

Ribo-seq

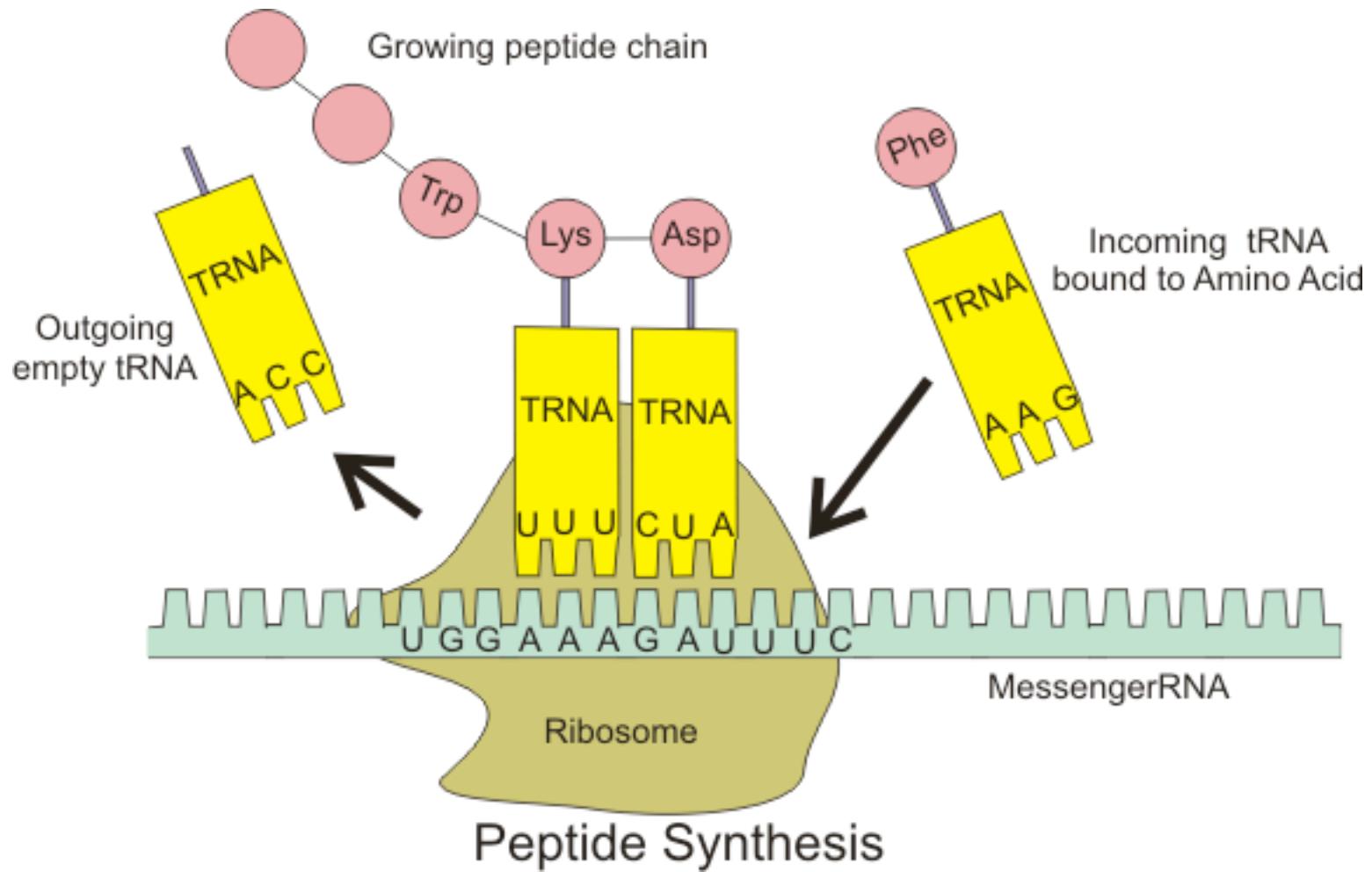
What is being translated
in the cell?

"Words
travel
worlds.
Translators
do the
driving."

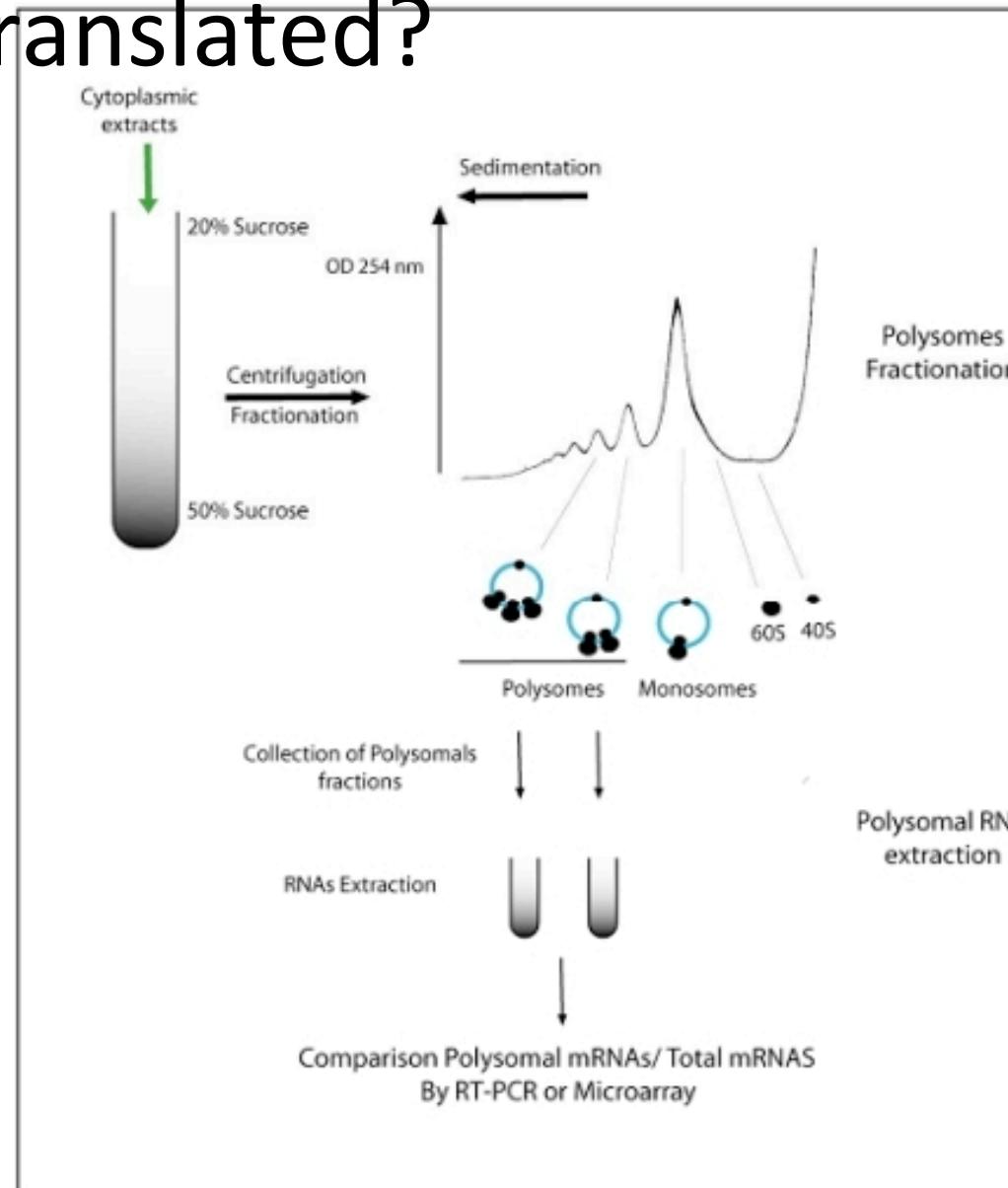
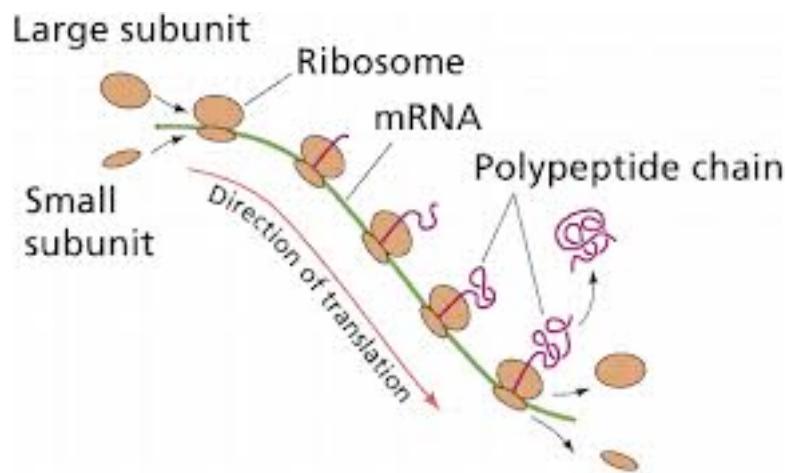
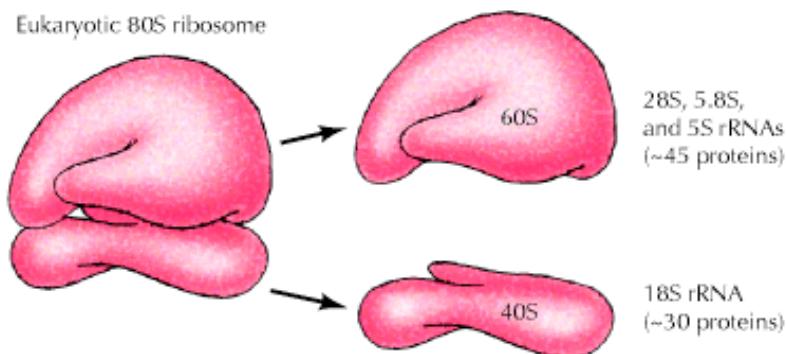
Anna Rusconi, translator.

www.proz.com
the translation workplace

What translation looks like

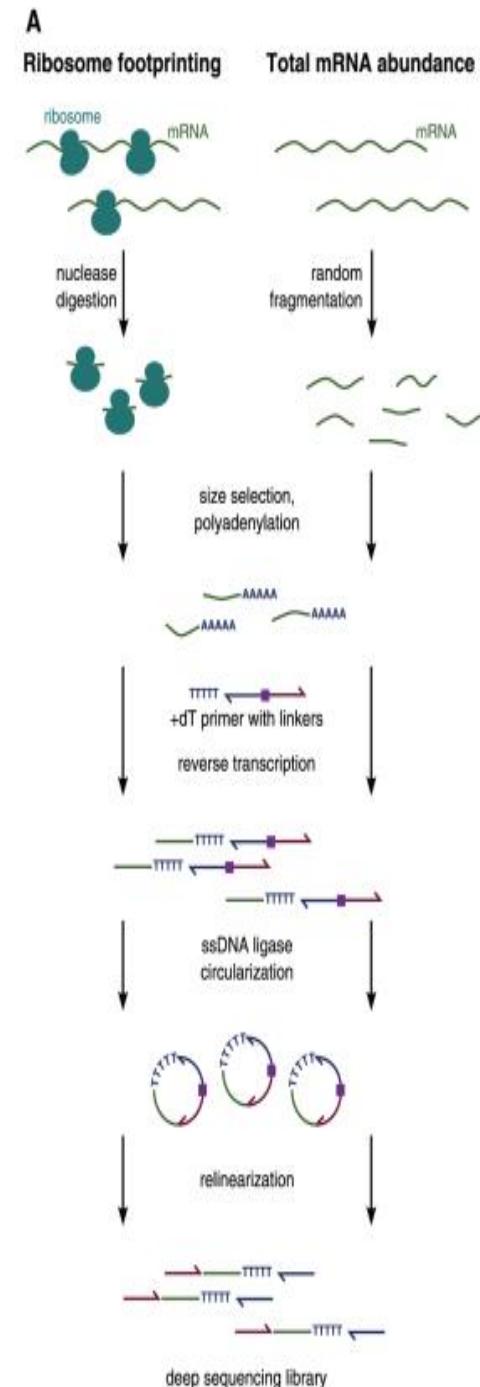


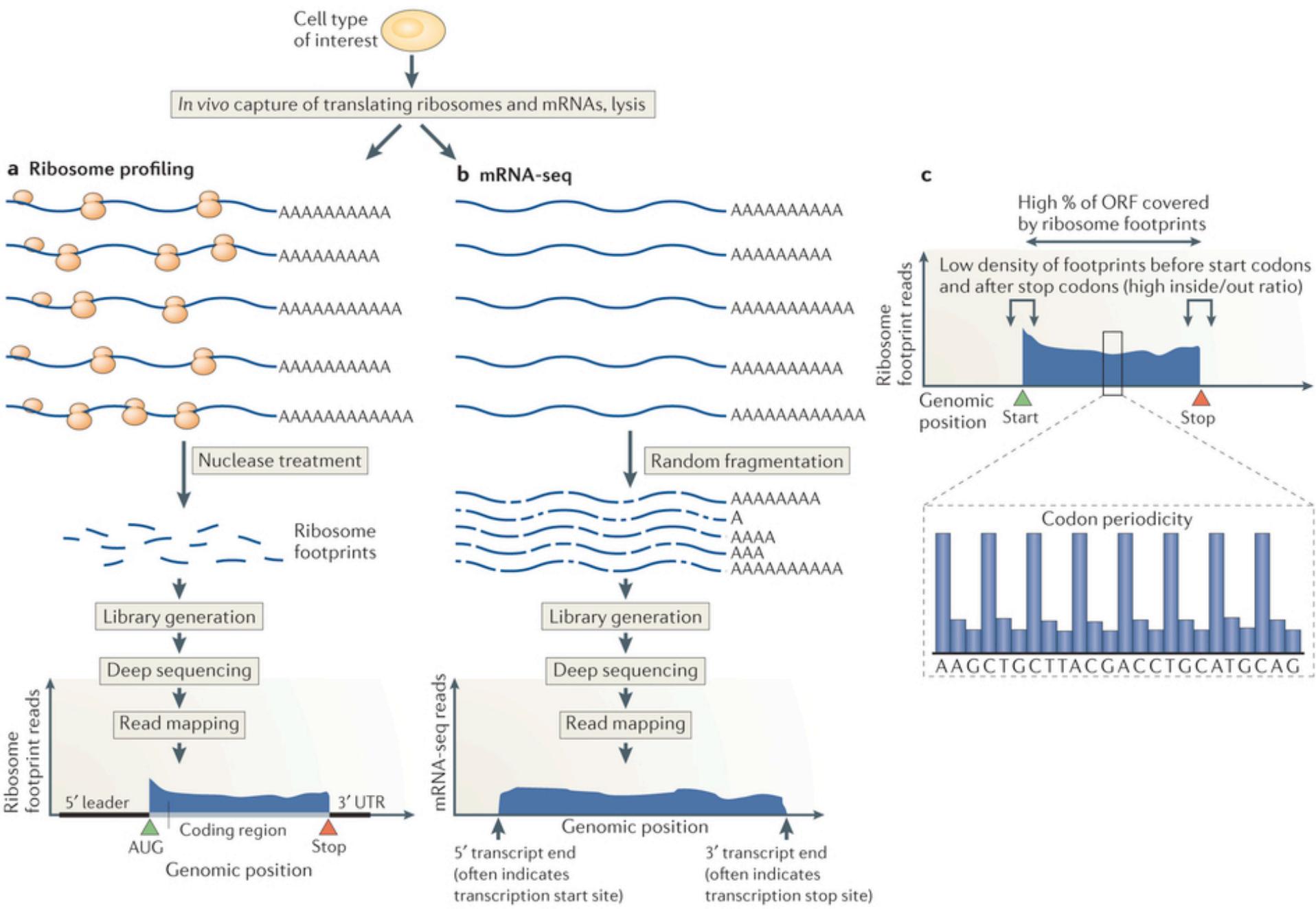
How can you tell how much RNA is being translated?



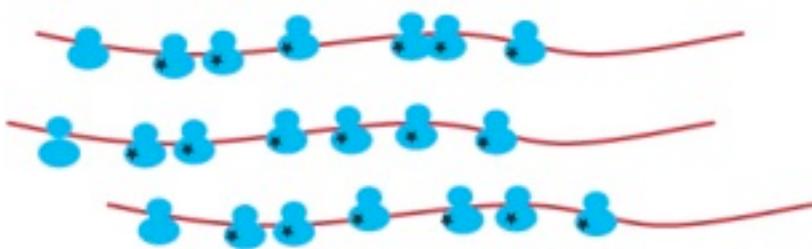
Ribo-seq

- Purpose:
 - mRNA level is not equivalent to protein level
 - proteins are hard to measure
- Goal:
 - Finding translation levels for all genes
 - Finding all translated genes



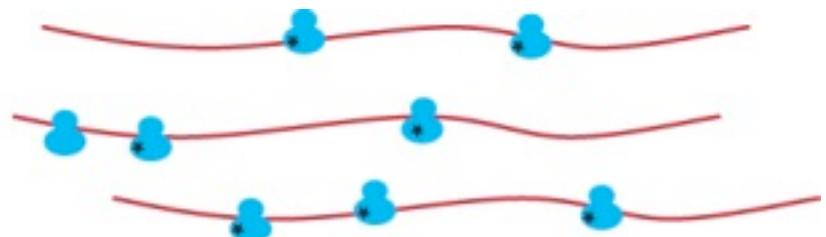


Translation Efficiency



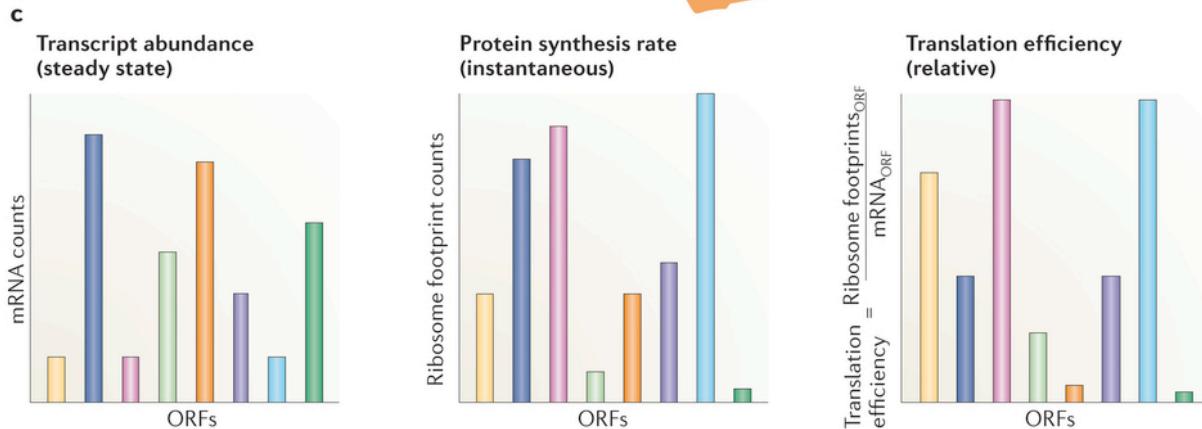
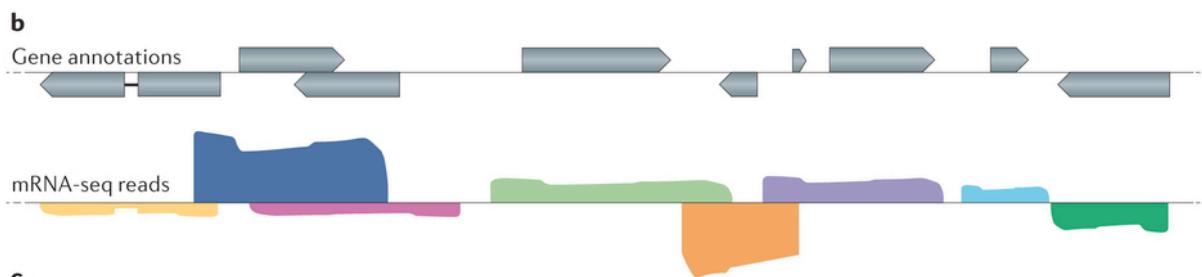
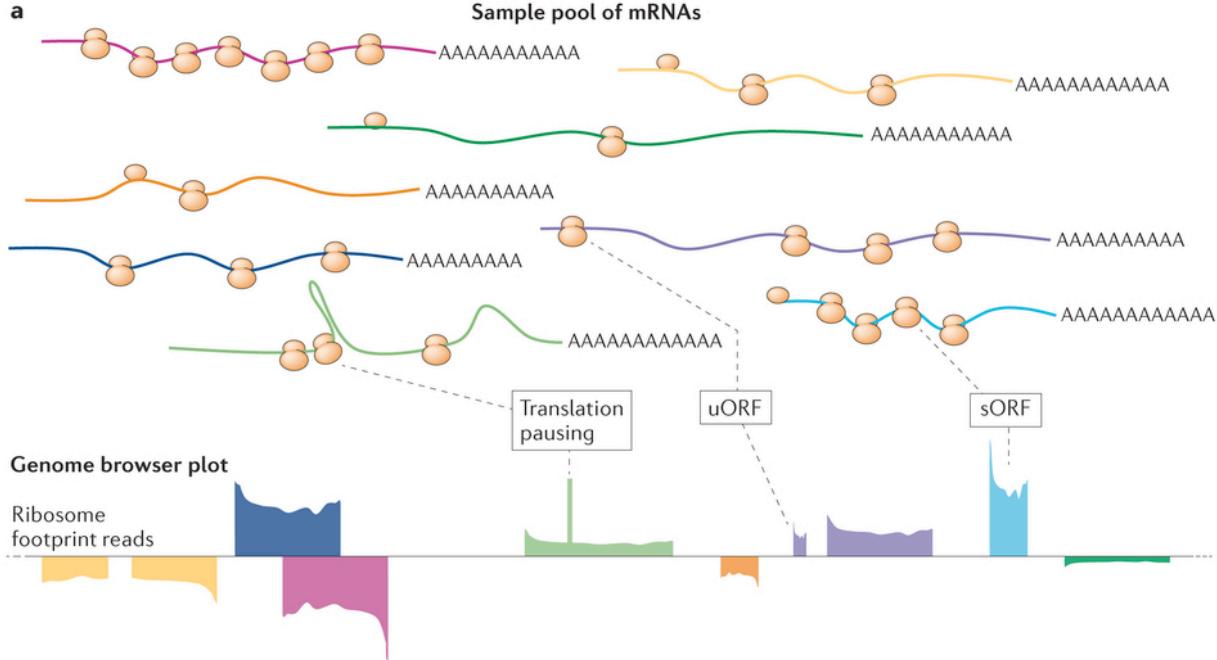
High

$$\text{Translation efficiency} = \frac{\text{# footprints}}{\text{# mRNA}}$$

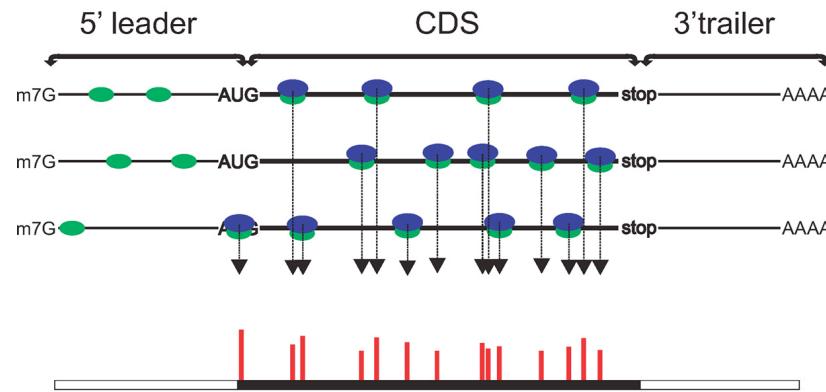


Low

$$\text{Translation efficiency} = \frac{\text{# footprints}}{\text{# mRNA}}$$

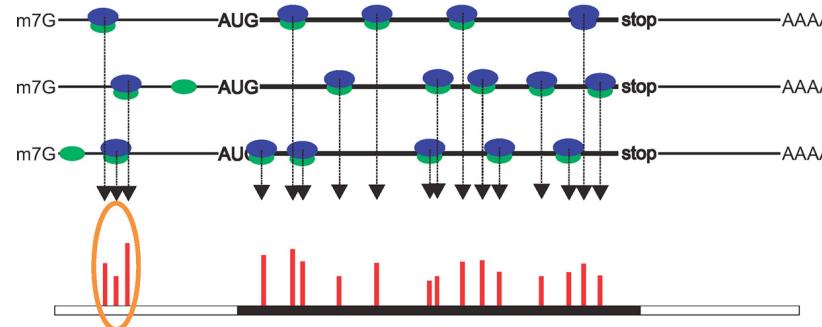


Expected

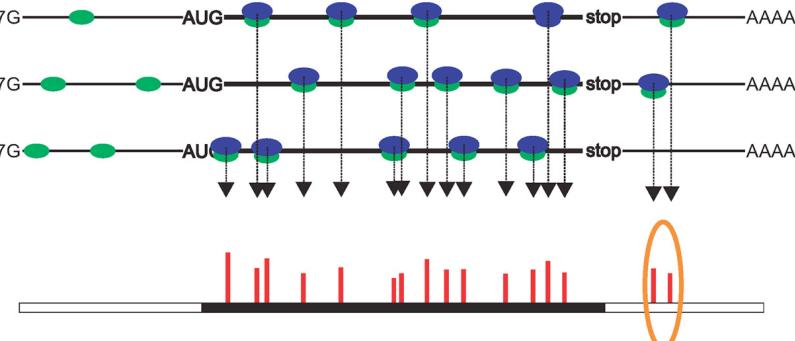


A Changes in translation efficiency

B Translation in 5'leader

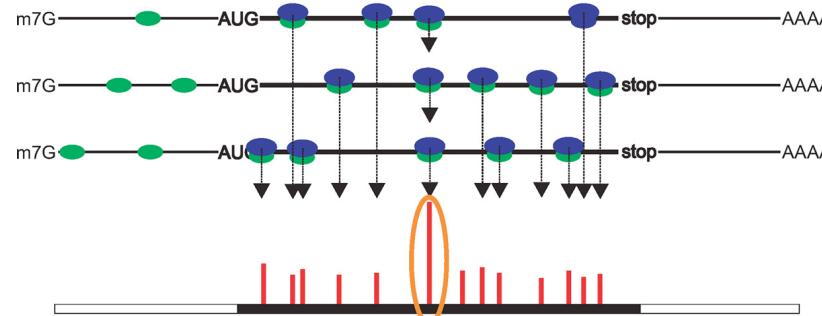


C Translation in 3'trailer

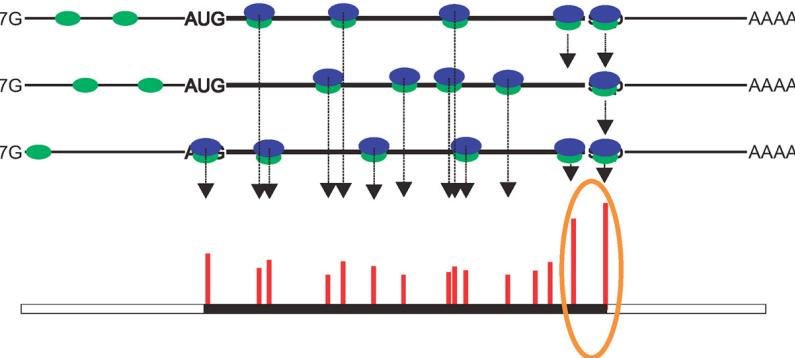


Interesting!

D Site-specific pause



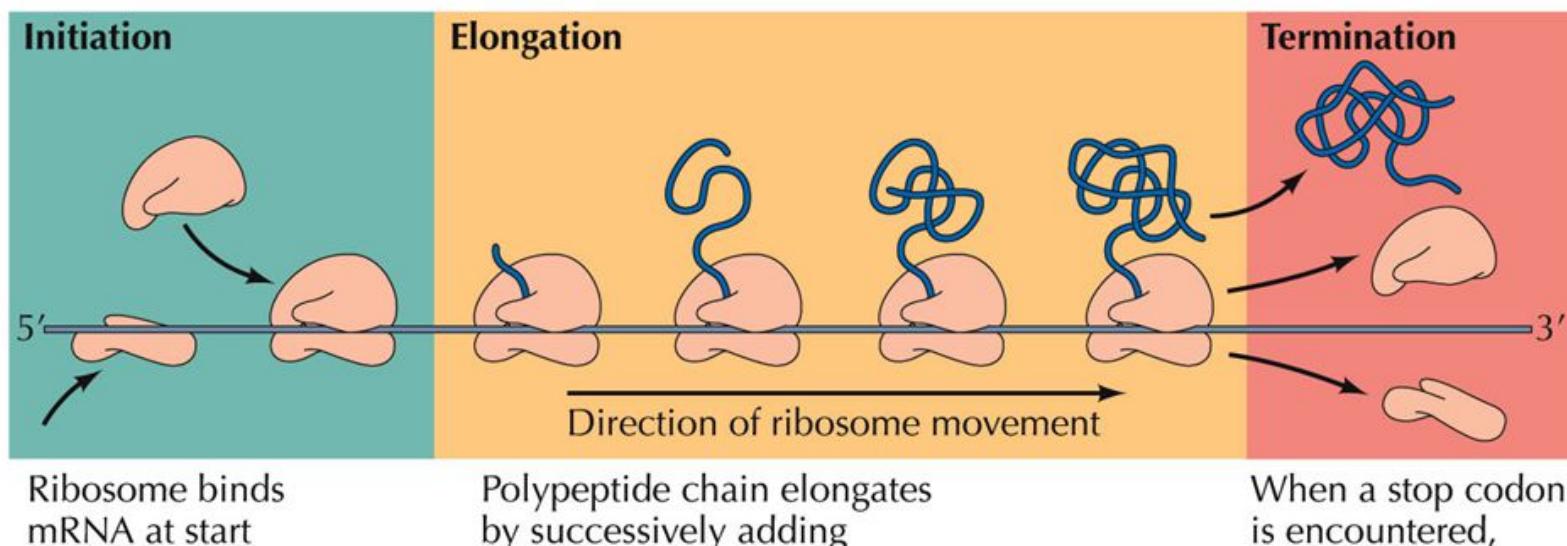
E Queuing at stop codon



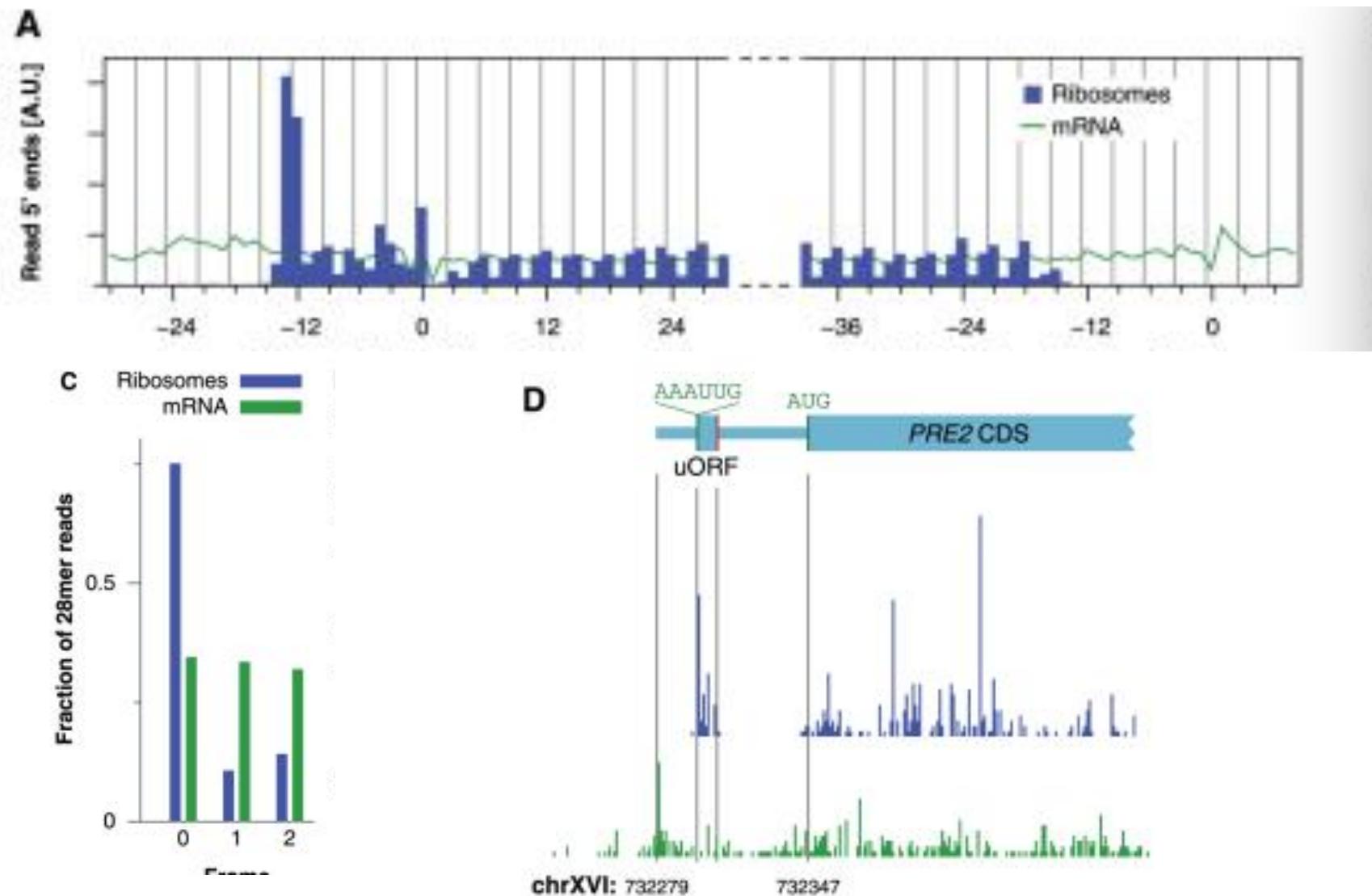
Three Phases of Translation

- **Initiation:** a ribosome binds to the mRNA strand at a start codon
- **Elongation:** tRNA carries the corresponding amino-acid to the ribosome
- **Termination:** ribosome releases amino-acid chain that is then folded into an active protein

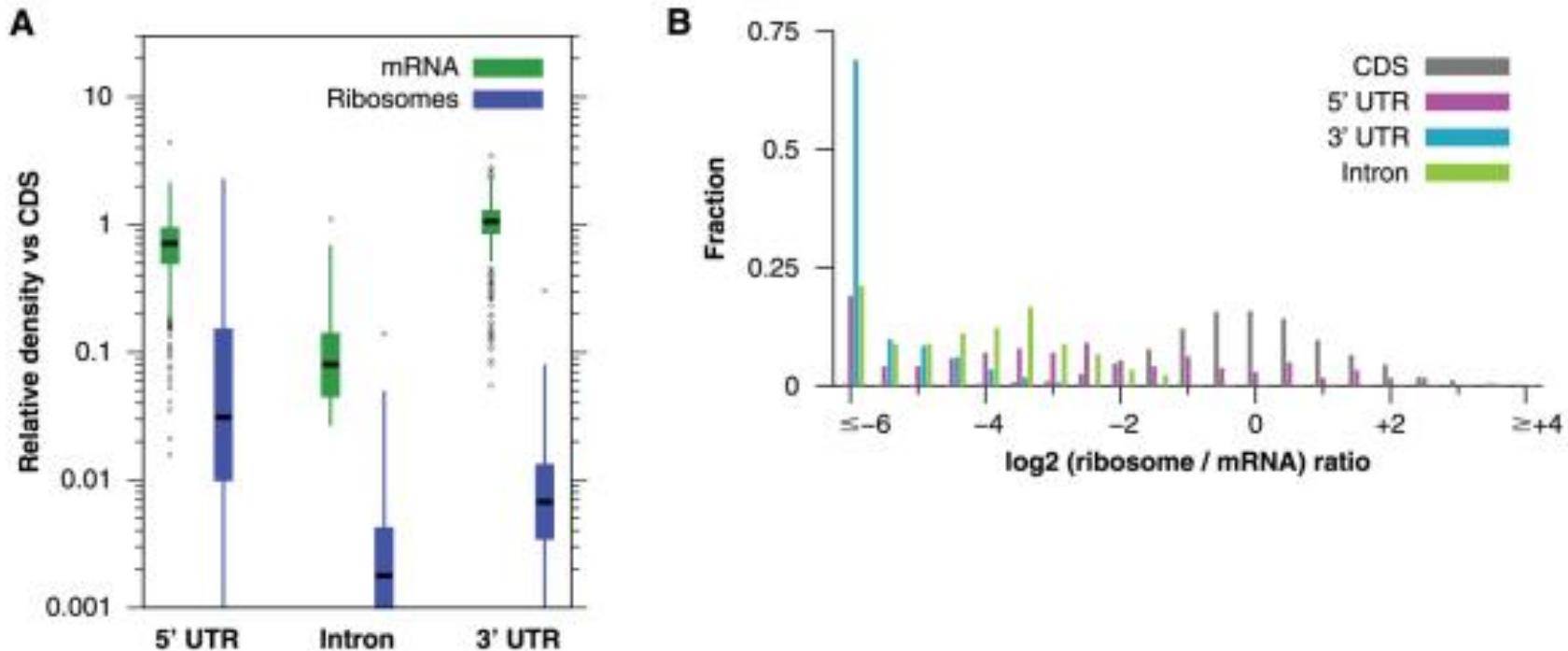
Large ribosomal unit joins, forming functional ribosome.



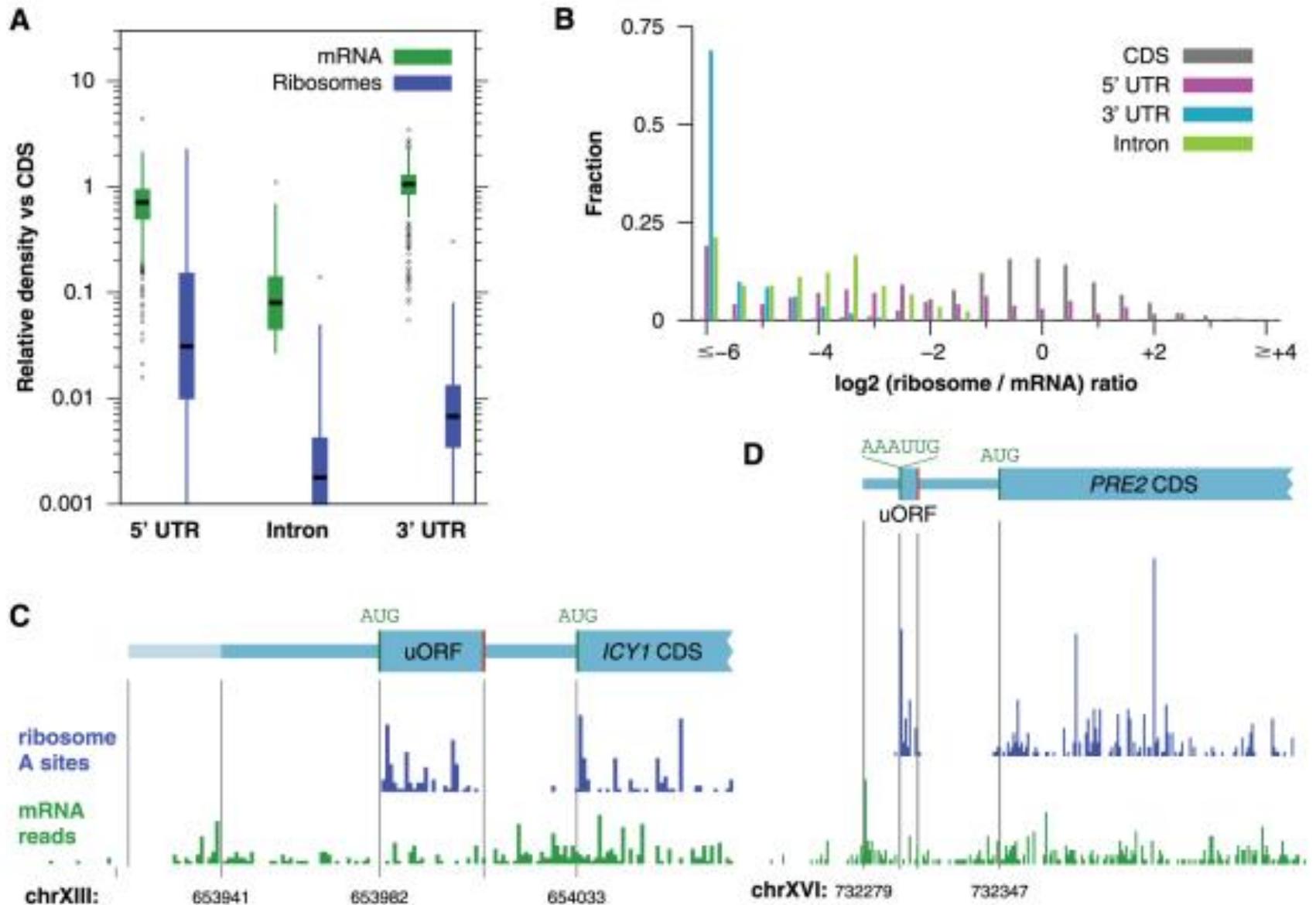
Ribo-seq, the gene and the meta-gene



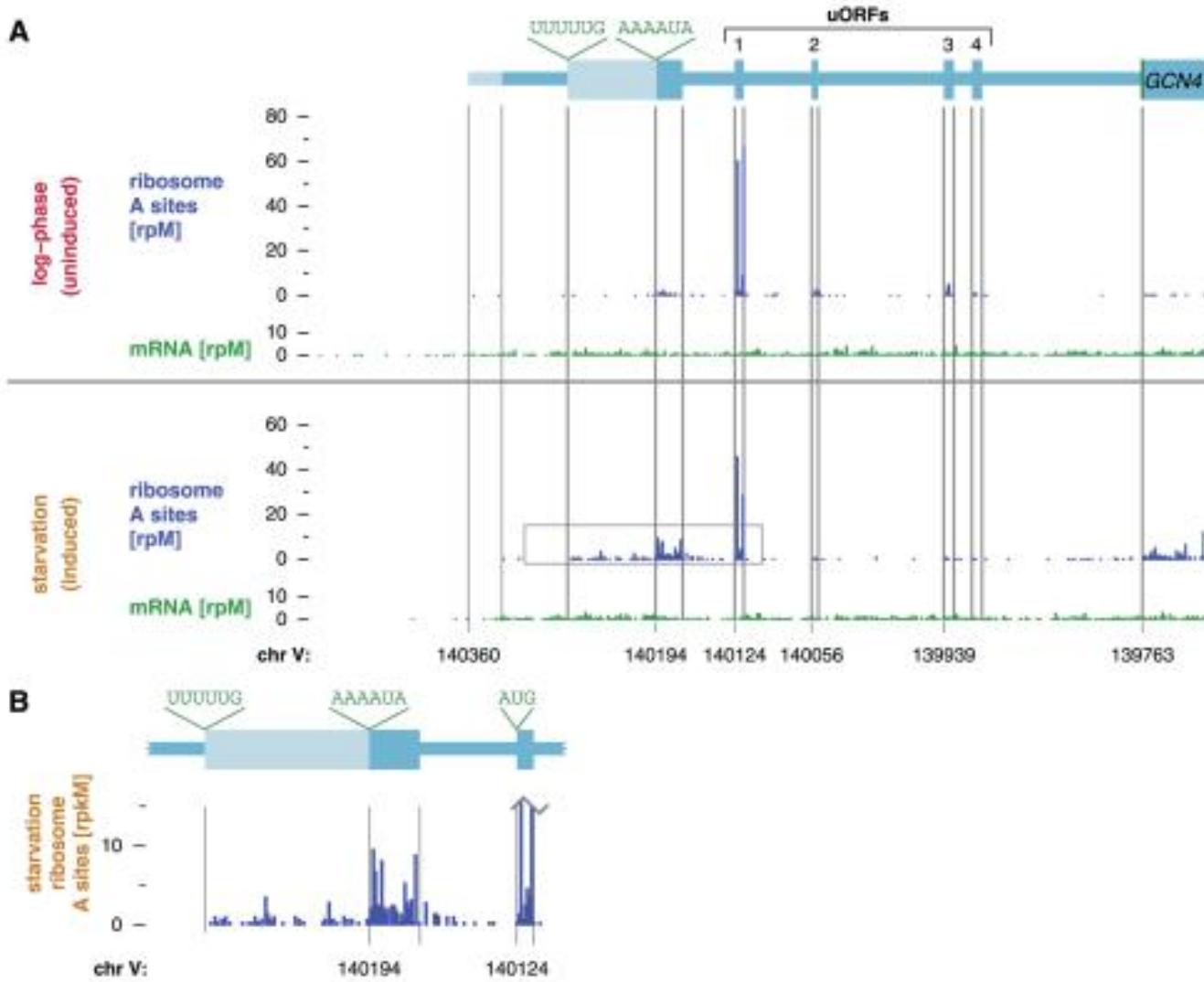
Interesting... uORFs

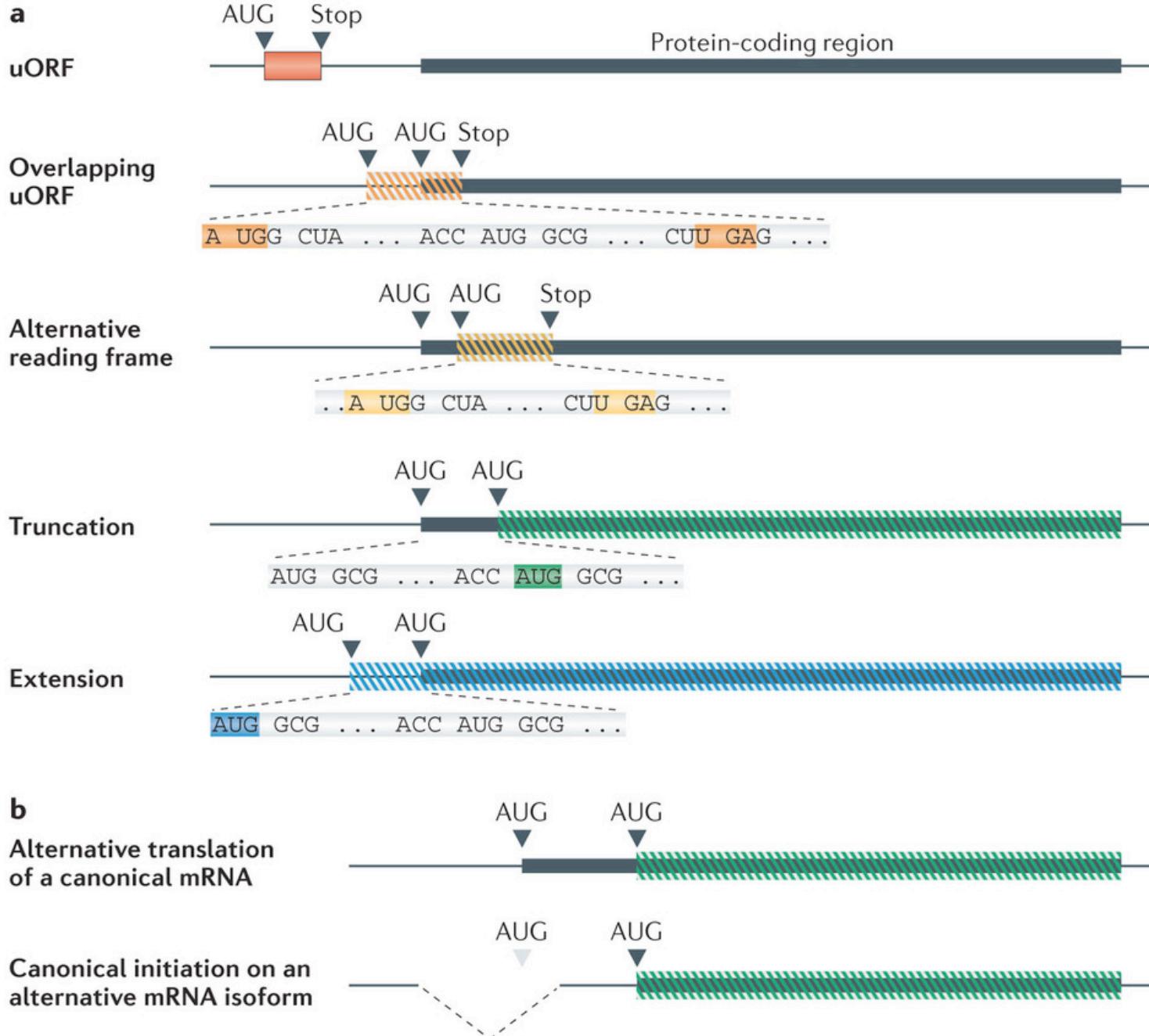


Interesting... uORFs

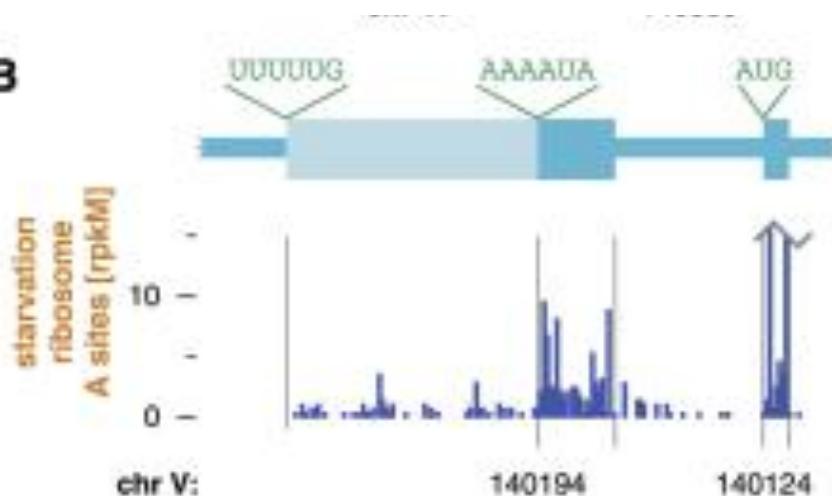
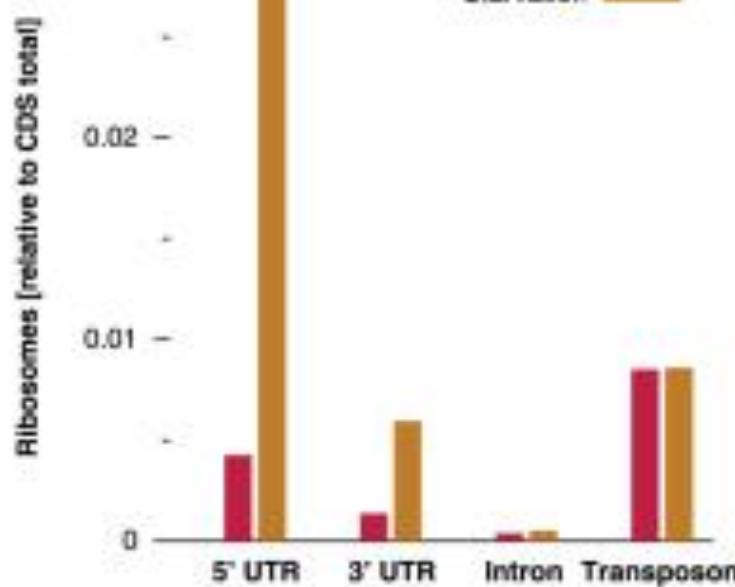
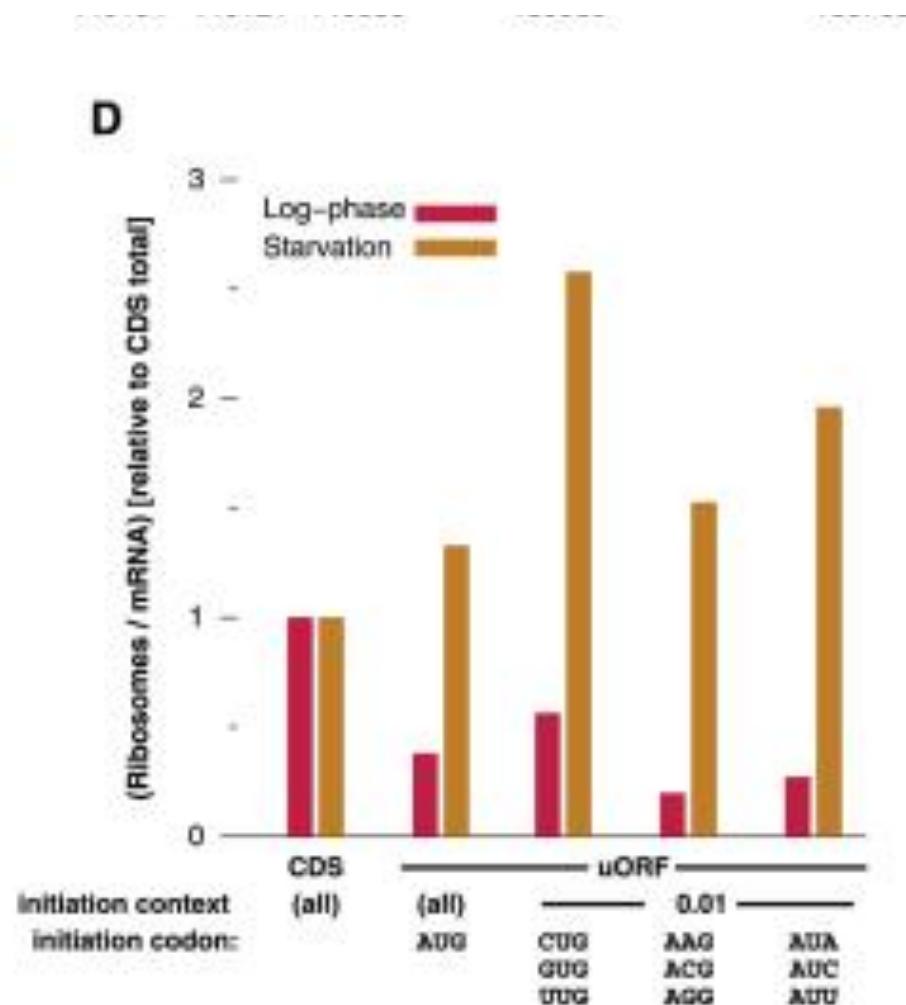


Non-canonical start

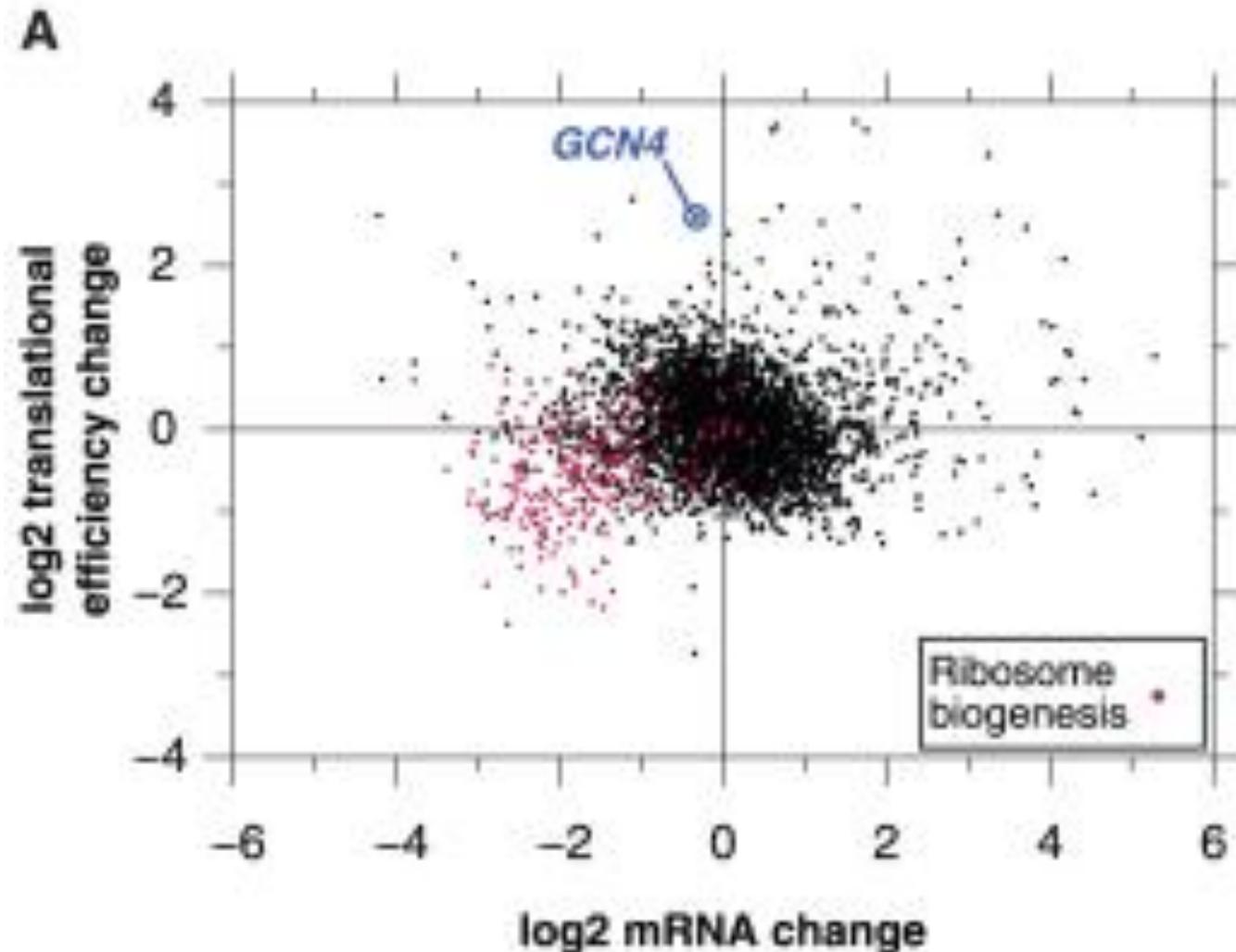




Non-canonical starts in uORFs

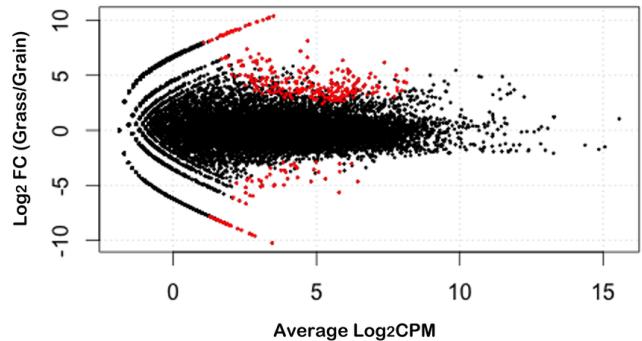
B**C****D**

Using Ribo-seq to determine which genes are most translated after perturbation

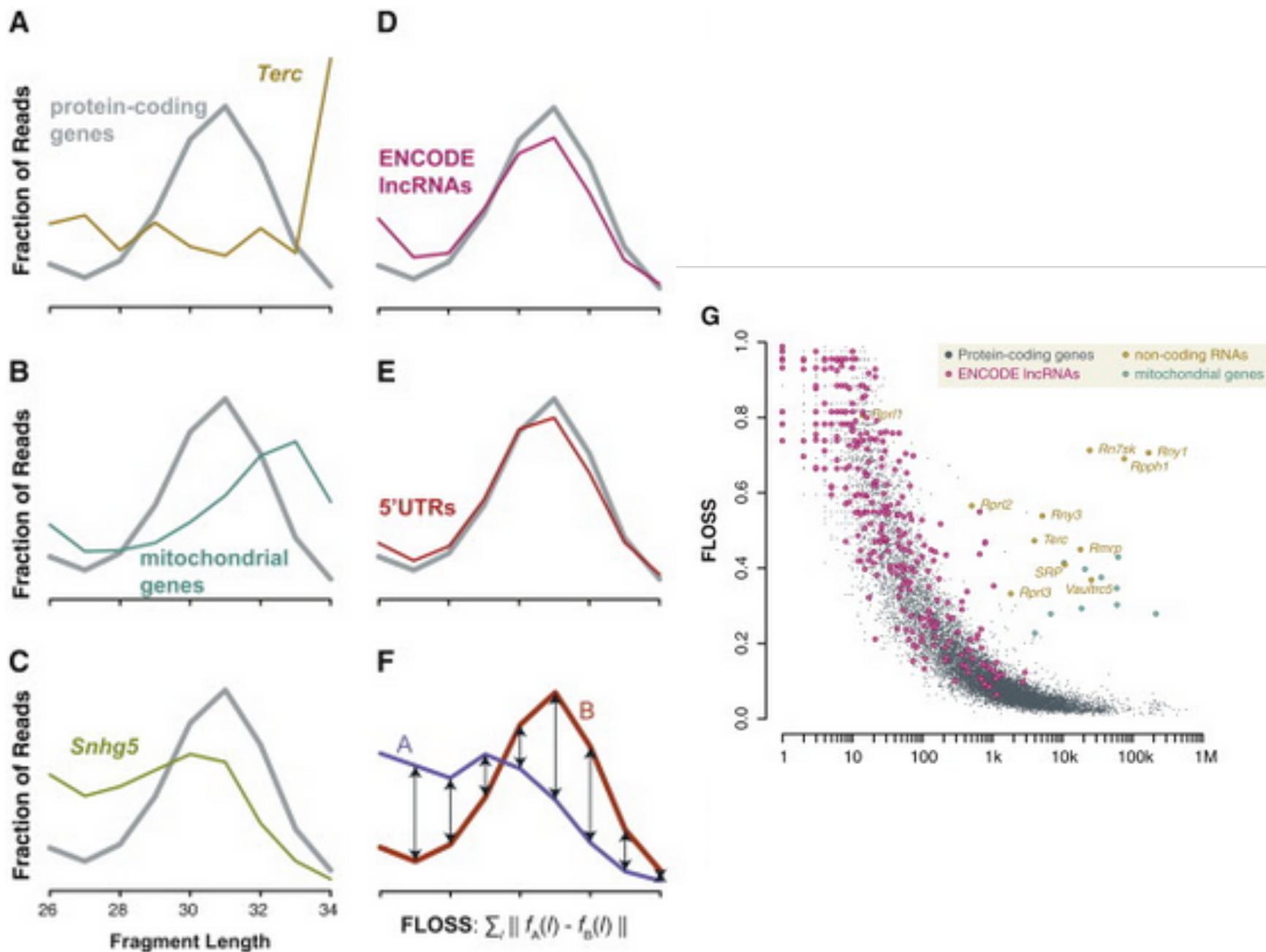


How do you know if your RNA (ORF) is being translated?

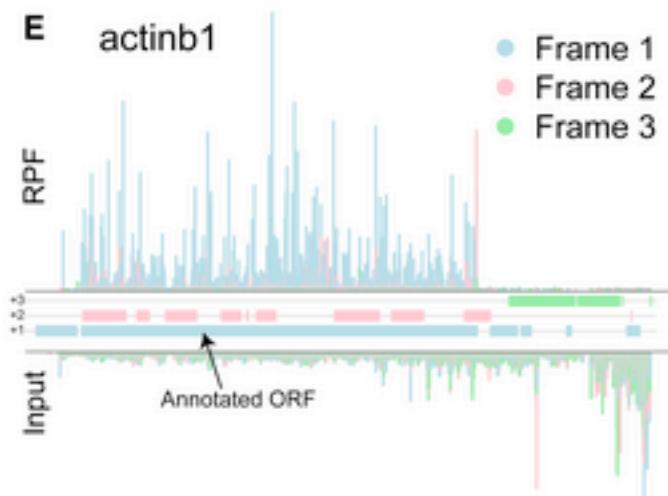
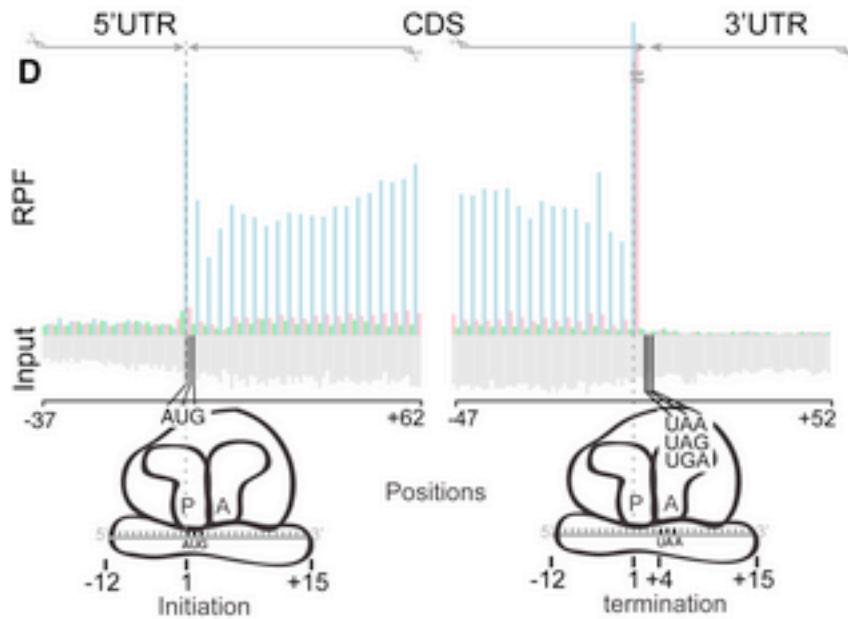
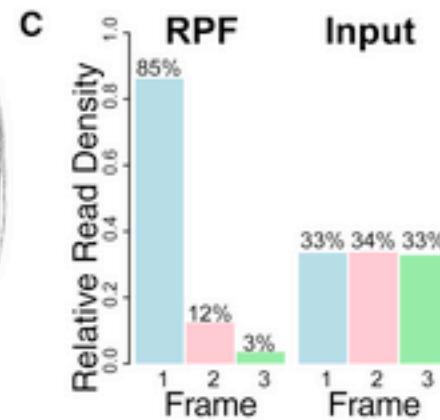
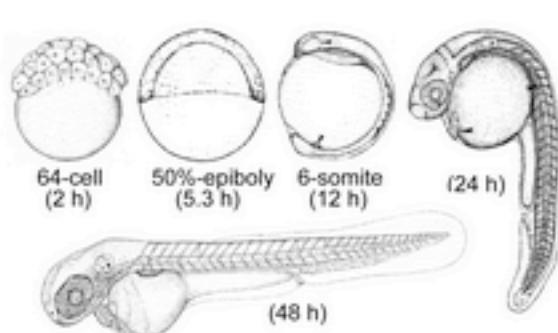
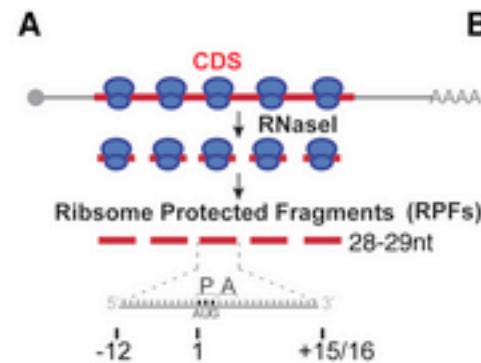
- Counts
 - Remember, fold change is bad!!!
 - But maybe differential expression is good enough?
 - » Lots of False positives!
- Score your ribo-seq differently
 - FLOSS
 - » fragment length organization similarity score
 - ORFscore
 - » Use the 3 nt pattern
- Check if the protein exists in databases
- Conservation of the ORF



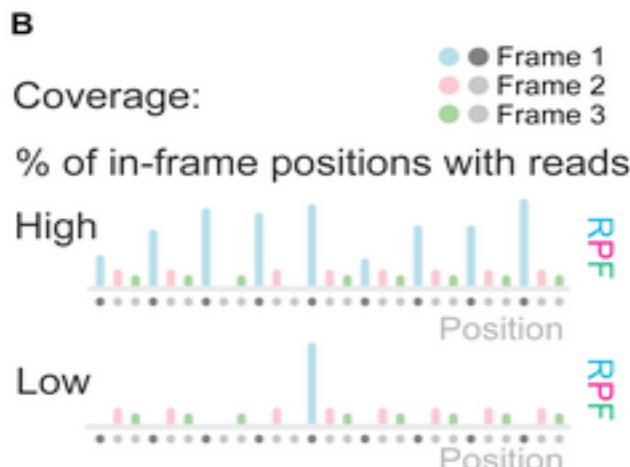
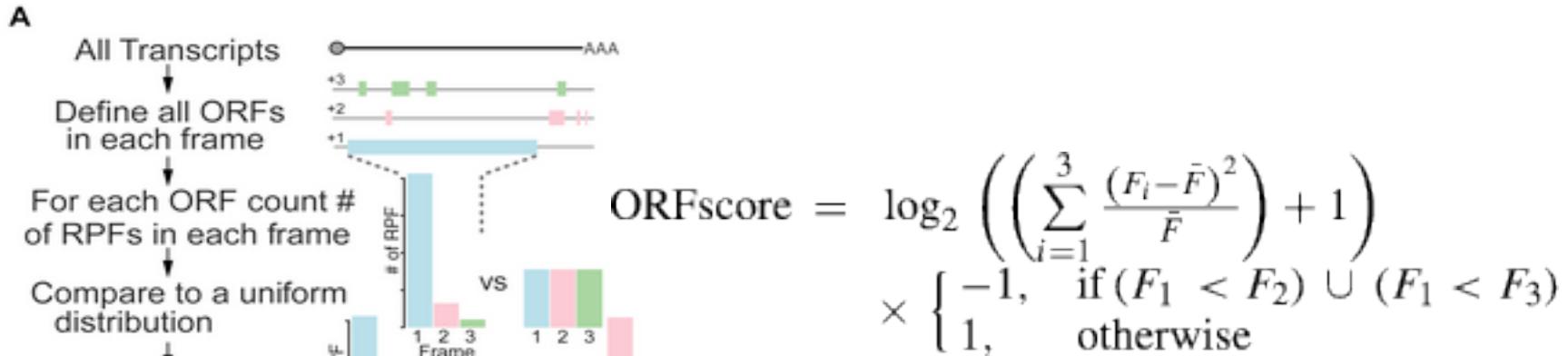
FLOSS-fragment length organization similarity score



ORFsco

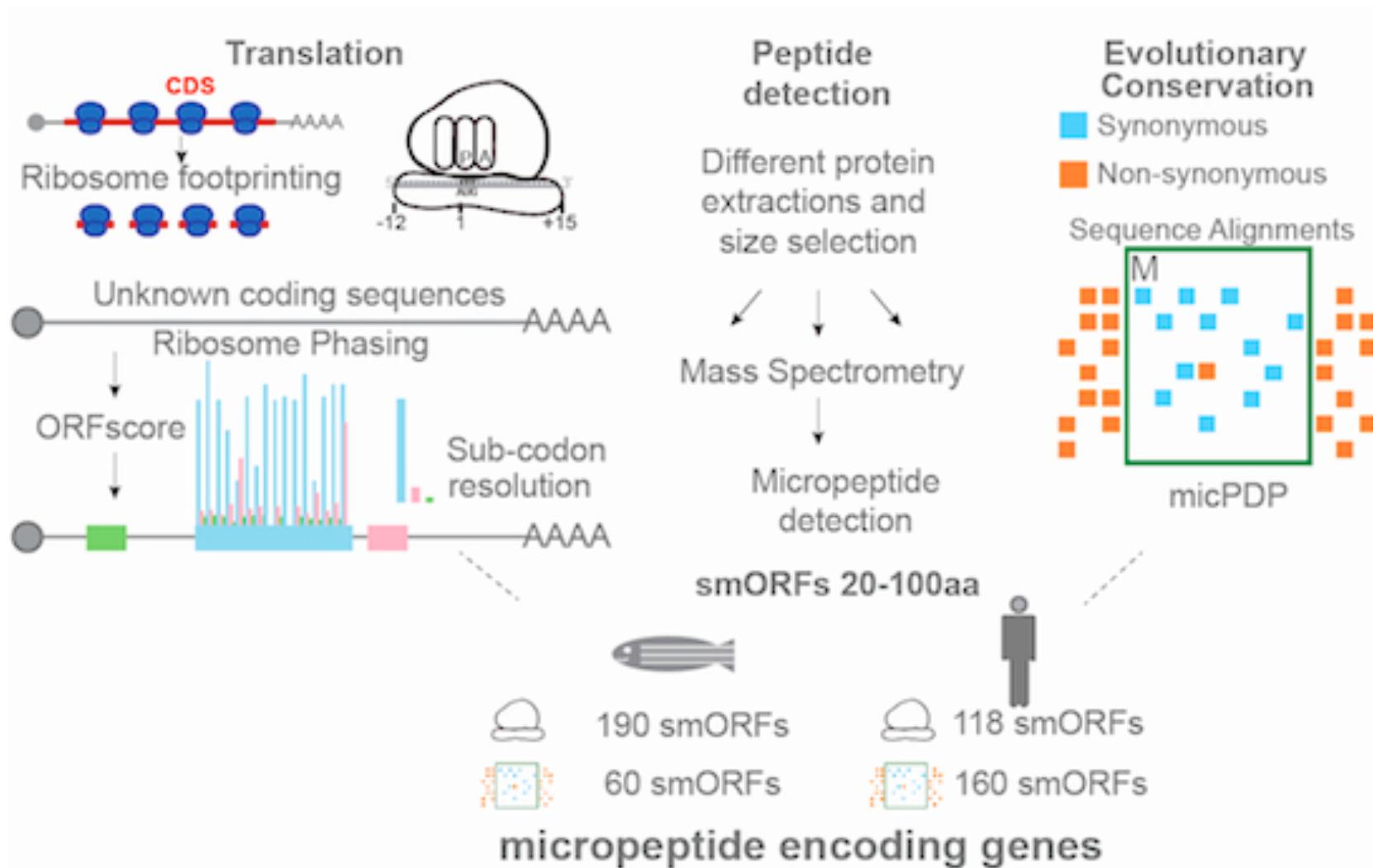


Calculating a ORFscore (are the frames uniform?)

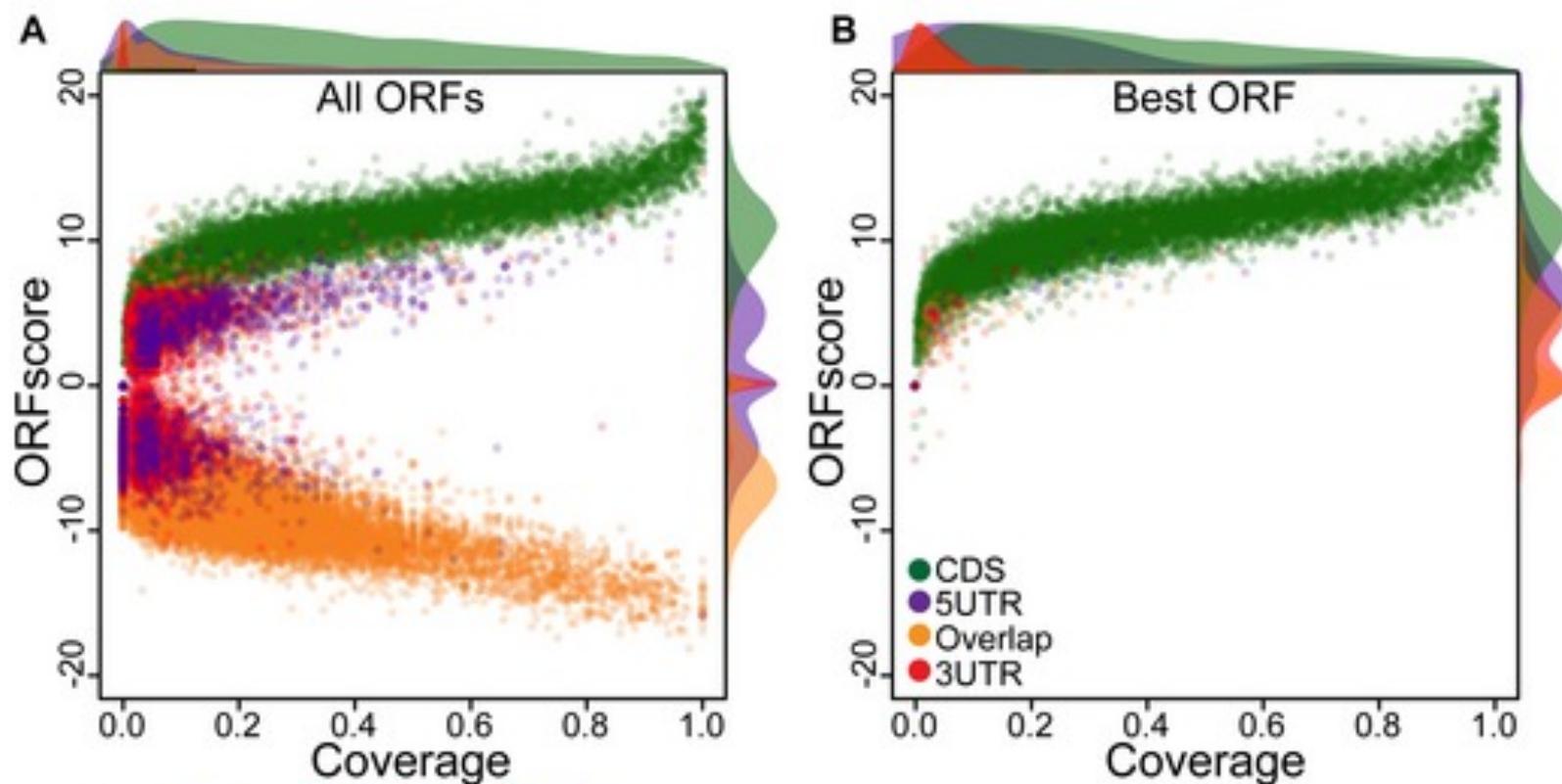


if uniform ORFscore = 0
else ORFscore
frame 1 ORFscore > 0
frame 2 or 3 ORFscore < 0

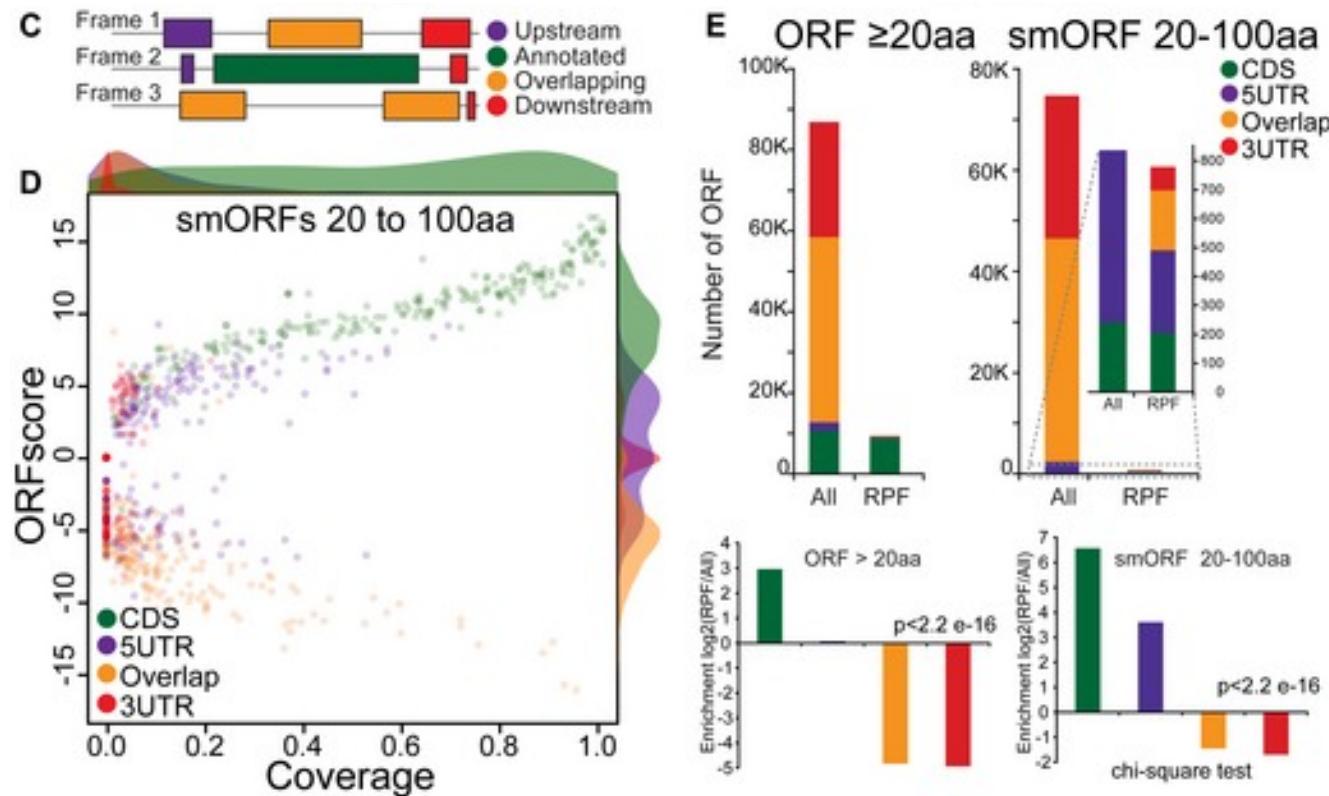
ORFscore can be combined with other evidence



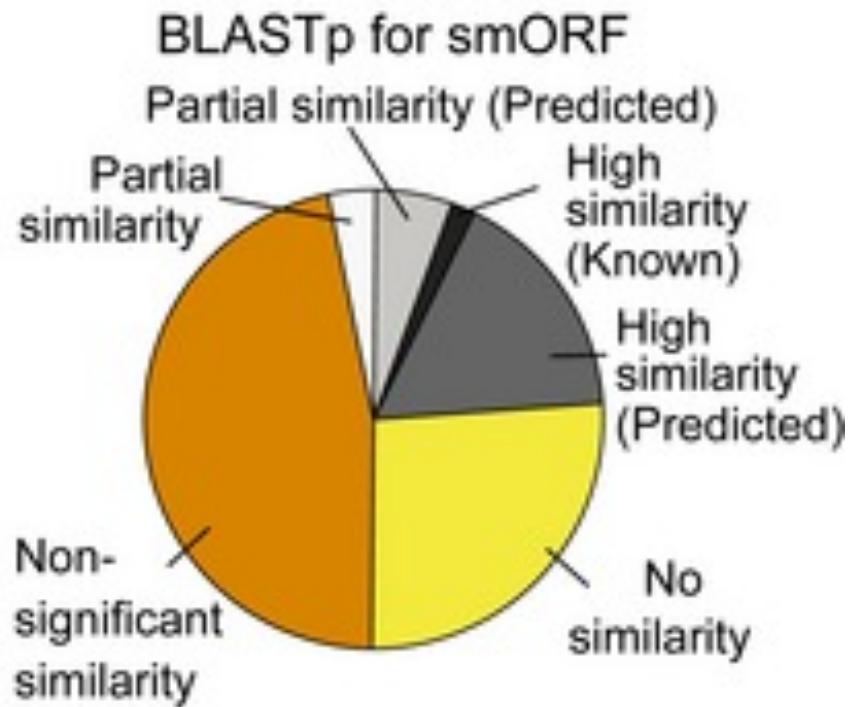
ORFscores mostly find known annotated genes



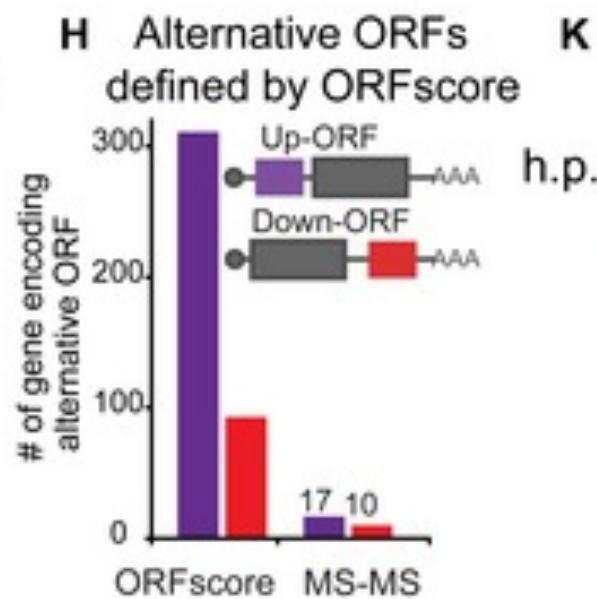
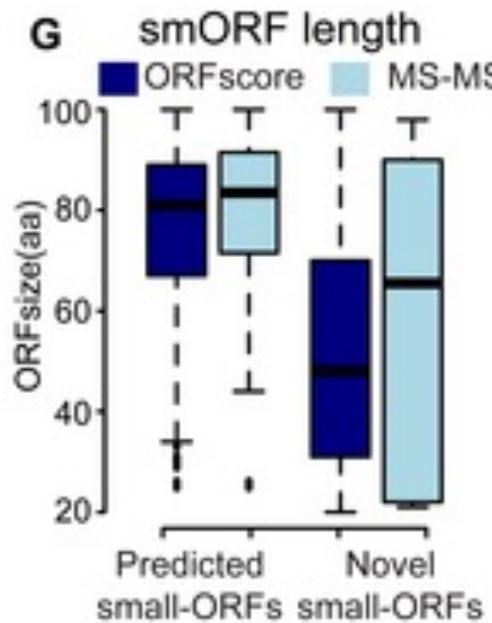
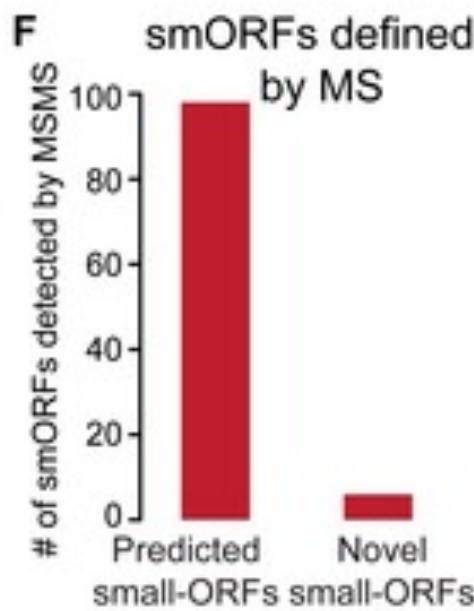
ORFscores mostly find known annotated genes



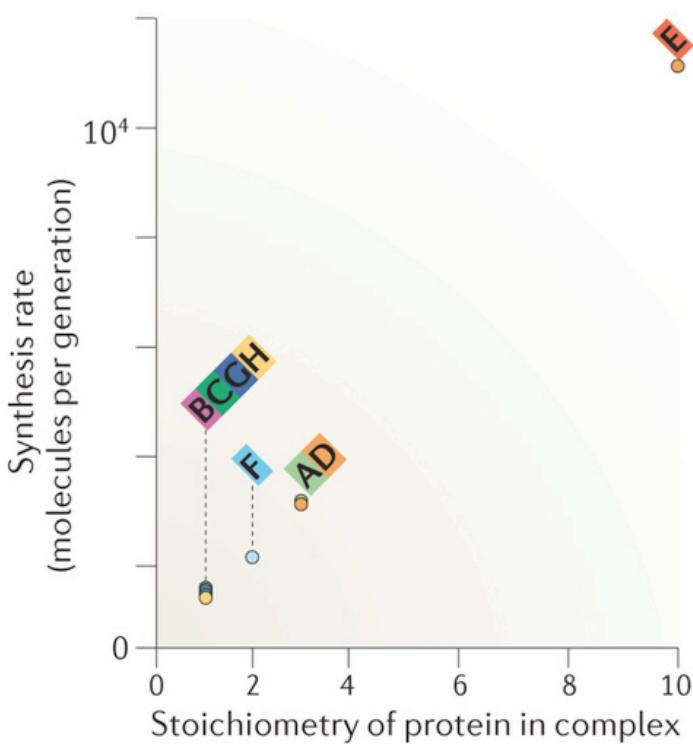
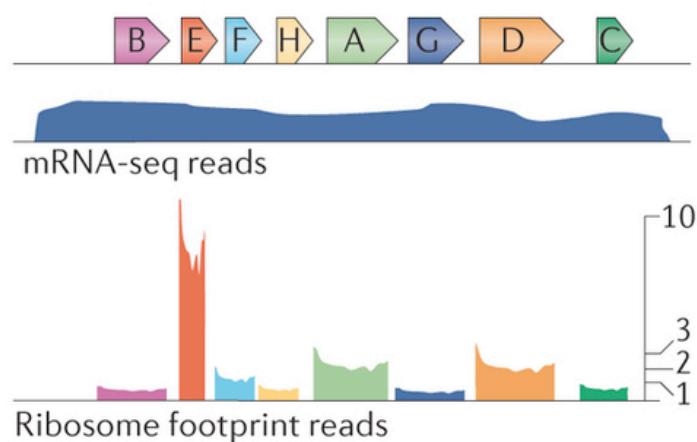
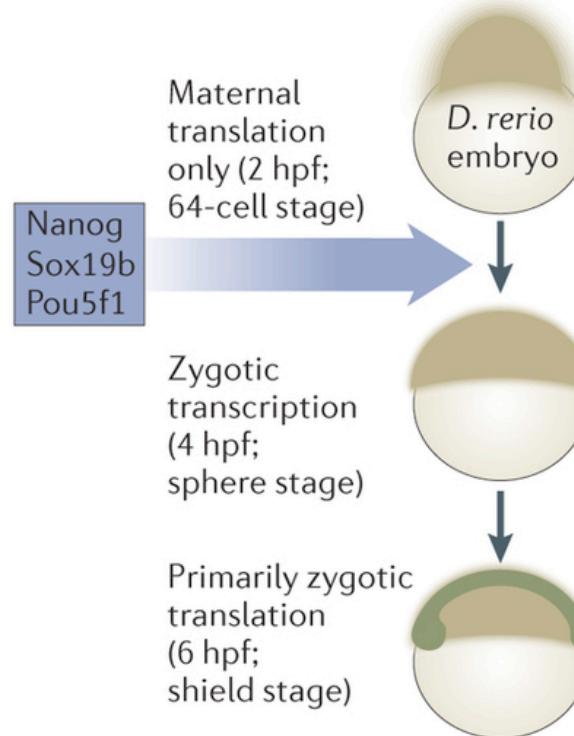
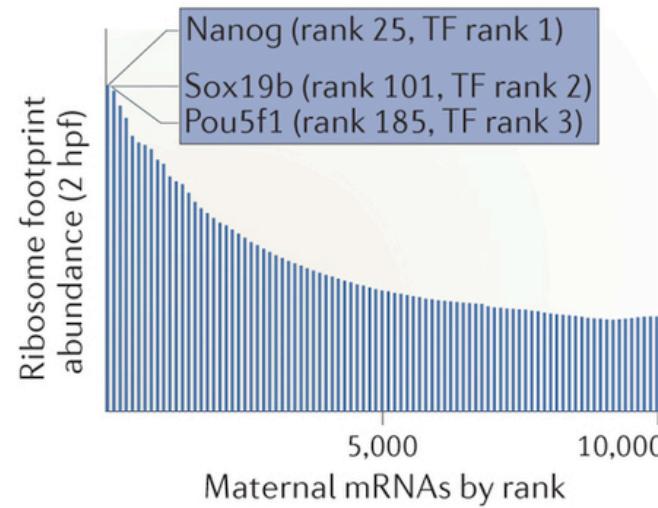
Confirm small ORFs by conservation



Confirm small ORFs by protein



What can we learn from
Ribo-seq?

a *E. coli* F_oF₁-ATP synthase operon**b *D. rerio* zygotic development**

Check this out!

The screenshot shows a web browser window with the URL <https://gwips.ucc.ie> in the address bar. The page title is "GWIPS-viz". The main content area features the text "Genome Wide Information on Protein Synthesis" and a navigation menu with links to "Genomes", "Table Browser", "Downloads", "Session", "Forum", and "Help". On the left, a sidebar lists links to "Genome Browser", "Table Browser", "RiboSeq.Org", "RiboGalaxy", and "Downloads". The "About GWIPS-viz" section describes the tool's purpose and its base on the UCSC Genome Browser. The "News" section contains three entries: one for October 2016 about RUST profiles, one for May 2016 about strand orientation support, and one for June 2015 about the hg38 global ribosome profiling track.

Genomes · Table Browser · Downloads · Session · Forum · Help

About GWIPS-viz

GWIPS-viz aims to provide on-line tools for the analysis and visualization of ribo-seq data obtained with the ribosome profiling technique, see [Ingolia et al \(2009\) Science](#).

GWIPS-viz is based on the [UCSC Genome Browser](#), developed by the Genome Informatics Group, Center for Biomolecular Science and Engineering, University of California, Santa Cruz.

News

Oct 2016
[RUST profiles](#) to check the quality of RiboSeq datasets are now available for many studies in GWIPS-viz. Just click on the individual study link in the *Ribosome Profiles - Elongating Ribosomes (A-site)* track group and then click on the *View RUST plots* button at the top left-hand side of the page. See [O'Connor et al. \(2016\)](#) for full details on RUST.

May 2016
The GWIPS-viz browser now supports strand orientation for bacterial studies.
Additionally we have applied a 3' prime offset to determine the A-site as mentioned in [Woolstenhulme et al. 2015](#).

June 2015
The GWIPS-viz hg38 global ribosome profiling track is now available on the [UCSC Genome Browser](#). Select *Full* view to see the **GWIPS-viz RiboSeq** track which is available under the *Expression* group.

