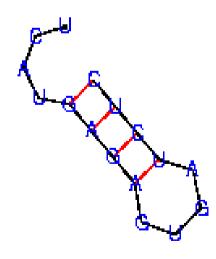
RNASDACE.OFG

Prediction & annotation of ncRNA

C. Gaspin

Unité de Mathématique et Informatique Appliqués Toulouse



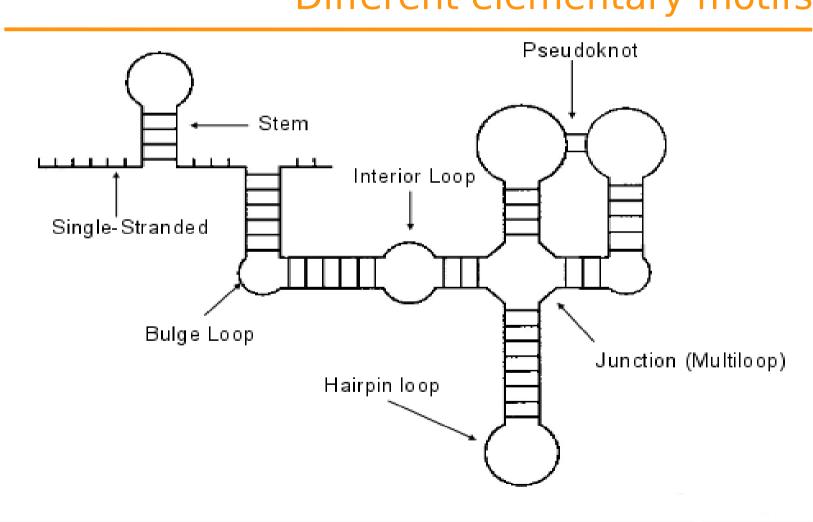
ncRNA background

RNA background

- RNA folds on itself by base pairing :
 - A with U: A-U, U-A
 - C with G: G-C, C-G
 - Sometimes G with U: U-G, G-U
- Folding = Secondary structure
- Structure related to function: ncRNA of the same family have a conserved structure CAA the family signature
- Sequence less conserved

RNASDACE.OFG

RNA background Different elementary motifs



The non coding protein RNA world A high diversity in size

Large non coding protein RNA

- >300 nt : rRNA, Xist, H19, ...
- Genome structure & expression

Small non coding protein RNA

- >30 nt : tRNA, snoRNA, snRNA...
- mRNA maturation, translation

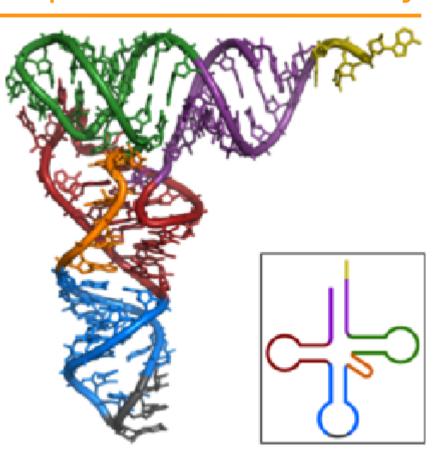
Micro non coding protein RNA

- 18-30 nt : miRNA, hc-siRNA, ta-siRNA, nat-siRNA, piRNA...
- PTGS, TGS, Genome stability, defense...

RNA background

Example: the tRNA family

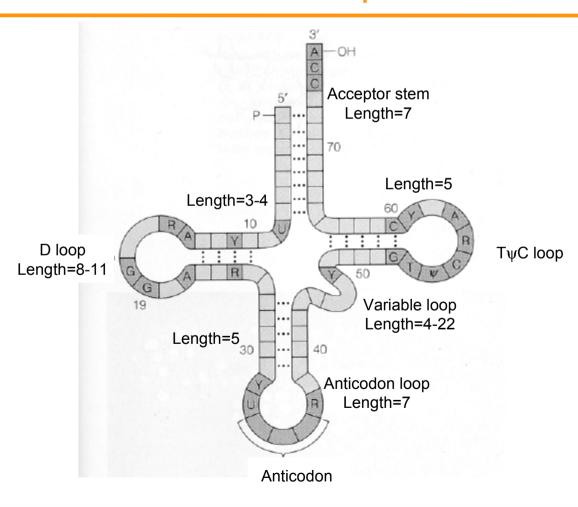
Copyright © 2006 Nature Publishing Group Nature Reviews | Microbiology



http://en.wikipedia.org/wiki/Transfer_RNA

RNA background

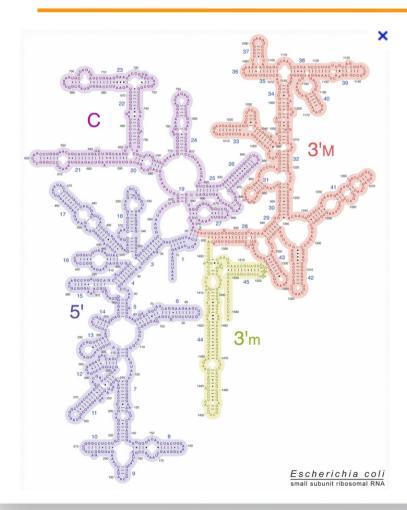
Example: the tRNA family

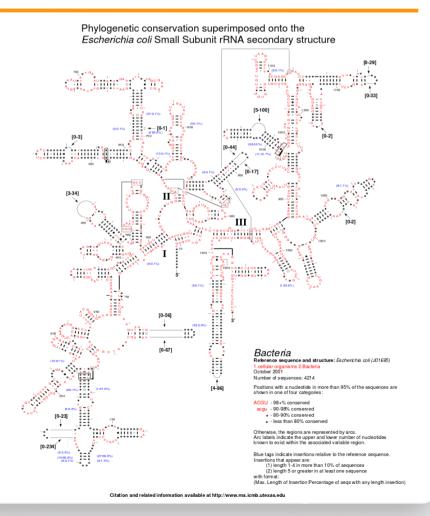


ACTUCIOCOCOTROTICORDOTISTICORDOTIST

RNASDACE.OF9

RNA background Example: 16S rRNA family





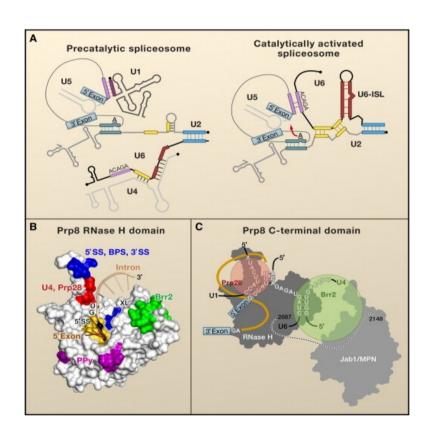
RNASDACE.OT9

RNA background

Example: snRNA family

```
stem/loop IV
```

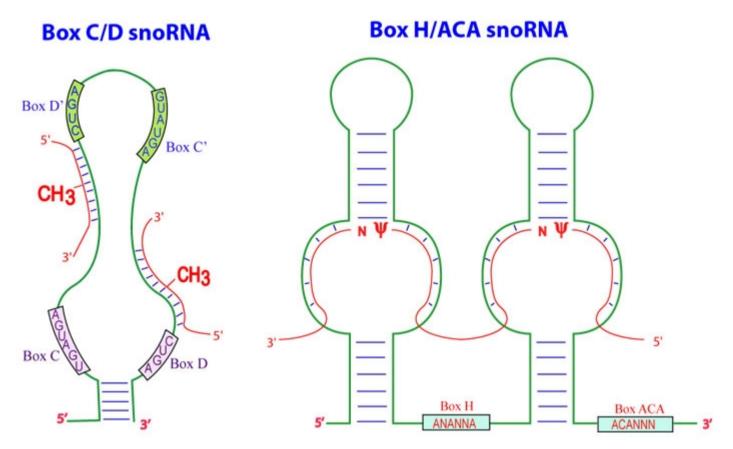
Breckenridge D G et al. PNAS 1999;96:852-856



Cell Volume 136, Issue 4 2009 701 - 718

RNA background

Example: snoRNA families



http://biochem.ncsu.edu/faculty/maxwell/snoRNA.jpg

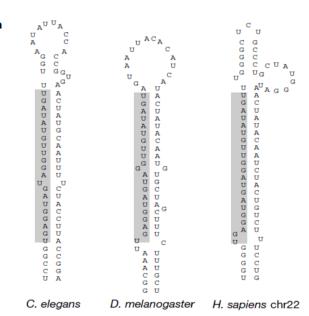
RNA background Example: miRNA family

Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA

Amy E. Pasquinelli*†, Brenda J. Reinhart*†, Frank Slack‡, Mark Q. Martindales, Mitzi I. Kurodall, Betsy Maller‡, David C. Haywards, Eldon E. Ball¶, Bernard Degnan#, Peter Müller*, Jürg Spring*, Ashok Srinivasan**, Mark Fishman**, John Finnerty††, Joseph Corbo‡‡, Michael Levine‡‡, Patrick Leahy§§, Eric Davidson§§ & Gary Ruvkun*

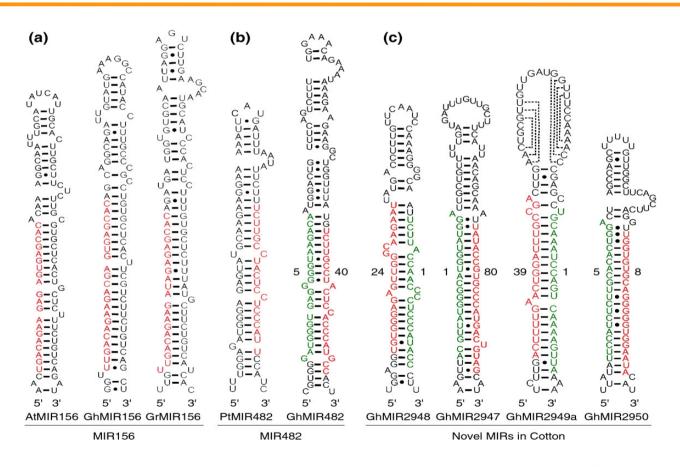
- * Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114,
- ‡ Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA
- § Kewalo Marine Lab, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96813, USA
- || Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030, USA





RNA background

Example: miRNA family

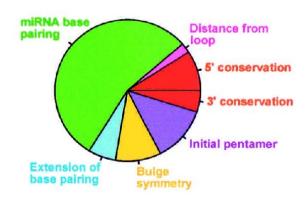


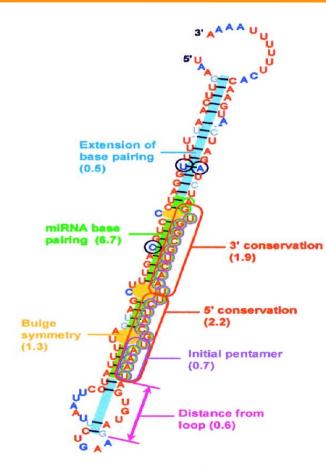
Pang et al. Genome Biology 2009 10:R122 doi:10.1186/gb-2009-10-11-r122

RNA background

Example: miRNA family

- MiRscan examines several features and computes a score
- The score is the sum of the evidence scores, computed independently for each feature

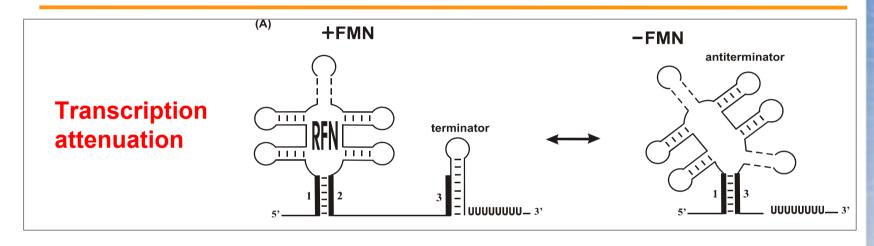


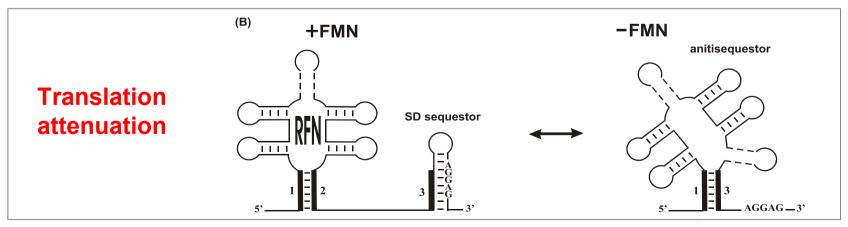


Lim et al, Genes and Development 2003

RNA background

Example: RFN riboswitch family

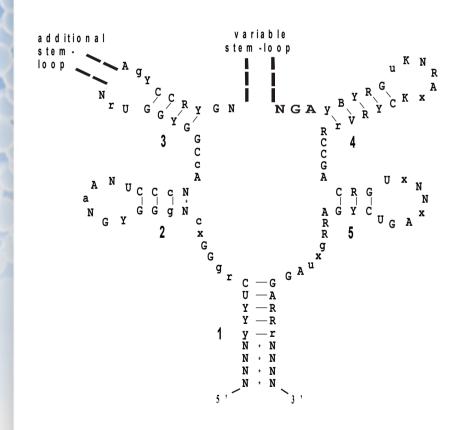




From Gelfand, Paris, 2004

RNA background

Example: RFN riboswitch family



Capitals: invariant positions.

Lower case: strongly conserved positions.

Dashes and stars: obligatory and facultative base pairs

Degenerate positions:

R = A or G

Y = C or U

K = G or U

B= not A

V = not U

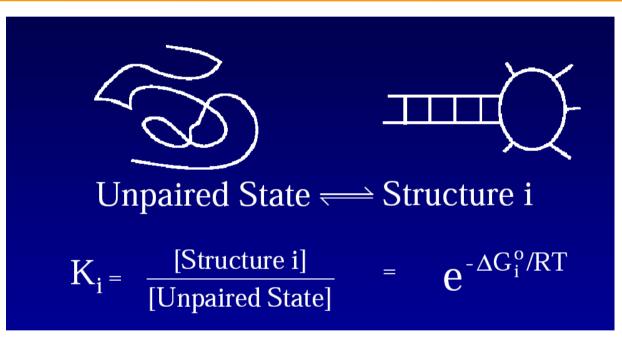
N: any nucleotide

X: any nucleotide or deletion

From Gelfand, Paris, 2004

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RNA background ncRNA and free energy



- K=equilibrium constant giving the ratio of concentrations for folded, S, and unfolded, U, species at equilibrium
- ΔG° = standard free energy difference between S and U
- R = gas constant
- T = temperature in kelvins

RNA background ncRNA and free energy

RNA background ncRNA and free energy

It is admitted

- The right secondary structure is that minimizing the free energy
- 18^N possible secondary structures for a sequence of length N
- For N=100 : **3x10**²⁵ structures to compute

Efficient software to do that

– RNAfold (Hofacker, 2003)

http://www.tbi.univie.ac.at/~ivo/RNA/

Mfold (Zucker, Science, 1989)

http://frontend.bioinfo.rpi.edu/zukerm/export/mfold-3.html

RNASDACE.OF9

ncRNA background

Where are ncRNA located?

ncRNA background

Where are they in eucaryotes?

- Generally in non coding regions
- But also in:
 - Inter-ORF
 - Introns
 - snoRNA, miRNA, tRNA
 - Coding regions
 - Anti-sens of (non) coding regions

ncRNA background

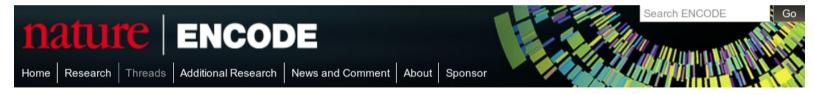
Where are they in bacteria and archea?

- Generally in non coding regions
 - All organisms, all families
- But also in :
 - Cis-reg: 5'UTR of mRNA
 - Antisens of (non) coding regions

ncRNA background Where are they in Human?

ncRNA in the ENCODE project

http://www.nature.com/encode/threads/non-coding-rna-characterization





A thread is a way to follow a specific scientific theme that brings together relevant sections extracted from the co-published ENCODE papers



Non-coding RNA characterization

Many novel and previously known non-coding RNA species are characterized in ENCODE

nature

An Integrated Encyclopedia of DNA Elements in

In addition, we annotated 8,801 automatically derived small RNAs and 9,640 manually curated long non-coding RNA (IncRNA) loci ¹⁷. Comparing IncRNAs to other ENCODE data indicates

ncRNA background Where are they in Human?

IncRNA

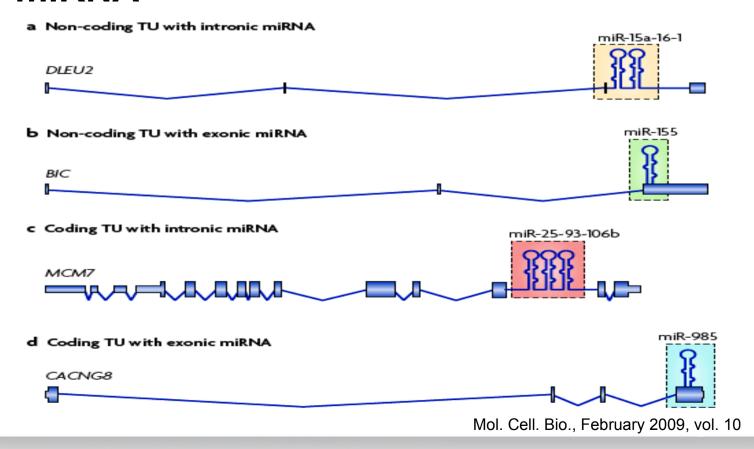
chromatin marks have been identified for 13.9% (Derrien *et al.* 2012). These IncRNAs can been further reclassified into the following locus biotypes based on their location with respect to protein-coding genes:

- Antisense RNAs: Locus that has at least one transcripts that intersect any exon of a
 protein-coding locus on the opposite strand, or published evidence of antisense regulation
 of a coding gene.
- 2. LincRNA: Locus is intergenic non-coding RNA loci.
- 3. Sense overlapping: Locus contains a coding gene within an intron on the same strand.
- 4. **Sense intronic:** Locus resides within intron of a coding gene, but does not intersect any exons on the same strand.
- Processed transcript: Locus where non of its transcripts contain an open reading frame (ORF) and cannot be placed in any of the other categories because of complexity in their structure.

In summary the IncRNAs data set in GENCODE 7 consists of 5,058 lincRNA loci, 3,214 antisense loci, 378 sense intronic loci and 930 processed transcripts loci. Manually evaluating

ncRNA background Where are they in eucaryotes?

miRNA



ncRNA annotation

Existing in International Repositories

Genomes and ncRNA annotation

The reference: Genbank

http://www.ncbi.nlm.nih.gov/genbank/genomesubmit/

Annotation

While annotation is optional for incomplete WGS submissions, complete genome submissions must be annotated. You can annotate the genome yourself, following the instructions on these pages. Alternatively, you can request that your genome submission be annotated by NCBI's <u>Prokaryotic Genomes Annotation Pipeline</u> (PGAAP) that is available for genomes being submitted to GenBank. **New in Dec. 2012:** Generate files for genome submission to GenBank and request PGAAP annotation in the Private Comments box during the submission of that genome. (There is no longer a separate PGAAP submission process.)

If you annotate yourself, several features are the minimal required annotation, but there are many additional features that can be included. It is our hope that the annotation present on any genome will evolve over time as more is known about the biology. In reviewing bacterial genome annotation, NCBI strives to ensure that the annotation is consistent throughout the submission and when compared to other genome submissions. We also strive to present information that is an accurate representation of the known biology. To do this we need your help. Please pay careful attention to the annotation instructions presented here and please review all your annotation before submitting your genome. Many genomes are annotated by automatic prediction programs and since these programs do make mistakes, it is up to all of us to try and ensure the information being presented is as accurate as possible. A summary of the required annotation is presented below, however please also refer to our detailed annotation instructions for our annotation expectations.

Required Annotation

- 1. Genes
 - locus tag
- 2. Coding regions of known proteins
 - · product (protein) names
- 3. structural RNAs (tRNAs and ribosomal RNAs)

structural RNAs

rRNA, tRNA, misc_RNA, and ncRNA are features used to annotate the various structural RNA genes. All RNA features must include a corresponding gene feature with a locus_tag qualifier. Only ribosomal RNAs (rRNA) and transfer RNAs (tRNA) are required.

Genomes and ncRNA annotation

Another reference : Ensembl

http://www.ensembl.org/info/docs/genebuild/index.html

The Ensembl Annotation Process

Genome assemblies

The Genome Assemblies page gives more information on where we get our genome assemblies from, how the sequence data for these genome assemblies are structured, and how we represent these data in Ensembl.

Protein-coding gene annotation

Protein-coding genes are automatically annotated using Ensembl's genebuild pipeline. All transcripts are based on mRNA and proteins in public scientific databases.

The human gene set is used as the GENCODE gene set. The human and mouse gene sets include all CCDS transcripts.

See the annotation article for more about the Ensembl genebuild pipeline, gene names and annotation.

Low-coverage genomes are annotated using a modified pipeline which attempts to locate genes across multiple scaffolds.

More genes

The Ensembl gene set also includes automatically-annotated pseudogenes and non-coding RNAs.

and <u>non-coding RNAs</u>.

or human and mouse, we include annotation from IMGT for Ig genes.

EST-based genes are predicted and displayed on the website but are not included in the final gene set

Paired-end Illumina RNA-seq data have been used to generate transcript models for many species including human, zebrafish and pig.

Alternative Splicing

Ensembl includes automatically-annotated Alternative splicing events for model organisms.

Ensembl release 71 - April 2013 @ WTSI / EBI

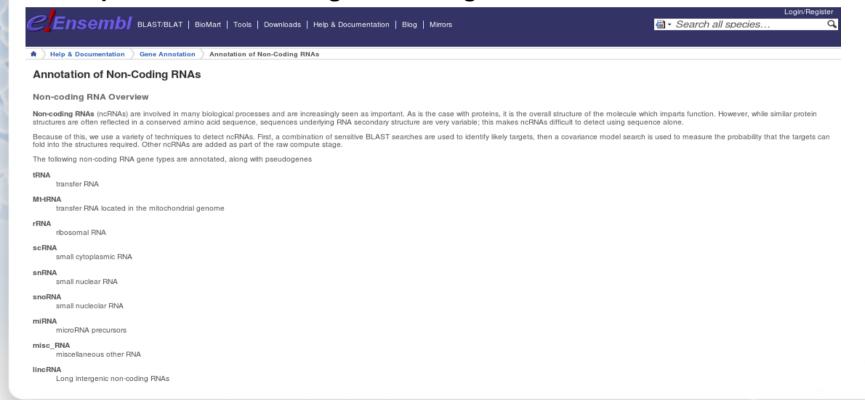
About Ensembl | Privacy Policy | Contact Us

Genomes and ncRNA annotation

запачностка подкостратовность.

Another reference: Ensembl

http://www.ensembl.org/info/docs/genebuild/ncrna.html



Genomes and ncRNA annotation

Another reference: Ensembl

http://www.ensembl.org/info/docs/genebuild/ncrna.html

Annotation Details

Most ncRNAs are annotated by aligning genomic sequence against RFAM using BLASTN. The BLAST hits are clustered and filtered by E value and are used to seed Infernal searches of the locus with the corresponding RFAM covariance models. The purpose of this is to reduce the search space required, as to scan the entire genome with all the RFAM covariance models would be extremely CPU-intensive. The resulting BLAST hits are then used as supporting evidence for ncRNA genes.

miRNAs are predicted by BLASTN of genomic sequence slices against miRBase sequences. All species are used. The BLAST hits are clustered and filtered by E value and the aligned genomic sequence is then checked for possible secondary structure using RNAFold. If evidence is found that the genomic sequence could form a stable hairpin structure, the locus is used to create a miRNA gene model. The resulting BLAST hit is used as supporting evidence for the miRNA gene.

Note: The miRNA identifier and name are only associated to the resulting Ensembl miRNA if they are of the same species.

tRNAs are annotated as part of the raw compute process using tRNAscan-SE.

lincRNA (Long intergenic non-coding RNAs) Ensembl gene annotation, cDNA alignments and chromatin-state map data from the Ensembl regulatory build are used to predict lincRNAs for human and mouse. We do not import the lincRNAs identified by Guttman et al [1], but their publication guided us to our current approach for automatically annotating lincRNAs. First, regions of chromatin methylation (H3K4me3 and H3K36me3) outside known protein-coding loci are identified. Next, cDNAs which overlap with H3K4me3 or H3K36me3 features are identified as candidate lincRNAs. A final evaluation step investigates if each candidate lincRNA has any protein-coding potential. Any candidate lincRNA containing a substantial open reading frame (ORF) covering 35% or more of its length and containing PFAM/tigrfam protein domains will be rejected. Candidate lincRNAs that pass the final evaluation step are included in the human or mouse gene set as lincRNA genes.

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Genomes and ncRNA annotation

Another reference : Ensembl

http://www.ensembl.org/info/data/ftp/index.html

Single species data

Popular sp	pecies are listed first. You can	customise this list via	our <u>nome page</u> .		1											
Show 10 - entries				Show/hide columns							Filter					
*	Species	DNA (FASTA)	cDNA (FASTA)	ncRNA (FASTA)	Protein sequence (FASTA)	Annotated sequence (EMBL)	Annotated sequence (GenBank)	Gene sets	Whole databases	Variation (EMF)	Variation (GVF)	Variation (VCF)	Variation (VEP)	Regulation (GFF)	Data files	BAM
Υ	Human Homo sapiens	FASTA	<u>FASTA</u>	<u>FASTA</u>	<u>FASTA</u>	EMBL	GenBank	GTF	MySQL	EMF	GVF	VCF	VEP	Regulation (GFF)	Regulation data files	BAM
Υ	Mouse Mus musculus	FASTA	<u>FASTA</u>	<u>FASTA</u>	<u>FASTA</u>	EMBL	GenBank	GTF	<u>MySQL</u>	<u>EMF</u>	<u>GVF</u>	VCF	<u>VEP</u>	Regulation (GFF)	Regulation data files	-
Υ	Zebrafish Danio rerio	FASTA	<u>FASTA</u>	<u>FASTA</u>	FASTA	EMBL	GenBank	GTF	MySQL	-	GVF	VCF	VEP	-	-	BAM
	Alpaca Vicugna pacos	FASTA	<u>FASTA</u>	<u>FASTA</u>	<u>FASTA</u>	<u>EMBL</u>	GenBank	GTF	MySQL	-						-
	Anole lizard Anolis carolinensis	<u>FASTA</u>	<u>FASTA</u>	<u>FASTA</u>	<u>FASTA</u>	EMBL	GenBank	GTF	MySQL	-	-	-	-	-	-	BAM
	Armadillo Dasypus novemcinctus	FASTA	FASTA	FASTA	FASTA	EMBL	GenBank	GTF	MySQL	-	-	-	-	-	-	-
	Bushbaby Otolemur garnettii	FASTA	<u>FASTA</u>	<u>FASTA</u>	FASTA	EMBL	GenBank	GTF	MySQL	-	-	-	-	-	-	-

ncRNA prediction

Versus

Coding RNA prediction

RNASDACE.OFG

ncRNA prediction and annotation

- Not predicted by gene prediction tools
 - No specific signal (start, stop, splicing sites...)
 - Multiple location (intergenic, intronic, coding, antisens)
 - Variable size
 - No strong sequence conservation in general
- A variety of existing approaches not always easy to integrate
 - Known family: Homology prediction
 - New family: De novo prediction

The non coding protein RNA world

Protein Approaches

- Statistically biased (codon triplets)
- Open Reading Frames

ncRNA Approaches

- High CG content (hyperthermophiles archaea)
- Orphan promoter/Terminator identification (bacteria)

The non coding protein RNA world

Comparative analysis: similarity searching

Proteins

- BLAST, Sequence Alignment (DP)
- Genes that code for proteins are conserved across genomes (e.g. low rate of mutation)

ncRNA

- Low sequence conservation
- Secondary structure usually conserved
- Alignment scoring based on structure can be imperative

RNASDACE.OFG

The non coding protein RNA world

Comparative analysis: similarity searching

A 1	IJ
G	Α
C<-> G-(C<->G
U-J	A
A-1	J
G-(C
A<->C-	3<->U
S1	

S2	AGAUCGAAAGAUCU
	* * * * *
S1	CGAUGGAUACAUCG
	* **
s 3	CCAUGGAUAGUUCG

Α	U
G	Α
G,	¢G
Ų,	⁺ U
A-	-U
C,	۲C
C-	-G
S 3	

RNASNACE.Org

ncRNA annotation

ncRNA databases

The non coding protein RNA world

Databases

Generalist databases

- No organism specificity
- No family specificity

Specific databases

- Groups of organisms : Plants, Animals, Human...
- ncRNA families: rRNA, tRNA, miRNA, snRNA, snoRNA, tmRNA...
- Both

The non coding protein RNA world

Generalist databases

RFAM

http://www.sanger.ac.uk/Software/Rfam/

NonCode

http://noncode.bioinfo.org.cn/index4.htm

RNAdb

http://jsm-research.imb.uq.edu.au/rnadb/

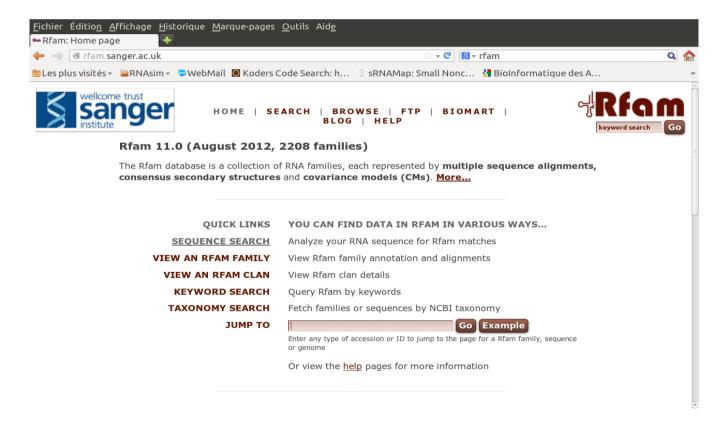
• fRNAdb

http://www.ncrna.org/frnadb/

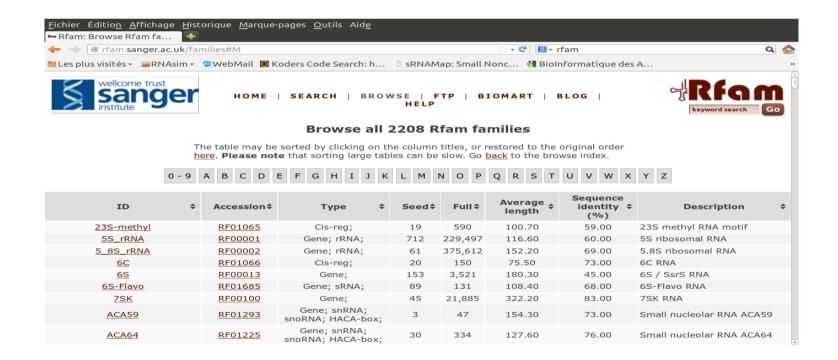
ncRNA

http://biobases.ibch.poznan.pl/ncRNA/

The non coding protein RNA world Generalist databases

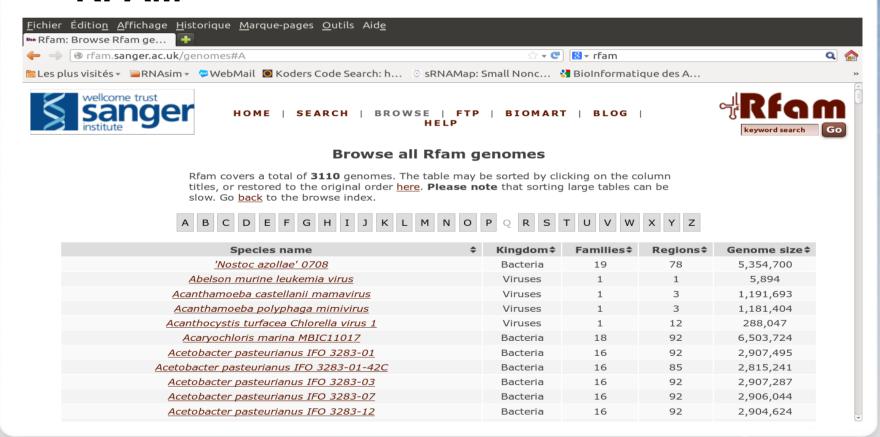


The non coding protein RNA world Generalist databases



The non coding protein RNA world

Generalist databases



The non coding protein RNA world

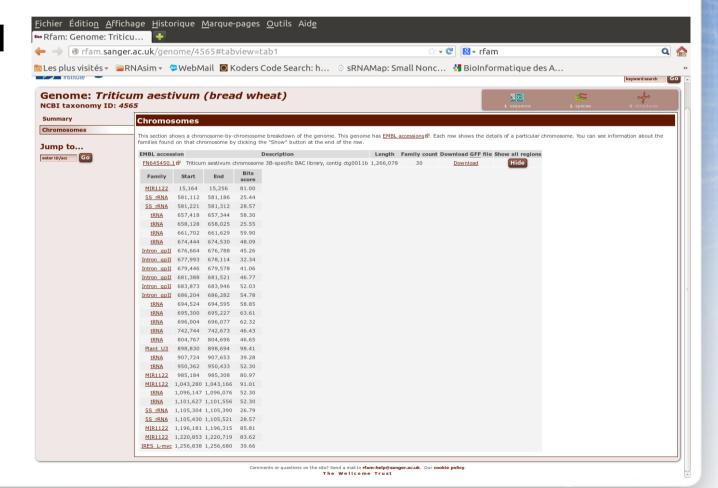
Generalist databases

RFAM



Comments or questions on the site? Send a mail to ${\tt rfam-help@sanger.ac.uk}$. Our ${\tt cookie\ policy}$ ${\tt The\ Wellcome\ Trust}$

The non coding protein RNA world Generalist databases



The non coding protein RNA world

Generalist databases

fRNAdb



A comprehensive non-coding RNA sequence database ver. 3.4

fRNAdb is Web Service (SOAP, REST) Ready.

Total: 510,055 entries









Please input some query keywords for retrieving RNAs via simple text search e.g. "miRNA" or "snoRNA".

Try add disease/tissue name to make your search more specific e.g. "miRNA oncogene".

Or can be more specific e.g. "miRNA oncogene human".

submit 🕡

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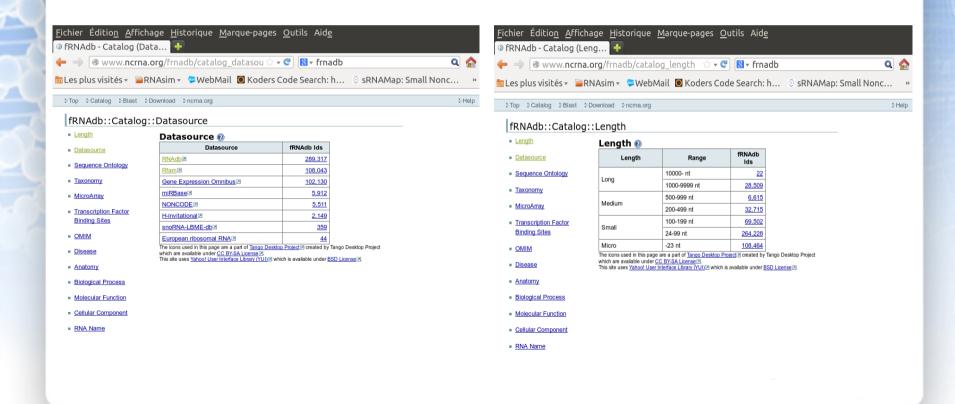


fRNAdb is licensed under a Creative Commons 表示-非當利-改変禁止 2.1 日本 License. Based on a work at www.ncma.org.

The non coding protein RNA world Generalist databases

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fRNAdb



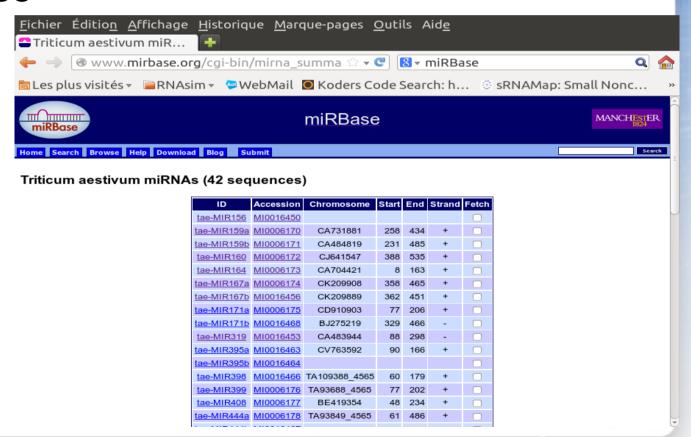
The non coding protein RNA world Specific databases

miRBase



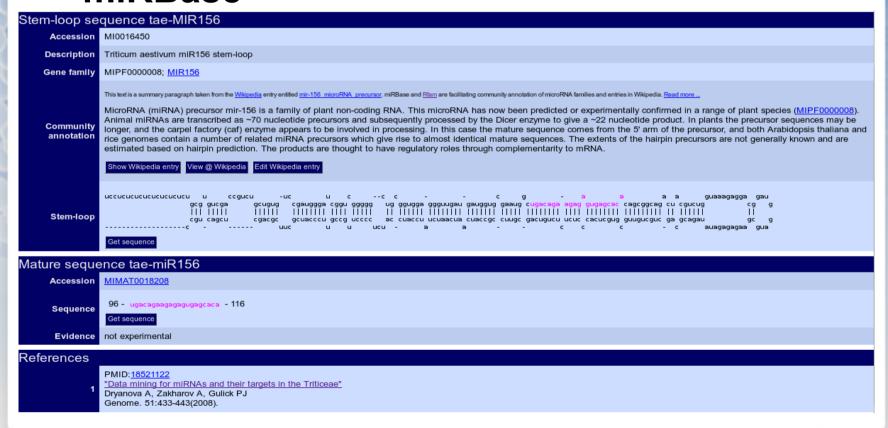
The non coding protein RNA world Specific databases

miRBase



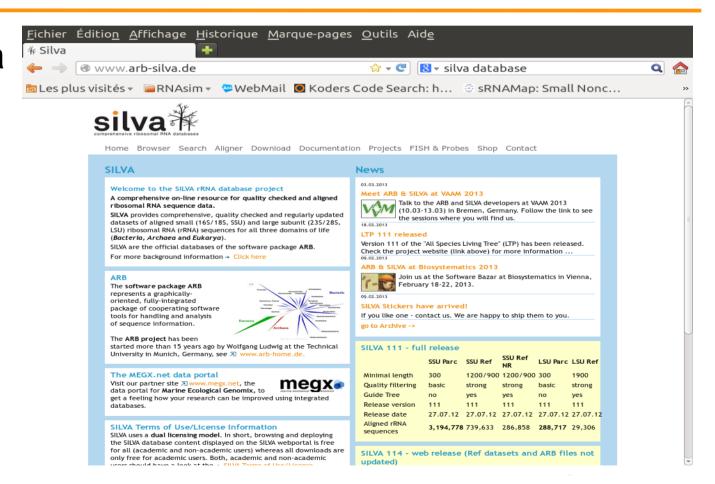
The non coding protein RNA world Specific databases

miRBase



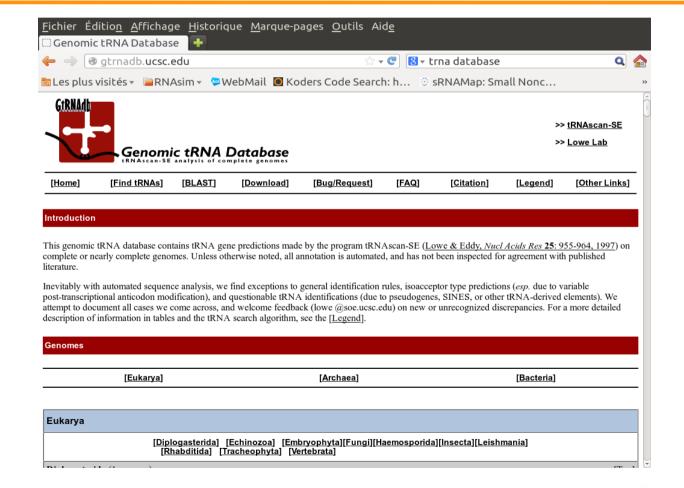
The non coding protein RNA world Specific databases

Silva



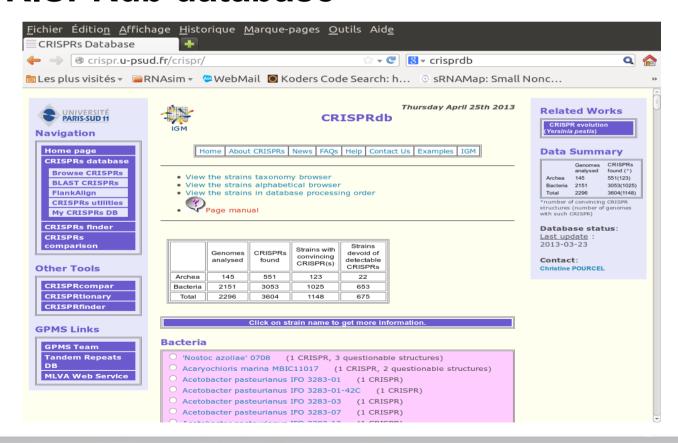
The non coding protein RNA world Specific databases

tRNA



The non coding protein RNA world Specific databases

CRISPRdb database



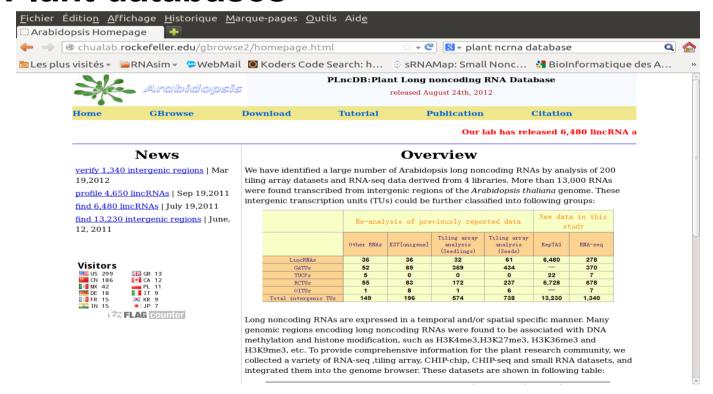
The non coding protein RNA world Specific databases

snoRNA database



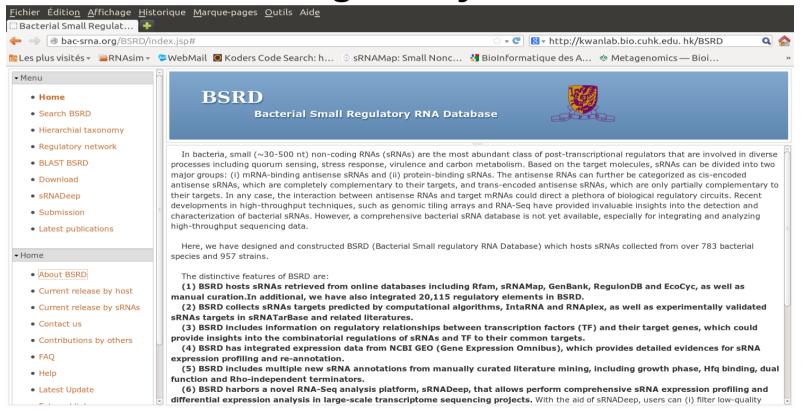
The non coding protein RNA world Specific databases

Plant databases



The non coding protein RNA world Specific databases

Bacteria small regulatory database



ncRNA annotation

Methods and tools for ncRNA prediction and annotation

The non coding protein RNA world Methods and tools

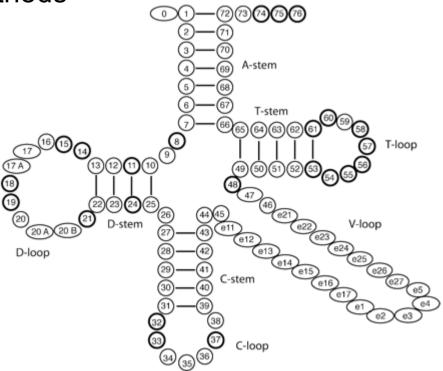
- A variety of existing approaches not always easy to integrate
 - Known family: Homology prediction
 - Specific family methods
 - Generalist
 - New family: De novo prediction
 - Orphan promoter/terminator
 - Comparative analysis of related organisms
 - Bias composition detection between coding and non coding

The non coding protein RNA world Methods and tools

Known family: Homology prediction

Specific family methods

• tRNA



The non coding protein RNA world Methods and tools

- Known family: Homology prediction
 - Specific family methods
 - tRNA
 - tRNAscan-SE (1997)

© 1997 Oxford University Press

Nucleic Acids Research, 1997, Vol. 25, No. 5

955-964

tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence

Todd M. Lowe and Sean R. Eddy*

Department of Genetics, Washington University School of Medicine, 660 South Euclid, Box 8232, St Louis, MO 63110, USA

The non coding protein RNA world Methods and tools

Known family: Homology prediction

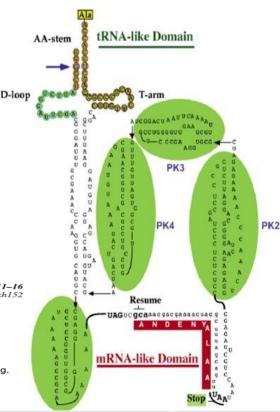
- Specific family methods
 - tRNA
 - TRNAscan-SE (1997)
 - tRNA+tmRNA (bacteria)
 - ARAGORN (2004)
 - Single chain tmRNA (ssra)

Nucleic Acids Research, 2004, Vol. 32, No. 1 11–16
DOI: 10.1093/nar/skh152

ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences

Dean Laslett and Bjorn Canback1,*

Murdoch University, Perth, Western Australia, Australia and ¹Department of Microbial Ecology, Ecology Building, Lund University, S-223 62, Sweden



The non coding protein RNA world Methods and tools

- Known family: Homology prediction
 - Specific family methods
 - rRNA
 - Bacteria and archaea :
 - » 16S rRNA
 - » 23S rRNA
 - » 5S rRNA
 - Eucaryotes
 - » 18S rRNA
 - » 28S rRNA
 - » 5.8S rRNA
 - » 5S rRNA

The non coding protein RNA world Methods and tools

Known family: Homology prediction

- Specific family methods
 - rRNA
 - 5S, 16S/18S, 23S/28S
 - Alignment+HMM

3100–3108 Nucleic Acids Research, 2007, Vol. 35, No. 9 doi:10.1093/nar/gkm160

Published online 22 April 2007

RNAmmer: consistent and rapid annotation of ribosomal RNA genes

Karin Lagesen^{1,2,*}, Peter Hallin³, Einar Andreas Rødland^{1,2,4,5}, Hans-Henrik Stærfeldt³, Torbjørn Rognes^{1,2,4} and David W. Ussery^{1,2,3}

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The non coding protein RNA world Methods and tools

goticrorogramagaanaargaaatik

- Known family: Homology prediction
 - Generic methods
 - Alignment + Secondary structure

The non coding protein RNA world

Methods and tools

Known family: homology prediction

- Generic methods
- Sequence alignment versus structural alignent

primary sequence alignment scoring:

structure + sequence alignment scoring:



The non coding protein RNA world Methods and tools

Known family: homology prediction

- Generic methods
- Sequence alignment & structure alignment
- At the core of Rfam database (http://rfam.sanger.ac.uk/)
 - A database of covariance models (probabilistic models of sequence/structure alignments)
 - Sequence alignment tool : Blastn
 - Structure alignment tool : Infernal (cmsearch)

D136-D140 Nucleic Acids Research, 2009, Vol. 37, Database issue

Published online 25 October 2008

Rfam: updates to the RNA families database

Paul P. Gardner^{1,*}, Jennifer Daub¹, John G. Tate¹, Eric P. Nawrocki², Diana L. Kolbe², Stinus Lindgreen³, Adam C. Wilkinson¹, Robert D. Finn¹, Sam Griffiths-Jones⁴, Sean R. Eddy² and Alex Bateman¹

¹Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK, ²Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, Virginia, USA, ³Center for Bioinformatics, Department of Biology, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen N, Denmark and ⁴Faculty of Life Sciences, The University of Manchester, Manchester M13 9PL, UK



The non coding protein RNA world Methods and tools

Known family: homology prediction

- Generic methods
- Descriptor-based methods
 - Subjective and painful descriptor generation
 - Subtle constraints not easily expressed
 - Yes/no answer (no scoring)

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Nucleic Acids Research, 1996, Vol. 24, No. 8 1395-1

Constraints (2008) 13:91–109 DOI 10.1007/s10601-007-9033-9

Palingol: a declarative programming language to describe nucleic acids' secondary structures and to scan sequence databases

Bernard Billoud*, Milutin Kontic and Alain Viari

Atelier de Bio-Informatique U

BIOINFORMATICS

ORIGINAL PAPER

D Vol. 2

Matthias Zytnicki · Christine Gaspin · Thomas Schiex

for RNA Motif Localization

DARN! A Weighted Constraint Solver

Genome analysis

Searching RNA motifs and their intermolecular contacts with constraint networks

P. Thébault^{1,2}, S. de Givry¹, T. Schiex¹ and C. Gaspin^{1,*}

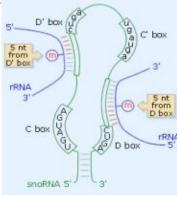
¹Unité de Biométrie & Intelligence Artificielle INRA, Chemin de Borde Rouge, Auzeville, BP 52627, 31326
Castanet-Tolosan, France and ²Plateforme Bioinformatique, INRA, Chemin de Borde Rouge,
Auzeville, BP 52627, 31326, Castanet-Tolosan, France

The non coding protein RNA world Methods and tools

н.	volcanii	1781375-1781262
н.	Marismortuii	1925960-1926073
н.	sp. NRC-1	1937209-1937273
н.	salinarum Rl	1925326-1925416
н.	lacusprofundi ATCC	408532-408467
Ν.	pharaonis DSM 2160	341490-341553
н.	walsbyi DSM 16790	2476861-2476796
Μ.	marisnigri	657340-657401
М.	vannielii	279347-279440
Μ.	maripaludis C5	729115-729022
Μ.	maripaludis C6	1600143-1600236
Μ.	maripaludis S2	941692-941599
Μ.	maripaludis C7	212028-211935
Μ.	aeolicus Nankai-3	860499-860588
Μ.	labreanum	378814-378919
Μ.	burtonii	2085602-2085512
Μ.	barkeri	1034452-1034513
Μ.	mazei	1703784-1703695
Μ.	acetivorans	161870-161781
C.	Methanoregula	646118-646064
М.	jannaschii	1606357-1606286
Ρ.	horikoshii	1732055-1731997
Ρ.	abyssi	1755931-1755873
Ρ.	furiosus	1849545-1849603
т.	kodakarensis KOD1	1226900-1226841

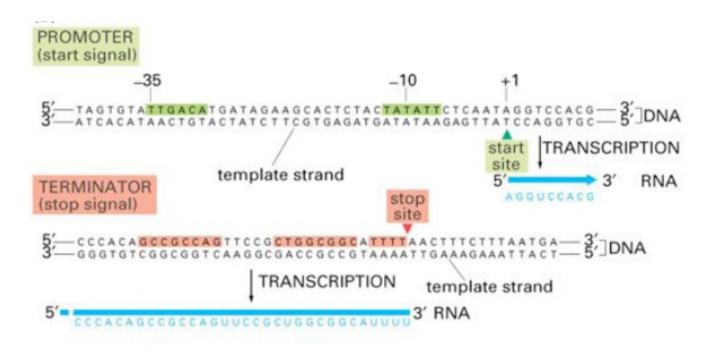
GGUGGCG	Α	UGACGA	AGGUUACCCCCA	CUCA
UGGUGGCG	Α	UGACGA	GAUGUUACCCCCG	CUGA
GGCGACG	G	UGACGA	GGAGUUACCCCCU	CCGA
GGCGACG	G	UGACGA	GGAGUUACCCCCU	CCGA
GGUGGCC	Α	UGACGA	CGUGUUACCCCCA	CUGA
GGUGGCG	G	UGACGA	CAGUUACCCCCU	CCGA
<u>UGGUGGC</u> C	Α	UGAUGA	CAGUUACCCCCA	CUGA
GGCGGCAG	Α	UGAUGA	GAGUUACCCCCA	CUGA
GC <u>UG</u> U	Α	UGAUGA	CAGUUACCCCCG	CUGA
CGU <u>UG</u> U	Α	UGAUGA	CAGUUACCCCCG	CUGA
CGU <u>UG</u> U	Α	UGAUGA	CAGUUACCCCCG	CUGA
		UGAUGA	CAGUUACCCCCG	CUGA
CGU <u>UG</u> U	Α	UGAUGA	CAGUUACCCCCG	CUGA
CAGCGA			CAGUUACCCCCG	CGGA
<u>GGACA</u> G	Α	UGAUGA	AAGUUACCCCCG	CUGA
GCGGCG	Α	UGAUGA	GAGUUACCCCGA	CCGA
UGC <u>GGC</u> G			GAGUUACCCCAA	CUGA
UG <u>CGG</u> CG	Α	UGAUGA	AAGUUACCCCGG	CUGA
UG <u>CGG</u> CG	Α	UGAUGA	GAGUUACCCCAG	CUGA
GG <u>CA</u> A	Α	UGAUGA	AAGUUACCCCCA	CUGA
<u>ACCUC</u> G	Α	UGAUGA	AAGUUACCCCCA	CUGA
<u>AAGCC</u> G	Α	UGAGGA	UCGUUAGCCACG	CUGA
C <u>AGCC</u> G	Α	UGAGGA	CCGUUAGCCACG	
U <u>GACC</u> G	Α	UGAGGA	GCGUUAGCCACG	CUGA
C <u>GGCC</u> G	Α	UGACGA	GCGUUAGCCACG	CUGA

GCCCCUUG	UGAUGA	UAGAUUUCAU	CCGA	GCCACC
GCCCCUUG	UGACGA	UGGACUUUUC	CUGA	GCCACCA
GCCCCUUG	UGACGA	GGCCGUUCUC	CCGA	GUCGCC
GCCCCUUG	UGACGA	GGCCGUUCUC	CCGA	<u>GUCGCC</u> C
GCCCCUUG	UGAUGA	GGGUUUUCCA	CCGA	GCCACC
GCCCCUUG	UGACGA	GGCACUCAUU	CCGA	GCCACC
GCCCCUUG	UGAUGU	GGUAUUUUAU	CUGA	GCCACCA
UCCGG	UGAUGA	AGCAGGGGG	CUGA	UGUCGCU
CUGGAUUAACUGCUUUAAAGGCUAUUUUUUCGGGA	UGACGA	UAUCAGCACUAU	CUGA	CAAAGCU.
CUAAAUUCAUGAUUUUCAUGAUUUUUGGGA	UGAAGA	CAUCAGCACUAU	CUGA	<u>CA</u> CAGC
CUAAAUUCGUGAUUUUCAUGAUUUUAGGA	UGACGA	CAUCAGCACUAU	CUGA	CACAGCU.
CUAAAUUCGUGAUUUUCAUGAUUUUGGGA	UGAAGA	CAUCAGCACUAU	CUGA	CACAGCU.
CUAAAUUCGUGAUUUUCAUGAUUUUUGGGA	UGAAGA	CAUCAGCACUAU	CUGA	CACAGCU.
. CUGAAACAAUAUUUAUUUUUGUUUCAGGA				
		UGCGUGGUC		
		UAAACUCUAA		
		GAACAAUUUC		
GCAAAAGA	UGAUGA	GAACAAUUUC	CUGA	U <u>GCC</u> GC
		GAACAAUUUC		
		AGCAGGGGA		
		AAUCGGCACUGU		
		UAAGAGGGUUAG		
		UAAGAGGGUUAG		
		CAAGAGGGUUAG		
CUGA	UGAGGA	AAAGAGGGUUAG	CCGA	GGCCA
285				



The non coding protein RNA world Methods and tools

- Unknown family
 - Orphan promoter/terminator



The non coding protein RNA world

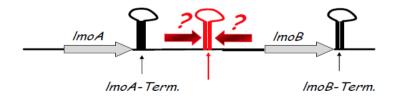
Methods and tools

Unknown family

Orphan promoter/terminator

Identification by "orphan" terminators prediction





p-independent terminators are identified by standard pattern-searching methods.

The non coding protein RNA world Methods and tools

Unknown family

- Orphan promoter/terminator
- Successfully applied in E. coli

Novel small RNA-encoding genes in the intergenic regions of Escherichia coli

Liron Argaman**, Ruth Hershberg**, Jörg Vogel**, Gill Bejerano*, E. Gerhart H. Wagner*, Hanah Margalit* and Shoshy Altuvia*

Background: Small, untranslated RNA molecules were identified initially in bacteria, but examples can be found in all kingdoms of life. These RNAs carry out diverse functions, and many of them are regulators of gene expression. Genes encoding small, untranslated RNAs are difficult to detect experimentally or to predict by traditional sequence analysis approaches. Thus, in spite of the rising recognition that such RNAs may play key roles in bacterial physiology, many of the small RNAs known to date were discovered fortuitously.

Results: To search the Escherichia coli genome sequence for genes encoding small RNAs, we developed a computational strategy employing transcription signals and genomic features of the known small RNA-encoding genes. The search, for which we used rather restrictive criteria, has led to the prediction of 24 putative sRNA-encoding genes, of which 23 were tested experimentally. Here we report on the discovery of 14 genes encoding novel small RNAs in E. coli and their expression patterns under a variety of physiological conditions. Most of the newly discovered RNAs are abundant. Interestingly, the expression level of a significant number of these RNAs increases upon entry into stationary phase.

Conclusions: Based on our results, we conclude that small RNAs are much more widespread than previously imagined and that these versatile molecules may play important roles in the fine-tuning of cell responses to changing environments.

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*These authors contributed equally to this work.

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The non coding protein RNA world Methods and tools

Unknown family

Orphan promoter/terminator

Research

Open Access

Rapid, accurate, computational discovery of Rho-independent transcription terminators illuminates their relationship to DNA uptake

Carleton L Kingsford, Kunmi Ayanbule and Steven L Salzberg

Address: Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD 20742, USA.

Correspondence: Carleton L Kingsford, Email: carlk@umiacs.umd.edu

Published: 21 February 2007

Genome Biology 2007, 8:R22 (doi:10.1186/gb-2007-8-2-r22)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2007/8/2/R22

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Nucleic Acids Research, 2011, Vol. 39, No. 14 5845–5852 doi:10.1093/nar/gkr168

RNIE: genome-wide prediction of bacterial intrinsic terminators

Paul P. $Gardner^{1,*}$, Lars $Barquist^1$, $Alex Bateman^1$, $Eric P. Nawrocki^2$ and $Zasha Weinberg^3$

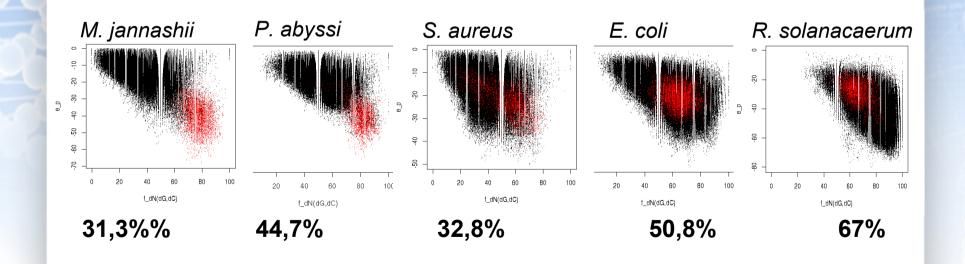
¹Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA0, UK, ²Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147 and ³Howard Hughes Medical Institute, Yale University, Box 208103, New Haven, CT 06520, USA

The non coding protein RNA world

Methods and tools

Unknown family

- Bias composition analysis
 - Schattner, NAR, 2002, Klein & Eddy, PNAS, 2002



The non coding protein RNA world

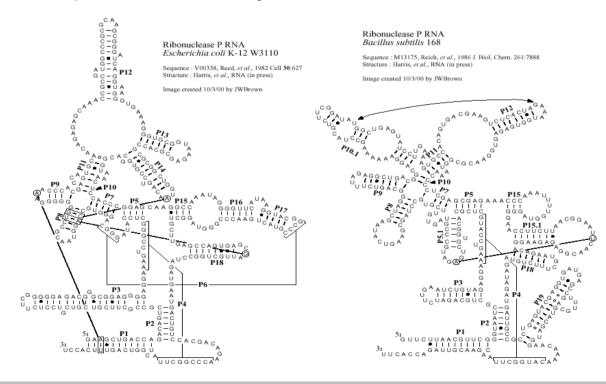
Methods and tools

Unknown family

- Comparative analysis
 - Sequence alignment
 - Structure alignment

The non coding protein RNA world Methods and tools

- Unknown family
 - Comparative analysis



RNASnace.org

The non coding protein RNA world Methods and tools

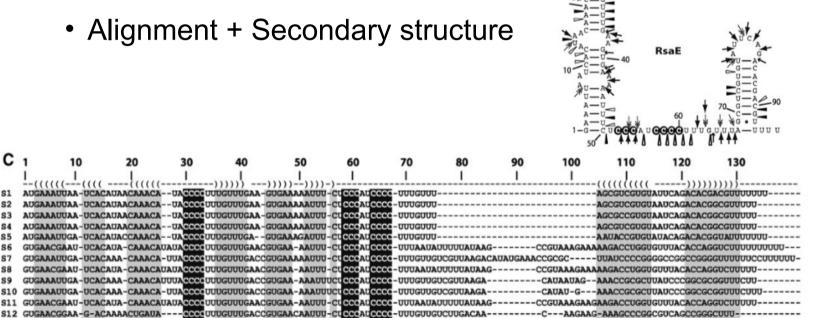
-UGUAAGUUGAAUACAAA-CAAAUA----<u>CCCC</u>UUUUGUUUGAUCAUGA-AACUUUCU <u>CCC</u>AU <u>CCCC</u>CUUUGUAGAUAUACAAAG------ACGGAAA-UAGGAUAUAUGCUGCCCCGUAUAUCCUUU------

Unknown family: Homology prediction

Generic methods

AUGAAAUUAA-UCACAUAACAACA--UACCCCUUUUGUUUGAA-GUGAAAAUUU-C AUGAAAUUAA-UCACAUAACAAACA--UACCCCUUUUGUUUGAA-GUGAAAAAUUU-C AUGAAAUUAA-UCACAUAACAACA--UACCCCUUUUGUUUGAA-GUGAAAAUUU-C GUGAACGAAU-UCACAUA-CAAACAUAUA-CCCUUUUGUUUGAACGUGAA-AAUUU-C GUGAAAUUGA-UCACAAA-CAAACA-UUACCCUUGUUUGACCGUGAAAAAUUU-C

Alignment + Secondary structure



Geissmann T et al. Nucl. Acids Res. 2009;37:7239-7257

RNASDACE.OFG

The non coding protein RNA world Methods and tools

Unknown family: Homology prediction

- RNAz (Washietl et al., 2004)

www.tbi.univie.ac.at/~wash/RNAz

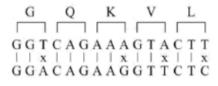
- Start with an alignment of homologous sequences
- Compute:
 - Mean free energy of aligned sequences
 - Structure conservation score
 - Mean pairwise identity
 - Number of sequences in the alignment
- Use a SVM to classify candidates

RNASDace.org

The non coding protein RNA world

Methods and tools

- Unknown family: Homology prediction
 - Q-RNA (Rivas & Eddy, 2001)
 - Start with a blast alignment
 - Models to assess coding/non coding
 - •Model for protein coding gene



P(GGT-GGA)*P(CAG-CAG)*...

Synonymous mutations

Model for ncRNA

(also include loop probabilities obtained from training set of real ncRNA)



P(T-T)*P(T-T)*P(GC-GC)*P(TA-AT)*...

Compensatory mutations

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The non coding protein RNA world Methods and tools

- miRNA prediction
 - De novo prediction
 - Search for the hairpin structure of the pre-miRNA
 - Hairpin search at the genome scale
 - Exploit conservation between organisms

Example of bacterial sRNA prediction & annotation with RNAspace

RNAS na C e. O r g ncRNA prediction and annotation RNAspace



RNAS nace. Or 9 ncRNA prediction and annotation RNAspace



RNAS na C e. O r g ncRNA prediction and annotation RNAspace

RNA The non-co					tform	Home 1.Load data 2.Predict 3.Explore
er the genomic sequ I the replicon. [?] nark:There is a glob Upload seque	al size limitat		_		of sequences is limit	led to 300 per multiFasta). For each sequence, you can describe the name, the domain the species, the st
Sequence name: Domain:	seq_ bact	000001 erla <u>*</u>			ed sequence(s) in FA	STA format from a file: Parcourir
Optional information Species: Strain: Replicon:	unkn unkn unkn	own				
						Clear Example Upload this sequence
Sequence name sample	Size 100001	Domain bacteria	Species E.coli	Strain K12	Replicon Chromosome	Header Sample sequence Escherichia coli str. K-12 substr. MG1655 4156417:4256417
						Email address(es) [?]: Submit

RNAS na C e. O r g ncRNA prediction and annotation RNAspace

	on platform	7				the at
		Home	1.Load	data	2. Predict	3. Explore
the sake of clarity, available annotation tools are organized nark: Maximum allowed running time for a gene finder is 8 I	hours.					
Homology search These tools identify regions	that are similar to known no	on-coding RNAs. Similarity is de	etected at the	e sequence lev	el and/or at the stru	ucture level. [?]
BLAST (sequence homology) [more]	Database:	Rfam_10.0_seed	▼ ps	arameters		
✓ Darn (RNA motif search) [more]	Descriptor:	snoRNA-CDbox [A]	•			
ERPIN (RNA motif search) [more]	Training set:	All [domain]	•			
INFERNAL (RNA motif search) [more]	Descriptor:	23S-methyl [RF01065	5]			
RNAmmer (specialized) [more]			po	arameters		
tRNAscan-SE (specialized) [more]			ps	arameters		
YASS (sequence homology) [more]	Database:	Rfam_10.0_seed	▼ ps	arameters		
Comparative Analysis You can compare yo secondary structure. (1. Select a set of organisms (at most four)	our data to a selection of g Only bacterial and archeal	genome sequences from differe genomes are available by now	ent species v. [?]	to find out sign	ificantly conserved	regions that exhibit a consens
secondary structure. 1. Select a set of organisms (at most four) Select an organism	our data to a selection of g Only bacterial and archeal	genomes are available by now	ent species v. [?]	to find out sign	ificantly conserved	regions that exhibit a consens
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RNASnace.org

ncRNA prediction and annotation

P. abyssi - 2 contigs of Triticum aestivum

P. abyssi

Known families

Homology search

- Blast → RFAM
- Darn! → C/D box sRNA
- RNAmmer
- tRNAscan-SE

New families

Comparative analysis

- P. furiosus
- T. kodakarensis

Bias composition

T. aestivum

Known families

Homology search

- Blast → RFAM
- RNAmmer
- tRNAscan-SF

RNASnace.org

ncRNA prediction and annotation

P. abyssi - 2 contigs of Triticum aestivum

P. abyssi

Known families

Homology search

- Blast → RFAM :161
- Darn! → C/D box sRNA: 123
- RNAmmer: 4
- tRNAscan-SE: 46

New families

Comparative analysis: 20

- P. furiosus
- T. kodakarensis

Bias composition: 101

T. aestivum

Known families

Homology search: 118

- Blast → RFAM : 118
 - * miR1122 : → Infernal :57
 - * 5S rRNA: → Infernal: 1
 - * tRNA:1 → Infernal: 2
 - * U4 :1 → Infernal : 1
 - * Intron gr II
- RNAmmer: 0
- tRNAscan-SE : 0

RNASRACE.OTS ncRNA prediction and annotation P. abyssi

Run or user identifier Description Number of RNAs Query sequence(s)	hromosome	
r01 Combine -74+36 BLAST/Rfam_10.0_seed 161 RNAmmer 4 IRNAscan-SE 46 Dam 123 BLAST/CG-seq/csRNAc 20	hromosome	
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000001 SegArchae snoRNA-CDbox 8633 8668 36 - PAbyssi archaea chromosome Darn	0	r01
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000392 SeqArchae unknown 15220 15275 56 PAbyssi archaea chromosome atypicalGC 000393 SeqArchae unknown 26561 26624 64 PAbyssi archaea chromosome atypicalGC 000394 SeqArchae unknown 30198 30302 105 PAbyssi archaea chromosome atypicalGC 000395 SeqArchae unknown 56891 57217 327 PAbyssi archaea chromosome BLAST/GG-seq/caRNAc 000355 SeqArchae unknown 56903 57229 327 PAbyssi archaea chromosome BLAST/GG-seq/caRNAc 000356 SeqArchae SRP_euk_arch 56923 57215 293 - PAbyssi archaea chromosome [combine:BLAST/Rfam_1] 000209 SeqArchae SRP_bact 57002 57046 45 - PAbyssi archaea chromosome BLAST/Rfam_10.0_seed 000188 SeqArchae sR46 57376 5	0 0 0 1 3 5 4 0	r02 r02 r01 r01 r01 r01 r02 r01
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RNASRACE.OTS ncRNA prediction and annotation P. abyssi

000212	SeqArchae	HgcC	163322	163358	37	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	1	r0
000400	SeqArchae	unknown	165348	165400	53		PAbyssi	archaea	chromosome	atypicalGC	0	r0
000401	SeqArchae	unknown	174473	174770	298		PAbyssi	archaea	chromosome	atypicalGC	0	rC
000178	SeqArchae	RNaseP_arch	174477	174806	330	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	5	r(
000402	SeqArchae	unknown	204807	206988	2182		PAbyssi	archaea	chromosome	atypicalGC	0	r
000334	SeqArchae	16s_rRNA	205051	206547	1497	+	PAbyssi	archaea	chromosome	RNAmmer	0	r
000358	SeqArchae	tRNA-Ala	206606	206683	78	+	PAbyssi	archaea	chromosome	[combine:BLAST/Rfam_1]	5	r
000331	SeqArchae	23s_rRNA	206816	209855	3040	+	PAbyssi	archaea	chromosome	RNAmmer	0	r
000403	SeqArchae	unknown	207037	207307	271		PAbyssi	archaea	chromosome	atypicalGC	0	r
000404	SeqArchae	unknown	207309	207985	677		PAbyssi	archaea	chromosome	atypicalGC	0	r
000405	SeqArchae	unknown	208012	208831	820		PAbyssi	archaea	chromosome	atypicalGC	0	
000406	SeqArchae	unknown	208882	209890	1009		PAbyssi	archaea	chromosome	atypicalGC	0	
000220	SeqArchae	PK-G12rRNA	209204	209313	110	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	5	
000190	SeqArchae	snoR9	230449	230575	127	-	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	5	
000014	SeqArchae	snoRNA-CDbox	230464	230513	50	-	PAbyssi	archaea	chromosome	Dam	0	
000193	SeqArchae	snoPyro_CD	230633	230690	58	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	3	
000015	SeqArchae	snoRNA-CDbox	230637	230687	51	+	PAbyssi	archaea	chromosome	Dam	0	
000230	SeqArchae	sR49	235441	235496	56	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	2	-
000016	SeqArchae	snoRNA-CDbox	235445	235494	50	+	PAbyssi	archaea	chromosome	Dam	0	
000208	SeqArchae	sR13	245976	246030	55	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	4	
000017	SeqArchae	snoRNA-CDbox	245980	246028	49	+	PAbyssi	archaea	chromosome	Dam	0	
000233	SeqArchae	sR43	250933	250988	56	-	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	3	
000018	SeqArchae	snoRNA-CDbox	250935	250984	50	-	PAbyssi	archaea	chromosome	Dam	0	
000194	SeqArchae	snoPyro_CD	258067	258122	56	+	PAbyssi	archaea	chromosome	BLAST/Rfam_10.0_seed	2	-
000019	SeqArchae	snoRNA-CDbox	258071	258119	49	+	PAbyssi	archaea	chromosome	Dam	0	
000337	SeqArchae	unknown	258075	258119	45		PAbyssi	archaea	chromosome	BLAST/CG-seq/caRNAc	1	
000020	SeqArchae	snoRNA-CDbox	279210	279261	52	+	PAbyssi	archaea	chromosome	Dam	0	
000359	SeqArchae	tRNA-His	296677	296753	77	+	PAbyssi	archaea	chromosome	[combine:BLAST/Rfam_1]	1	
000021	SeqArchae	snoRNA-CDbox	301375	301421	47	-	PAbyssi	archaea	chromosome	Dam	0	
000022	SeqArchae	snoRNA-CDbox	303264	303317	54	+	PAbyssi	archaea	chromosome	Dam	0	
000407	SeqArchae	unknown	303630	303731	102		PAbyssi	archaea	chromosome	atypicalGC	0	
000408	SeqArchae	unknown	305598	305716	119		PAbyssi	archaea	chromosome	atypicalGC	0	-

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P. abyssi

User sequence(s)

ID	SeqName	Family	Begin	End	Size	Strand	Software
000348	SeqArchae	unknown	1083324	1083412	89		[BLAST/CG-seq/c]

Database sequence(s)

Sequence Description	database	Begin	End
gi 57639935 ref NC_006624.1 _Thermococcus_kodakarensis_KOD1,_complete_genome_2	NC_006624	914605	914704
gi 18976372 ref NC_003413.1 _Pyrococcus_furiosus_DSM_3638,_complete_genome_1	NC_003413	1022786	1022875

Alignment produced by Carnac + Gardenia

000348 %gi 57639935 re %gi 18976372 re	UUCUG-UAAGCAC-AAAUCGAUAAAUUUUUAUAUACCUUCAUUAUUAGA <mark>CAAGU</mark> UUCUCACGAUAAAAUCGCUGGCCUAAACCGAUAAAUUUUUAUAUACUCAUCACACUA <mark>GUUGGGU</mark> UUCUG-UAAUUACAC-AGACAGAUAAAUUUUUAUAUACUUUUCACCCUA <mark>AUUUAGU</mark> **** * * * * * * * * * * * * * * * * *
000348 %gi 57639935 re %gi 18976372 re	CAAAAAAGUGUACUACAAAAAUCUGUACUUGGUGGU CAGAAAAUUGUACUACAAAACUCUGUACCCGGUGGU CAGAAAAAUGUACUACAAAAAUUUGUACUAGGUGGG ** **** ********* * ***** *****
000348 %gi 57639935 re %gi 18976372 re	UUCU G U AA C C A A GAUAAAUUUUUAUAUAC UA GUA
000348 %gi 57639935 re %gi 18976372 re	(((())))))) (((())))))))) (((())))))))

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000259	contig	MIR1122	221919	222033	115	+	Wheat	eukaryote	unknown	INFERNAL	0	r05
000265	contig	MIR1122	221919	222033	115	-	Wheat	eukaryote	unknown	INFERNAL	0	r05
000020	contig	MIR1122	221922	222032	111	-	Wheat	eukaryote	unknown	BLAST/Rfam_10.0_seed	2	r01
000012	contig	MIR1122	221923	222031	109	+	Wheat	eukaryote	unknown	BLAST/Rfam_10.0_seed	2	r01
000193	contig	MIR1122	398065	398146	82	-	Wheat	eukaryote	unknown	[combine:BLAST/Rfam_1]	6	explore
000189	contig	MIR1122	398066	398146	81	+	Wheat	eukaryote	unknown	[combine:BLAST/Rfam_1]	6	explore
000257	contig	U4	607456	607607	152	+	Wheat	eukaryote	unknown	INFERNAL	0	r04
000199	contig	U4	607456	607586	131	+	Wheat	eukaryote	unknown	[combine:BLAST/Rfam_1]	10	explore
000264	contig	MIR1122	629622	629719	98	+	Wheat	eukaryote	unknown	INFERNAL	0	r05
000263	contig	MIR1122	631502	631599	98	+	Wheat	eukaryote	unknown	INFERNAL	0	r05
000198	contig	5s_rRNA	759594	759659	66	+	Wheat	eukaryote	unknown	[combine:BLAST/Rfam_1]	12	explore
000194	contig	MIR1122	795165	795289	125	-	Wheat	eukaryote	unknown	[combine:BLAST/Rfam_1]	7	explore
000260	contig	MIR1122	795166	795289	124	+	Wheat	eukaryote	unknown	INFERNAL	0	r05

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T. aestivum:U4?



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T. aestivum:U4?

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000199	contig	U4	607456	607586	131	+	[combine:BLAST/]	
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		Sequence Description	n			database	Begin	End
>U4 U302335.1/6979	_					Rfam_10.0_seed	1	139
>U4 M479189.1/4511-	_					Rfam_10.0_seed	1	138
	44768-44912_JRF00015					Rfam_10.0_seed	1	130
>U4 U302335.1/6979						Rfam_10.0_seed	1	139
	44768-44912_JRF00015					Rfam_10.0_seed	1	130
>U4 P004858.3/48993	_					Rfam_10.0_seed	1	130
	/42069-42219_JRF00015					Rfam_10.0_seed	1	137
>U4 M479189.1/4511-	-4661_JRF00015					Rfam_10.0_seed	1	138
>U4 ARH01003623.1/	/42069-42219_JRF00015					Rfam_10.0_seed	1	137
>U4 P004858.3/48993	3-49137_JRF00015					Rfam_10.0_seed	1	130
Top sequence: RNA Bottom sequence: R	fam_10.0_seed U4 U3023	35.1/69797-69946_JR	F00015 acgcacctagtgaggt					
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RNASDACE.OFG

T. aestivum:U4?



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RNASDACE.OTG

T. aestivum:U4?

		Н
RNA features		
ID:	000257	
Family:	U4	
Segmence name:	contig (<i>M/heat unknown</i> _eukanyote _unknown)	
Start:	607456 Update preview	
End:	607607	
Strand:		
Predicted by:	INFERNAL 1.0.2 with a 2.83e-19 score on 25-4-2013	
Sequence and s	structure(s)	
	1 ATTTTTGCGCTTGGGGCAATGACGCACCTAGTGAGGTAATACCGAGGCGCGTCAATTGCTGGTTGAAAACTATTTCCAAACTCCCTCTTTGGCCCTCACG 01 GGTCACTGAGAATTTGTGCAAAGGCTCCCTCTCCTTGGAGAGGGCAAGGGCC	
New secondary	structure	
	secondary structure in bracket-dot format. mpute the minimal free energy secondary structure with: Select a software	
	Add this structure in the preview page	
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	Reset illitial values Save Call	cci

RNASDACE.OTG

T. aestivum:U4?

				50-50-50		
RNA feature)s					
ID:	000	199				
Family:	U4					
Sequence name		ig (Wheat unknown - eukaryote -	unknown)			
Start:	607			Update p	review	
End:	607	586				
Strand: Predicted by:	+ comi	bine:BLAST/Rfam_10.0_seed - wi	ith a 0.0 score on 25-4-2013			
Genome con	сссттстт	GGAGATGCTCTTAA [000:	199] TCCTTGGAGAGGGC	AAGGGCCTACGAATTAAATAA		
	ary stru	cture dary structure in bracket-dot form the minimal free energy/secondar	et.		s structure in the preview pa	nge
Alignment (s		D alignment(s)	\			
			1		Reset initia	al values Save Cancel
00257	1	TTTGTTTGTT	GGTAGTCTGA	TTA GTCCCAC	CTCG GTAACT	GAGGCAGGTG
	51	GCAAGGGGGA	GCTAGGTATA	A GAACGGACC	ATGGGCGTAT	GGAAATGGGC
		ATTTTTGCGC				
	151	GTCAATTGCT	GGTTGAAAAC	TATTTCCAAA	CTCCCTCTTT	GGCCCTCACG
	201	GGTCACTGAG	$\Delta \Delta TTTGTGC\Delta$	AAGGCTCCCT	CTCCTTGGAG	AGGGCAAGGG
	201251	GGTCACTGAG CC	AATTTGTGCA	AAGGCTCCCT	CTCCTTGGAG	AGGGCAAGGG

RNASNACE.OTS sRNA prediction and annotation T. aestivum

						Home	1.Load	data	2.Predict	3.E	xplore
urrent re	sults for the 0057	39160f9a421 p	project: 179	9 putative	s RNAs pr	edicted.					
oftware tool:	s used and user action	s are summarized	in the left re-	sizable table	and query sequ	uence(s) in the rigi	nt re-sizable table. S	ee the project his	tory for more detail	s.	
R	un or user identifier	Des	scription	N	umber of RNA	As		Query sec	quence(s)		
r05		INFERNAL		57		contig	983767 nt	eukaryote	Wheat	unknown	unknown
r07		INFERNAL		2		contig_	914 2522860 n	t eukaryote	unknown	unknown	unknown
r06		INFERNAL		1							
r04		INFERNAL		1							
User	action	Combine		-136	+68						
r03		BLAST/Rfam	_10.0_seed	67							
r02		Combine		-0+0							
		INFERNAL		1							
User	action	Combine		-0+0							
-04		Combine		-0+0							
r01		Combine BLAST/Rfam	10.0	-2+1 119							
		RNAmmer	_10.0_seed	0							
		tRNAscan-SE	=	0							
Criterium Table viev			e (wildcards a	allowed)	Add/Updat	Result te 179/179 RN	As satisfy filter(s)		ayed putative RNAs		iters on the list
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Table view the table of the table [?].	Z Compar	CGview view	e value		lect predictions	te 179/179 RN	eck boxes in the left	displa	ayed putative RNAs	[?].	wn-drop lists b
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Table view he table of he table [?]. Predictions	JBrowse view results may be sorted 1 - 20 of 179 00018 contig 00261 contig	CGview view by clicking on the MIR1122 MIR1122	column titles. 21364 21	. You can sel	Display Ten	by ticking the chesse set ▼ Show Wheat e	ack boxes in the left 20 ukaryote unkno	column and perfo	ayed putative RNAs rm actions on them AST/Rfam_10.0_see INFERNAL	[?]. a using the do	wn-drop lists b
Table view he table of he table [?]. Predictions	JBrowse view results may be sorted 1 - 20 of 179 00018 contig 00261 contig 00267 contig	CGview view by clicking on the MIR1122 MIR1122 MIR1122	column titles. 21364 21 21364 21 21364 21	. You can sel 1441 78 1445 82 1445 82	Display Ten	by ticking the choose set Show Wheat e Wheat e	ack boxes in the left 20 v ukaryote unkno ukaryote unkno ukaryote unkno ukaryote unkno	column and performs BU	m actions on them AST/Rfam_10.0_sec INFERNAL	e [?]. a using the do Page	wn-drop lists b
Table view he table of he table [?]. Predictions 0 0 0 0	Compar V JBrowse view results may be sorted 1 - 20 of 179 00018 contig 000261 contig 00267 contig 00002 contig	CGview view by clicking on the MIR1122 MIR1122 MIR1122 SS_RNA	column titles. 21364 21 21364 21 21364 21 33257 33	. You can sel 1441 78 1445 82 1445 82 3337 81	Display Ten	by ticking the choose set Show Wheat e Wheat e Wheat e Wheat e	cck boxes in the left 20 ukaryote unkno ukaryote unkno ukaryote unkno ukaryote unkno	column and performance of the second	rm actions on them AST/Rfam_10.0_set INFERNAL INFERNAL AST/Rfam_10.0_set	e using the do Page	wn-drop lists b
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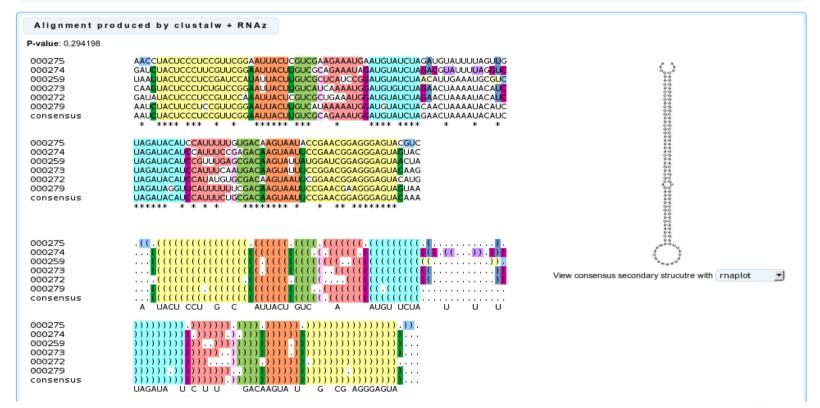
RNASDACE.OFG

T. aestivum:miR1122?

Page	1	\mathbf{I}	of	1

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User sequence(s)								
ID	SeqName	Family	Begin	End	Size	Strand	Software	
000275	contig_914	MIR1122	540570	540684	115	+	INFERNAL	
000274	contig_914	MIR1122	2231326	2231440	115	+	INFERNAL	
000259	contig	MIR1122	221919	222033	115	+	INFERNAL	
000273	contig_914	MIR1122	675545	675659	115	+	INFERNAL	
000272	contig_914	MIR1122	648743	648857	115	+	INFERNAL	
000279	contig_914	MIR1122	755563	755677	115	+	INFERNAL	



RNASDace.org

T. aestivum:miR1122?



Search Results

We found 3 unique results for your query ("mir1122"), in 4 sections of the database.

Section	Description	Number of hits
miRNA name	match the accession or ID of a hairpin precursor entry	3
Previous ID	match the previous ID of a hairpin precursor entry	0
Mature name	match the accession or ID of a mature miRNA sequence	3
Previous Mature ID	match the previous mature ID of a mature entry	0
Dead entry	match the accession or ID of a dead entry	0
Dead entry previous ID	match the accession or ID of a dead entry	0
Gene symbol	find miRNA entries based on gene symbols	0
Description	search miRNA entry description	3
Comments	search miRNA entry comments	1
PubMed ID	find miRNA entries based on literature reference PubMed ID	0
Literature reference	search title and authors of associated literature references	0

The above key shows a brief description of each of the database sections, along with the number of hits found in each one. Only unique miRNA entries are shown in the results table below. Click the column headings to sort the results table, or restore to the original order.

Accession∜	ID∜	miRNA name [⊕]	Mature name	Description∜	Comments∜
MI0006184	tae-MIR1122	✓	✓	✓	✓
MI0011568	bdi-MIR1122	✓	✓	✓	
MI0016607	far-MIR1122	✓	✓	✓	

RNASnace.org

ncRNA prediction and annotation

Prediction is different of validation !!!

miRNA prediction

- Homology search
 - Sequence alignment: very good conservation of the mature miRNA: Blastn against miRBase
- Be careful with plants !!!
- Pre-miRNA structure is to verify
- Take care of false positives

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ncRNA prediction and annotation Prediction is different of validation!!!

