From evolutionary theory to RNA therapeutics

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Outline

A start in biotechnology
(2002 – 2006)

Pivot to ecology, evolutionary biology and behavior
(2005 – 2011)

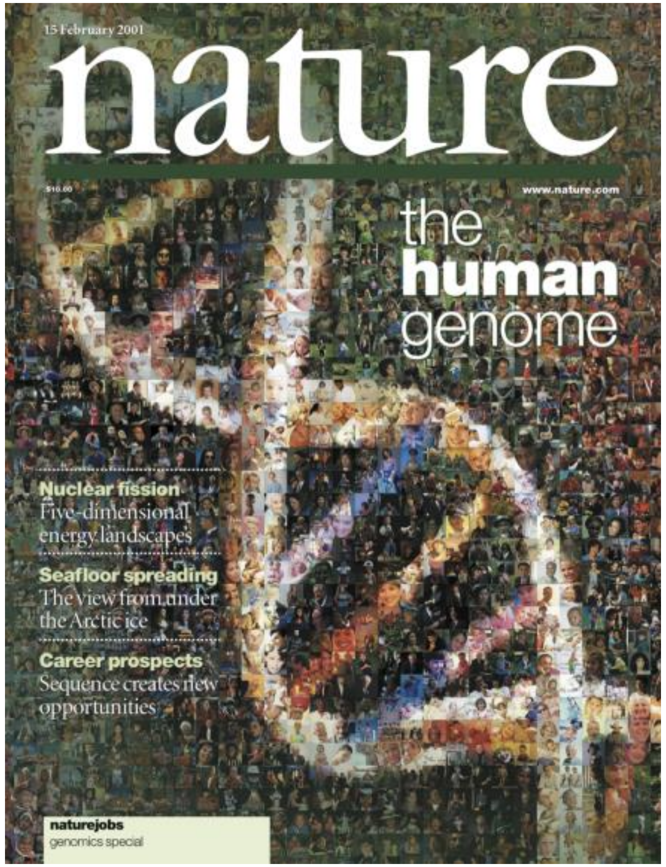
Finding a niche – a narrow intersection between disparate disciplines
(2011 – 2015)

Getting my hands dirty – leading a hybrid group
(2016 – 2022)

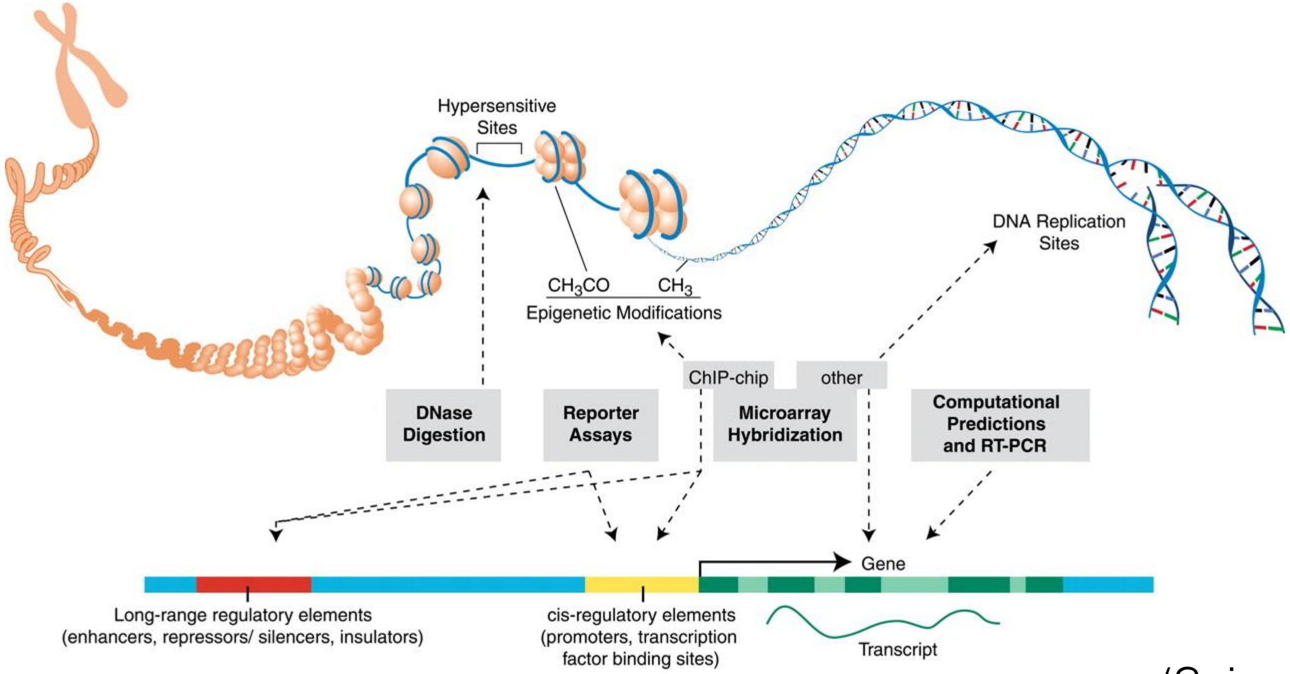
Leaving behind academia – a jump into the exciting world of RNA therapeutics
(2022 – Now)

A start in biotechnology
Anna University - Chennai

How I decided to stop worrying and learned to code



ENCODE Project

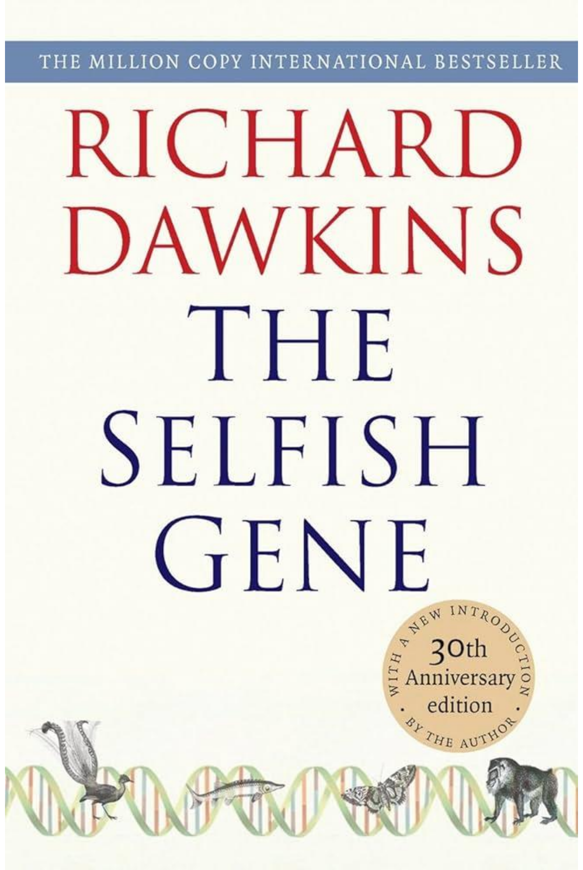


(Science 2004)

How I decided to stop worrying and learned to code

- Pick a (hard) problem to focus on
- Find an equally motivated partner
- Language matters less than you may think (at least when you are getting started)

A pivot to Ecology, Evolution, and Behavior


THE MILLION COPY INTERNATIONAL BESTSELLER

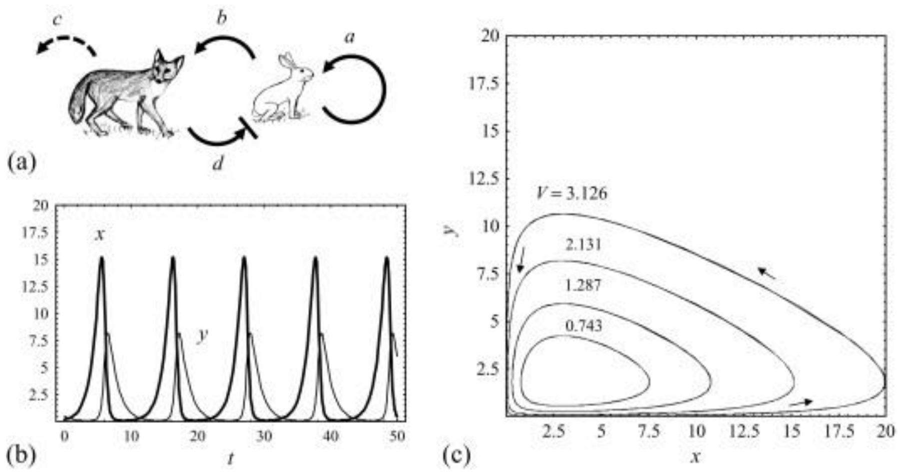
RICHARD DAWKINS
THE SELFISH GENE

30th Anniversary edition
WITH A NEW INTRODUCTION BY THE AUTHOR

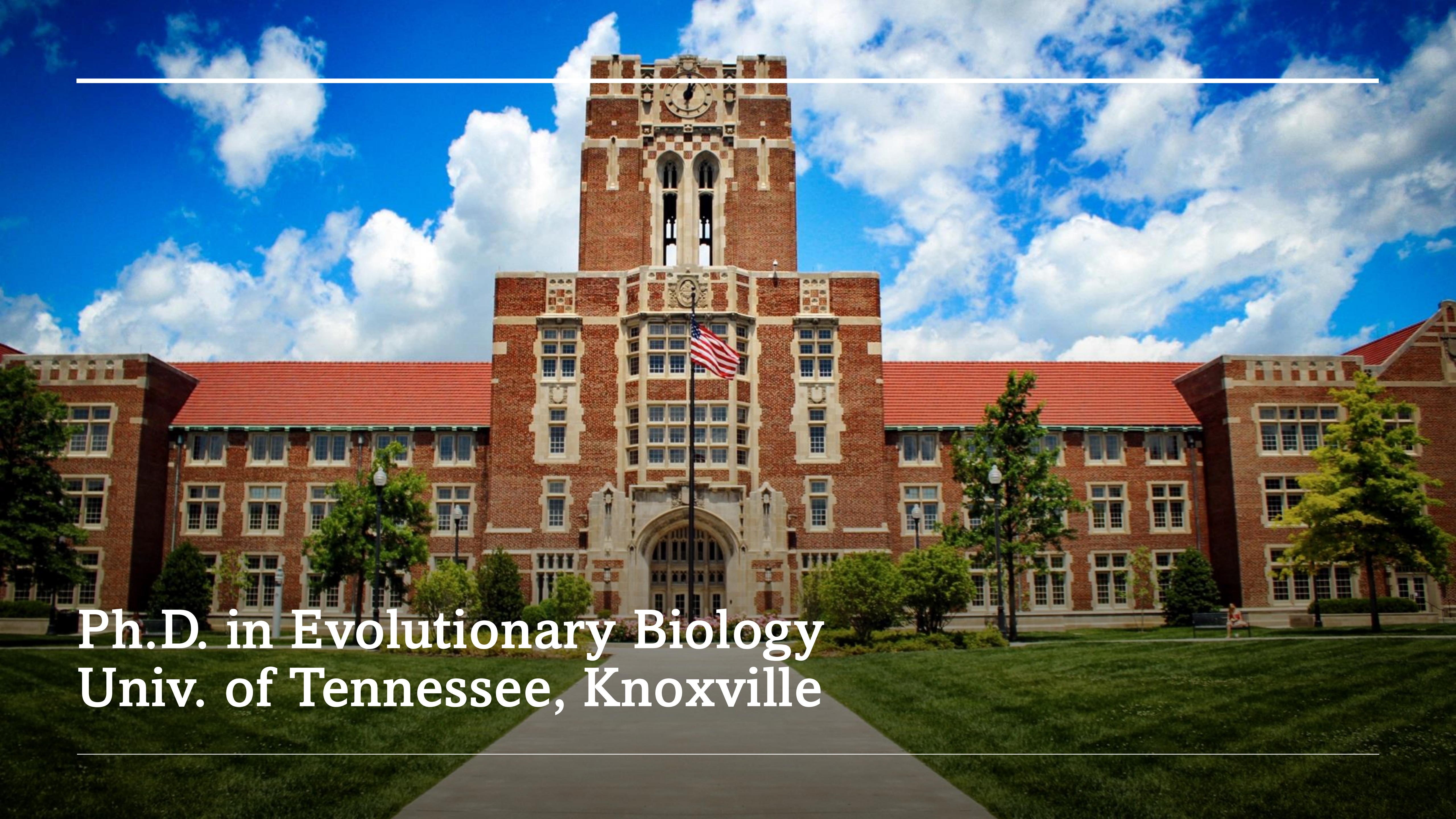
Brood care in primitively eusocial ants


PC:EurekaAlert

Lotka-Volterra models in ecology



Kinoshita 2013



Ph.D. in Evolutionary Biology
Univ. of Tennessee, Knoxville

A series of lucky breaks

- Accepted into a Ph.D. program in Ecology, Evolution, and Behavior without having taken a single formal class in either Ecology, Evolution, or Behavior
- A desperate grad student and a patient mentor
- Funding not tied to the lab = freedom to dabble in disconnected projects

On the evolution of codon usage bias

Genetic code is redundant

	U	C	A	G	
U	UUU F	UCU S	UAU Y	UGU C	U
	UUC F	UCC S	UAC Y	UGC C	C
	UUA L	UCA S	UAA X	UGA X	A
	UUG L	UCG S	UAG X	UGG W	G
C	CUU L	CCU P	CAU H	CGU R	U
	CUC L	CCC P	CAC H	CGC R	C
	CUA L	CCA P	CAA Q	CGA R	A
	CUG L	CCG P	CAG Q	CGG R	G
A	AUU I	ACU T	AAU N	AGU S	U
	AUC I	ACC T	AAC N	AGC S	C
	AUA I	ACA T	AAA K	AGA R	A
	AUG M	ACG T	AAG K	AGG R	G
G	GUU V	GCU A	GAU D	GGU G	U
	GUC V	GCC A	GAC D	GGC G	C
	GUA V	GCA A	GAA E	GGA G	A
	GUG V	GCG A	GAG E	GGG G	G

Population-Genetics framework

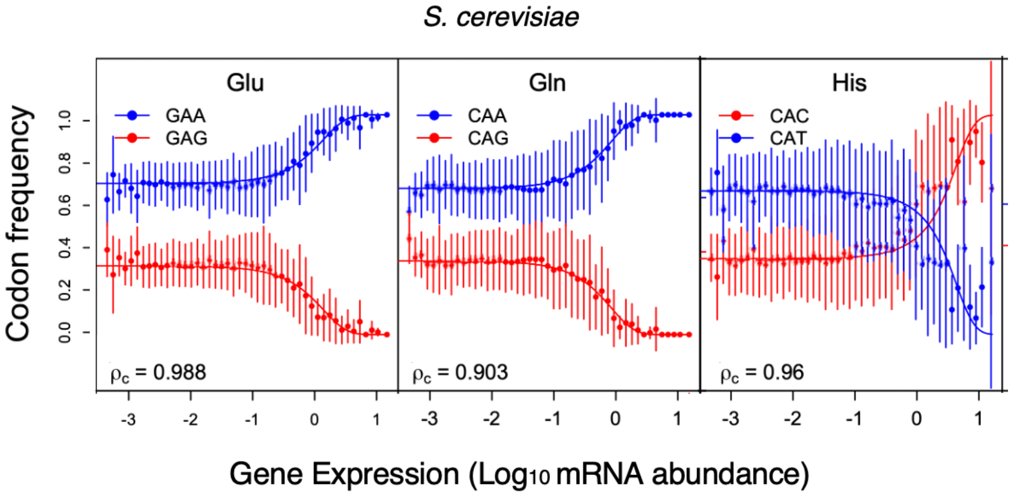
Mechanistic model

Stationary distribution

$$P(\vec{x}|\phi) = \frac{\overbrace{w(\vec{x}|\phi)}^{\text{Selection}}{}^{\overbrace{N_e}^{\text{Genetic Drift}}}\overbrace{\prod_{i=1}^{61}\mu_i^{x_i}}^{\text{Mutation Bias}}}{\sum_{y\in S_c} w(\vec{y}|\phi)^{N_e}\prod_{i=1}^{61}\mu_i^{y_i}}$$

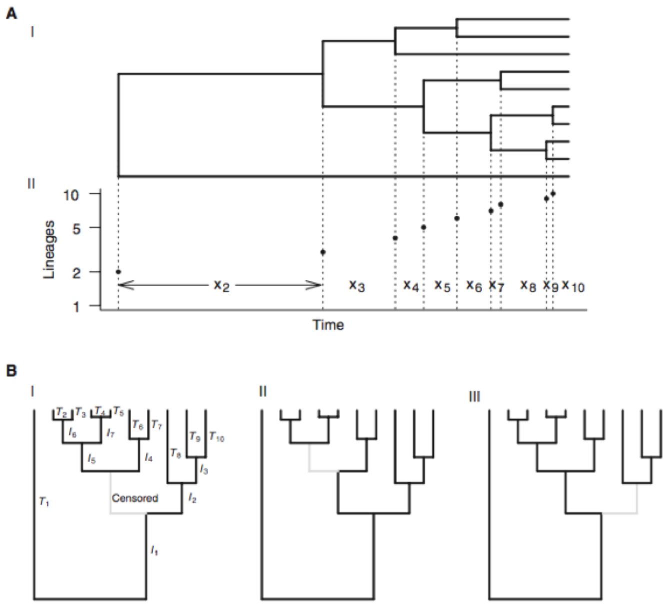
N_e = Effective population size
S_c = Synonymous genotype space
μ = Codon-specific mutation rate

For 300 codons,
$S_c \approx 10^{140}$

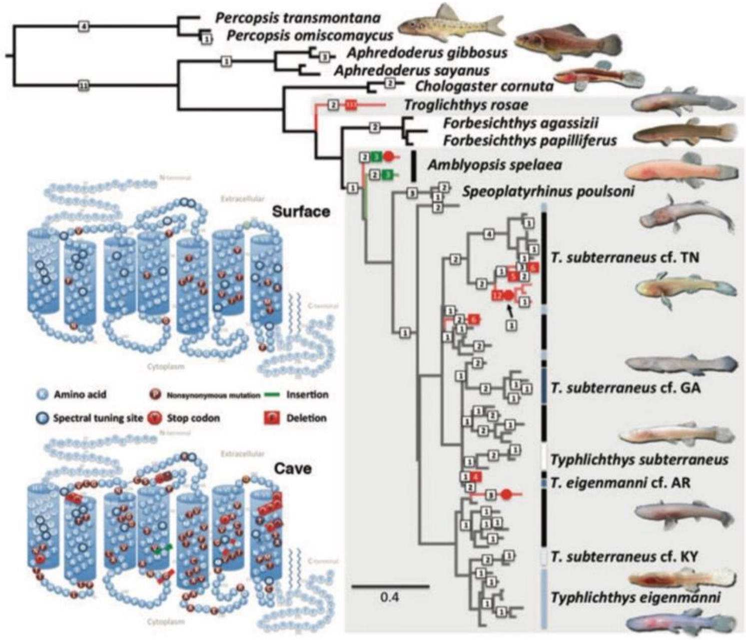


Shah & Gilchrist, *PNAS* 2011
Gilchrist *et al.*, *GBE* 2015
Cope & Shah, *PLOS Genetics* 2022
Cope & Shah, PNAS 2025

Beyond dissertation projects



Shah et al, *Evolution* 2013
Fordyce *et al.*, *Evol. Bioinfo* 2014



Niemiller et al, *Evolution* 2013

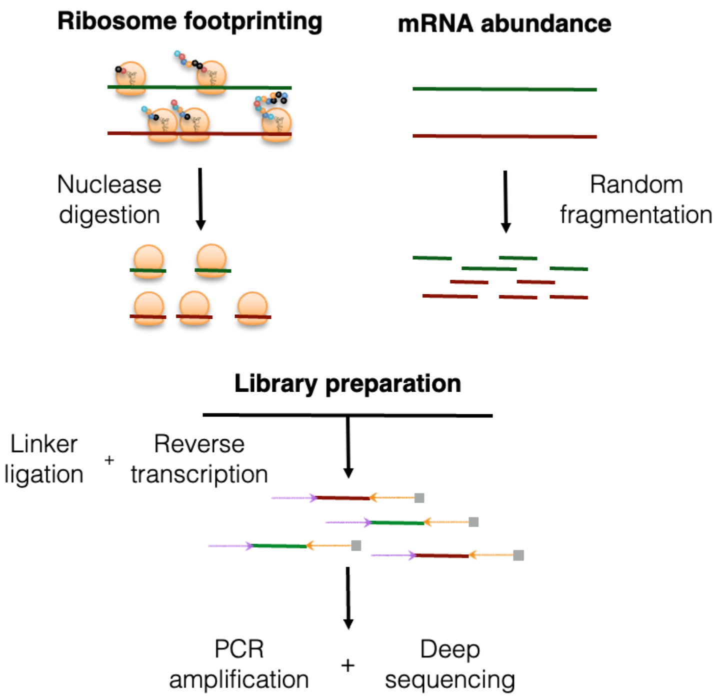
Succeeding in grad school and beyond

- Mentors matter – a lot
- Finding a mentor is easier than you think – Just ask (no, seriously)
- Breadth of knowledge is as important (if not more) as depth that the Ph.D. prepares you for

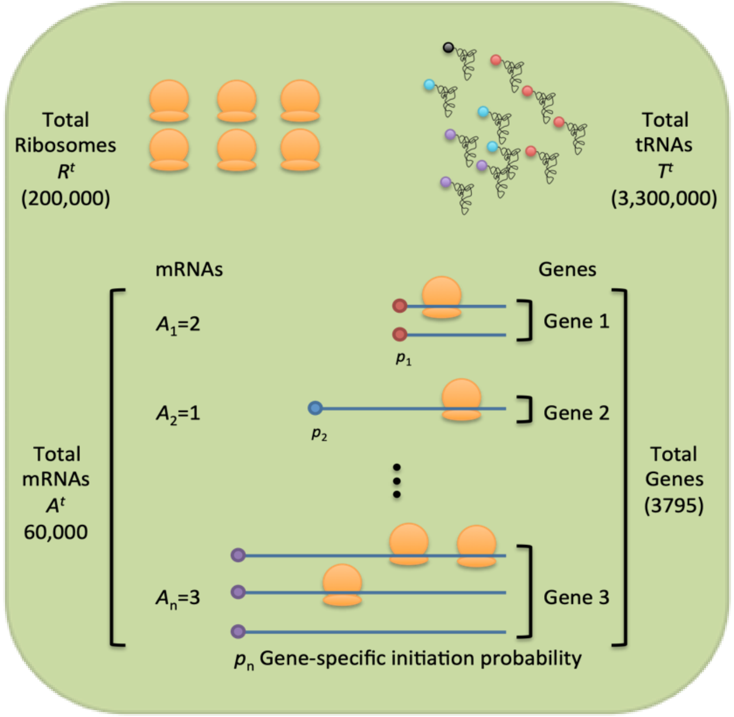
Finding a niche at the intersection of evolution and translation

Univ. of Pennsylvania

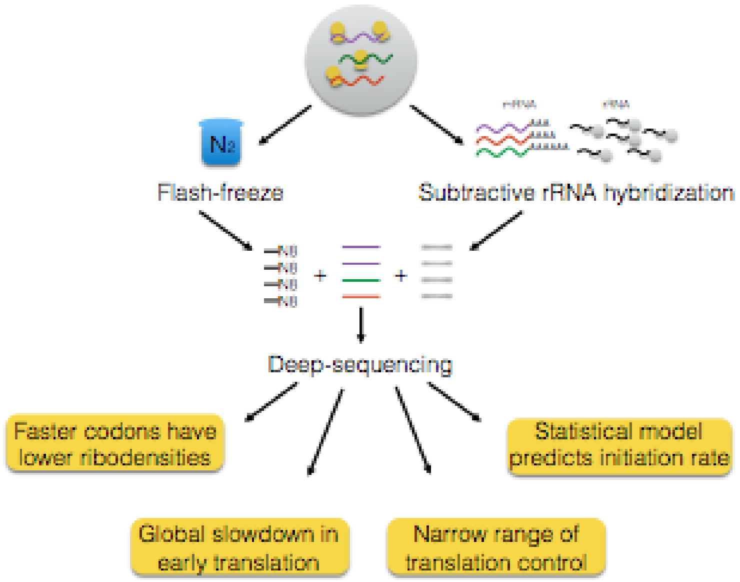
Resolving conflicts between evolutionary theories and new sequencing technologies



Ingolia et al., *Science* 2009



Shah et al., *Cell* 2013

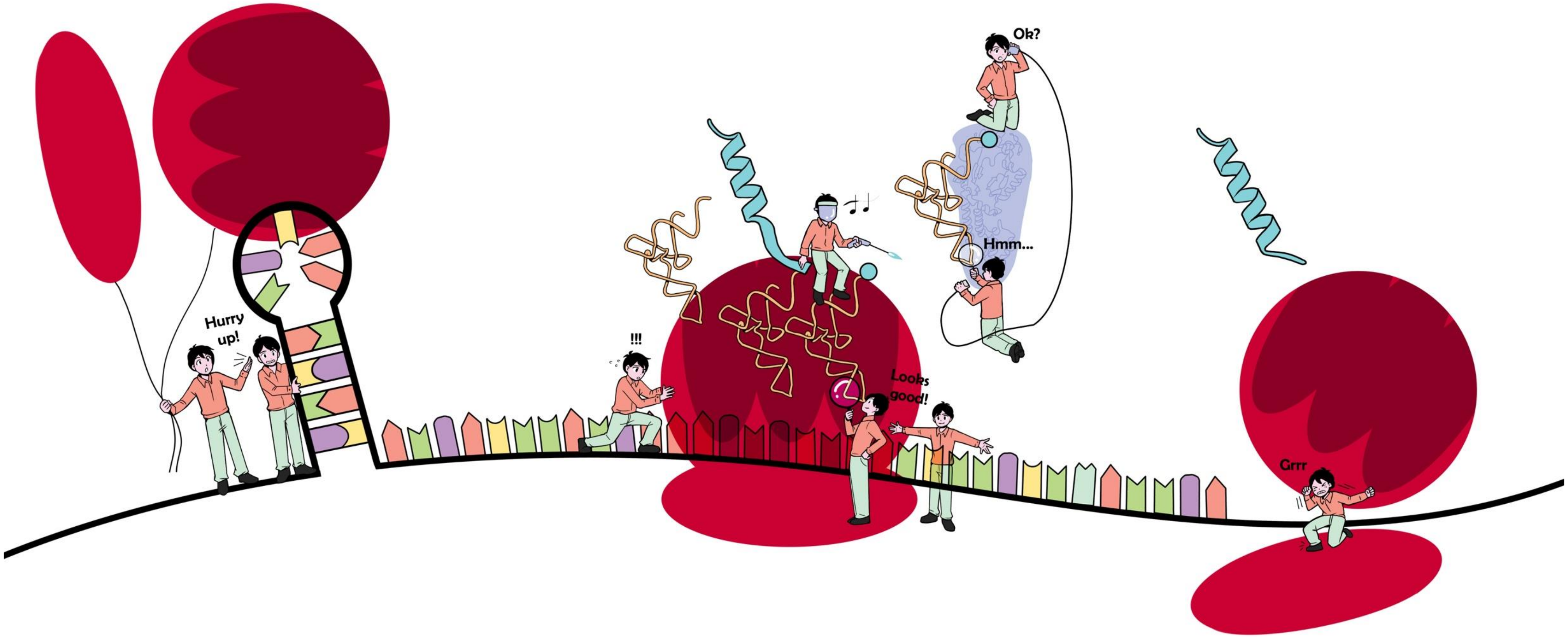


Weinberg & Shah et al., *Cell Reports* 2015

How ~~to find your~~ I found my niche

- Right place at the right time
- Opportunity to work in completely different fields
- Freedom to own my work and build on it
- Leveraging the breadth of experience

Leading a hybrid group
Rutgers University

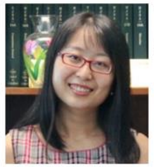


The people who made it all happen


Alex Cope
Postdoctoral researcher


Sangeevan Vellappan
Graduate Student (jointly with Yadavalli lab)

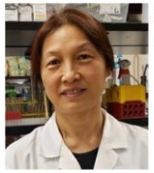

Sukanya Das
Graduate Student


Tongji Xing
Graduate Student


John Favate
Graduate Student


Theresa Rogers
Postdoctoral researcher


Peter Tilton
Undergraduate Researcher


Shun Liang
Lab manager


Alexandra Logerfo
Graduate Student


Anna MacKinnon
Undergraduate Researcher


Sadhana Chidambaran
Undergraduate Researcher


Alexander Salibi
Masters Student

Kevin Shen
Undergraduate Researcher


Sagara Wijeratne
Lab assistant


Kirti Gupta
Postdoctoral researcher


Darya Pavlenko
Undergraduate Researcher


Albert Sultan
Undergraduate Researcher


Madhuri Bhupathiraju
Undergraduate Researcher


Yasasvi Talagadadeevi
Undergraduate Researcher


Razan Elhag
Undergraduate Researcher


Kush Patel
Undergraduate Researcher


Anjali Patel
Undergraduate Researcher

Building analytical tools for new sequencing technologies

Explore datasets

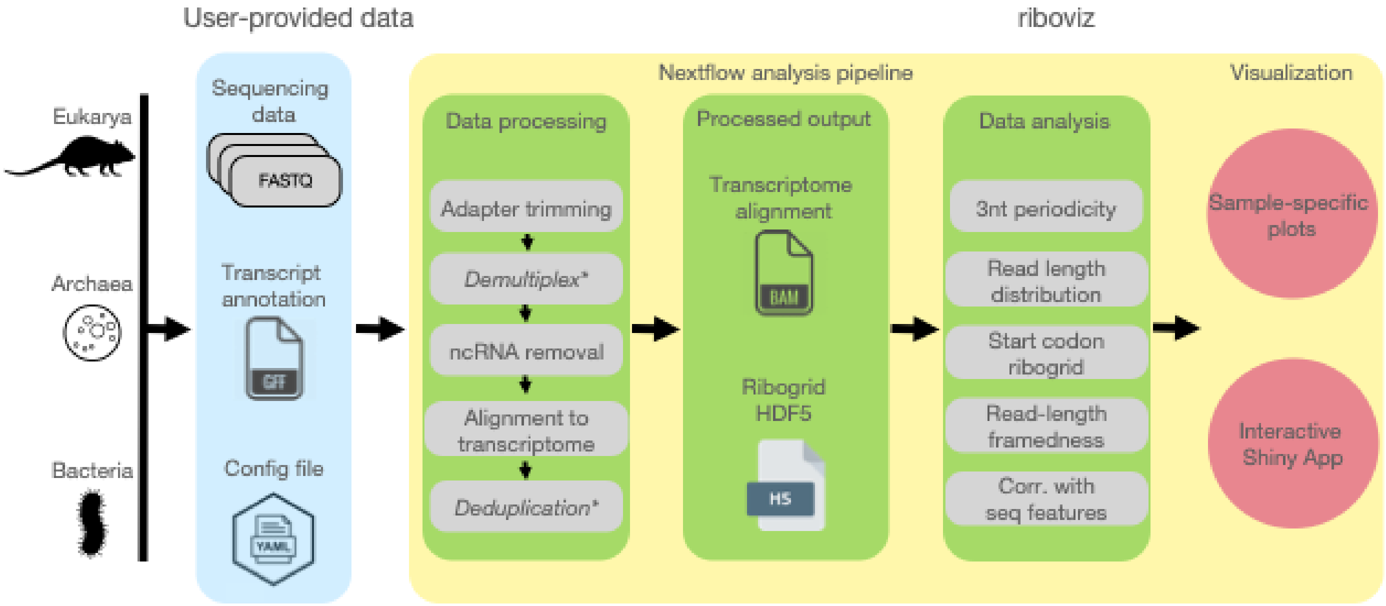
Metagenomic Analyses

3 nucleotide periodicity

Analysis of all mapped reads to visualize the periodicity of mapped reads along the ORFs. Ribosome-footprinting datasets should have a 3nt periodicity as well as accumulation at the start and stop codons.

Read count histogram

lDistribution of lengths of reads mapped to coding sequences.



User-provided data

riboviz

Eukarya

Archaea

Bacteria

Sequencing data

FASTQ

Transcript annotation

GFF

Config file

YAML

Nextflow analysis pipeline

Data processing

Adapter trimming

*Demultiplex**

ncRNA removal

Alignment to transcriptome

*Deduplication**

Processed output

Transcriptome alignment

BAM

Ribogrid HDF5

H5

Data analysis

3nt periodicity

Read length distribution

Start codon ribogrid

Read-length framedness

Corr. with seq features

Visualization

Sample-specific plots

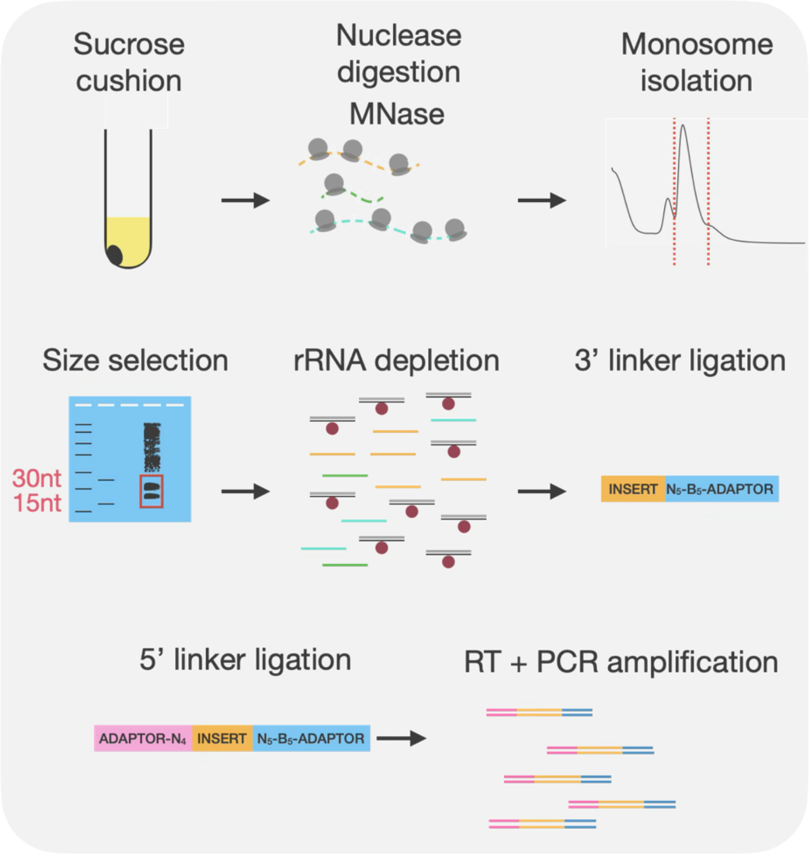
Interactive Shiny App

Carja *et al.*, *BMC Bioinformatics* 2017

Cope *et al.*, *Bioinformatics* 2022

Building genomic tools

Improved Ribo-seq methods



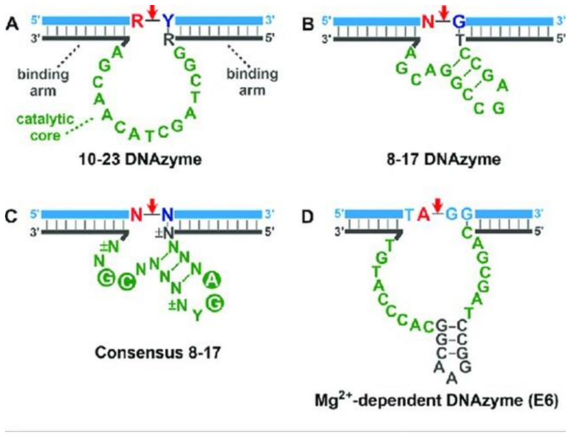
Sucrose cushion → Nuclease digestion MNase → Monosome isolation

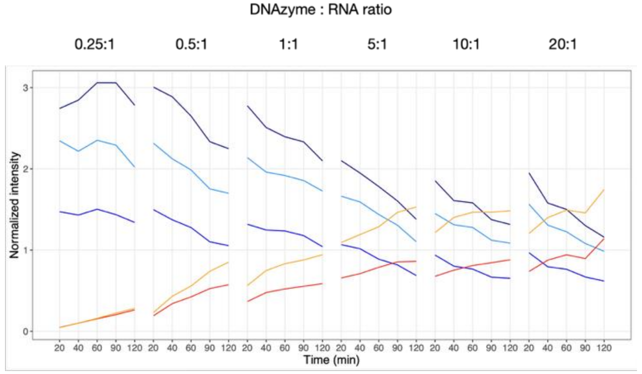
Size selection → rRNA depletion → 3’ linker ligation

5’ linker ligation → RT + PCR amplification

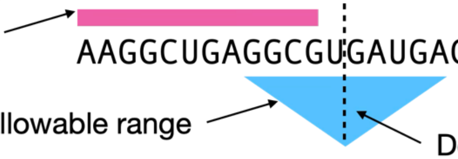
Cope *et al.*, *Methods in Mol Bio* 2022

DNAzyme-based tools for NGS libraries





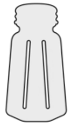
Minimum homology arm size



AAGGCUGAGGCGUGAUGACGAGGCAC

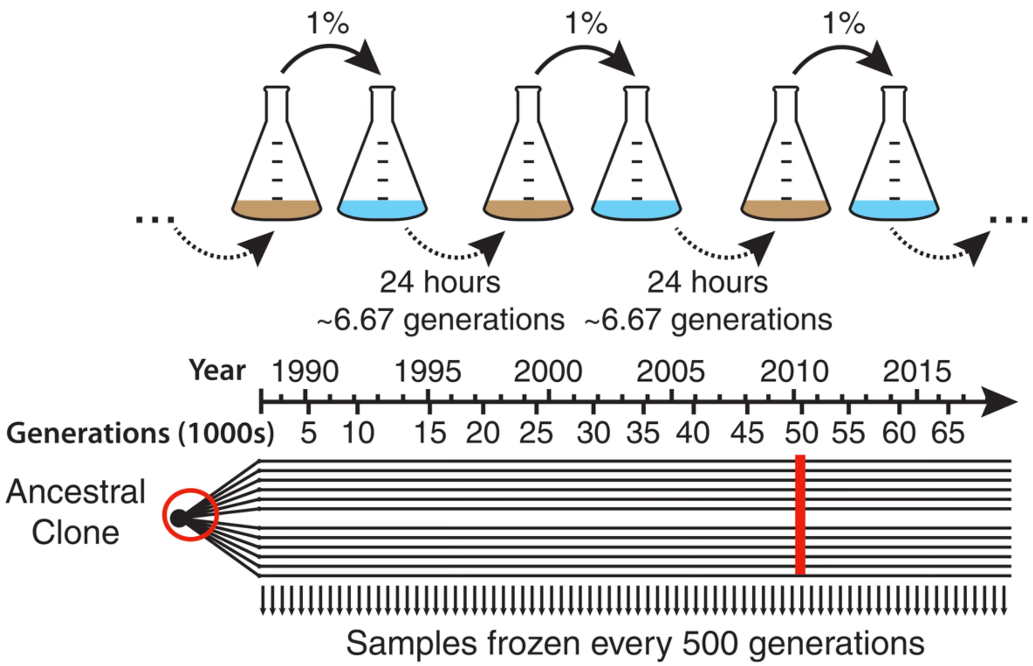
Allowable range

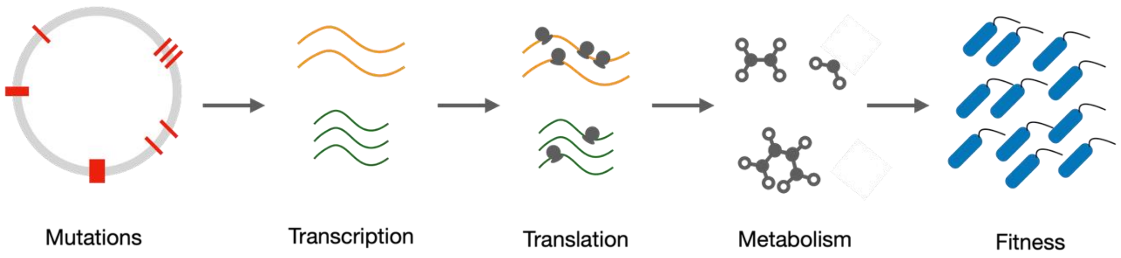
Desired cut site

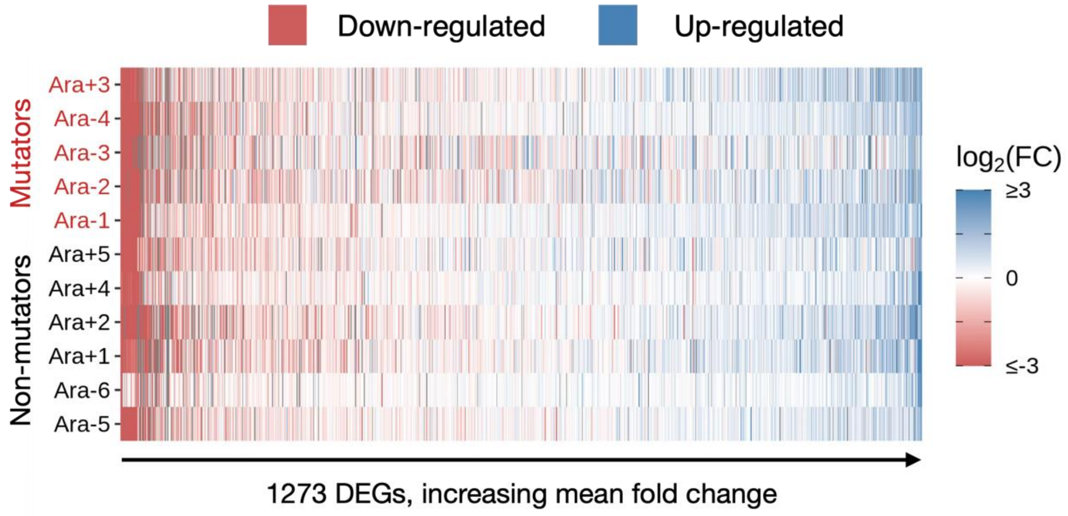


Salt concentrations

Mapping the landscape of molecular changes over 22 years of bacterial adaptation





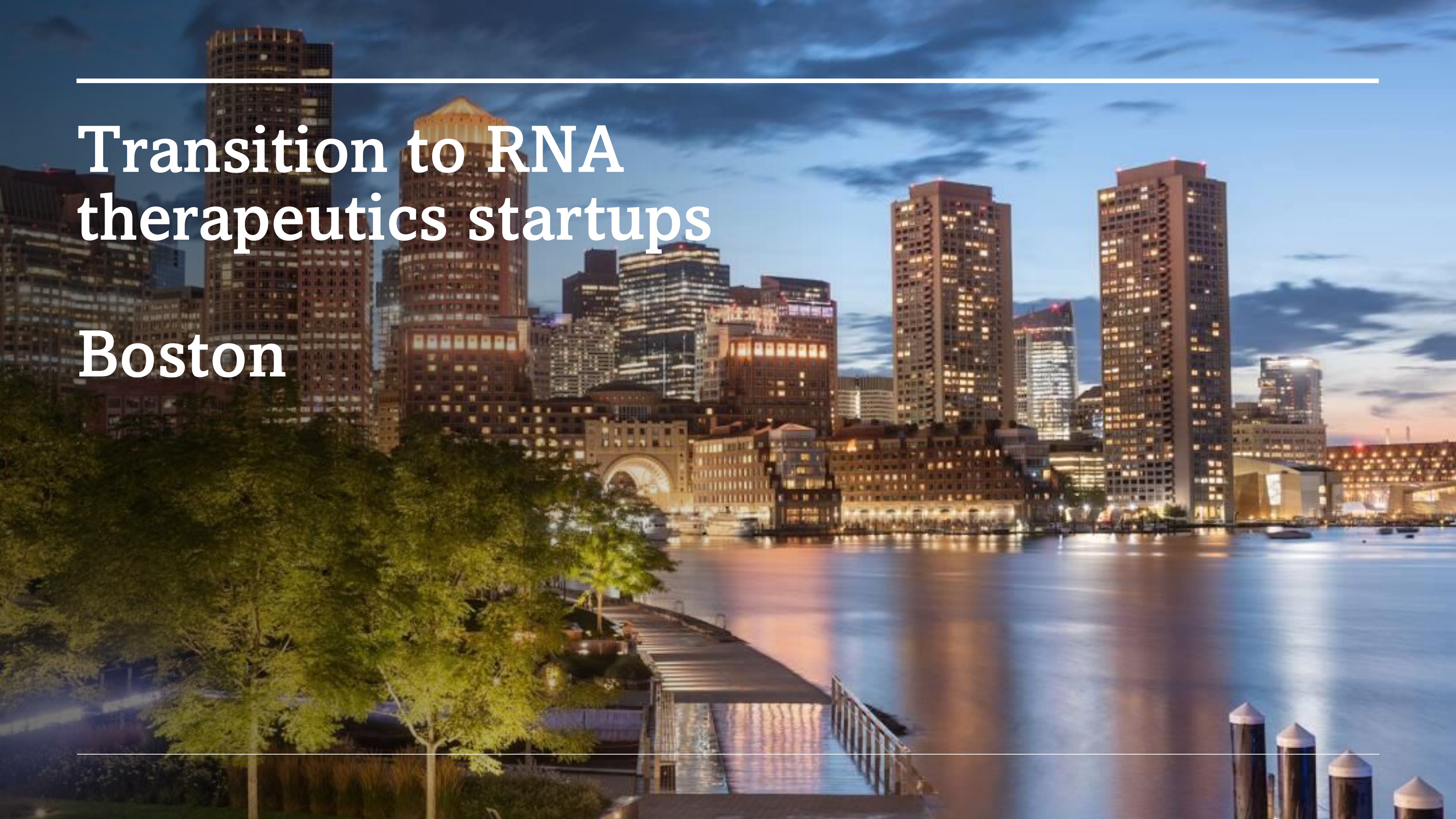


Lenski et al., 1991; Blount et. al. 2018

Favate *et al.*, eLife 2022; Favate *et al.* eLife 2023

A few notes on building a successful team

- Recruit people who are smarter than you
- Willingness to learn is more critical than skill
- Willingness to teach/share is even more important
- Patience is key – a single wrong hire can set back an entire team



Transition to RNA therapeutics startups

Boston

Multiple modalities targeting hard-to-drug targets

Ananke THERAPEUTICS	Small molecules therapies targeting RNA structures
convergenceBIO Better mRNA	More stable and better translated mRNAs
SanegeneBio	Next generation of RNAi therapies

Transition to biotech startups

- Why?
  - A feeling of moving away from the science to a purely managerial role
  - Harder to sustain a hybrid program despite funding
  - Midlife crisis?
- Why then?
  - Post-COVID excitement around RNA-based therapies
  - A two-year unpaid sabbatical
- How?
  - Mostly unremarkable
  - Recruiters can be helpful
  - Honest conversations about strengths and limitations

What do I do?

- Build computational pipelines
  - To design sequences – mRNAs, siRNAs
  - To analyze high-throughput genomic datasets
- Build informatics infrastructure
  - Mine publicly available large datasets and integrate them with internal data to build a comprehensive knowledgebase
  - Deploy and maintain LIMS+ELN systems
- Lead and support target discovery and nomination efforts
- Manage external collaborations and partnerships

Computational Biology in the era of vibe coding

- Vibe coding is here to stay
- Simple scripts/pipelines to production-grade code
- Integrated and automated workflows
- Leaner teams

- Challenges:
  - Over-complicated and fragile
  - Edge-case handling becomes harder
  - IP considerations when designing molecules