Comparing RNA & Protein Abundance

M Gerstein
&
P Emani

Outline: Comparing Protein & RNA Abundance

- **Past** Context:
  to work in the Center
  - Quantifying the moderate **statistical correlation between protein & RNA**
  - PARE server
- **EMpire** (Current result)
  - Leveraging the correlation to **better assign peptides to isoforms**
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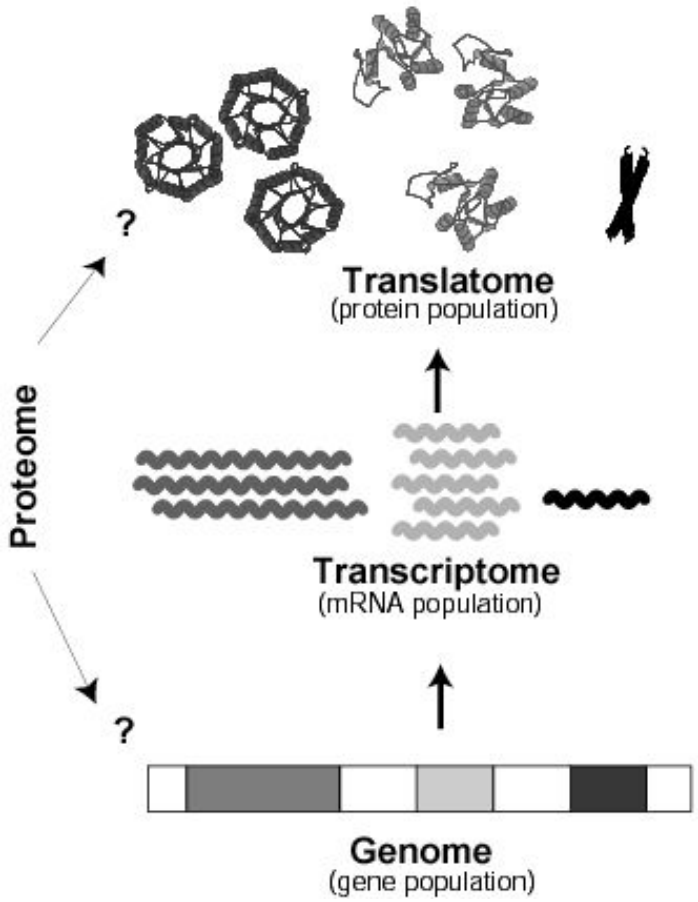
Why relate amounts of protein & mRNA?



[Greenbaum et al. *Bioinformatics* 2002, *18*, 587]

Gene expression -
major place for **regulation**
(easy to measure)

vs.

Concentration of protein -
major determinant of **activity**

Expectations from simple kinetic models:

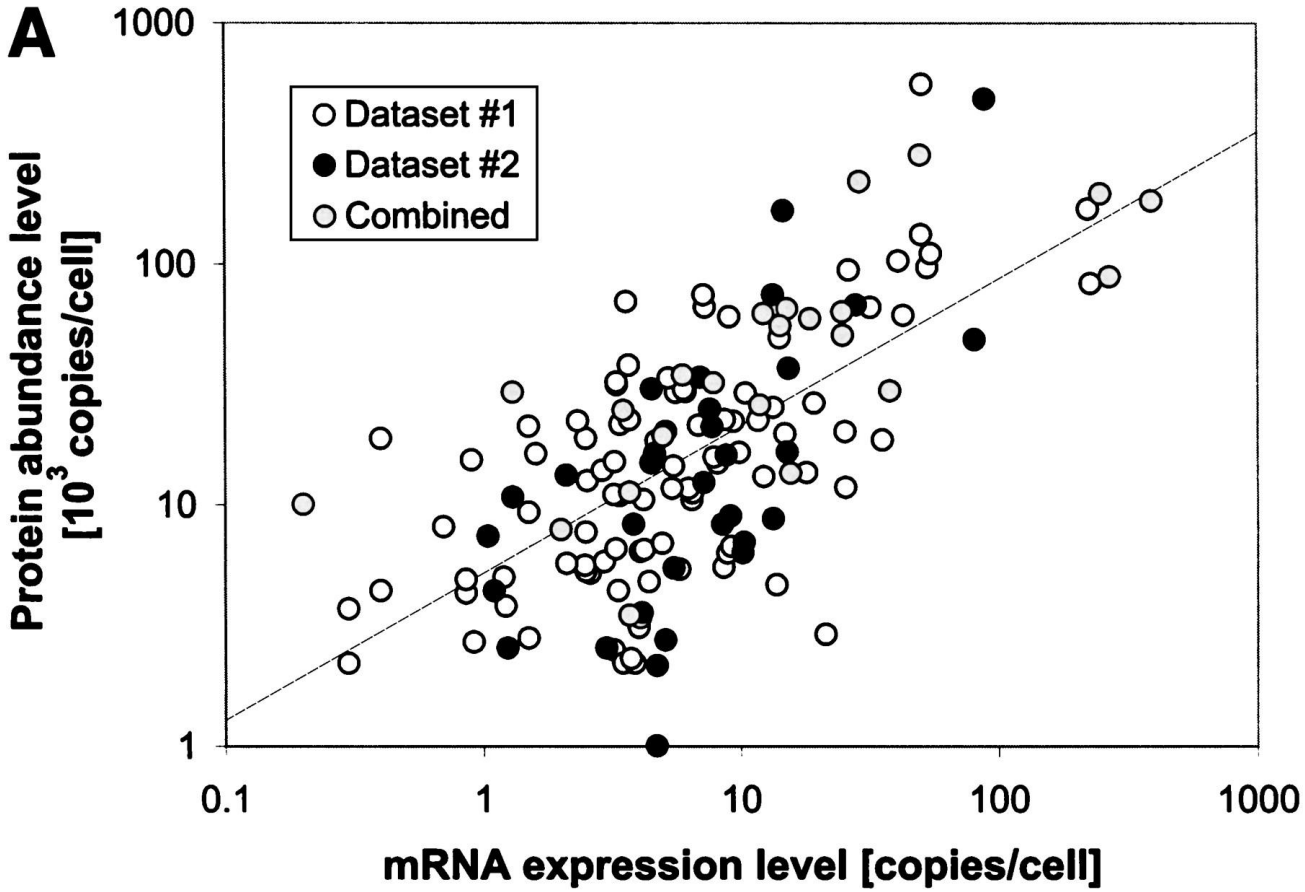
$$\frac{dP_i}{dt} = k_{s,i}[mRNA_i] - k_{d,i}P_i$$

At steady state: $P_i = \dfrac{k_{s;i}[mRNA_i]}{k_{d,i}}$

where $k_{s,i}$ and $k_{d,i}$ are the protein synthesis and degradation rate constants

Outliers from trend interesting

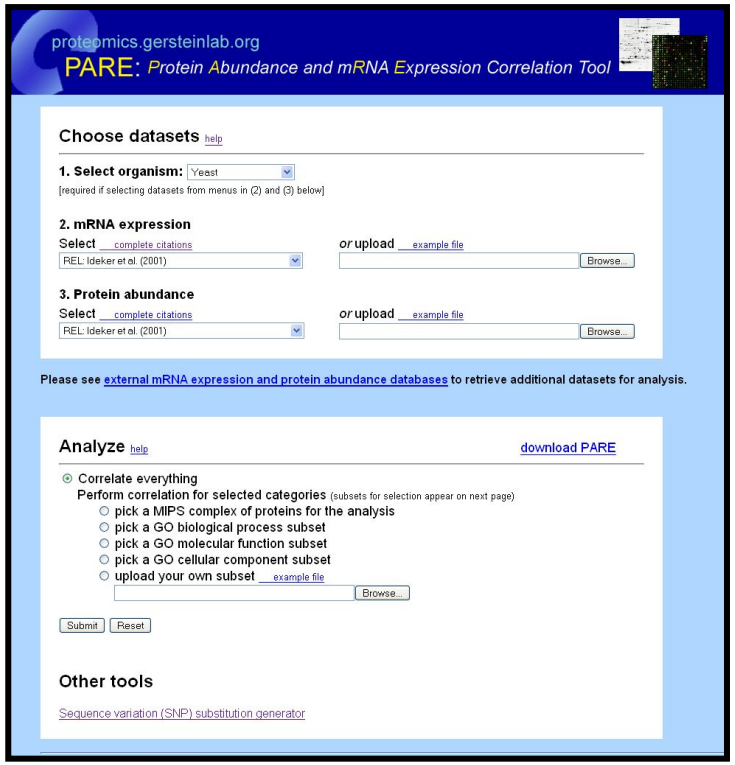
Early result on mRNA vs Protein, using 2D gels



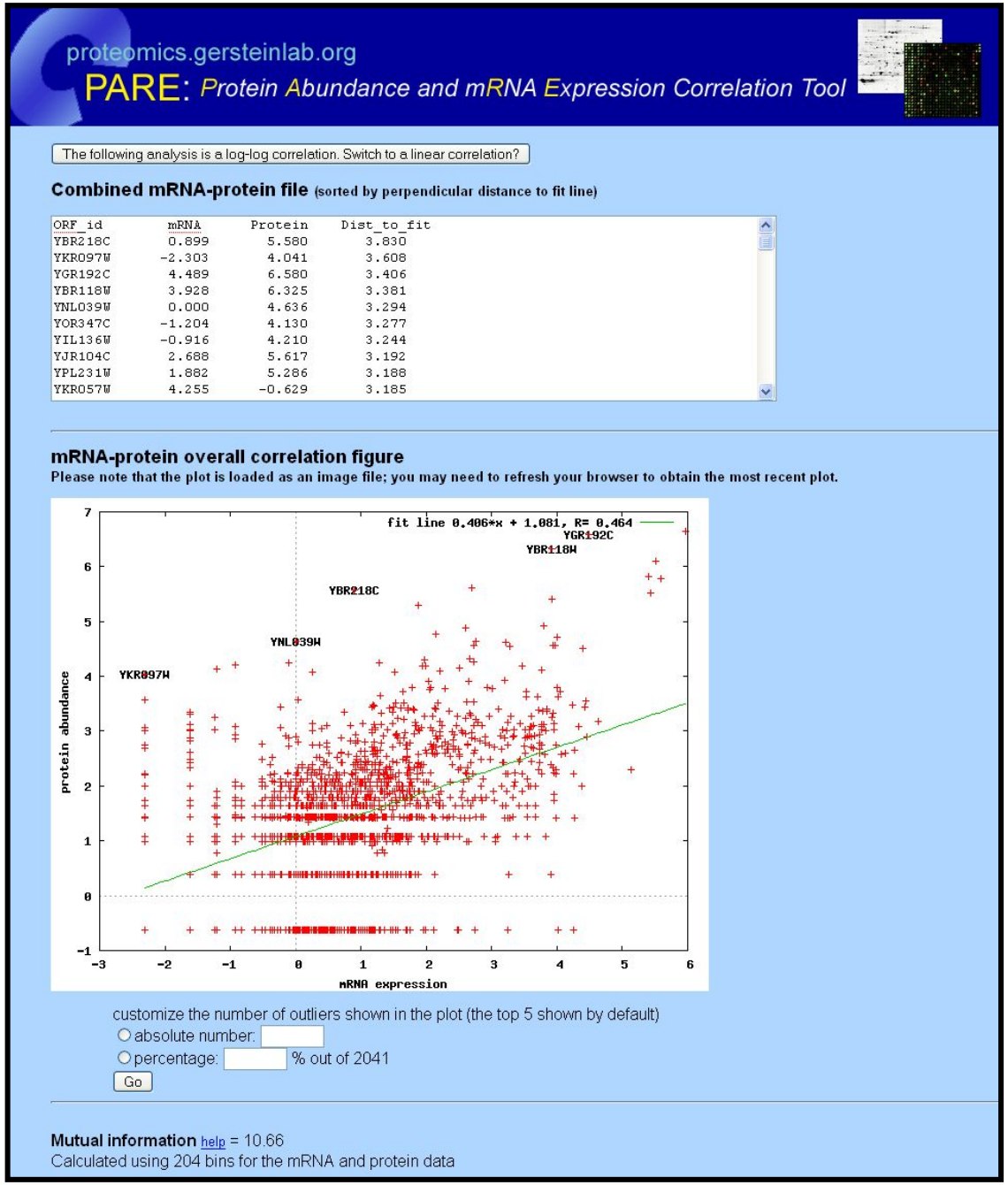
r =0.67

[Greenbaum et al. *Bioinformatics* 2002, *18*, 587]

PARE



[Yu et al., BMC Bioinfo. '07]



Open-source code
Downloadable

Analyze all or GO subset

Log-log plot of correlation
- -linear fit
- -outliers labeled

Calculation of mutual information

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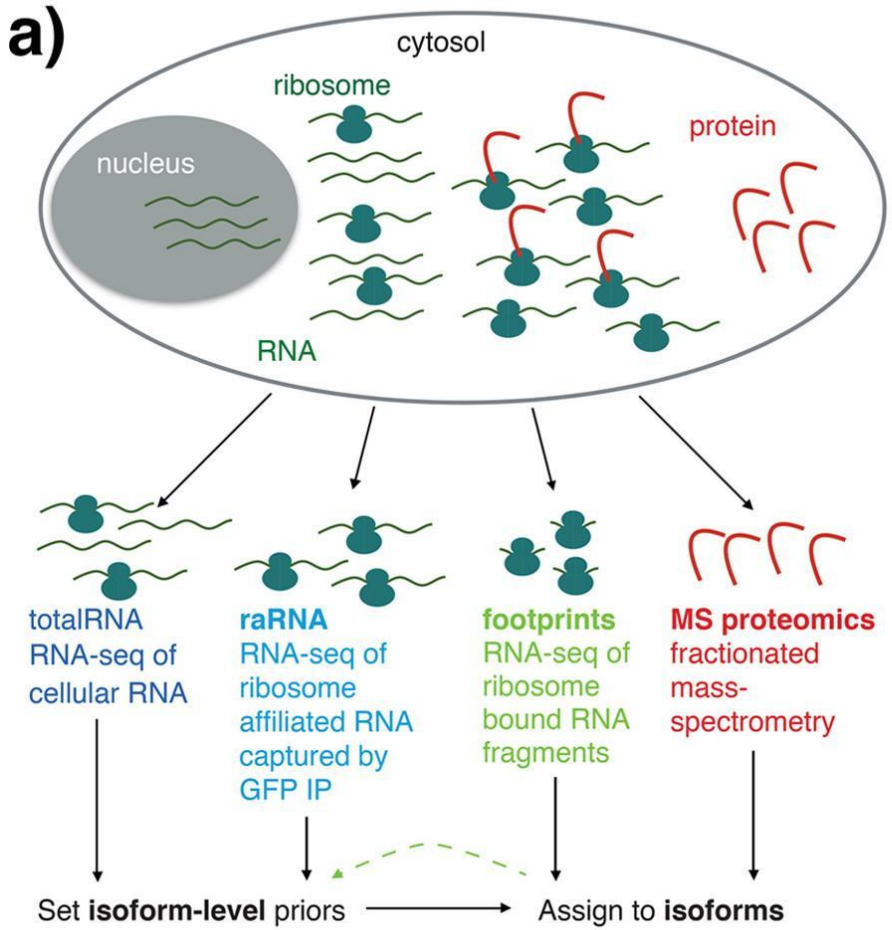
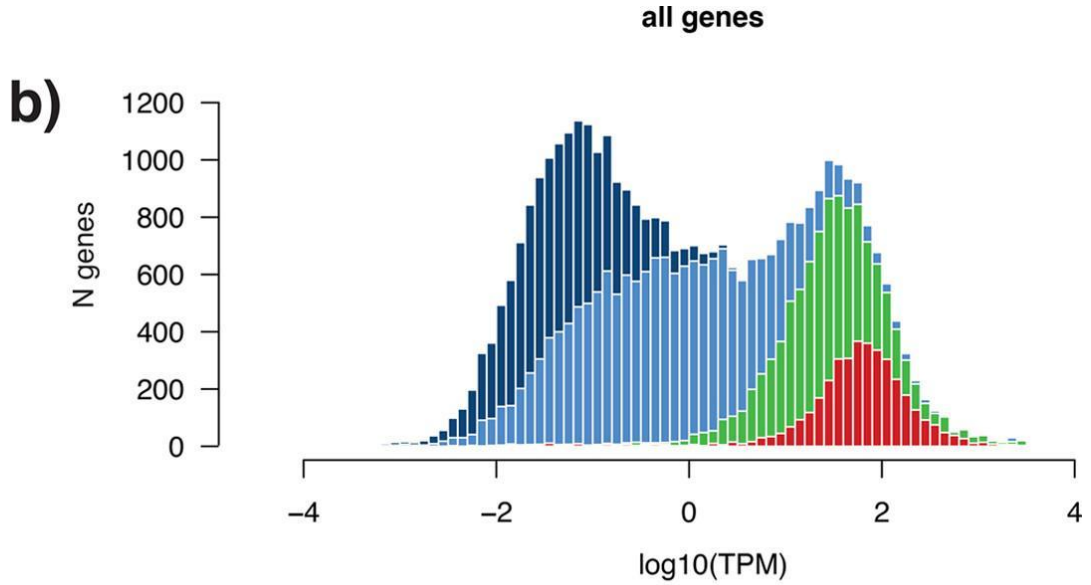
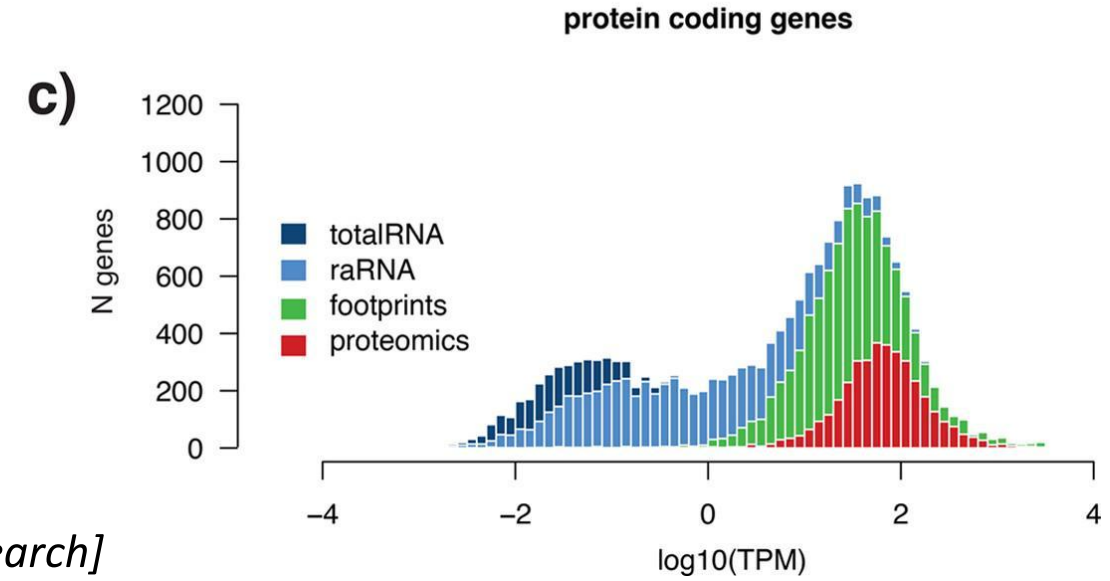
Integration of RNA-seq and Proteomic Data for Isoform Interpretation





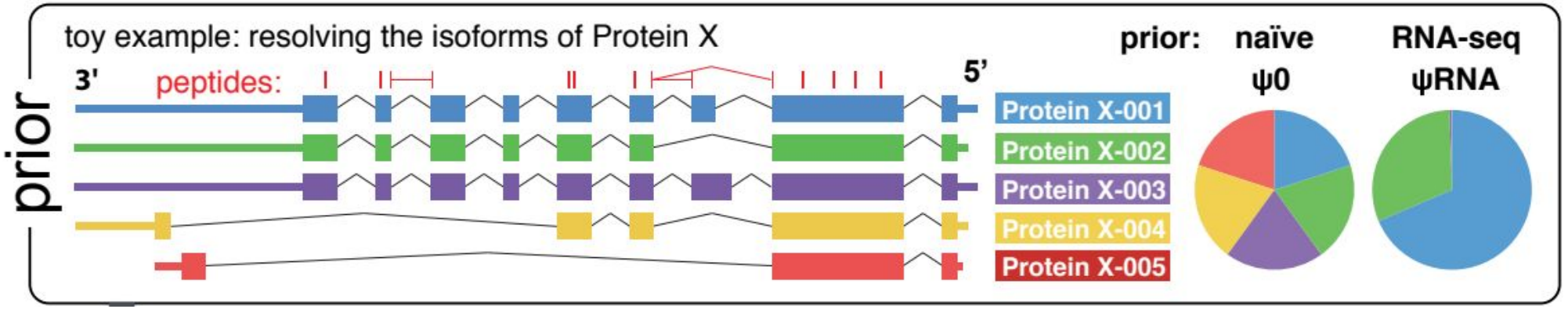


[Carlyle, Kitchen et al. (2018) *Journal of Proteome Research]*

Challenge for Isoform-Level Interpretation of Proteomics Data

Multimapping

- Different assays reflecting expression at various levels
- More reads at earlier stage assay (RNA-Seq > FP > MS)
- Leverage other assays for better estimation



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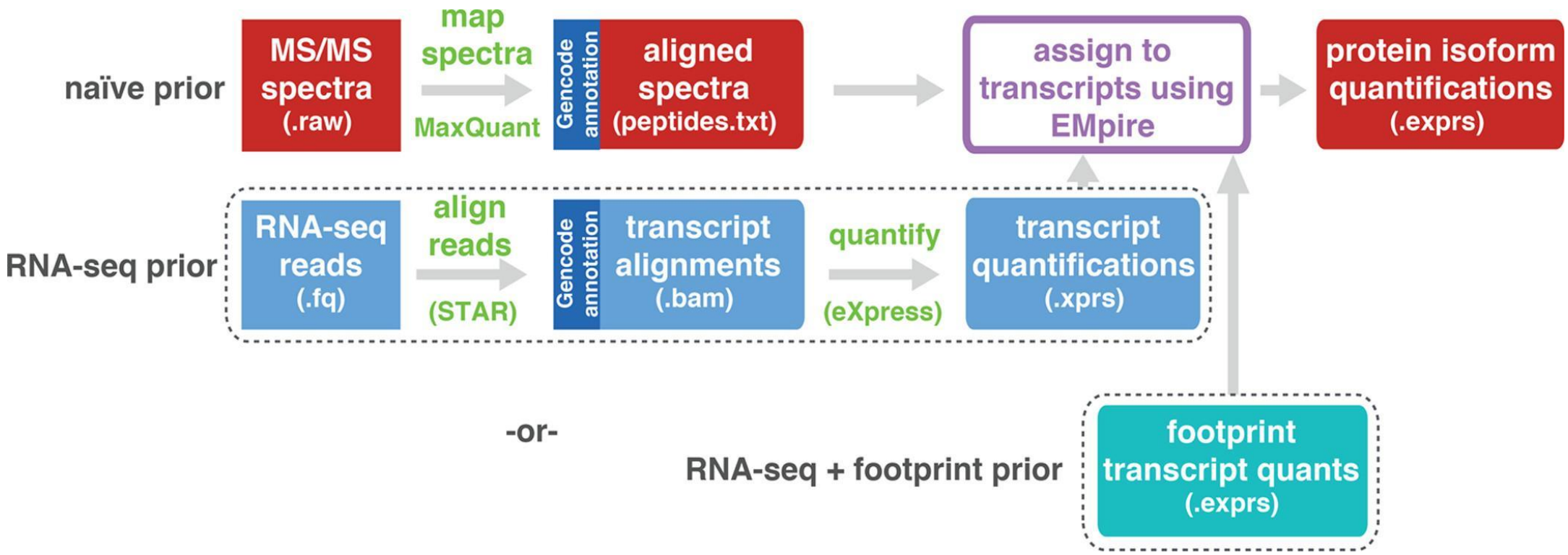
EMpire (Expectation Maximisation Propagation of Isoform abundance from RNA Expression)



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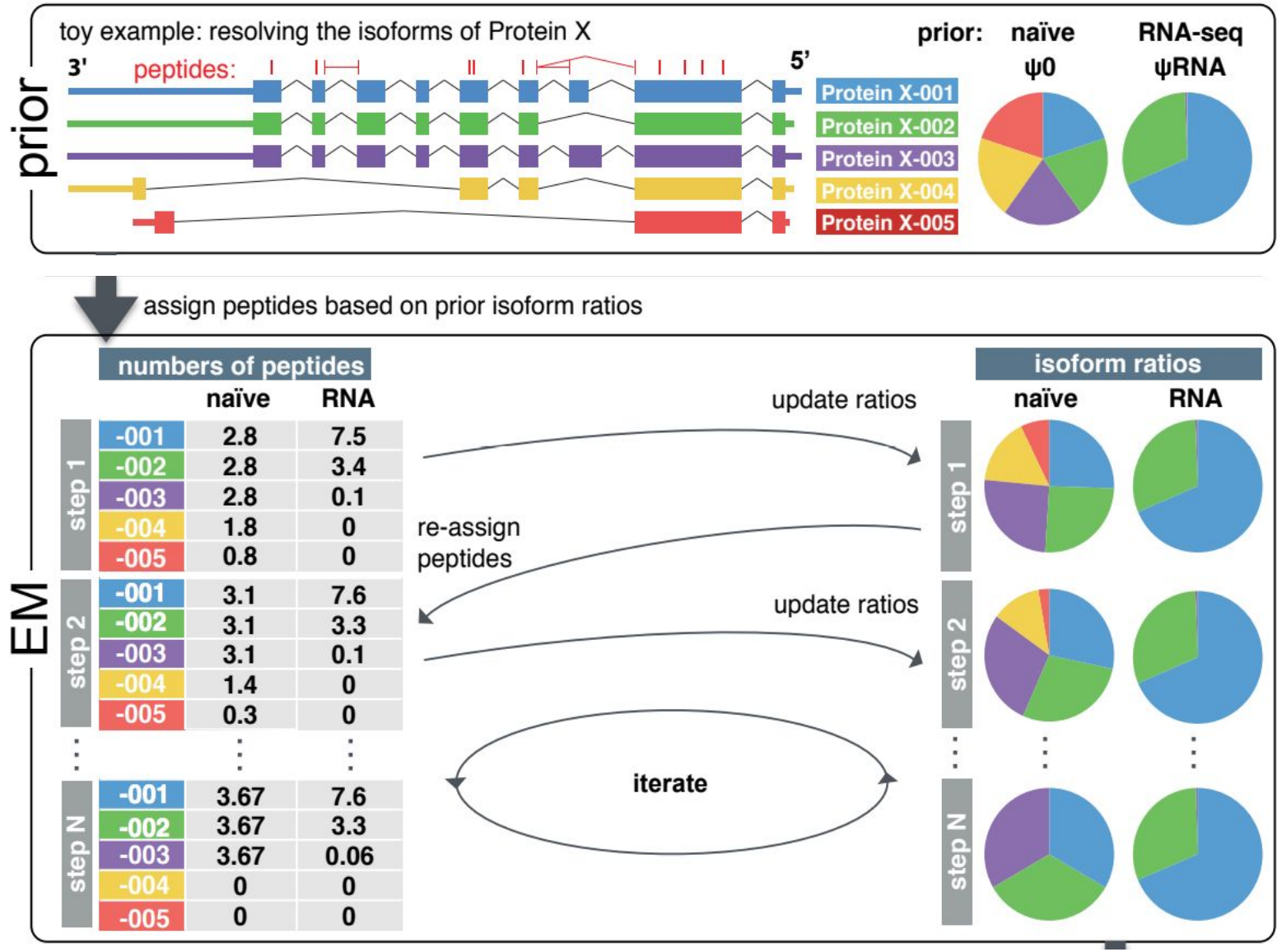
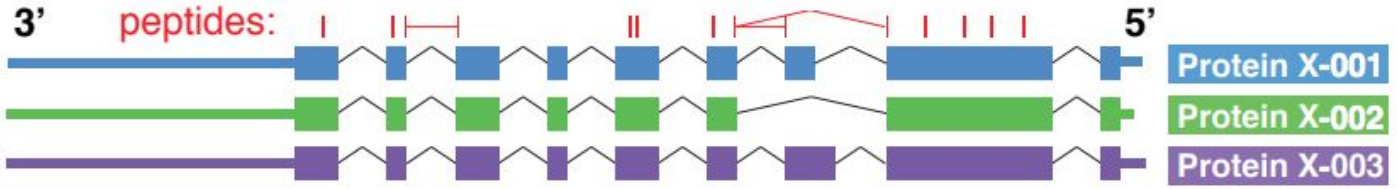


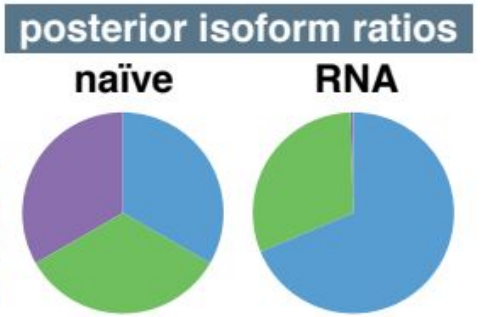
[Carlyle, Kitchen et al. (2018) *Journal of Proteome Research]*

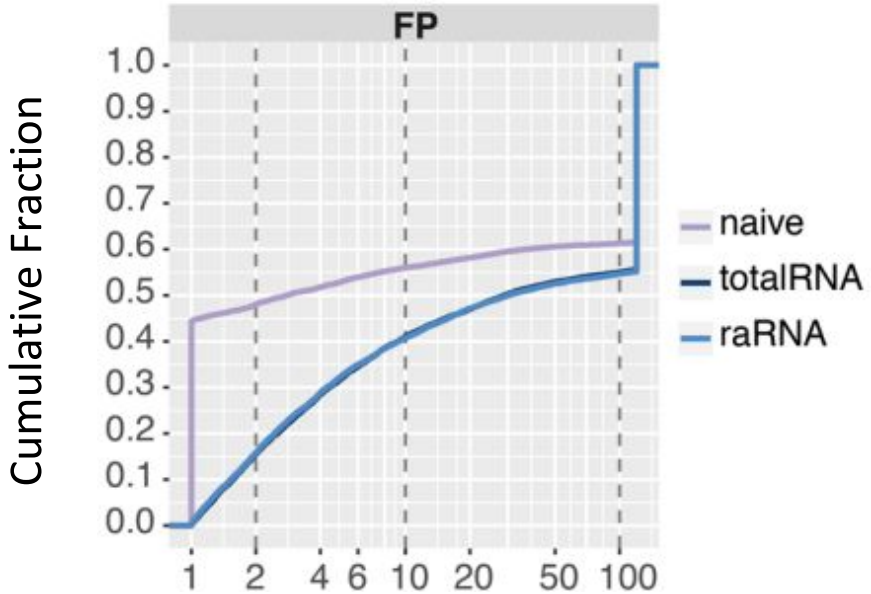
[Carlyle, Kitchen et al. (2018) *Journal of Proteome Research*]

result

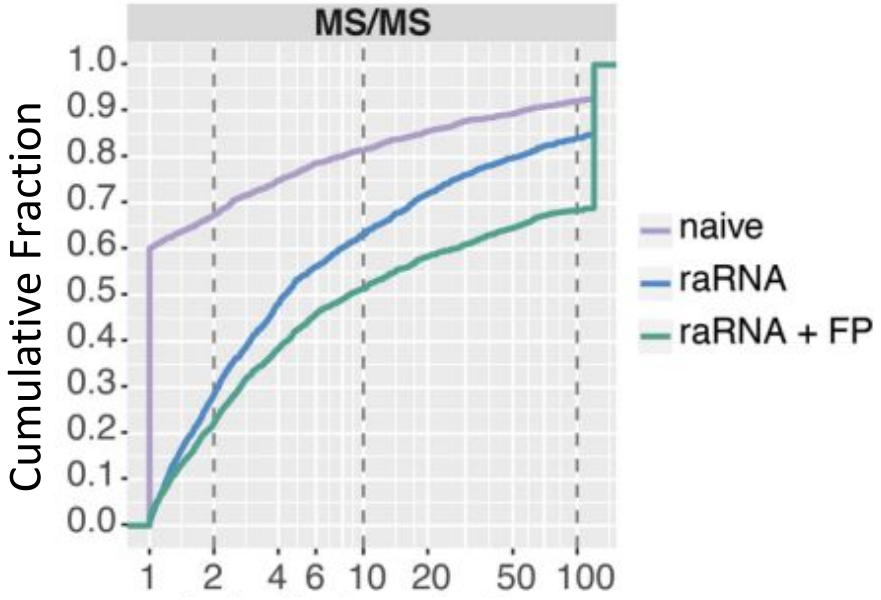
The RNA prior supports **Protein X-001** as the >2-fold dominant isoform, while the naïve prior gets stuck with three equally likely isoforms:







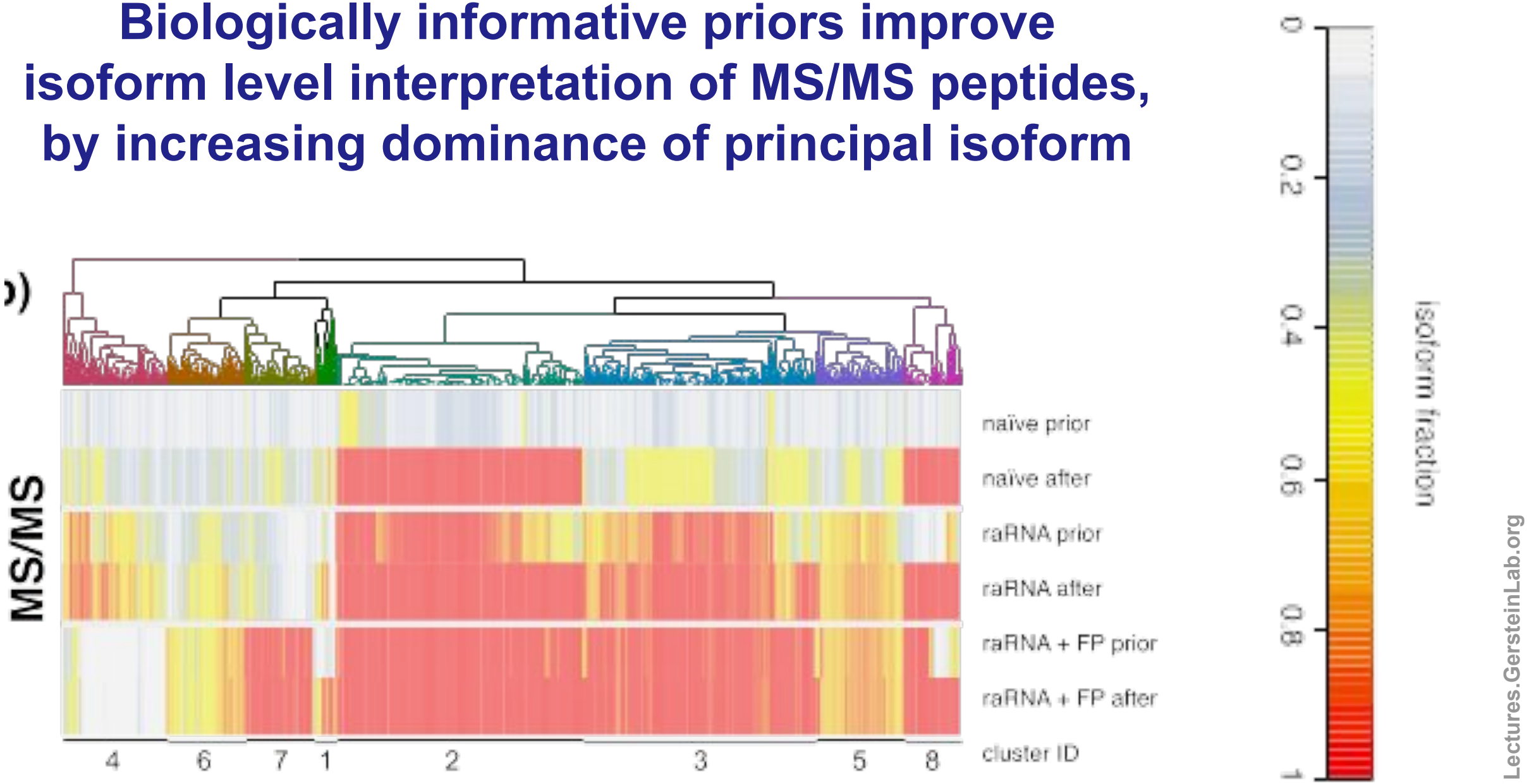
≤ principal isoform dominance
=principal isoform/second isoform



≤ principal isoform dominance
=principal isoform/second isoform

Larger principal isoform dominance = **Less** ambiguity in major isoform identification

Biologically informative priors improve isoform level interpretation of MS/MS peptides, by increasing dominance of principal isoform



[**Carlyle, Kitchen** et al. (2018) *Journal of Proteome Research*]

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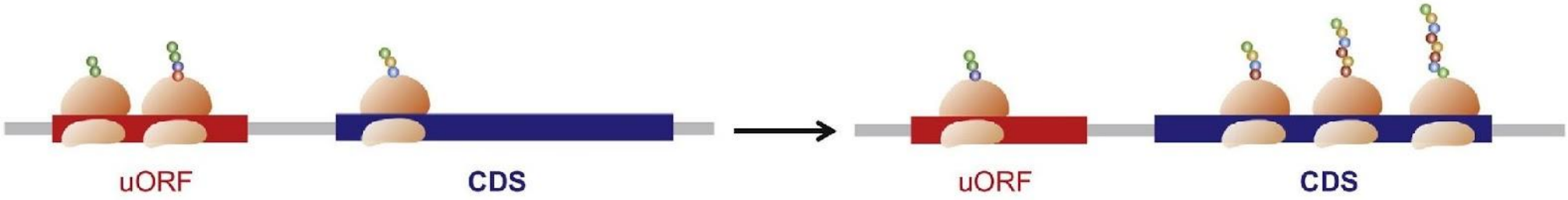
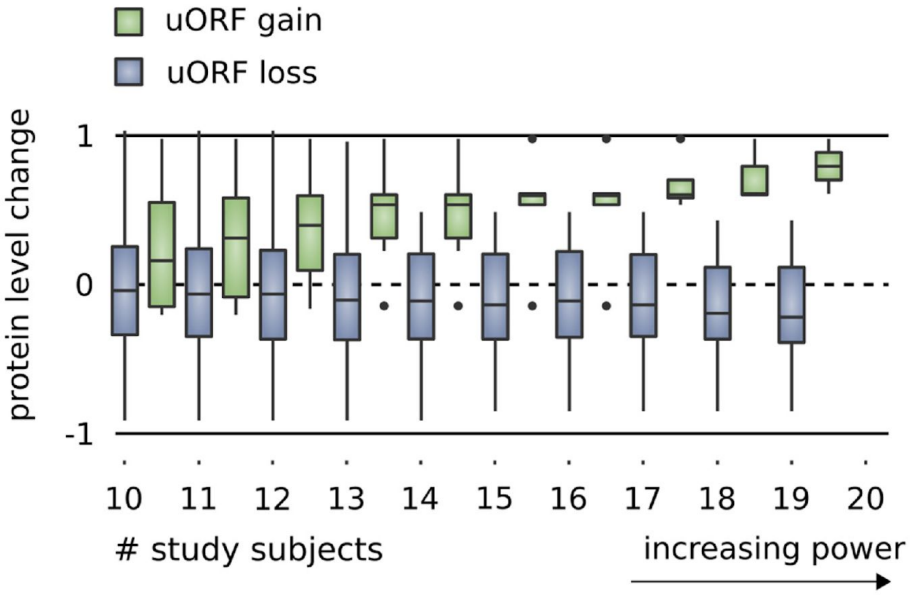
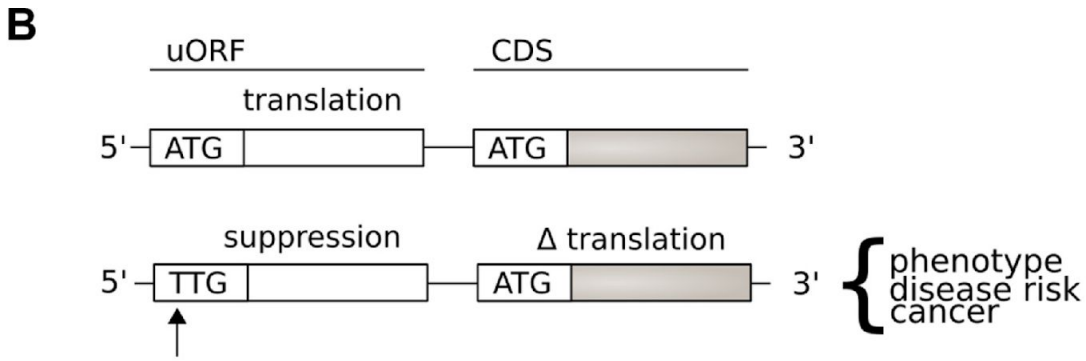
Upstream open reading frames (uORFs) may shift the expected balance between mRNA & protein



[Zhang et al., Trends in Biochemical Sciences ('19)]

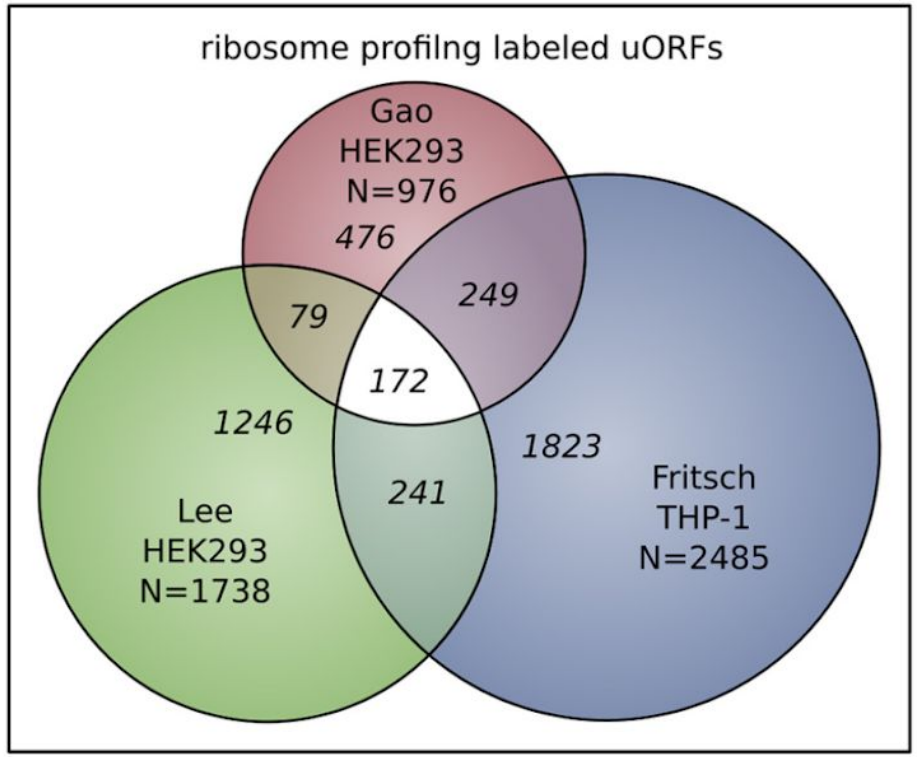


In Battle et al. 2014 data uORF gain & loss assoc. protein level change.



uORF regulation can be affected by mutation

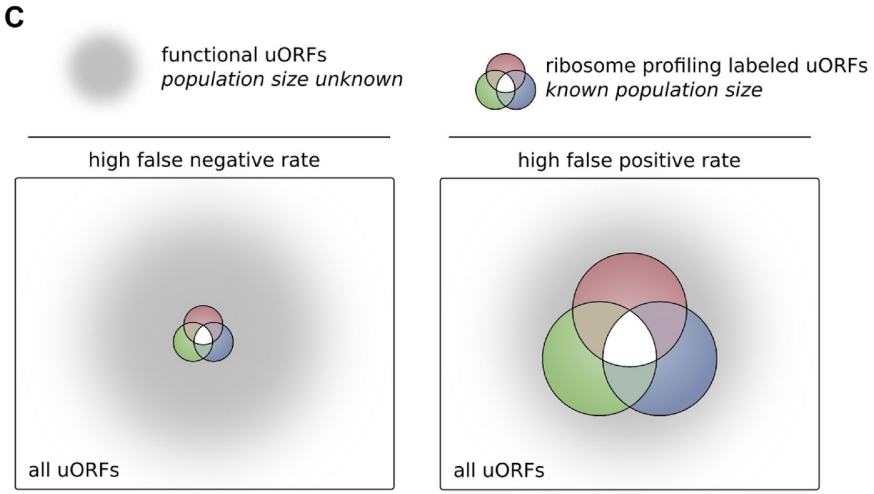
[McGillivray et al., *NAR* ('18)]



genome-wide uORFs
N = 1.3 million

From a “Universe” of 1.3 M pot. uORFs

The population of functional uORFs may be significant

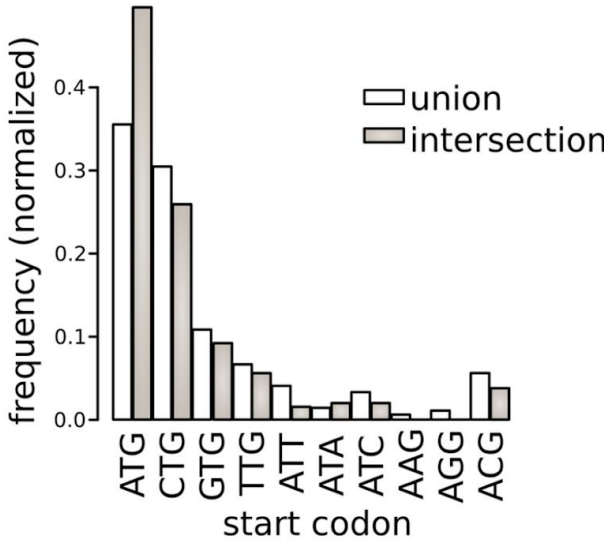


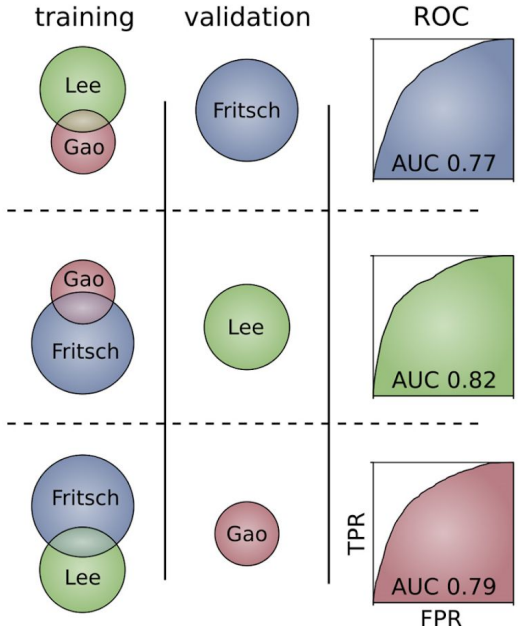
- Ribosome profiling experiments have low overlap in identified uORFs.
- This suggests high false-negative rate, and more functional uORFs than currently known.

[McGillivray et al., *NAR* (‘18)]

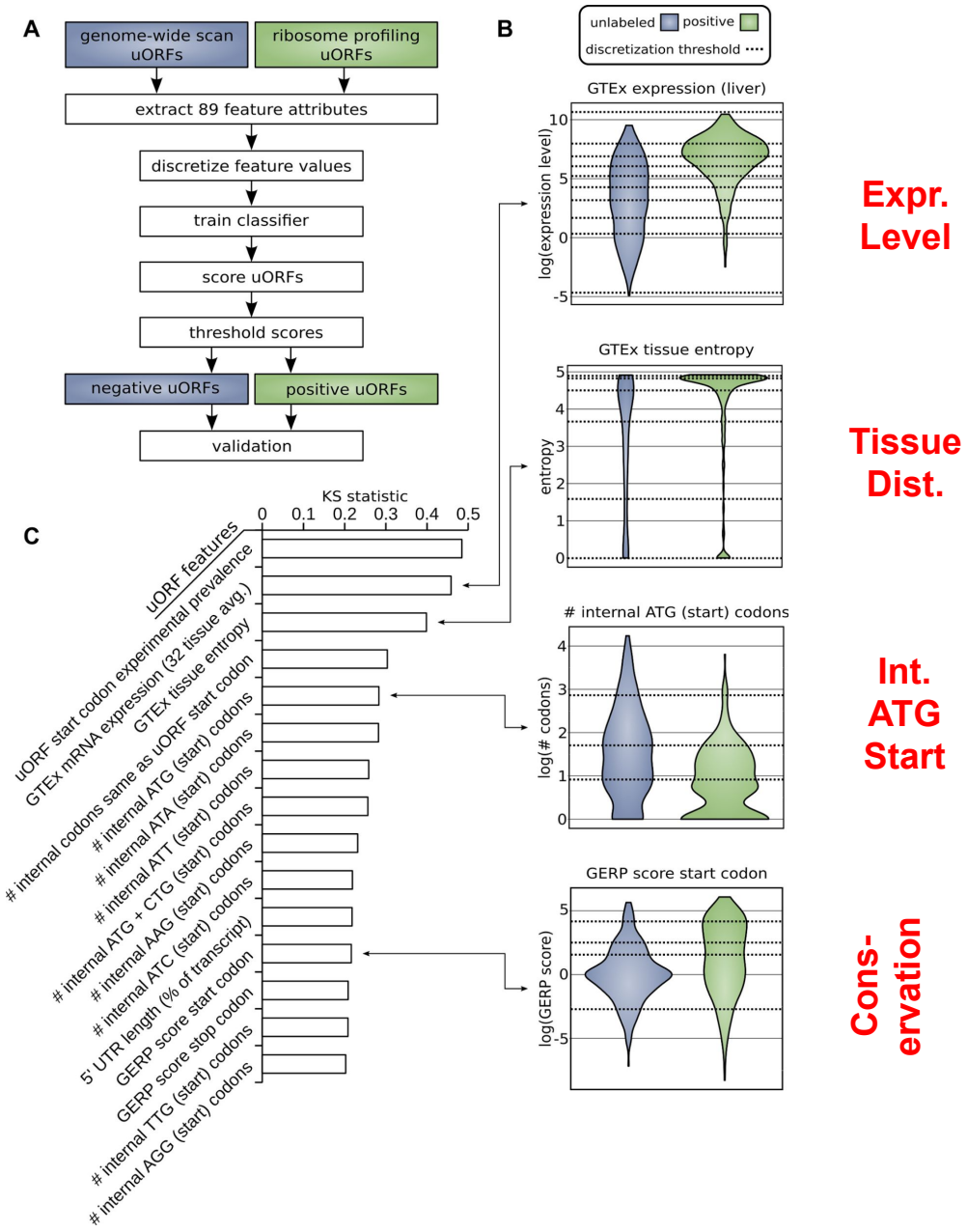
Prediction & validation of functional uORFs using 89 features

- All near-cognate start codons predicted.
- Cross-validation on independent ribosome profiling datasets and validation using in vivo protein levels and ribosome occupancy in humans (Battle et al. 2014).





[McGillivray et al., *NAR* ('18)]



Expr. Level

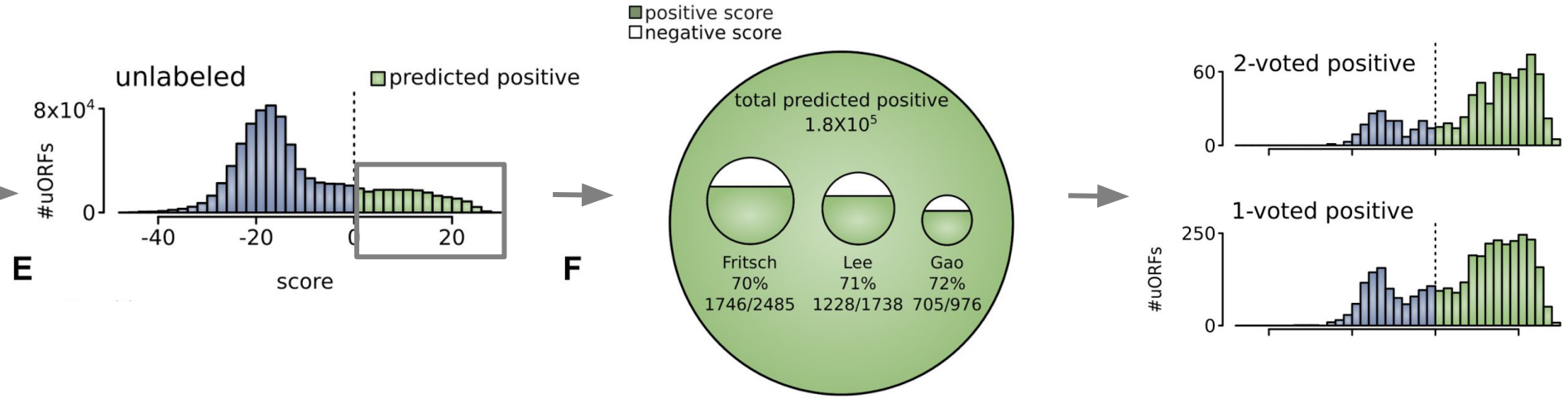
Tissue Dist.

Int. ATG Start

Cons-ervation

A comprehensive catalog of functional uORFs

Universe of **1.3M** uORFs scored via Simple Bayes algo.



- Predicted functional uORFs may be intersected with disease associated variants.

[McGillivray et al., *NAR* ('18)]

- **180K**: Large predicted positive set likely to affect translation
- Calibration on gold standards, suggests getting **~70%** of known

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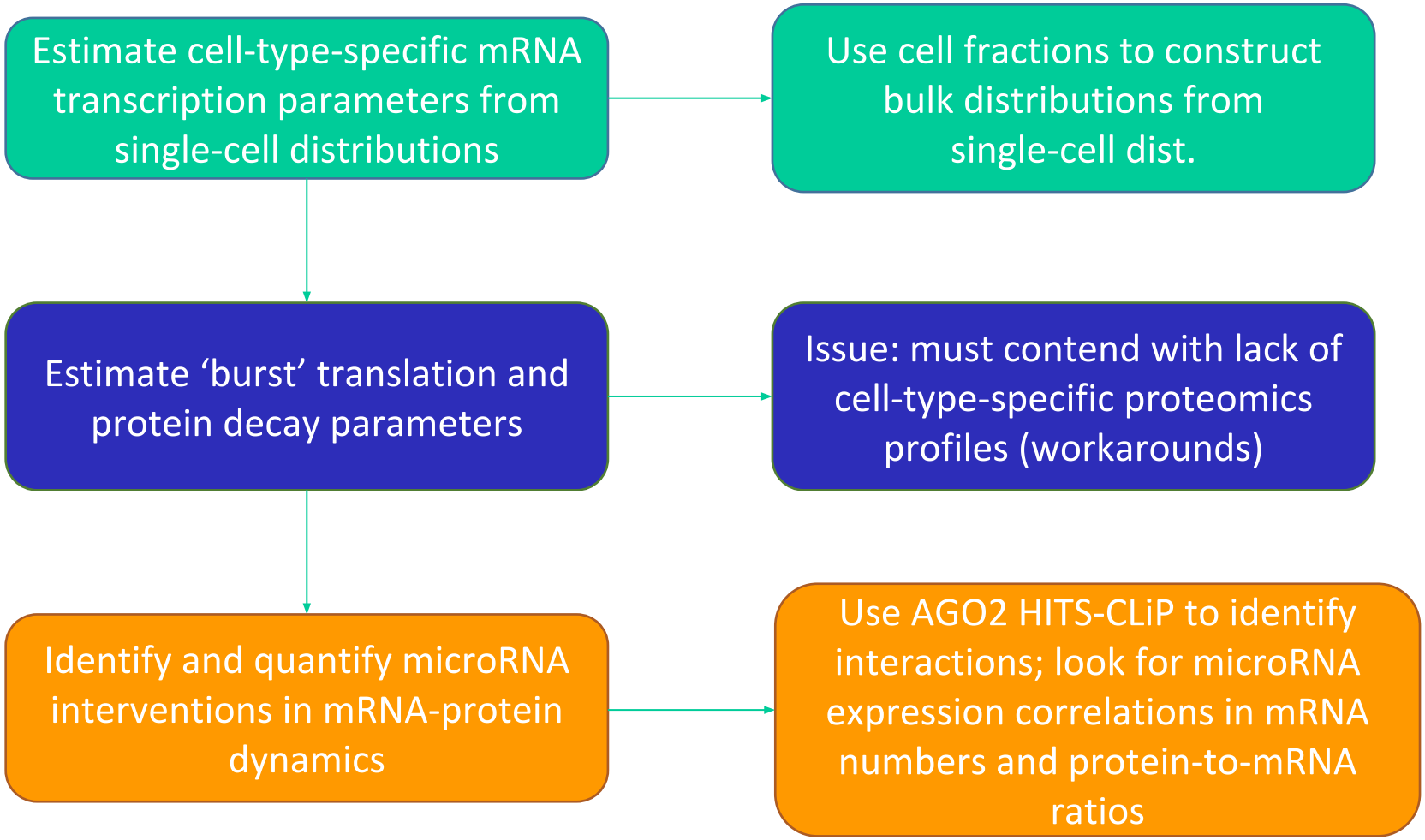
Leveraging New Datasets

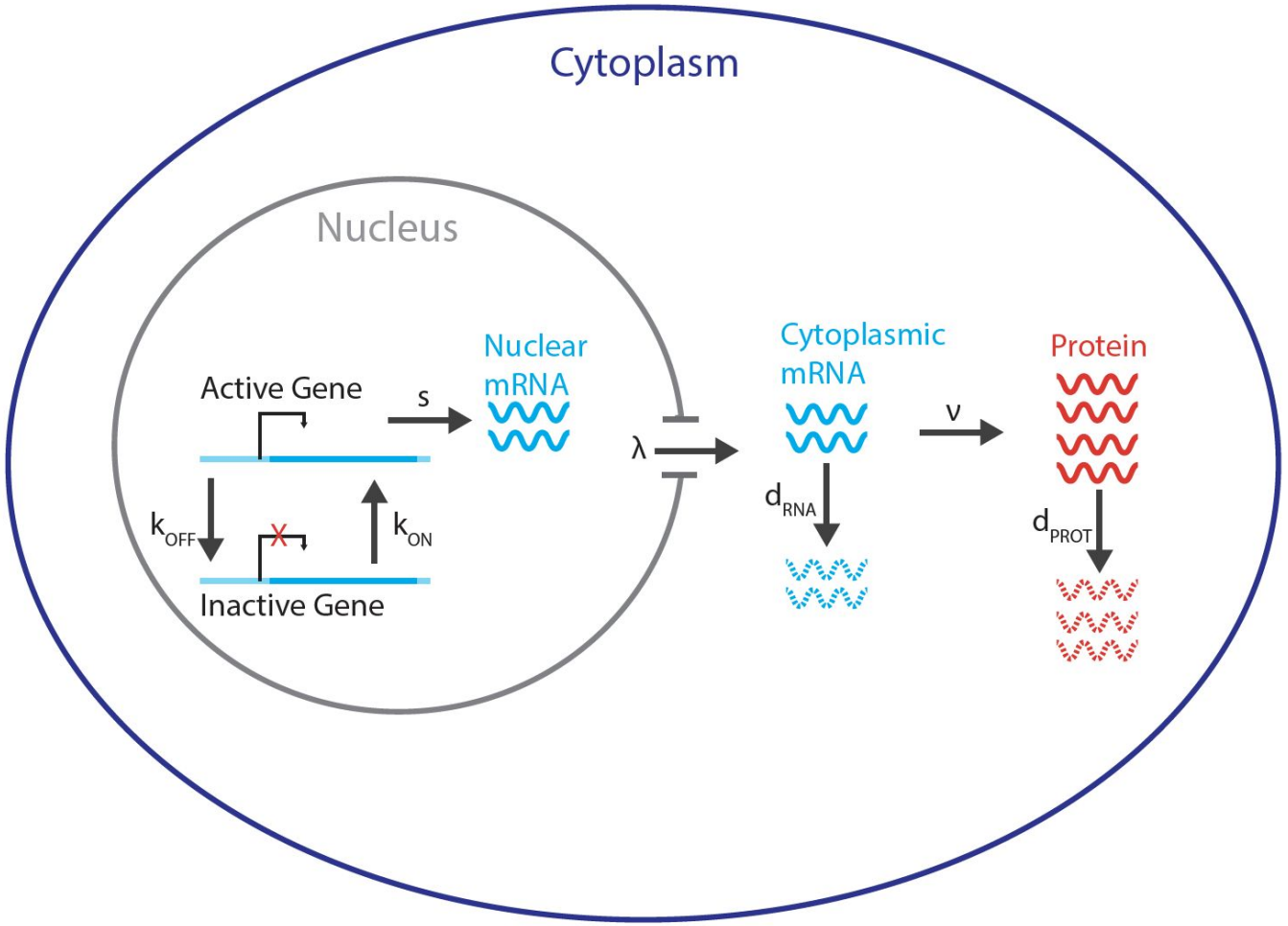
1. Availability of single-cell RNA-seq datasets warrants revisiting mRNA-protein dynamics with an eye towards cell-type-specific characterizations:
   - Can the rich information on mRNA distributions from single-cell data provide clues to the origin of mRNA-protein discrepancies in the bulk data?
   - What can we learn about cell-type specifics of the mRNA-to-protein relationship?
2. The Brainspan dataset considered in the NIDA grant research represents a unique opportunity for individual-specific study:
   - Individual-matched bulk RNA-seq, smallRNA-seq and proteomics data are available for several individuals in several brain regions
   - AGO2 HITS-CLIP data is also available to link microRNA to potential mRNA targets

Combining all these modalities into a coherent framework suggests a return to first principles

Schematic workflow



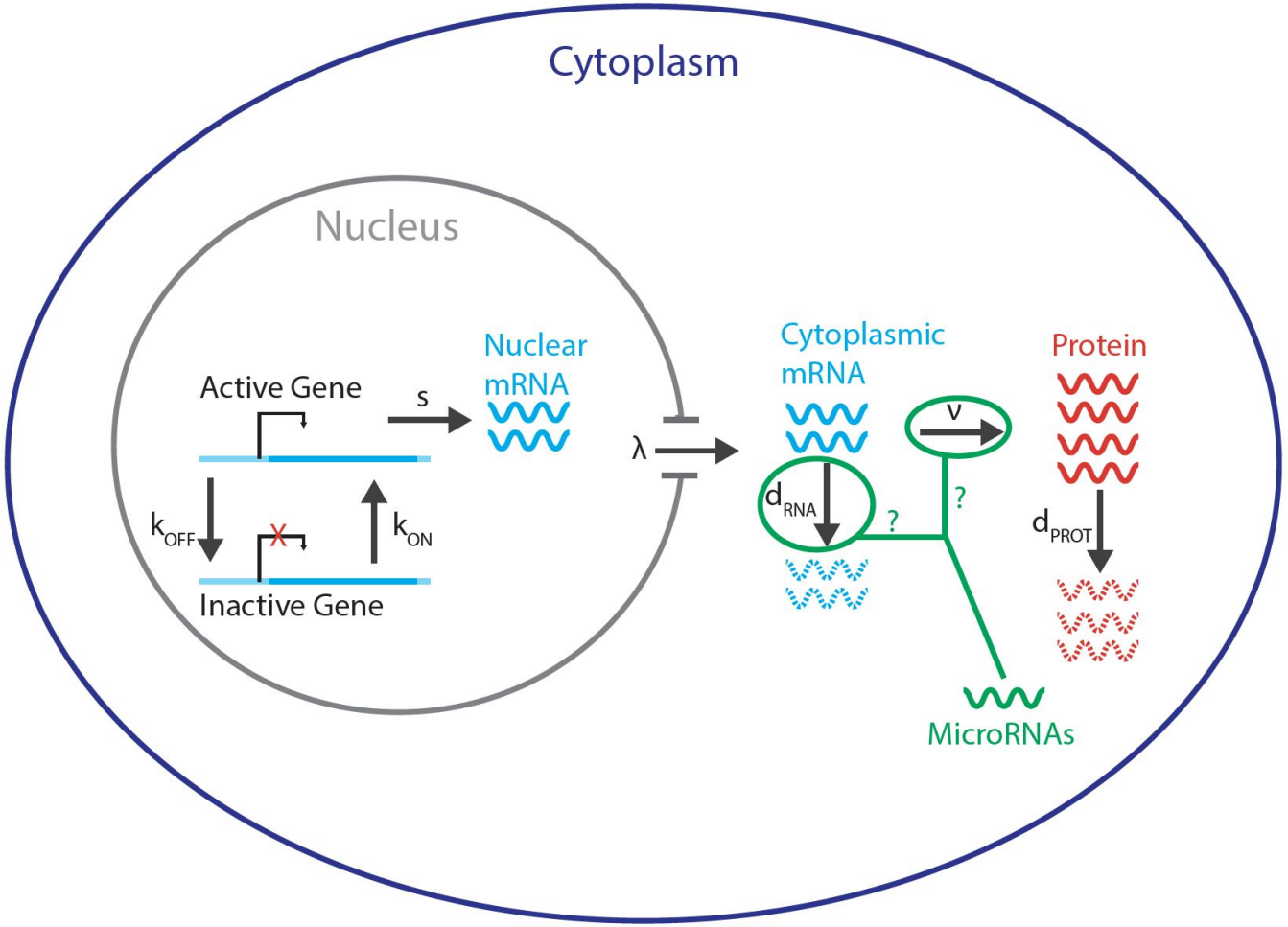
mRNA-Protein dynamics



1. <u>Use normalized single-cell mRNA distributions:</u>
   a. Infer cell-type-specific k_{ON}, k_{OFF} and s (Kim and Marioni 2013) using Poisson-Beta model of 'burst' transcription
   b. Replace decay rate by nuclear export rate λ
2. <u>Use two-state model of protein translation (Shahrezaei and Swain 2008):</u>
   a. Obtain cell-type-specific cyto. RNA and Protein numbers in terms of λ, v, d_{RNA}, d_{PROT}.
   b. Use bulk mRNA and Protein data as constraints

Kim and Marioni, *Genome Biology* **2013**, 14:R7.
Shahrezaei and Swain, *PNAS* **2008**, 105(45), Pgs. 17256–17261.

MicroRNA intervention



Sousa et al., *Science* **2017**, 358, Pgs. 1027–1032.

1. Use AGO2 HITS-CLIP binding data on microRNA-mRNA interactions in the brain (Sousa et al 2017):
   a. Compare mRNAs with multiple targeting microRNAs to those with one or none; expression levels and inferred parameters
2. Use smallRNA-seq expression data:
   a. Directly correlate with protein-to-mRNA ratios and mRNA expression levels
   b. Correlate with inferred parameters to determine whether microRNA impacts mRNA degradation, protein translation, or both

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Acknowledgments!

genecensus.org/expression/translatome
D **Greenbaum**, R Jansen, M Gerstein

Proteomics.gersteinlab.org **(PARE)**
E **Yu**, A Burba, M Gerstein

github.com/rkitchen/**EMpire**
B **Carlyle**, R **Kitchen**, J Zhang, R Wilson, T Lam, J Rozowsky,
K Williams, N Sestan, M Gerstein, A Nairn

github.gersteinlab.org/**uORFs**
P **McGillivray**, R Ault, M Pawashe, R Kitchen,
S Balasubramanian, M Gerstein

Brainspan data
P **Emani,** T Galeev, N Sestan, A Nairn