Help:

RNAlien is a tool for automatic construction of RNA family models from a single sequence. The sections of this help document cover the use of the webservice and the commandline tool, as well as details of the pipeline used in the backend.
If you look for source code or a installation guide for the tool please refer to the Tool subpage.
Please note that constructions can take up to 24h, if you want to construct multiple families please use the tool instead of the webservice.
This manual is included with the Tool as manual.pdf. (https://github.com/eggzilla/RNAlien/raw/master/manual.pdf)

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Webservice:

Input:

RNAlien Webservice accepts a fasta file with a single sequence as obligatory input. Example GlmS fasta file content:

```
>AARQ02000011.1/391-585
AAUUGAAUAGAAGCGCCAGAACUGAUUGGGACGAAAAUGCUUGAAGGUGAAAUCCCUGAA
AAGUAUCGAUCAGUUGACGAGGAGGAGAUUAAUCGAAGUUUCGGCGGGAGUCUCCCGGCU
GUGCAUGCAGUCGUUAAGUCUUACUUACAAAUCAUUUGGGUGACCAAGUGGACAGAGUAG
UAAUGAAACAUGCUU
```

The organism where this sequence originates from can either be supplied via the autocomplete function, as name or via the NCBI Taxononomy id. In out current example that would be the organism (Listeria monocytogenes FSL N3-165) or Taxononomy id(393124). The id can be retrieved as described here (Retrieve Taxonomy id).

Output:

RNAlien collects potential family members in multiple iterations. While the construction process continues, the progress and intermediate results are summarized in a table. Each iteration goes through multiple states (loading, sequence search, sequence retrieval, candidate alignment, candidate filtering, query selection, model calibration, done).

Progress:

Iteration	Upper taxonomic treshold id	Aligned Sequences	Alignment Link	Covariance Model Link	Status
0	205922	0	loading	loading	*done*
1	205922	0	stockholm-format	covariance-model	*done*
2	294	3	stockholm-format	covariance-model	*done*
3	136843	8	stockholm-format	covariance-model	*model calibration*

Once the construction is finished all result files are available in the result table. The organisms of found sequences are visualized in the taxonomic overview.

Results:

Log	Fasta	Stockholm Alignment	Covariance Model	Rnaz Output	cmstat Output	Zip Archive

The evaluation results for the constructed covariance model and the stockholm alignment used to build it are summarized in the evaluation table.

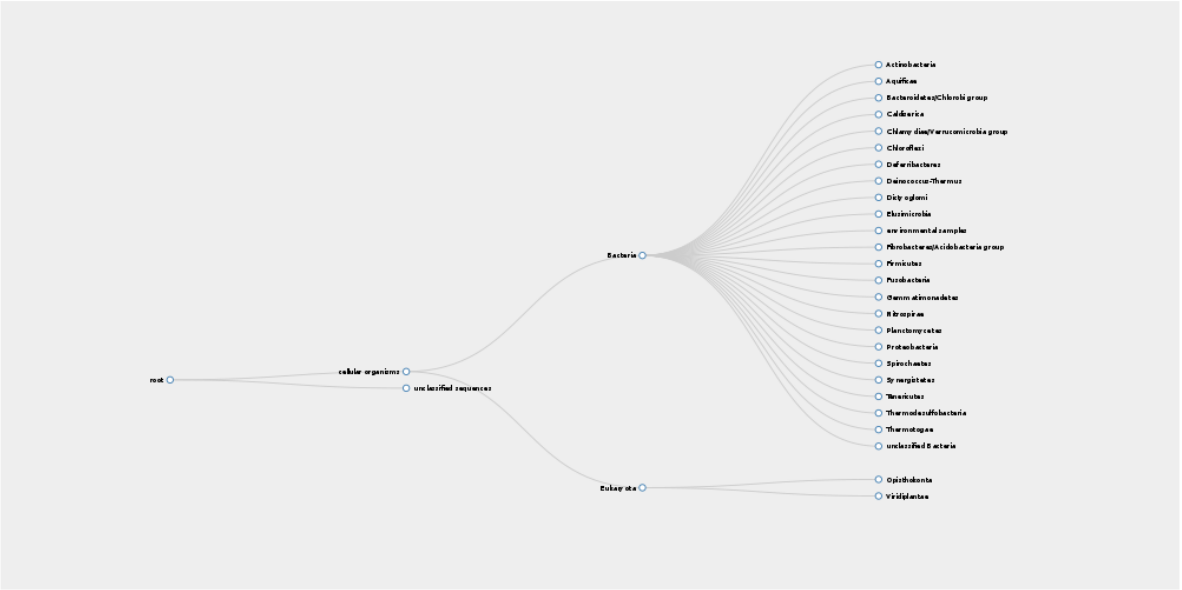
Evaluation Results

CMstat statistics for result.cm	
Sequence Number	1604
Effective Sequences	15.75
Consensus length	336
Expected maximum hit-length	742
Basepairs	67
Bifurcations	5
Modeltype	"cm"
Relative Entropy CM	0.59
Relative Entropy HMM	0.46

RNAz statistics for result alignment:	
Mean pairwise identity	70.09
Shannon entropy	0.52075
GC content	0.5253
Mean single sequence minimum free energy	-121.74
Consensus minimum free energy	-79.69
Energy contribution	-73.3
Covariance contribution	-6.39
Combinations pair	1.62
Mean z-score	-0.4
Structure conservation index	0.65
Background model	dinucleotide
Decision model	structural RNA alignment quality
SVM decision value	-0.64
SVM class propability	0.149028
Prediction	OTHER

The taxonomnic tree of the organsims the included sequences originated from can be found in the zoom and collapsable taxonomy tree

Taxonomy overview



Finally all included sequences are listed in the included sequences table.

Included Sequences

Taxonomy Id	Included in Iteration	Entry Header
136843	1	gb|CP008896.1|:c4840158-4839764 Pseudomonas fluorescens strain UK4, complete genome
86264	1	gi|559137252:1-396 TPA: Pseudomonas brassicacearum subsp. brassicacearum NFM421 tmRNA gene Psmon_brass_NFM421
294	1	gi|559137242:1-394 TPA: Pseudomonas fluorescens NZI7 tmRNA gene Psmon_prote_Pf5
380021	2	gi|610523943:c953216-952821 Pseudomonas protegens Cab57 DNA, complete genomecmS_3_396_'+'
200451	2	gb|CP004045.1|:3880333-3880731 Pseudomonas poae RE*1-1-14, complete genomecmS_3_399_'+'
136843	2	gb|CP011507.1|:c4107953-4107555 Pseudomonas trivialis strain IHBB745, complete genomecmS_3_399_'+'
29442	2	gi|559137212:1-236 TPA: Pseudomonas tolaasii NCPPB 2192 tmRNA gene Psmon_fluor_SBW25cmS_3_236_'+'
294	2	gi|559137242:1-394 TPA: Pseudomonas fluorescens NZI7 tmRNA gene Psmon_prote_Pf5cmS_1_394_'+'
1232139	3	gi|652789639:3292883-3293275 Pseudomonas pseudoalcaligenes genome assembly Ppseudo_Pac, chromosome : IcmS_2_393_'+'
587851	3	gb|AY911521.1|:1-348 Pseudomonas chlororaphis tmRNA (ssrA) gene, partial sequencecmS_1_346_'+'
587753	3	gb|CP009290.1|:5735684-5736076 Pseudomonas chlororaphis subsp. aurantiaca strain JD37, complete genomecmS_2_393_'+'
578833	3	gb|CP007441.1|:c964480-964104 Pseudomonas stutzeri strain 28a24, complete genomecmS_2_377_'+'
312306	3	gi|559136889:1-392 TPA: Pseudomonas entomophila L48 tmRNA gene Psmon_entom_L48cmS_1_392_'+'
251722	3	gi|559137038:1-387 TPA: Pseudomonas syringae pv. aesculi str. 0893_23 tmRNA gene Psmon_syrin_1448AcmS_1_387_'+'
208964	3	gb|CP008749.1|:c901873-901516 Pseudomonas aeruginosa PAO1H2O genomecmS_2_354_'+'
136845	3	gb|AY911522.1|:1-349 Pseudomonas putida tmRNA (ssrA) gene, partial sequencecmS_1_347_'+'
136842	3	gb|CP011110.1|:5753906-5754298 Pseudomonas chlororaphis strain PCL1606, complete genomecmS_2_393_'+'
136841	3	gb|CP012679.1|:4779561-4779918 Pseudomonas aeruginosa strain PA1RG, complete genomecmS_2_354_'+'
103796	3	gb|CP011972.1|:5087900-5088288 Pseudomonas syringae pv. actinidiae ICMP 18884, complete genomecmS_3_389_'+'
86264	3	gb|CP002585.1|:c970721-970325 Pseudomonas brassicacearum subsp. brassicacearum NFM421, complete genomecmS_3_397_'+'

Tool:

Input:

The RNAlien command line utility accepts several command line parameters(see table). Please note that RNAlien requires a active and uninterrupted internet connection, which it also verifies before starting.

Parameter	Switch	Required	Description	Default value
inputFastaFilePath	-i	yes	Path to input fasta file	
outputPath	-o	yes	Path to output directory	
inputTaxId	-t	no	NCBI taxonomy ID number of input RNA organism	
			Only candidate sequences with a nSCI higher than	

inputnSCICutoff	-z	no	this value are accepted.	1.0
inputEvalueCutoff	-e	no	Evalue cutoff for cmsearch filtering.	0.001
inputBlastDatabase	-b	no	Specify name of blast database to use.	nt
coverageFilter	-a	no	Filter blast hits by coverage of at least 80%.	True
singleHitperTax	-s	no	Only the best blast hit per taxonomic entry is considered.	False
blastsoftmasking	-f	no	Toggles blast softmasking, meaning exclusion of low complexity (repetative) regions in lookup table.	True
inputqueryselectionmethod	-m	no	Method for selection of queries (filtering,clustering).	filtering
threads	-c	no	Number of available cpu slots/cores.	1
taxonomyRestriction	-r	no	Restrict search space to taxonomic kingdom (bacteria,archea,eukaryia).	
sessionIdentificator	-d	no	Optional session id that is used instead of automatically generated one.	
Help	-?	no	Print this help	

Example call for RNAlien:

```
RNAlien -i /home/user/newrna.fa -c 5 -t 562 -o /home/user/temp/ -d construction1
```

This command starts RNAlien with the input fasta file /home/user/newrna.fa, 5 cores, the taxonomy id set to E. coli. RNAlien will create a directory called construction1 in /home/user/temp/. On how to obtain the taxonomy id for the organism the sequence originates from see Retrieve Taxonomy id.

Output:

RNAlien creates a output folder which contains all files used in the construction and log files. The most relevant output files and the logfile are directly deposited in the main folder. Each step of model expansion has an own subfolder. Following is a description of the output folder contents, of the different iteration directories that are created during model construction (empty,initial model construction, model expansion), the blast log directory that is contained in each iteration directory and the Log file content.

+**RNAlien output folder**
|
|--Log file: Summary for construction process, tool versions, iteration info, evaluation results (see Log file)
|--result.cm: Result covariance model
|--result.stockholm: Result stockholm alignment
|--result.fa: Result fasta
|--result.csv
|--evaluation: contains RNAz and cmstat output for result-files
|--log: contains non-iteration specific log files
|--1 iteration directories (see description below)
|--2 ..
|--3 ..

+**iterationdirectory: initial model construction**
|
|--model.cm: Result covariance model
|--model.stockholm: Result stockholm alignment
|--model.fa: Result fasta
|--log: Raw and processed blast hits and accepted /rejected candidates (see iteration log directory)
|--input.fa: The input fasta sequence |--input.fold: RNAfold output of the input fasta sequence |--1.fa: fasta

file for first candidate |--1.alifold: RNAalifold file for the input sequence and the first candidate
|--1.fold: RNAfold output for the first candidate
|--2.fa: Fasta file for the second candidate
|-..

+**iterationdirectory: modelexpansion**
|
|--model.cm: Result covariance model
|--model.stockholm: Result stockholm alignment
|--model.fa: Result fasta
|--log: Directory that contains raw and processed blast hits (see blastdirectory)
|--1.fa: fasta file for first candidate
|--1.cmsearch
|--2.fa
|--2.cmsearch
|--..

+**iteration log directory**
|
|--1_1blastOutput: Raw blast output for first query, indicated by leading 1_
|--1_2blastHits: Parsed blast output
|--1_3blastHitsFilteredByLength: Blasthits filtered by exceeding 3* query length
|--1_3ablastHitsFilteredByLength: Blasthits filtered by having >80% coverage
|--1_4blastHitsFilteredByParentTaxId: Only one blasthit per parent taxid
|--1_5filteredBlastResult: Only one blasthit per taxid
|--1_6requestedSequenceElements: Blasthit derived sequences requested from Entrez
|--1_10afullSequencesWithSimilars: Sequences retrieved from Entrez
|--1_10fullSequences: fullSequencesWithSimilars filtered for only containing unique sequences
|--2_1blastOutput: Raw blast output for second query, indicated by leading 2_
|--..
|--11candidates: All query specific sequences merged
|--12candidatesFilteredByCollected: Filter for sequences not identical with collected
|--13selectedCandidates: Sequences selected either by nSCI or cmsearch for inclusion in model by set evalue cutoff
|--14rejectedCandidates: Sequences that were rejected
|--15potentialCandidates: Sequences that are within a 10^3 interval of the set evalue cutoff, will be reevaluated at end of modelconstruction

Log file

Benchmark

RNAfamilies constructed for benchmark are available as archive:

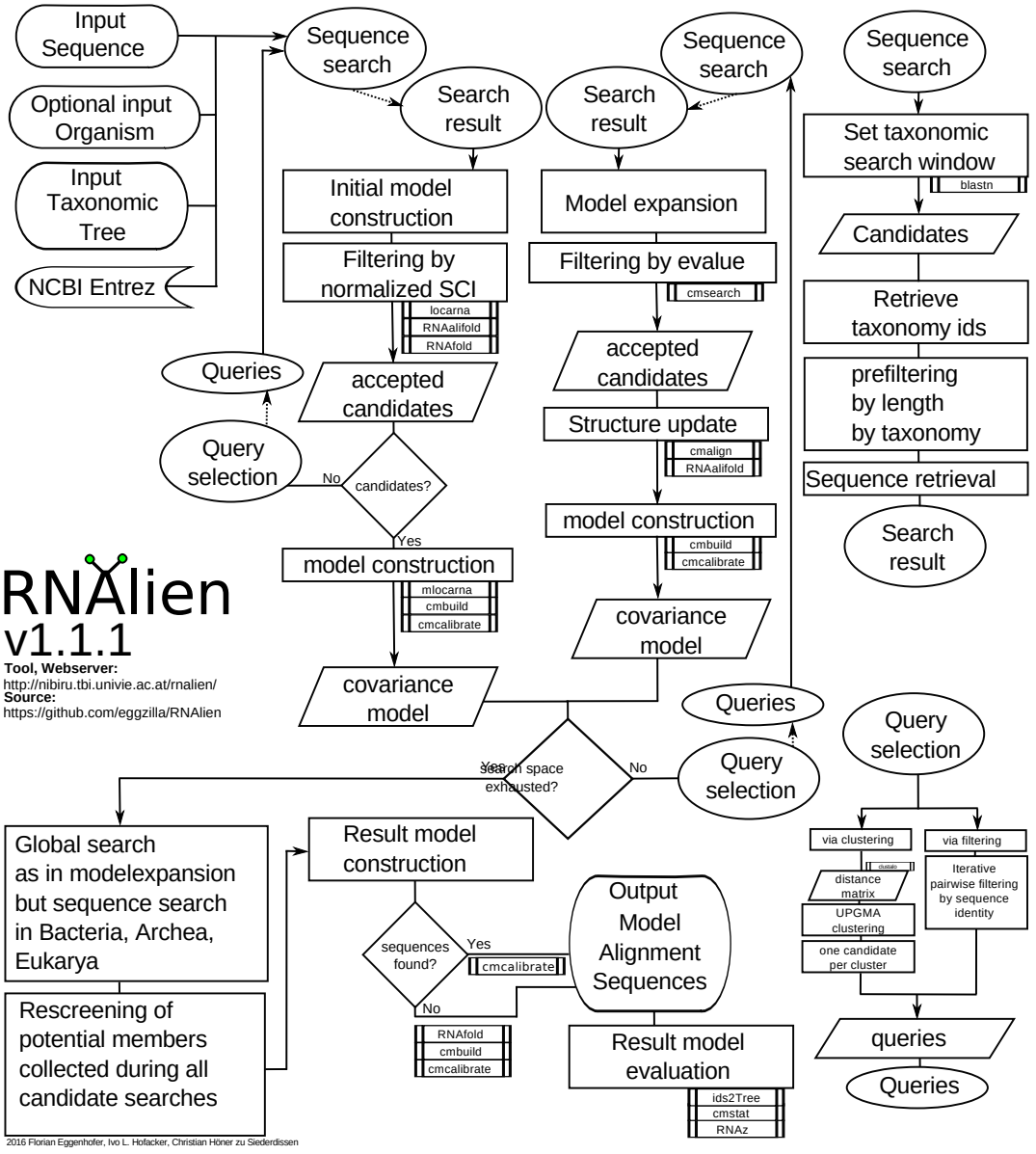
Rfam id	Family name	Archive link (.tar.gz)
RF00001	5S rRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/1.tar.gz)
RF00002	5_8S rRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/2.tar.gz)
RF00003	U1	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/3.tar.gz)
RF00004	U2	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/4.tar.gz)

RF00005	tRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/5.tar.gz)
RF00008	Hammerhead_3	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/6.tar.gz)
RF00010	RNaseP_bact_a	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/7.tar.gz)
RF00011	RNaseP_bact_b	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/8.tar.gz)
RF00017	Metazoa_SRP	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/9.tar.gz)
RF00023	tmRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/10.tar.gz)
RF00026	U6	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/11.tar.gz)
RF00028	Intron_gpI	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/12.tar.gz)
RF00029	Intron_gpII	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/13.tar.gz)
RF00032	Histone3	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/14.tar.gz)
RF00037	IRE_I	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/15.tar.gz)
RF00044	Phage_pRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/16.tar.gz)
RF00050	FMN	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/17.tar.gz)
RF00059	TPP	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/18.tar.gz)
RF00114	S15	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/19.tar.gz)
RF00162	SAM	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/20.tar.gz)
RF00164	s2m	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/21.tar.gz)
RF00167	Purine	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/22.tar.gz)
RF00168	Lysine	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/23.tar.gz)
RF00169	Bacteria_small_SRP	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/24.tar.gz)
RF00174	Cobalamin	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/25.tar.gz)
RF00175	HIV-1_DIS	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/26.tar.gz)
RF00177	SSU_rRNA_bacteria	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/27.tar.gz)
RF00207	K10_TLS	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/28.tar.gz)
RF00209	IRES_Pesti	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/29.tar.gz)
RF00234	glmS	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/30.tar.gz)
RF00374	Gammaretro_CES	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/31.tar.gz)
RF00380	ykoK	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/32.tar.gz)
RF00458	IRES_Cripavirus	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/33.tar.gz)
RF00480	HIV_FE	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/34.tar.gz)
RF00500	TCV_H5	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/35.tar.gz)
RF00504	Glycine	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/36.tar.gz)
RF00843	mir-228	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/37.tar.gz)
RF00871	mir-689	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/38.tar.gz)
RF01051	c-di-GMP-I	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/39.tar.gz)
RF01054	preQ1-II	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/40.tar.gz)
RF01073	GP_knot1	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/41.tar.gz)
RF01118	PK-G12rRNA	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/42.tar.gz)
RF01380	HIV-1_SD	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/44.tar.gz)
RF01510	MFR	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/45.tar.gz)
RF01689	AdoCbl-variant	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/46.tar.gz)
RF01734	crcB	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/47.tar.gz)
RF01786	c-di-GMP-II	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/48.tar.gz)
RF01831	THF	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/49.tar.gz)
RF01852	tRNA-Sec	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/50.tar.gz)
RF01856	Protozoa_SRP	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/51.tar.gz)
RF01857	Archaea_SRP	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/52.tar.gz)
RF01998	group-II-D1D4-1	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/53.tar.gz)
RF02001	group-II-D1D4-3	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/54.tar.gz)
RF02095	mir-2985-2	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/55.tar.gz)
RF02253	IRE_II	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/56.tar.gz)
RF02519	ToxI	archive (http://nibiru.tbi.univie.ac.at/rnalien_tmp/benchmark/57.tar.gz)

Values for the specificity and recall plots were computed with the RNAlienStatistics executable and the rnalienstatistics.pl (https://github.com/eggzilla/RNAlien/blob/master/scripts/alienresultstatistics.pl) script included in RNAlien.

RNAalien pipeline

RNAlien pipeline



Retrieve Taxnonomy id

To obtain the Taxonomy id go to NCBI Taxonomy (http://www.ncbi.nlm.nih.gov/taxonomy).
Enter the organisms name in the Taxonomy field (e.g Escherichia coli) and click search. You are then

redirected to a Summary page (http://www.ncbi.nlm.nih.gov/taxonomy/?term=Escherichia+coli). Click the organism name. Depending on how specific your search was you can either select from a list of organisms (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=562) or you are directed to a organism page Escherichia coli str. K-12 substr. MG1655star (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=879462&lvl=3&lin=f&keep=1&srchmode=1&unlock). You can find the taxonomy id right below the organisms name (Escherichia coli str. K-12 substr. MG1655star Taxonomy ID: 879462).

TBI - 2015