Prediction of structural non-coding RNAs by comparative sequence analysis

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Outline

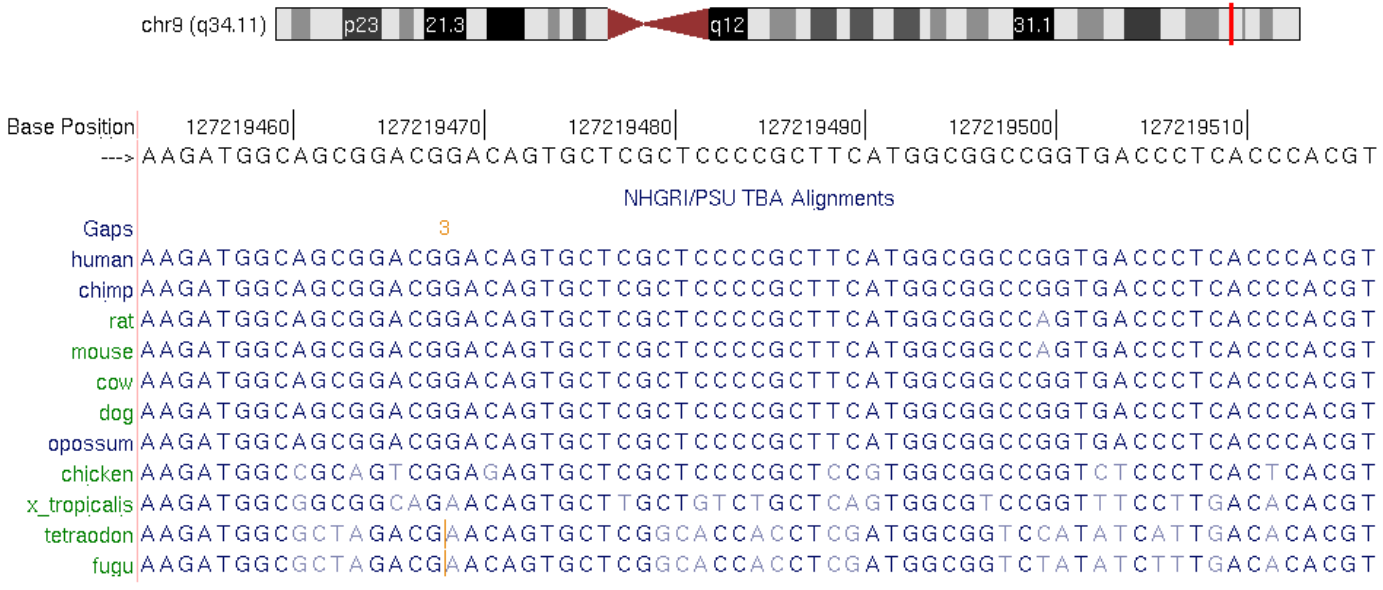
1. Introduction to non-coding RNAs and motivation of this work
2. New algorithms for detection of structural non-coding RNAs
3. A large scale screen of the human genome
4. Other applications

Non-coding RNAs

Non coding RNAs ("RNA genes") are transcripts that exert their function as RNA whithout being translated to protein.

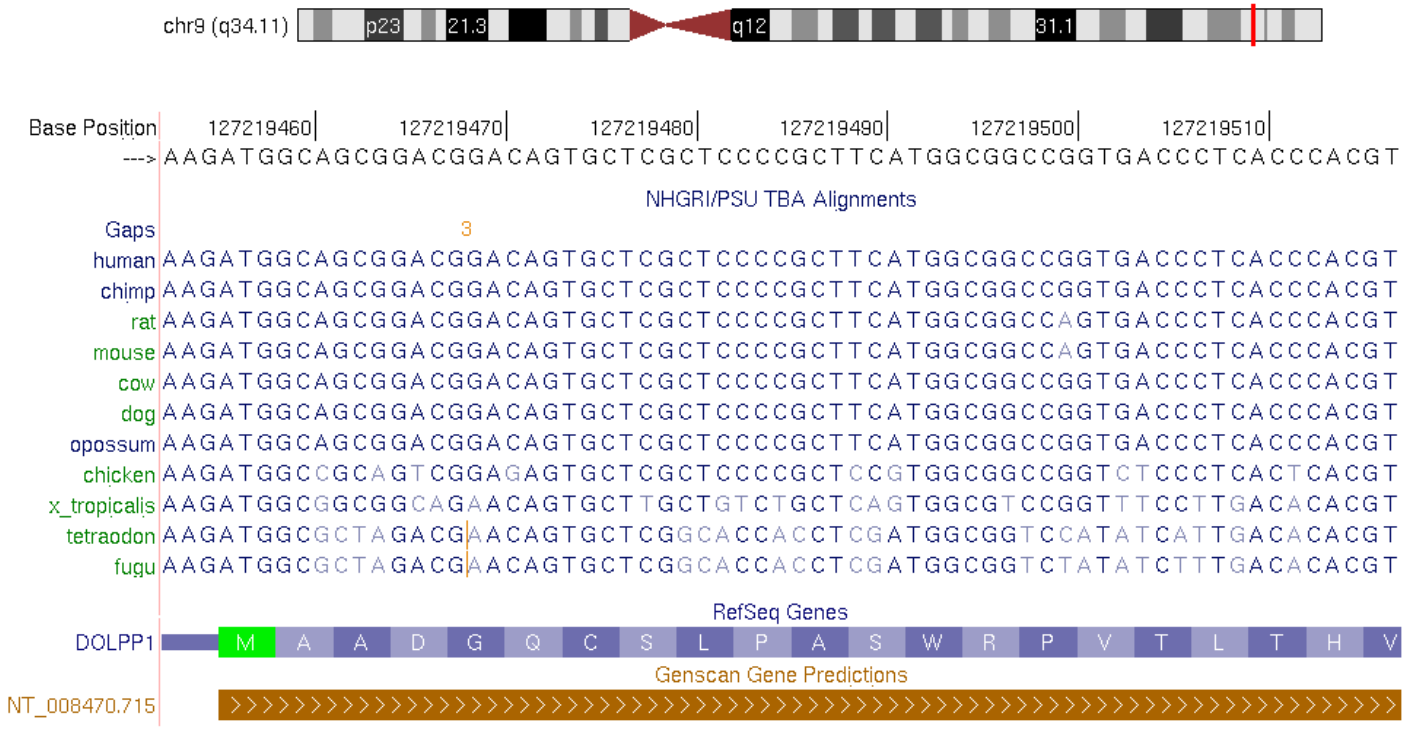
- "Classical" examples:
  - Protein expression: **transfer RNA, ribosomal RNA**
  - Pre-mRNA splicing: **spliceosomal RNAs**
  - tRNA maturation: **Ribonuclease P**
  - Protein export: **Signal recognition particle RNA**
- New abundant classes of small non-coding RNAs: **microRNAs, snoRNAs**
- Many other examples are currently emerging in all organisms studied.

Motivation

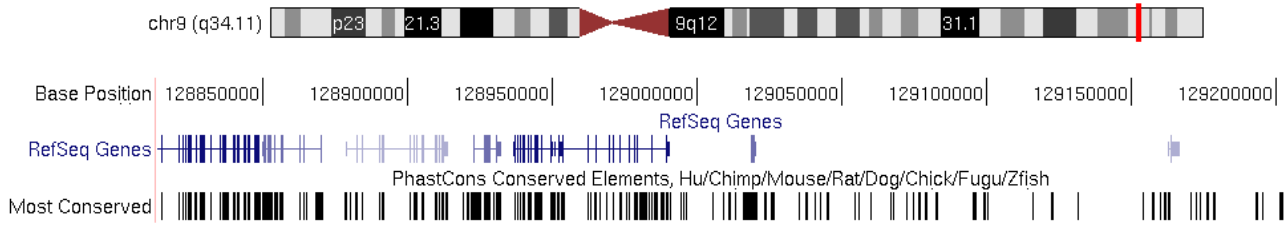


1. A vast amount of genomic data is available

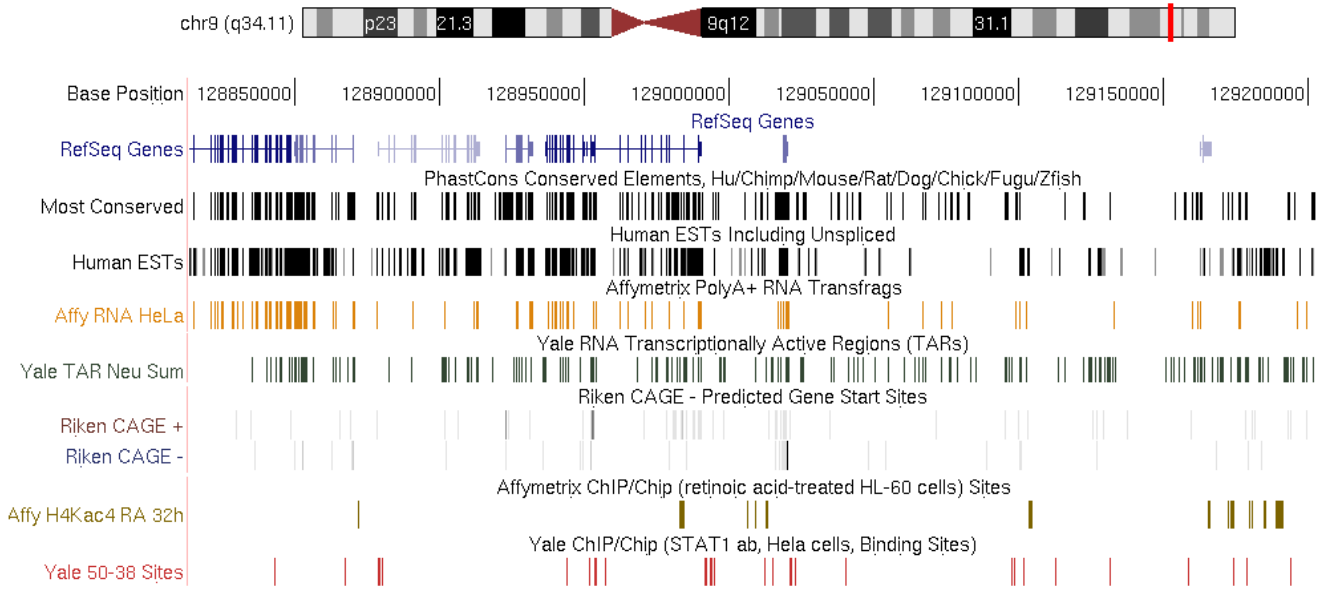
Motivation



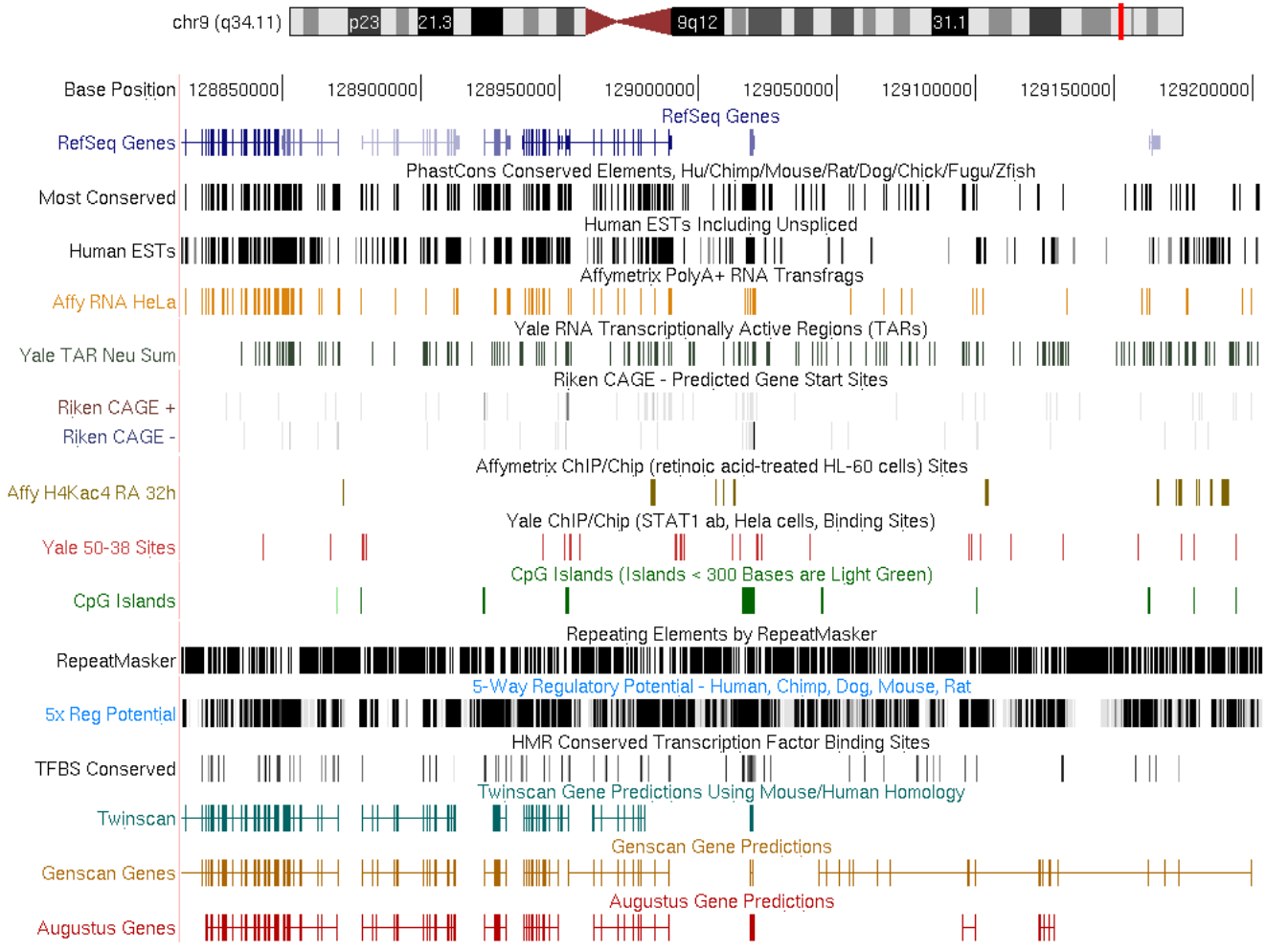
1. A vast amount of genomic data is available
2. There are fewer protein coding genes than expected



3. Highly conserved non-coding DNA awaits functional annotation.



3. Highly conserved non-coding DNA awaits functional annotation.
4. The transcriptional map of the human genome is much more complex than expected.



Non-coding RNAs ?

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4. The transcriptional map of the human genome is much more complex than expected.

Computational identification of non-coding RNAs

- Based on *a priori* knowledge: find members of known families
  - Sequence similiarity alone: `BLASTN`
  - Sequence and additional motif information: specialized programs for e.g. tRNA or snoRNAs
- *De novo* prediction: find new genes and families
  - Unlike protein coding genes (ORFs, codon bias,...) ncRNAs lack strong statistical signals in primary sequence
  - The function of many ncRNA depend on a defined secondary structure

Can secondary structure predictions be used for ncRNA detection?

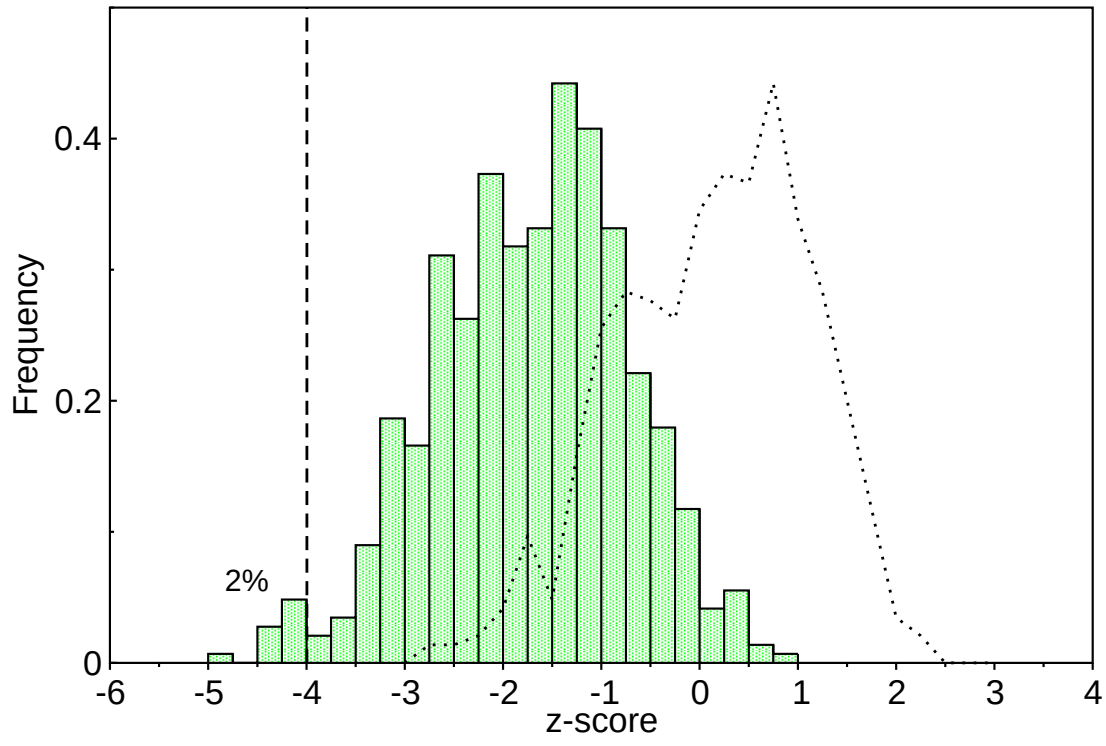
Significance of predicted RNA secondary structures: z-score statistics

- Has a natural occuring RNA sequence a lower MFE than random sequences of the same size and base composition?
  1. Calculate native MFE m.
  2. Calculate mean μ and standard deviation σ of MFEs of a large number of shuffled random sequences.
  3. Express significance in standard deviations from the mean as z-score

$$z = \frac{m - \mu}{\sigma}$$

- Negative z-scores indicate that the native RNA is more stable than the random RNAs.

z-scores for 579 tRNAs



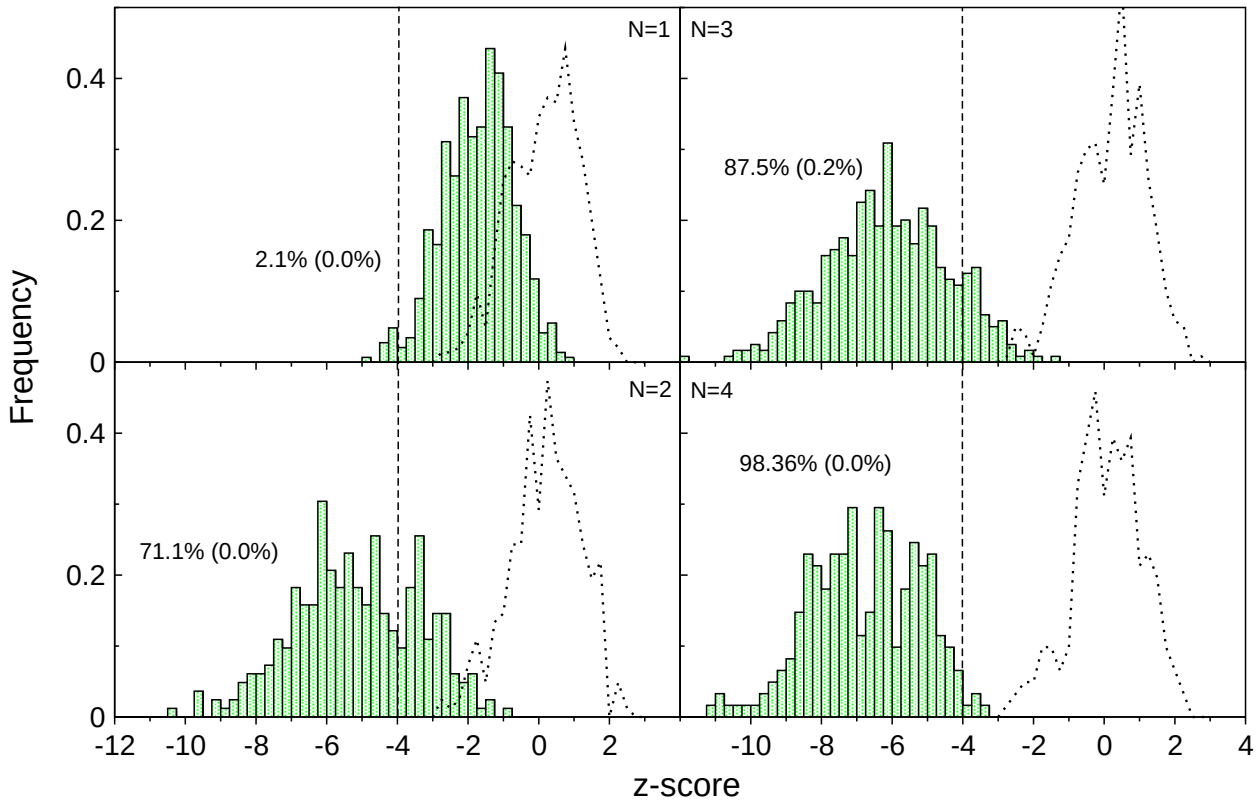
- Only 2% below a z-score threshold of -4.
- Native sequences are not clearly separated from the random bulk.

Consensus folding using RNAalifold

- `RNAalifold` uses the same algorithms and energy parameters as `RNAfold`
- Energy contributions of the single sequences are averaged
- Covariance information (e.g. compensatory mutations) is incorporated in the energy model.
- It calculates a consensus MFE consisting of an energy term and a covariance term:

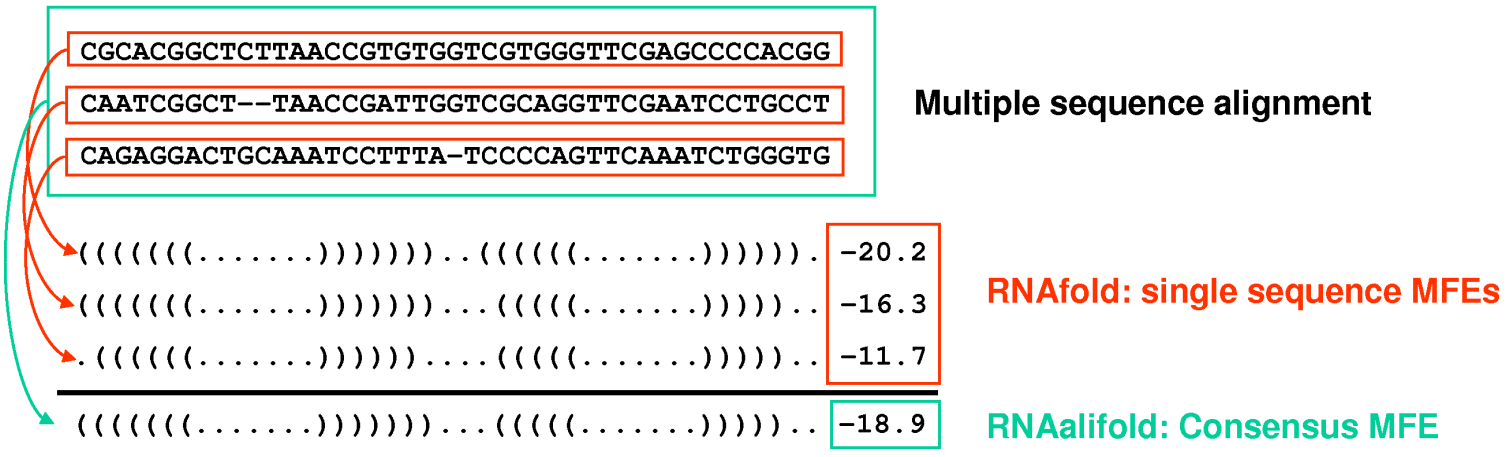
```
(((((((..(((.........))).(((((.......)))))).....(((((.......)))))))))).
GTTTCCGTAGTGTAGCGGTTATCACATTCGCCTCACACGCGAAAGGTCCCCGGTTCGATCCCGGGCGGAAACA
GTTTCCGTAGTGTAGTGGTTATCACGTTCGCCTAACACGCGAAAGGTCCCCGGTTCGAAACCGGGCGGAAACA
GTTTTCGTAGTGTAGTGGTTATCACGTGTGCTTCACACGCACAAGGTCCCCGGTTCGAACCCGGGCGAAAACA
**** ********** ********* *  ** * ******  ****************  ******* *****
(-24.76 = -23.43 +  -1.33)
```

z-scores of consensus MFEs for tRNA alignments



- `Alifoldz`: Additional information from aligned sequences shifts MFE predictions towards significant levels.

The structure conservation index

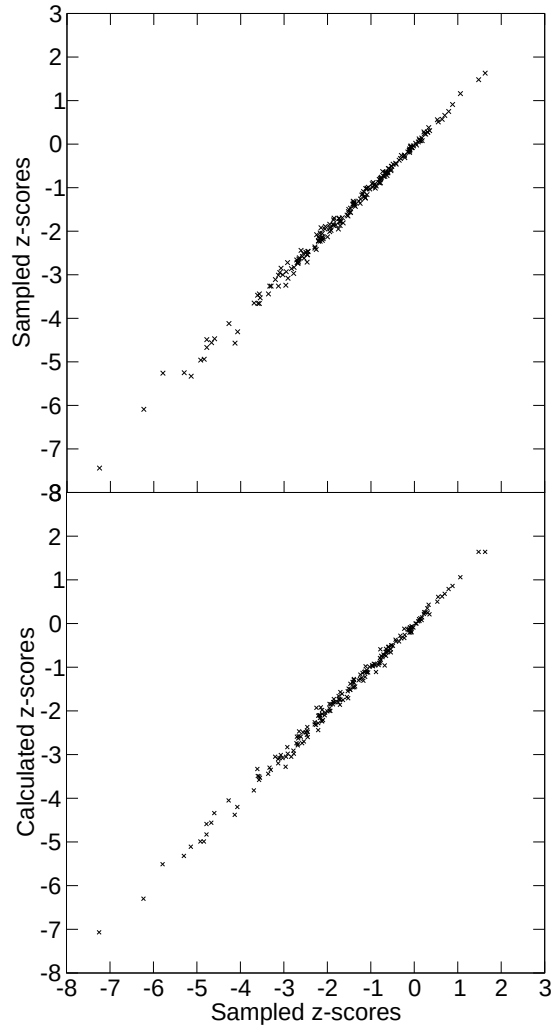


$$\text{SCI} = \frac{\text{Consensus MFE}}{\text{Mean single MFEs}}$$

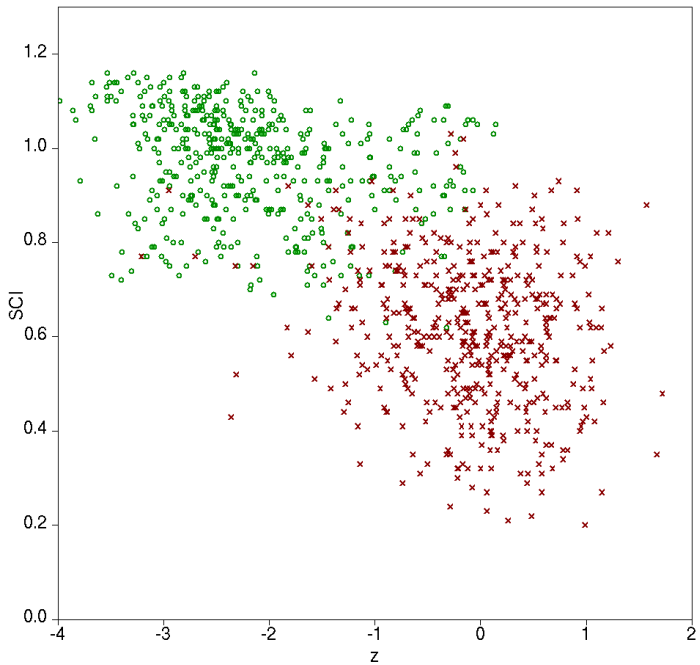
- The SCI is an efficient and convenient measure for secondary structure conservation.

Efficient calculation of stability z-scores

- The significance of a predicted MFE structure can be expressed as z-score which is normalized w.r.t. sequence length and base composition.
- Traditionally, z-scores are sampled by time-consuming random shuffling.
- The shuffling can be replaced by a Support Vector Machine regression calculation which is of the same accuracy.



SVM classification based on both scores



- Both scores separate native ncRNAs from controls in two dimensions.

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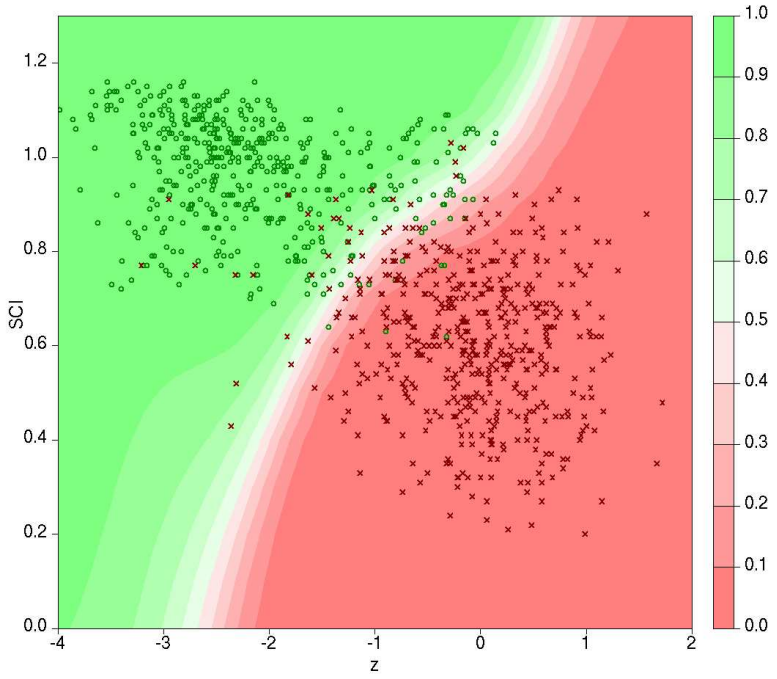

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- A support vector machine is used for classification.

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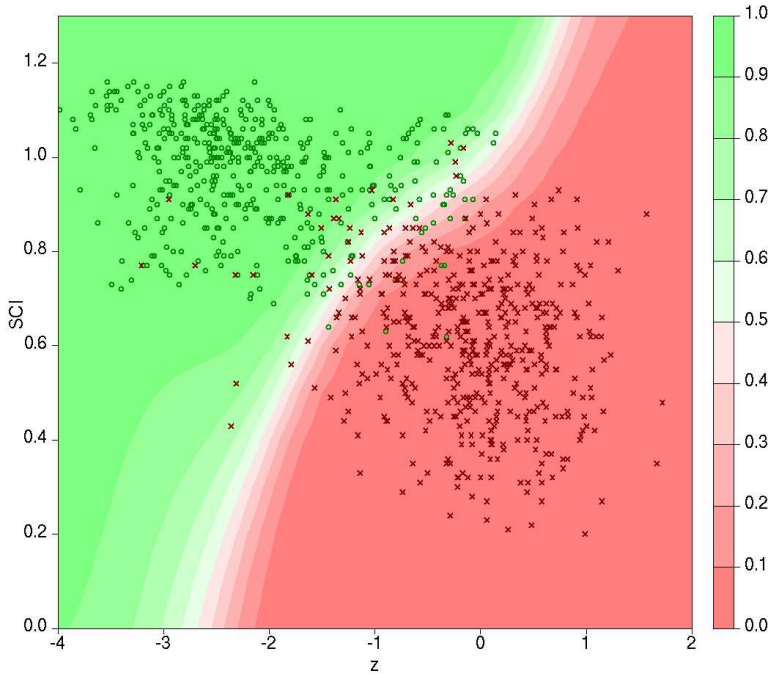
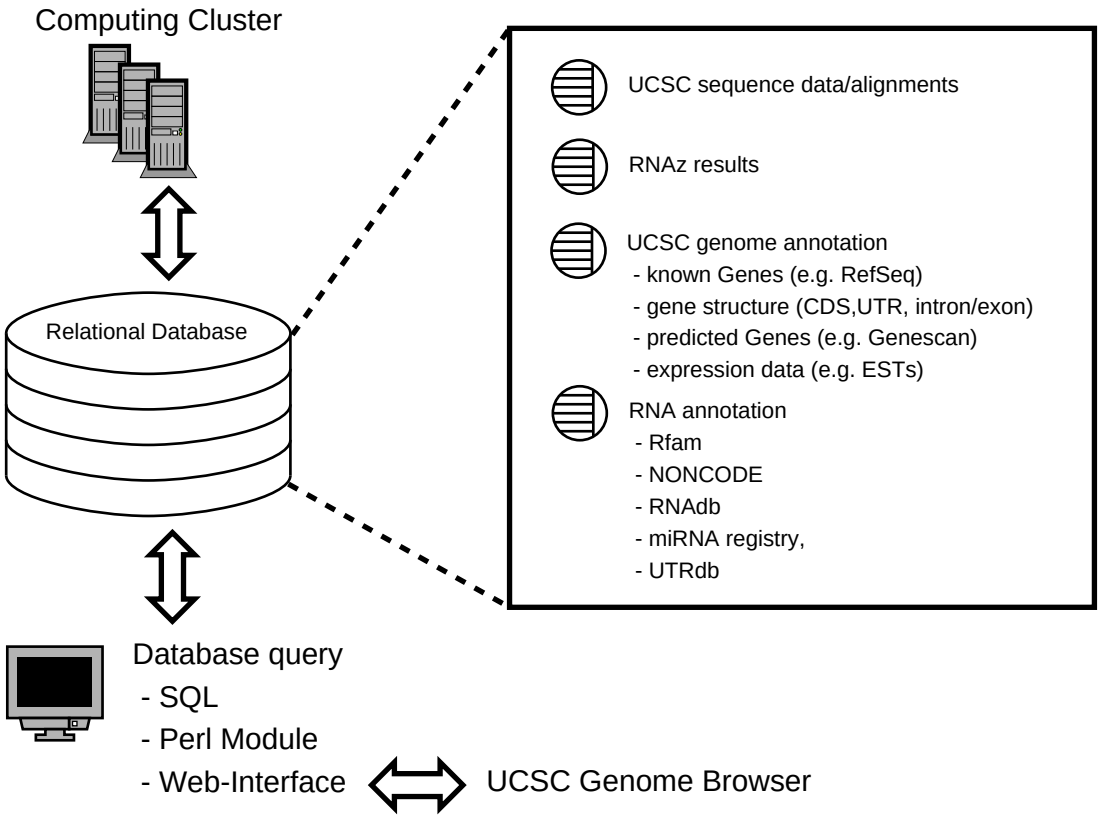


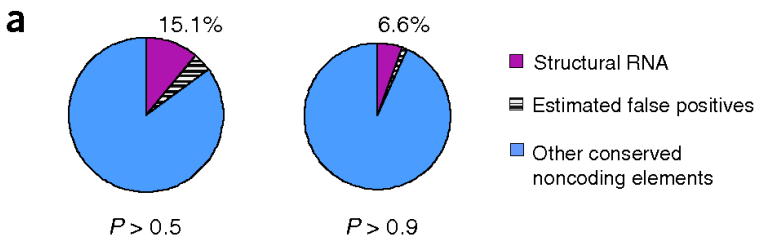
- Both scores separate native ncRNAs from controls in two dimensions.
- A support vector machine is used for classification.
- `RNAz`: more accurate and faster than any other available programs.

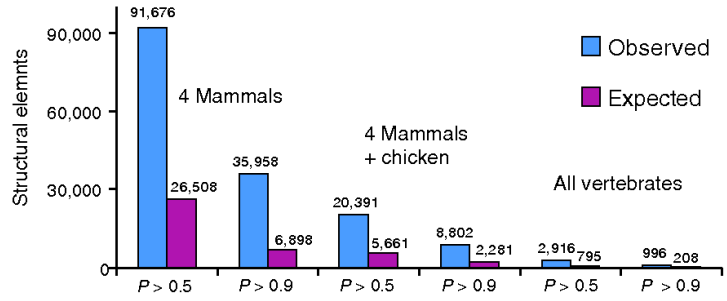
Screening the human genome

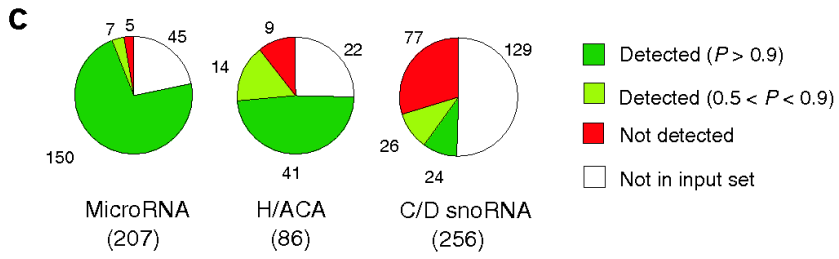


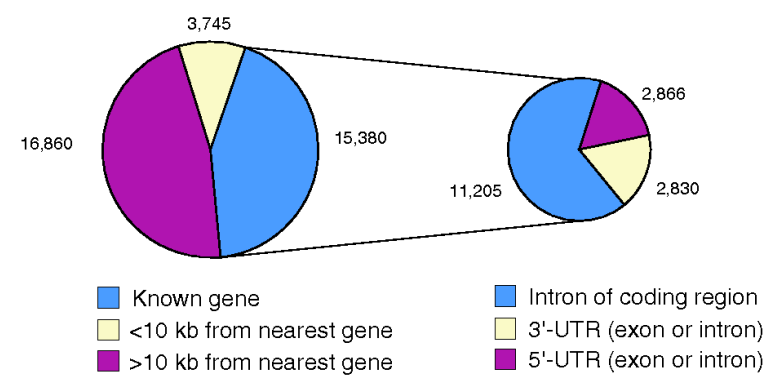
- Large scale comparative screen of mammals/vertebrates
- $\approx$ 5% of the best conserved non-coding regions
- $\longrightarrow$ 438,788 alignments covering 82.64 MB (2.88% of the genome)

Statistics of detected structures

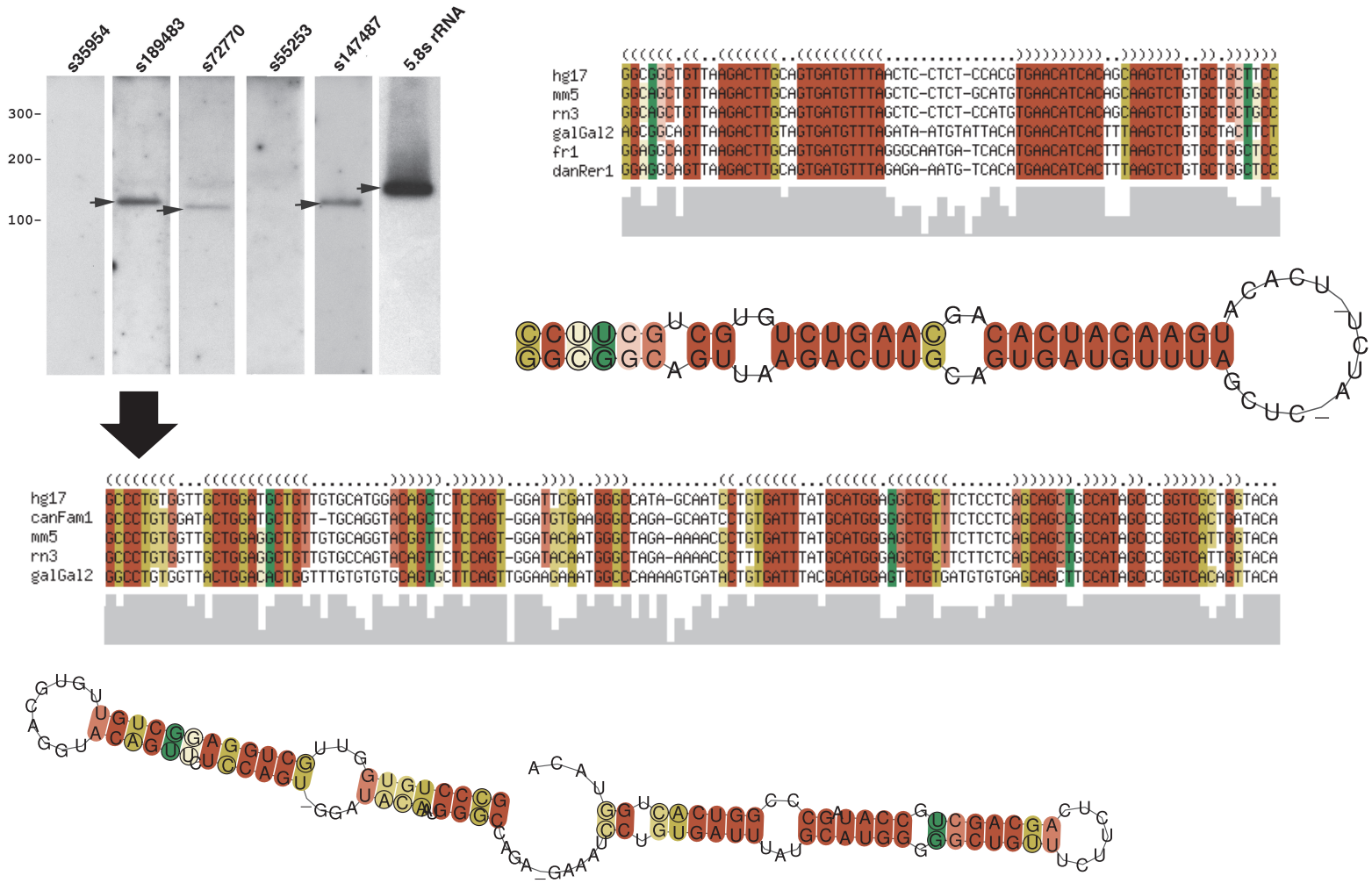




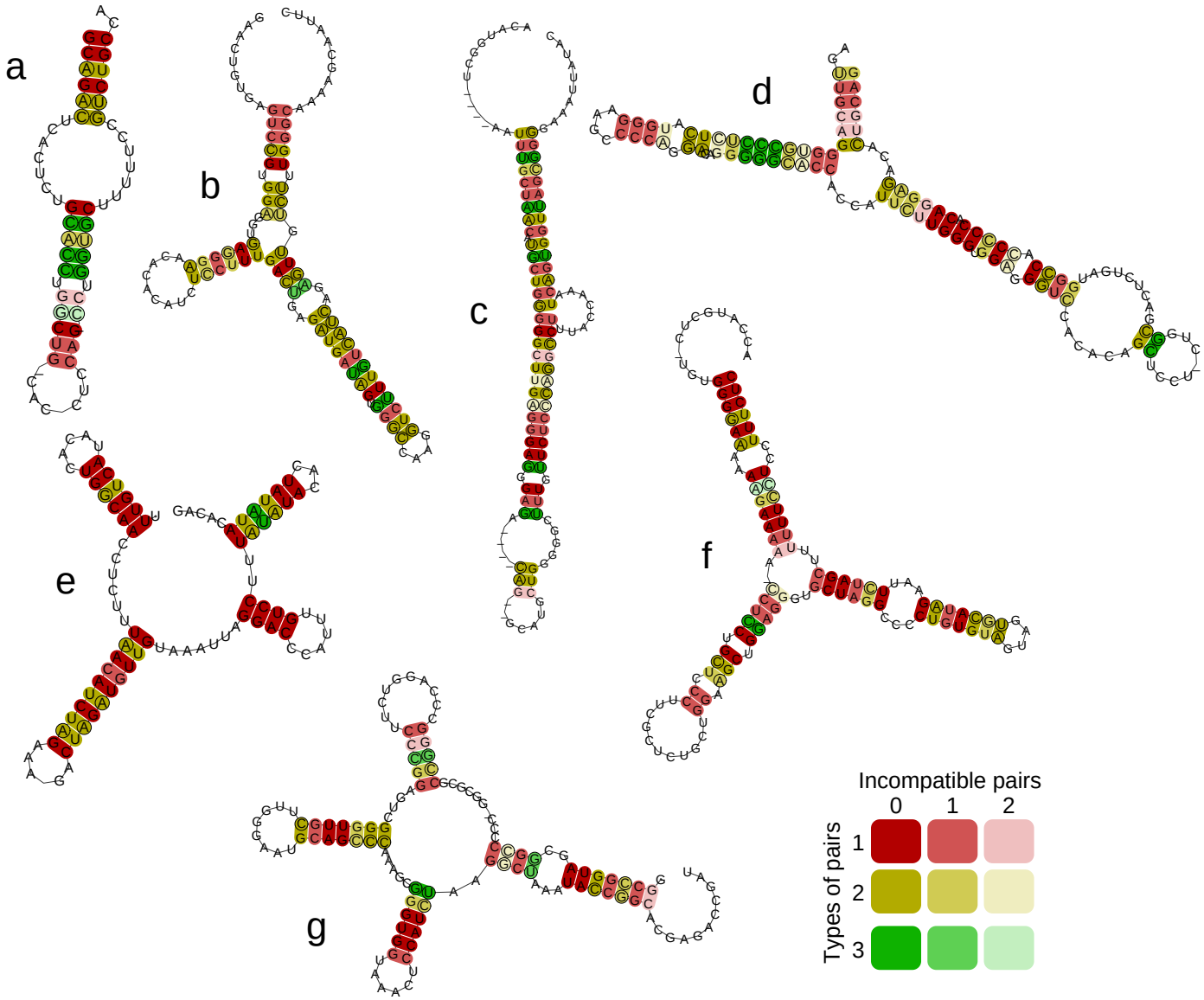




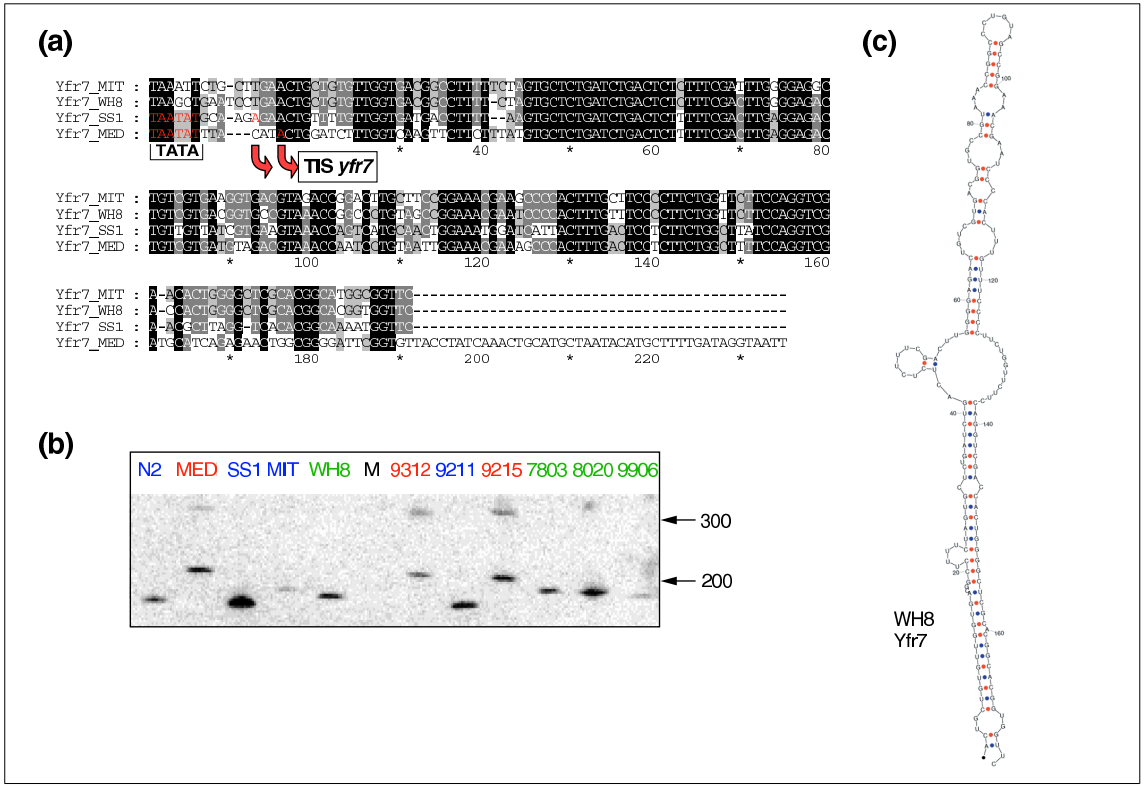
Novel structural RNAs of known classes: mirRNAs and H/ACA snoRNAs



Novel structures of unknown function

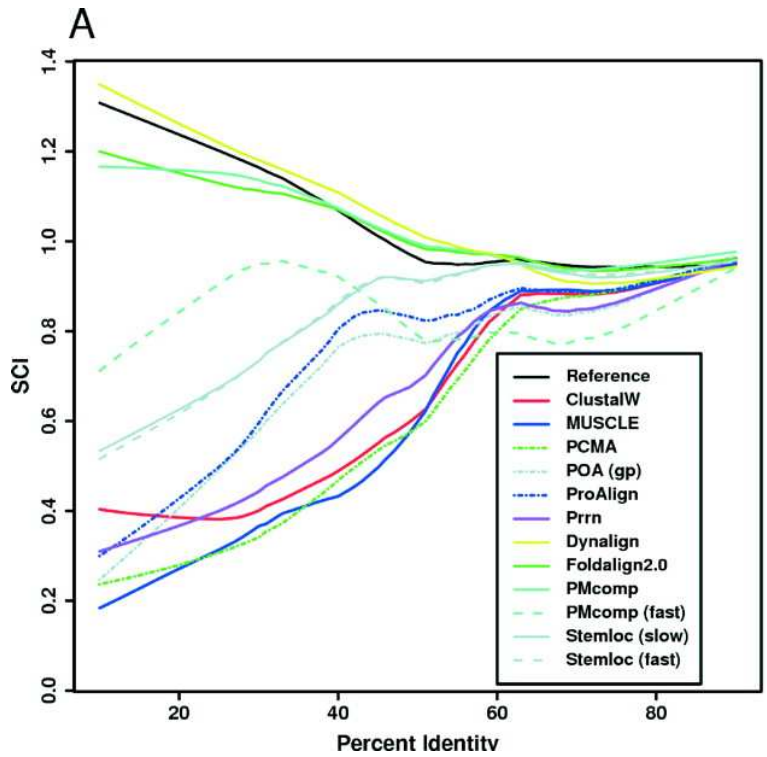


Other applications: Cyanobacterial ncRNAs



- I. Axmann, P. Kensche *et al.* (*Genome Biol.* **6**:R73, 2005) identified and characterized 7 novel ncRNAs in cyanobacteria using `Alifoldz`.

Other applications: Benchmarking alignment programs on structural RNAs



- The SCI can be used to assess the quality of an alignment of a structural RNA (P. Gardner, A. Wilm & S. Washietl *Nucleic Acids Res.* **33**:2433, 2005).

Other applications

- RNAz screen of urochordate genomes (K. Missal, D. Rose, P.F. Stadler *Bioinformatics* **21**: Suppl 2,ii77-ii78, 2005)
- RNAz screen of nematode genomes (K. Missal *et al. J. Exp. Zoolog. B*, in press).
- Prediction of putative miRNA precursors in the miRNAMap (Hsu *et al.*, submitted)

Summary and Conclusions

- *De novo* ncRNA prediction is notoriously difficult.
- Single sequence methods are of limited statistical significance.
- Comparative approaches dramatically improve accuracy.
- `RNAz` is an accurate and efficient approach for predicting ncRNAs.
- `RNAz` used for the first comprehensive annotation of conserved RNA secondary structures in the human genome.
- The data provides a strong basis for further computational and experimental studies.
- The programs and methods presented here were successfully used in a variety of other applications.

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