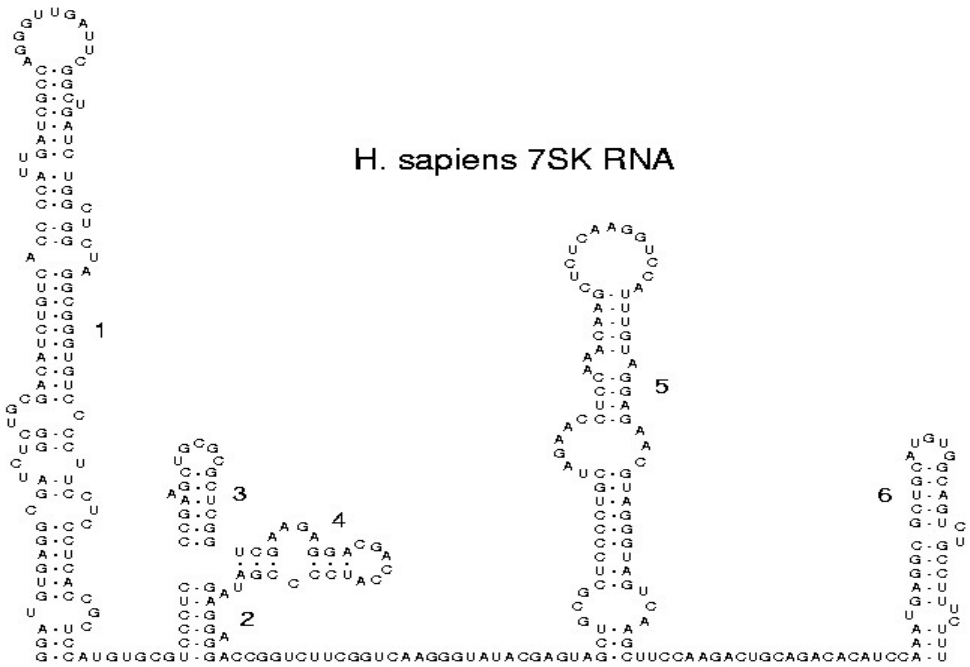
UE Praktikum Bioinformatik

WS 08/09

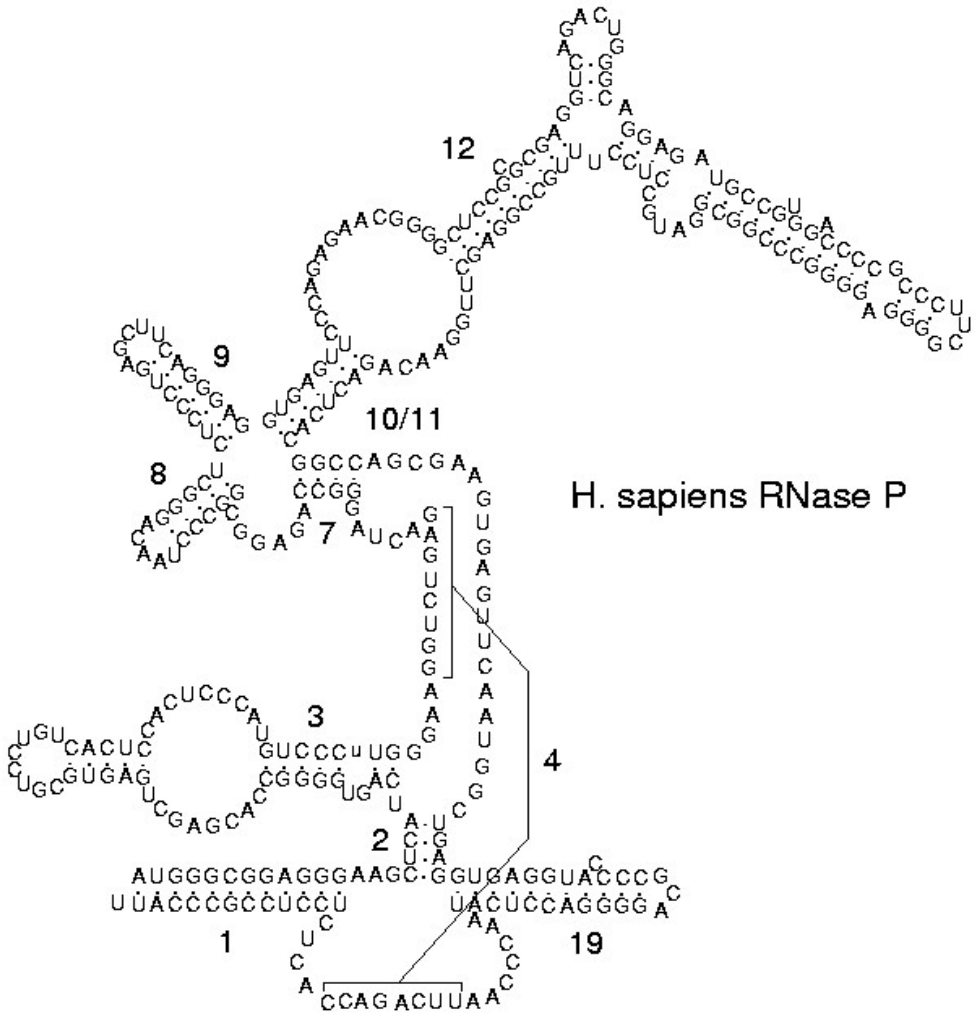
University of Vienna

7SK snRNA



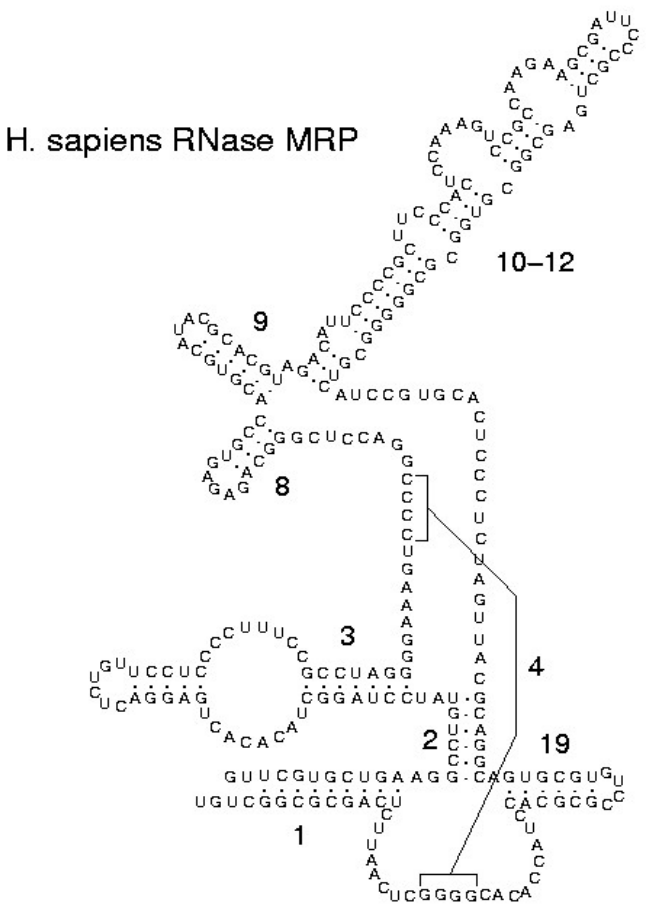
7SK was discovered as an abundant small nuclear RNA in the mid-70s but a possible function has only recently been suggested. Two independent studies have found that 7SK RNA binds the CDK9/cyclin T complex (known as elongation factor P-TEFb). P-TEFb activates transcription by phosphorylating the C-terminal domain of RNA polymerase II.

Nuclear RNase P



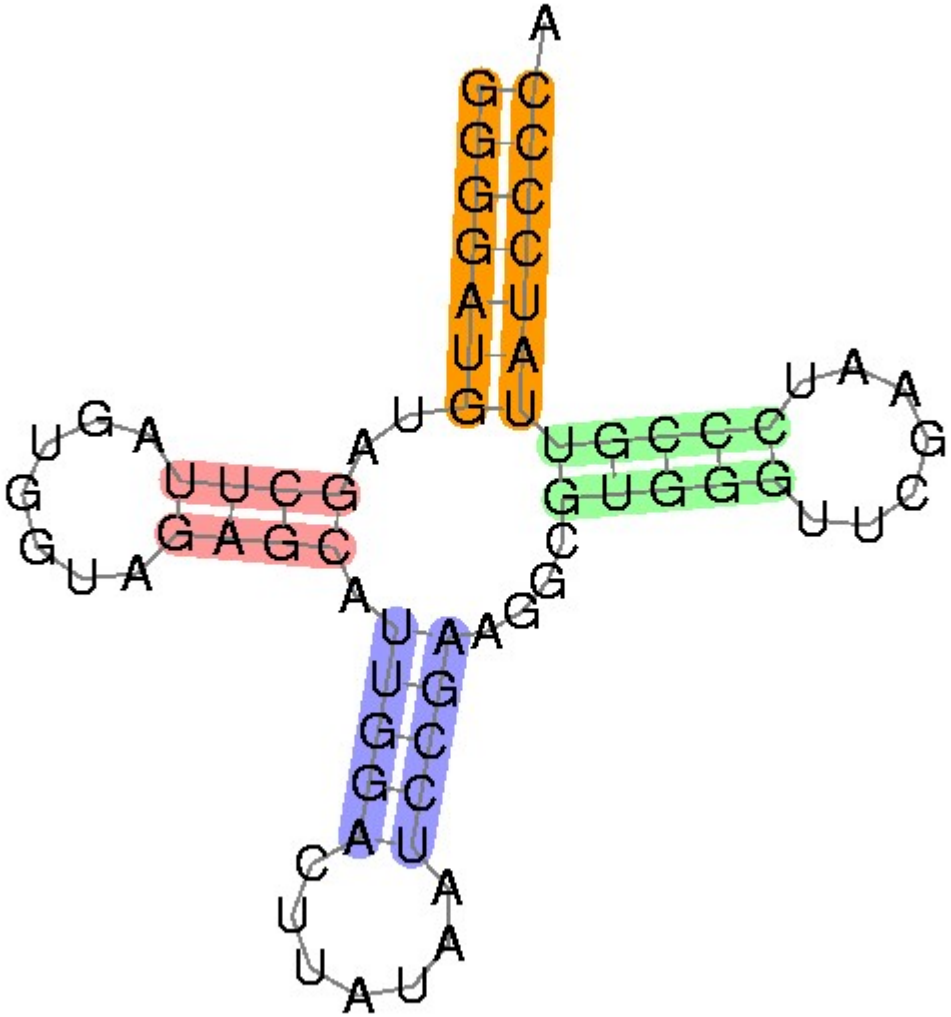
In molecular biology, nuclear ribonuclease P (RNase P) is a ubiquitous endoribonuclease, found in archaea, bacteria and eukarya as well as chloroplasts and mitochondria. Its best characterised enzyme activity is the generation of mature 5'-ends of tRNAs by cleaving the 5'-leader elements of precursor-tRNAs.

RNase MRP



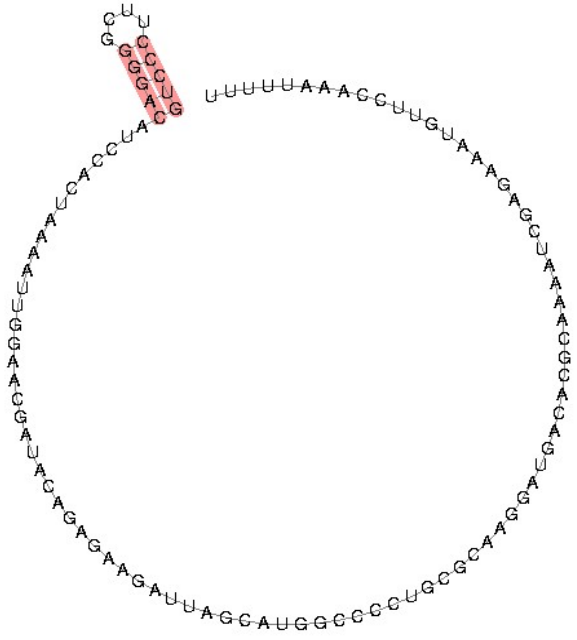
RNase MRP is an enzymatically active ribonucleoprotein with two distinct roles in eukaryotes. In mitochondria it plays a direct role in the initiation of mitochondrial DNA replication. In the nucleus it is involved in precursor rRNA processing, where it cleaves the internal transcribed spacer 1 between 18S and 5.8S rRNAs. Despite distinct functions, RNase MRP has been shown to be evolutionarily related to RNase P. Like eukaryotic RNase P, RNase MRP is not catalytically active without associated protein subunits.

tRNA-SeC



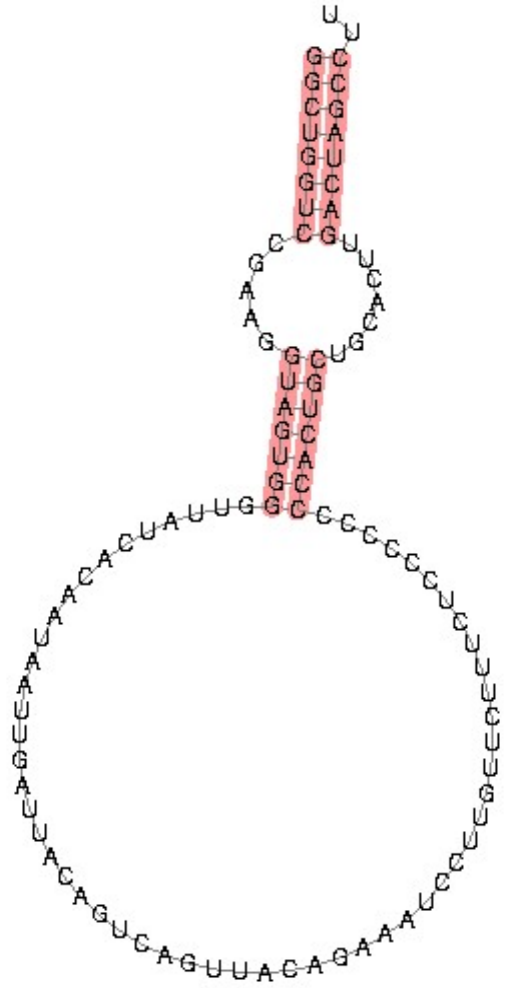
Like the other amino acids used by cells, selenocysteine has a specialized tRNA.

U6 snRNA



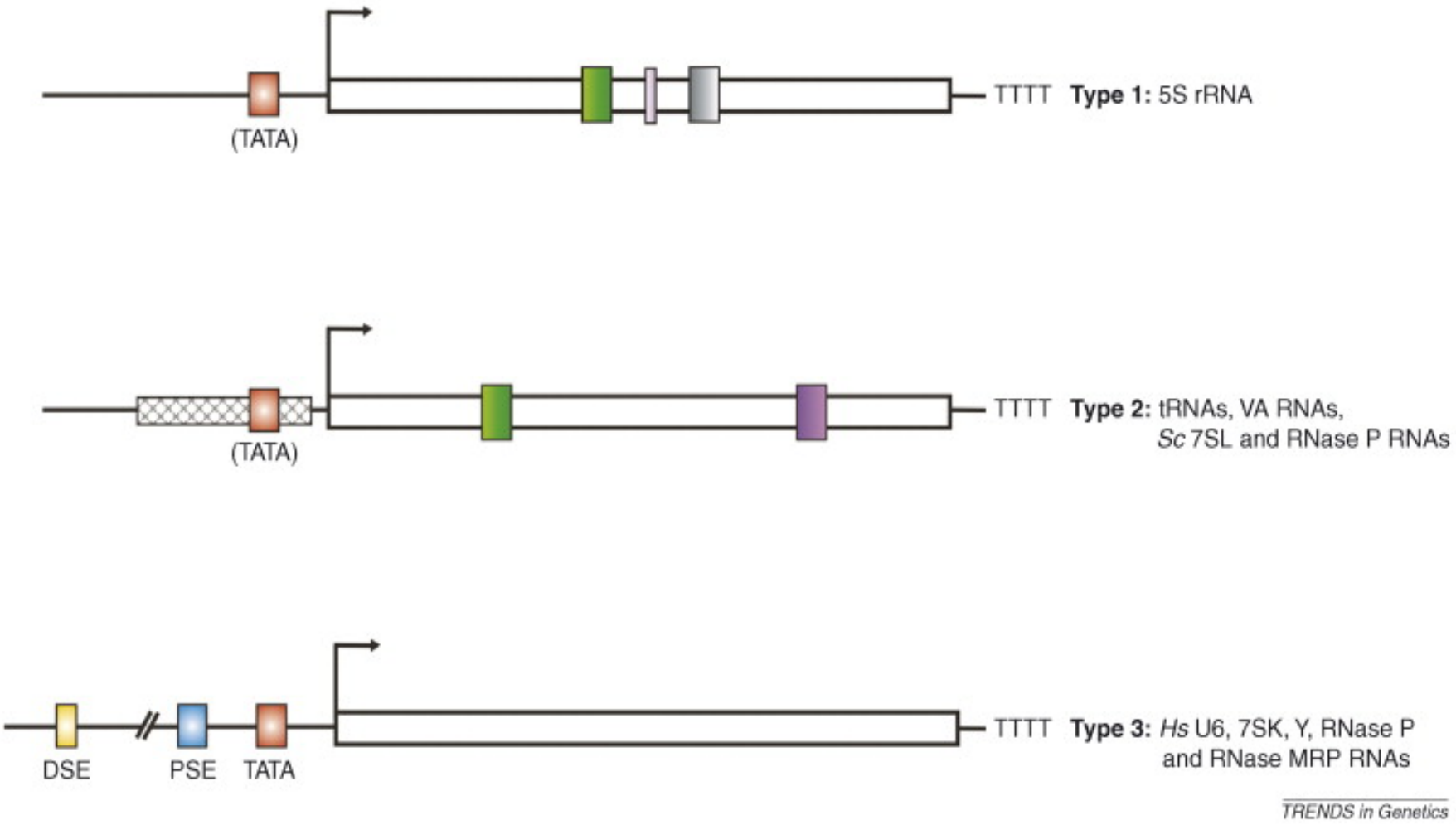
U6 snRNA is a non-coding RNA that is a component of the spliceosome which is involved in splicing pre-mRNA. The putative secondary structure consensus base pairing is confined to a short 5' stem-loop, but U6 snRNA is thought to form extensive base-pair interactions with U4 snRNA. There appear to be many copies of U6 derived pseudogenes or repeats in vertebrate genomes.

Y RNA



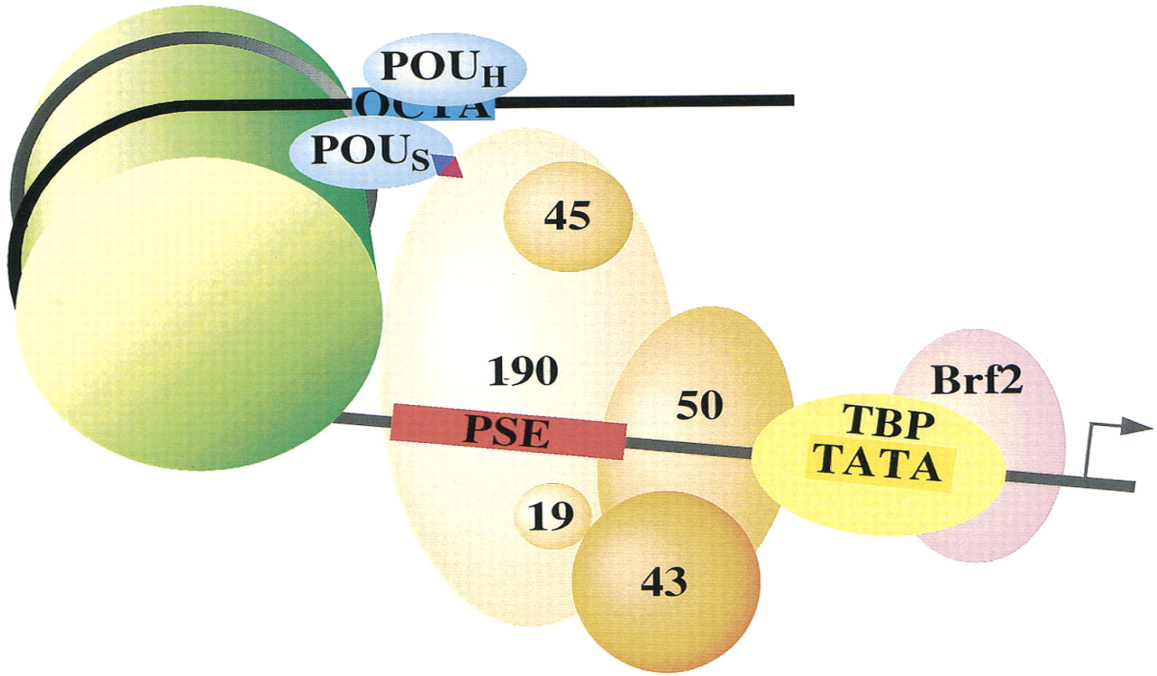
Two functions have been described for Y RNAs in the literature: In one line of evidence, Y RNA appears to function as a repressor of Ro. In its free state, Ro binds to a variety of misfolded RNAs including misfolded 5S rRNAs, and is thought to act as some sort of quality control mechanism. Secondly, it has been described recently that human Y RNAs are functionally required for DNA replication.

What do they have in common?



Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A. The expanding RNA polymerase III transcriptome. Trends Genet. 2007 Dec;23(12):614-22.

An example



Human U6 transcription initiation complex. TBP (yellow) and SNAPc (orange) bind cooperatively to the DNA, presumably through a direct protein–protein contact that involves a 50-amino-acid segment within the N-terminal region of SNAP190. TBP also binds cooperatively with Brf2. SNAPc and the Oct-1 POU domain (blue) bind cooperatively to DNA, through a direct protein–protein contact involving a glutamic acid at position 7 within the POUS domain (blue triangle) and a lysine at position 900 within SNAP190 (red triangle). This direct protein–protein interaction is mediated by a positioned nucleosome (green) that brings into close proximity the octamer sequence and the PSE.

Schramm L, Hernandez N. Recruitment of RNA polymerase III to its target promoters.Genes Dev. 2002 Oct 15;16(20):2593-620.

The Idea

Are there more Pol III transcripts with external promoters than the already known snRNAs?

Has never ever anyone done something like this?

- Myslinski E, Gérard MA, Krol A, Carbon P. **A genome scale location analysis of human Staf/ZNF143-binding sites suggests a widespread role for human Staf/ZNF143 in mammalian promoters.** J Biol Chem. 2006;281(52):39953-62.

- Pagano A, Castelnuovo M, Tortelli F, Ferrari R, Dieci G, Cancedda R. **New small nuclear RNA gene-like transcriptional units as sources of regulatory transcripts**. PLoS Genet. 2007 Feb 2;3(2):e1.

Here is the master plan ...

- Get the exact location of known Pol III transcript with external promoter elements in the genome (Literature, homology search, available annotation)
- Extract up to 400 nt of the upstream sequence
- Annotate and extract TATA-Box, PSE, DSE
- Build a `FRAGREP` model
- Use `FRAGREP` to search the genome
- Statistical analysis of the putative promoter regions (R project)
- Examine evolutionary conservation of the putative promoter region

Don't trust annotations!

- There are many *pseudogenes* out there.
- Compare the flanking regions (are the same genes upstream and downstream compared to other species)
- PSE and TATA are a very good sign!

Beyond the scope of this course ...

- Correlate putative promoters with expression data

- Machine learning approaches to narrow the number of putative promoters

- Examination of the possible transcripts