This is followed by a TAB-delimited list of predicted piRNA clusters where each line comprises the following information: Location, coordinates, size[bp], absolute hits, normalized hits, normalized hits per kb, normalized hits with 1T, normalized hits with 10A, normalized hits with typical piRNA size, normalized hits on the predicted main strand, predicted directionality, putative transcription factor binding sites. At the bottom you will see the total size of the predicted piRNA clusters (% of the genome), the number of non-identical sequences inside piRNA clusters (% of all non-identical sequences in the map file) and the number of sequence reads inside piRNA clusters (% of all sequence reads in the map file).

```

===== proTRAC =====
VERSION: 2.1 LAST MODIFIED: 02.OCTOBER 2015

Please cite:
Rosenkranz D, Zischler H. proTRAC - a software for probabilistic piRNA cluster
detection, visualization and analysis. 2012. BMC Bioinformatics 13:5.

Contact:
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email: rosenkrd@uni-mainz.de

You can find the latest proTRAC version at:
http://sourceforge.net/projects/protrac/files
=====

PARAMETERS:
Map file: .....piRNA.map
Genome file: .....Danio_rerio.Zv9.dna
RepeatMasker annotation: n.a.
GeneSet:.....n.a.

Significant (p<=0.01) hit density will be calculated based
on observed hit distribution.

Sliding window size: ..... 5000 bp
Sliding window increment: ..... 1000 bp
Normalize each hit by number of genomic hits: ..... yes
Normalize each hit by number of sequence reads: ..... yes
Normalize values (-> per million mapped reads): ..... yes
Min. fraction of hits with 1T(U) or 10A: ..... 0.75
Alternatively: Min. fraction of hits with 1T(U) and 10A: .... 0.5
Min. fraction of hits with typical piRNA length: ..... 0.75
Typical piRNA length: ..... 24-32 nt
Min. size of a piRNA cluster: ..... 5000 bp.
Min. number of hits (absolute): ..... 0
Min. number of hits (normalized): ..... 0
Min. fraction of hits on the mainstrand: ..... 0.5
Top fraction of mapped sequences (in terms of read counts): . 1%
Top fraction accounts for max. n% of sequence reads: ..... 90%
Min. fraction of hits on each arm of a bidirectional cluster: 0.1
Output image file for each cluster: ..... yes
Output a summary table: ..... yes

```



```
Output a FASTA file for each cluster (piRNA sequences): ..... yes
Output a FASTA file comprising cluster sequences: ..... yes
Search DNA motifs in clusters: ..... yes
Output flanking sequences: +/- ..... 0 bp
Output ~.pTi file: ..... no
=====
[LIST OF PREDICTED CLUSTERS AND RELATED INFORMATION]
Total size of 75 predicted piRNA clusters: 795922 bp (0.056%)
Non identical sequences that can be assigned to clusters: 437960 (76.32%)
Sequence reads that can be assigned to clusters: 690440 (70.07%)
```

6. Contact

If you have any questions or comments or found any bugs in the software please do not hesitate to contact:

David Rosenkranz
Institute of Anthropology, small RNA group
Johannes Gutenberg University Mainz, Germany
Email: rosenkranz@uni-mainz.de
Web: <http://www.smallRNAGroup.uni-mainz.de>

7. Citation

If you use the proTRAC software for your publication please cite the following paper:
Rosenkranz D, Zischler H. proTRAC - a software for probabilistic piRNA cluster detection, visualization and analysis. 2012. *BMC Bioinformatics* 13:5.

If you use proTRAC version 2.0 or later please cite the following paper in addition:
Rosenkranz D, Rudloff S, Bastuck K, Ketting RF, Zischler H. Tupaia small RNAs provide insights into function and evolution of RNAi-based transposon defense in mammals 2015. *RNA* 21(5).