

High-throughput experimental approaches mapping RNA structure to function

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Structure is a fundamental property of every RNA

Structure-dependent **activity**

- rRNA, tRNA, snRNA, ribozymes, ...

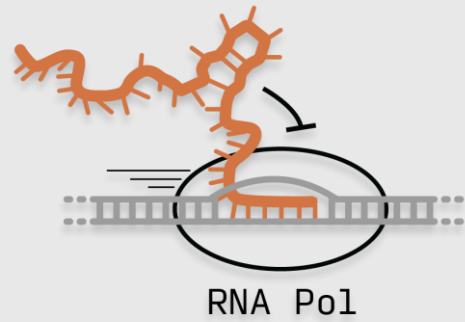
} *lots is known*

Structure-dependent **regulation**

- mRNA, eRNA, uaRNA, lncRNA, ...
- genomes are pervasively transcribed

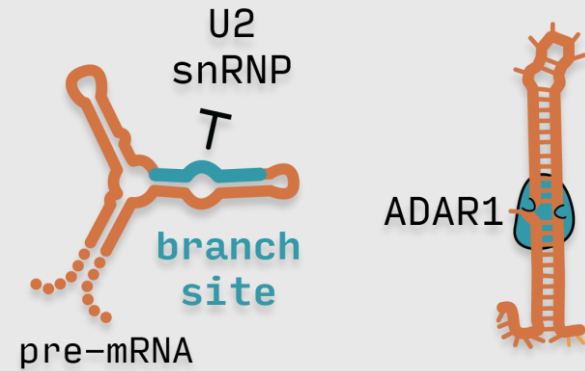
} *very little is known*

Transcription



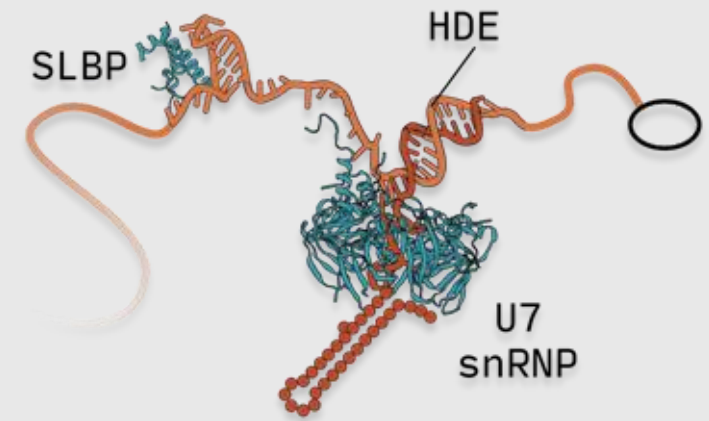
Turowski et al (2020) 32585128
 Zamft et al (2012) 22615360
 Schärffen et al (2019) 33811916

RNA Processing



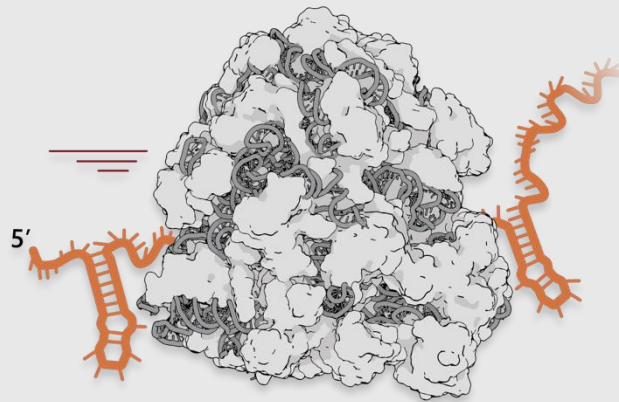
Li et al (2024) 38499485
 Matthews et al (2016) 27065196

Termination

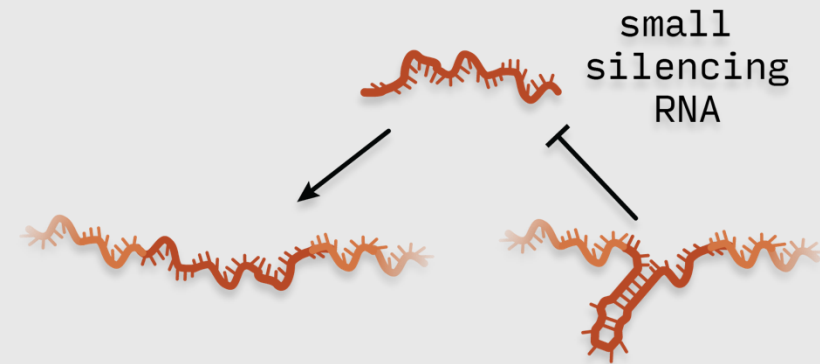


Sun et al (2020) 32029631
 Saldi et al (2018) 29483154

Translation



RNAi



Becker et al (2019) 31324449
 Ameres et al (2007) 17632058
 Reynolds et al (2004) 14758366

Multi-layered regulation of gene output

...GTGTTGTAATTATGATTGAGAGATTATAGTTGCGGATTCC... DNA

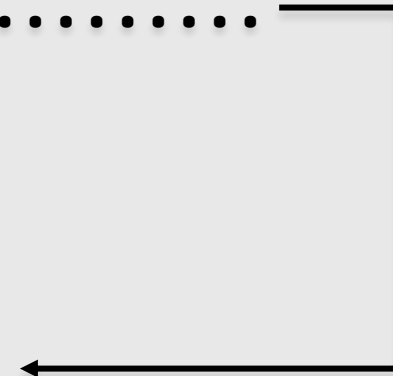
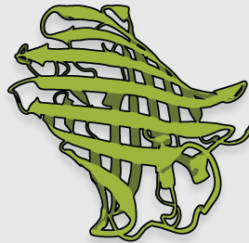


...GUGUUGUAAUUAUGAUUGAGAGAUUAUAGUUGCGGAUUCC... RNA

.....((((((((((((((((.....)))))))))))))).....



phenotype
= gene output



Overview

1. Measuring co-transcriptional folding

- high-resolution, RNA polymerase position-resolved DMS probing in yeast cells

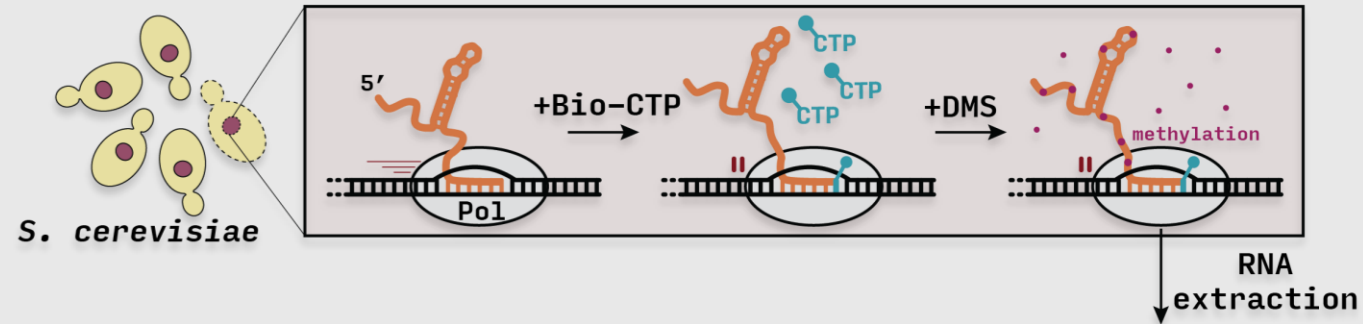
Schärfer et al (2025) 40139190

2. Intron RNA structure controls pre-mRNA splicing

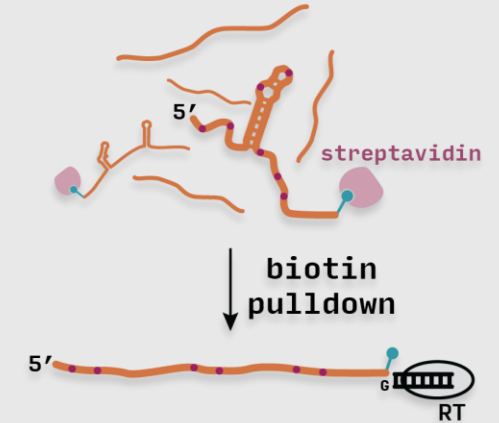
- massively parallel reporter assays map sequence to function
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Schärfer et al (2026) 41278789
preprint

Co-transcriptional structure tracking: CoSTseq



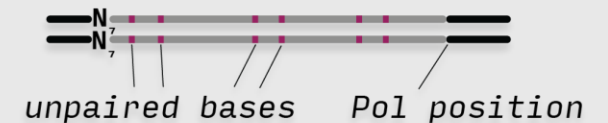
biotin run-on



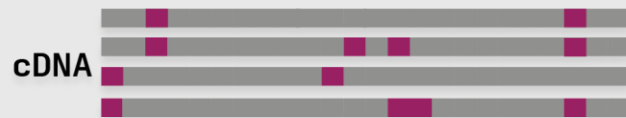
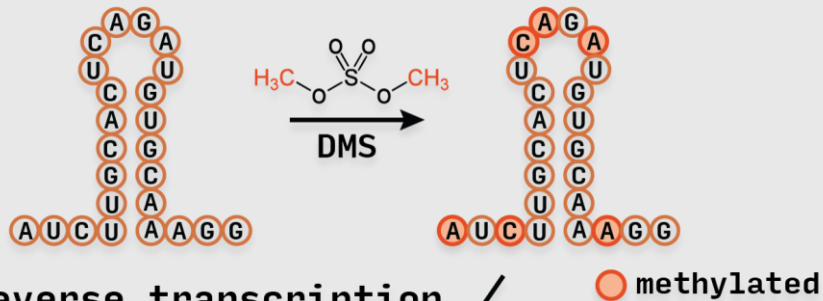
reverse transcription,
adapter ligation



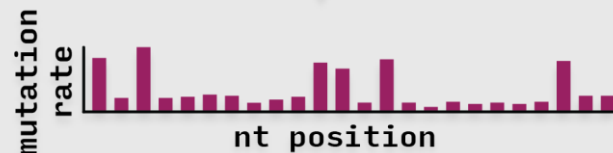
PCR,
2x150bp Illumina seq



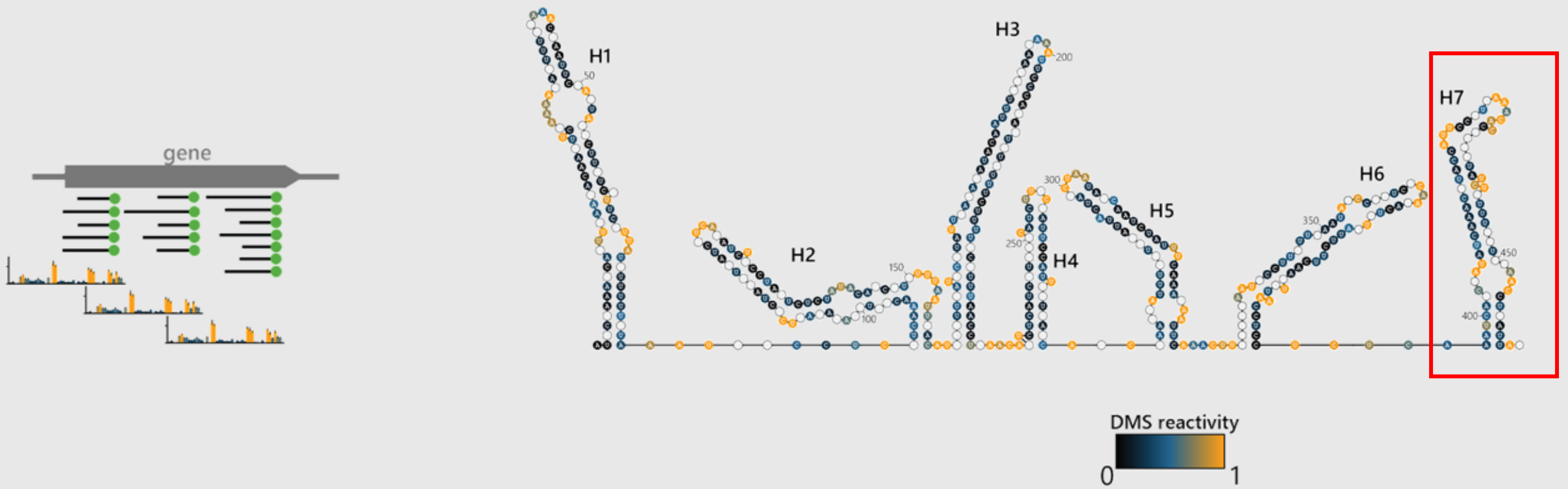
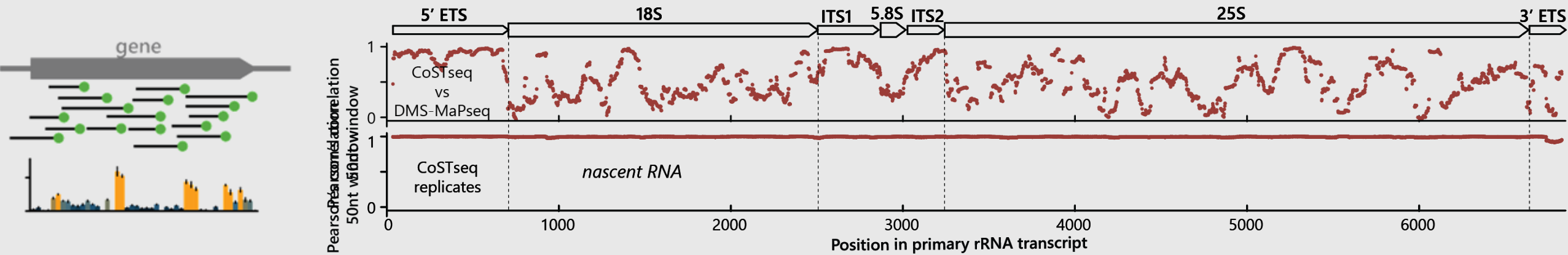
DMS-MaPseq

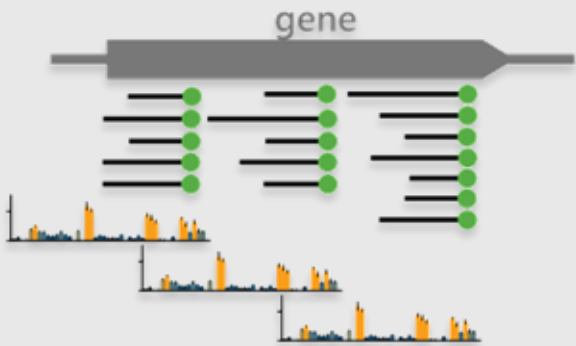


sequencing

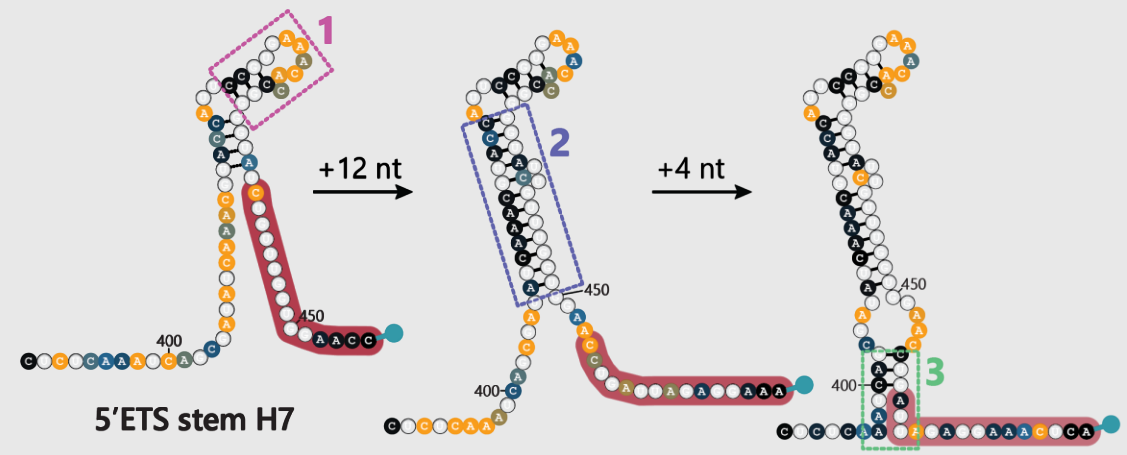
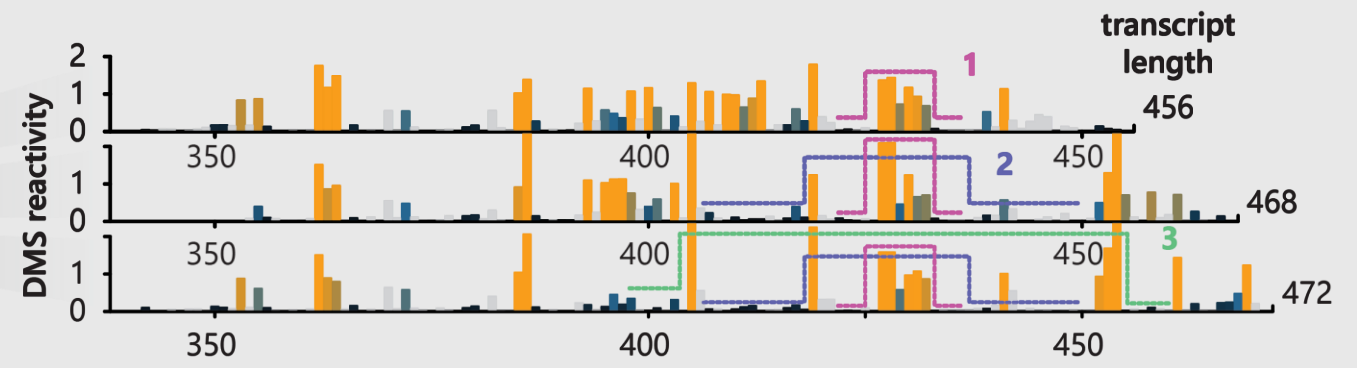
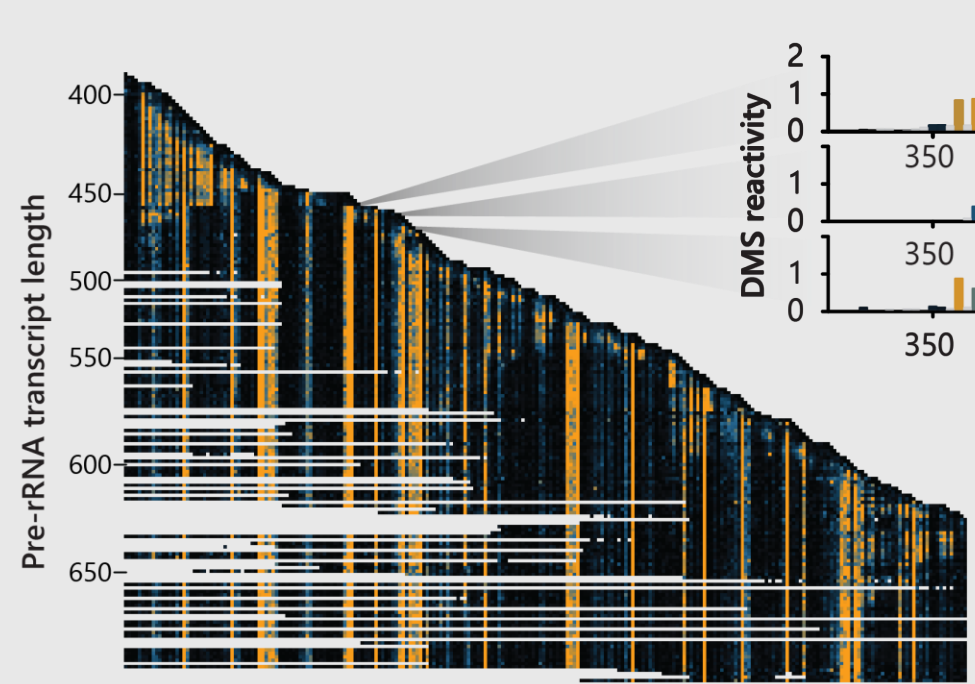
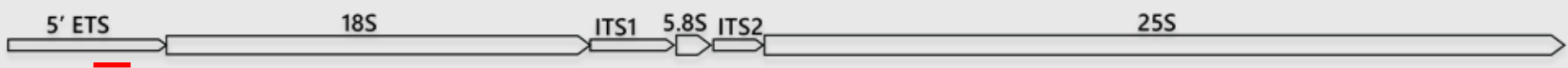


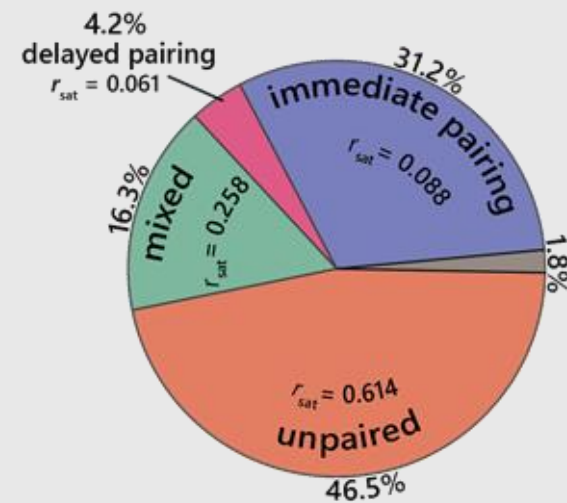
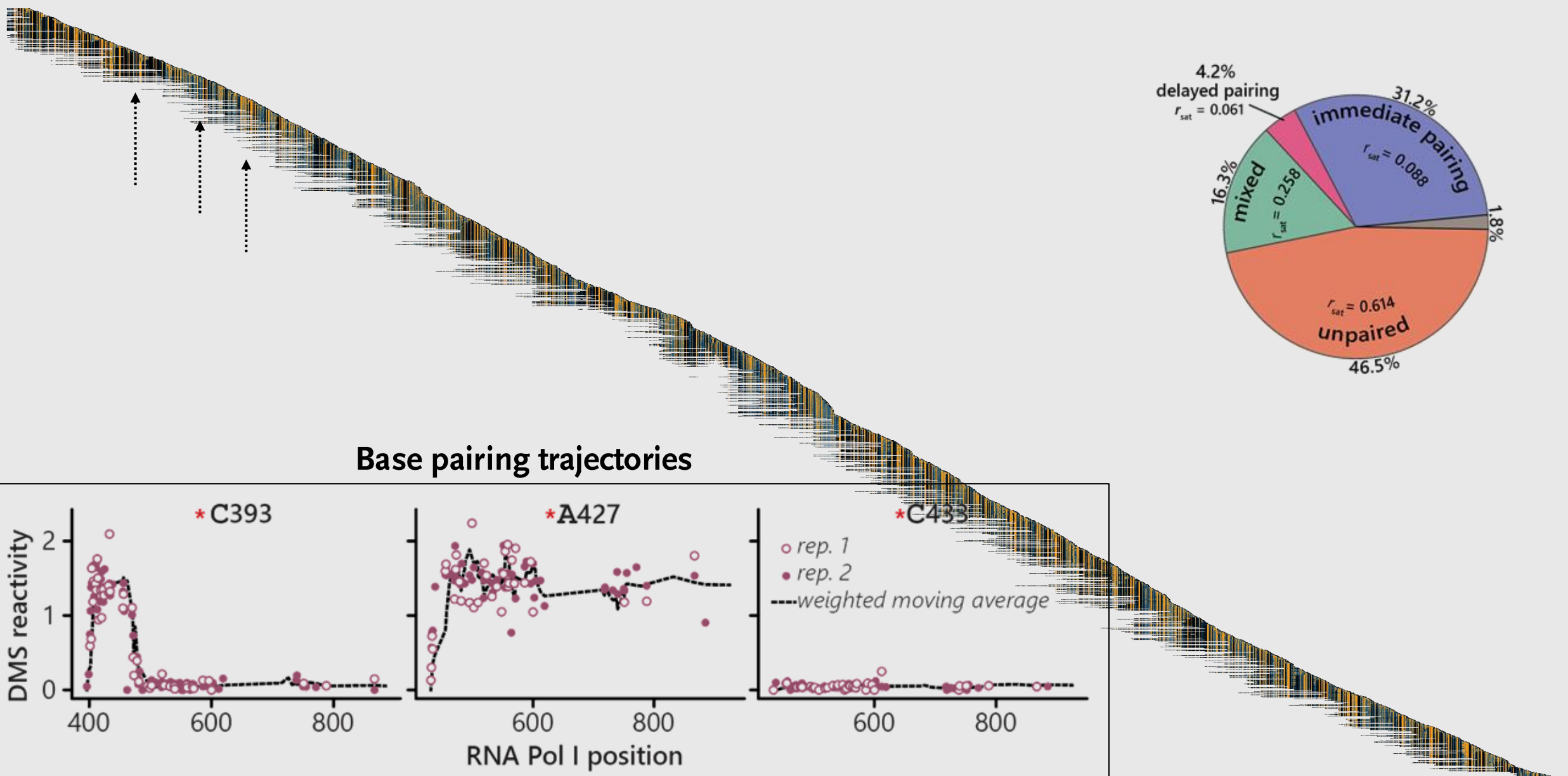
Nascent RNA base pairs upon exiting RNA Pol



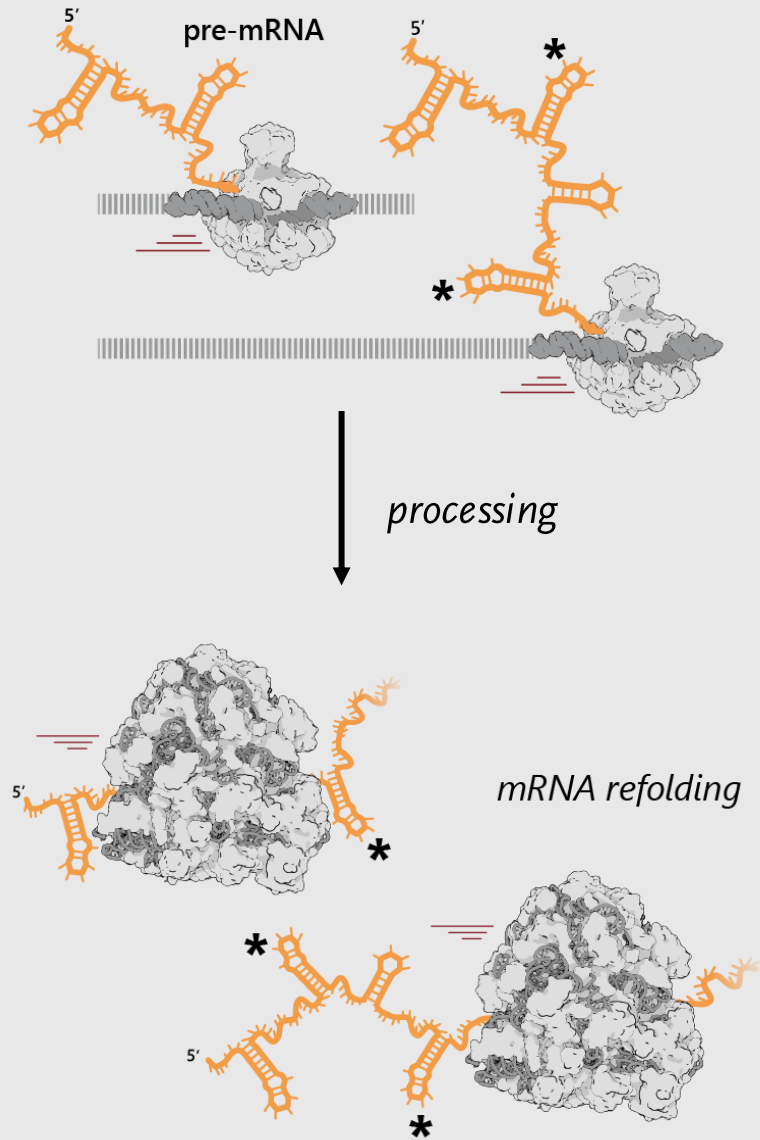
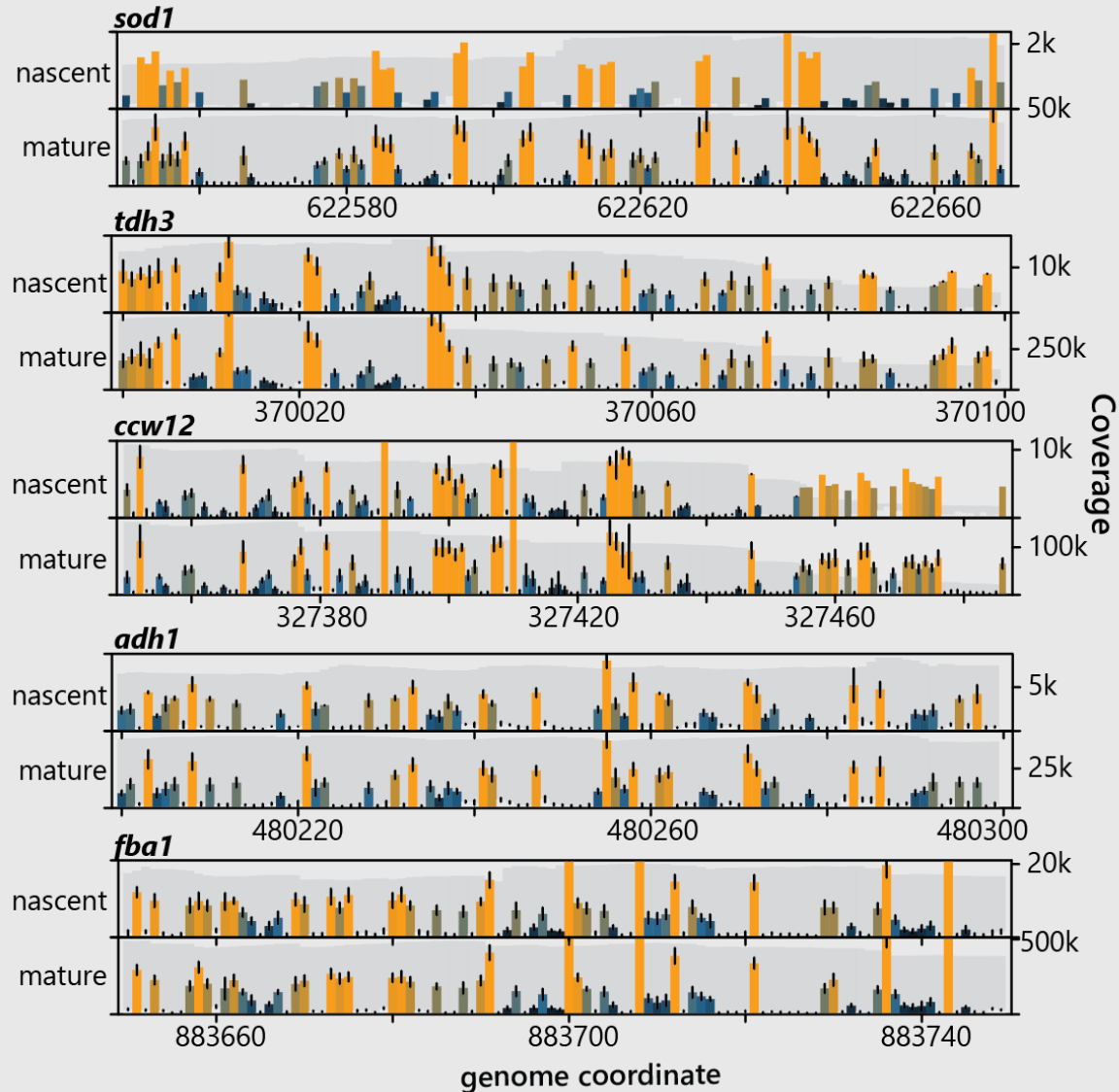


pre-rRNA:





Concordance of nascent and mature mRNA structure



Overview

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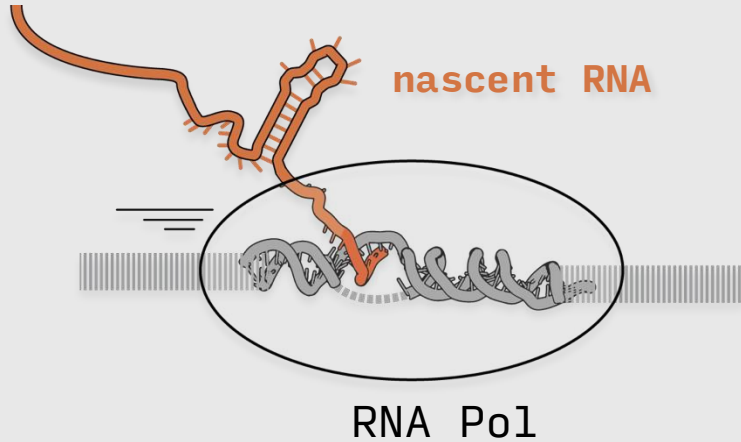
Schärfer et al (2025) 40139190

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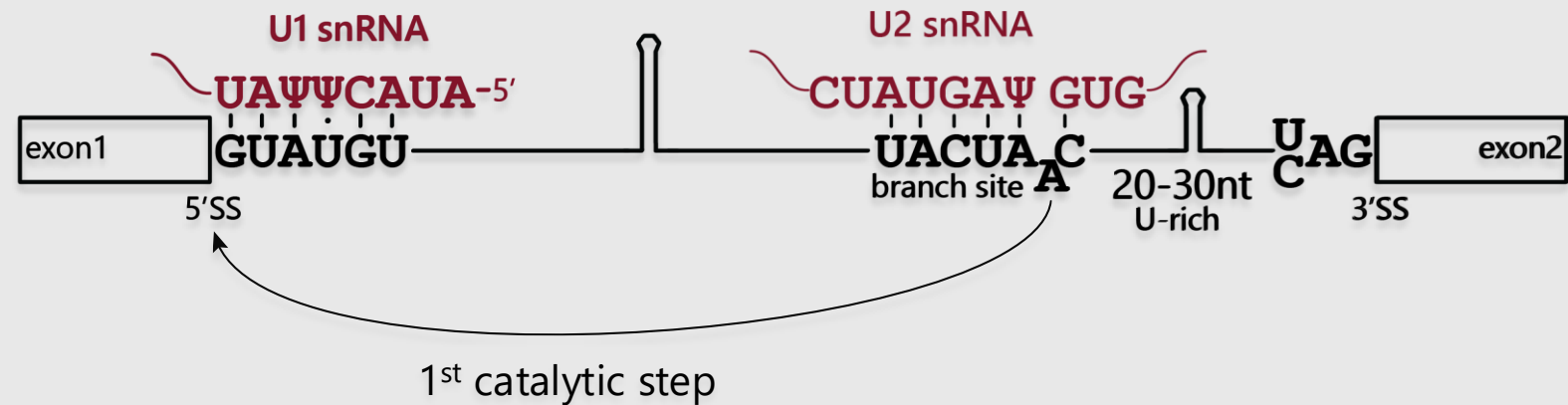
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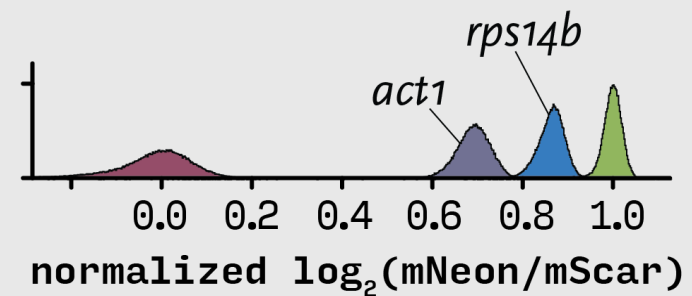
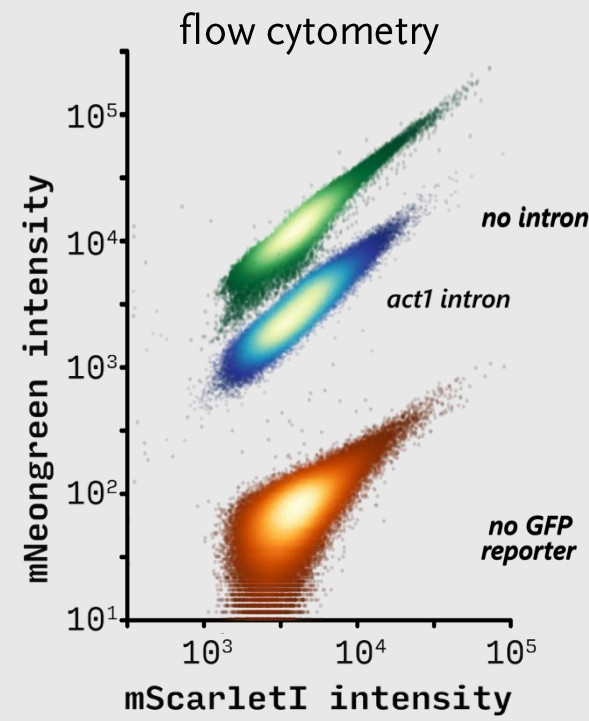
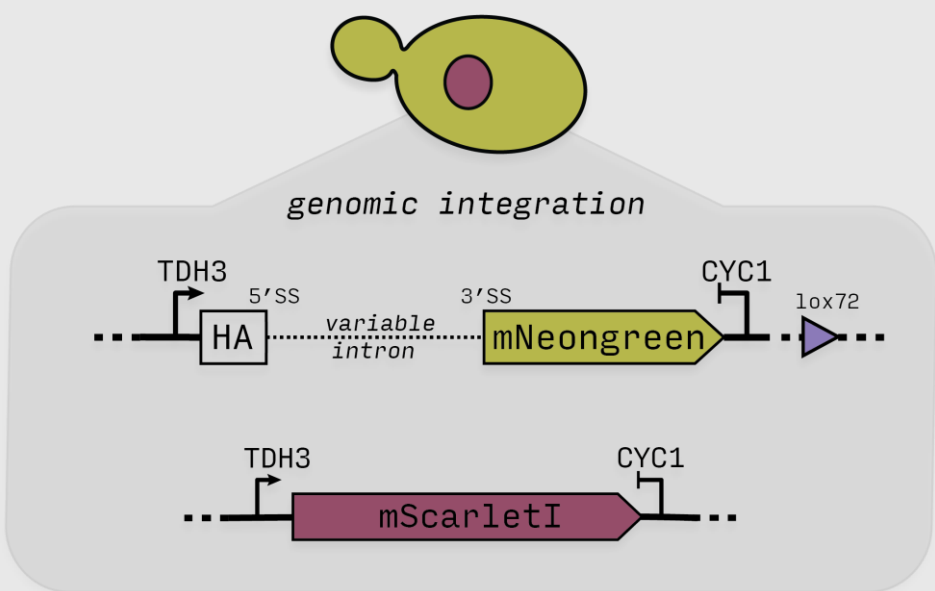
The spliceosome encounters folded RNA



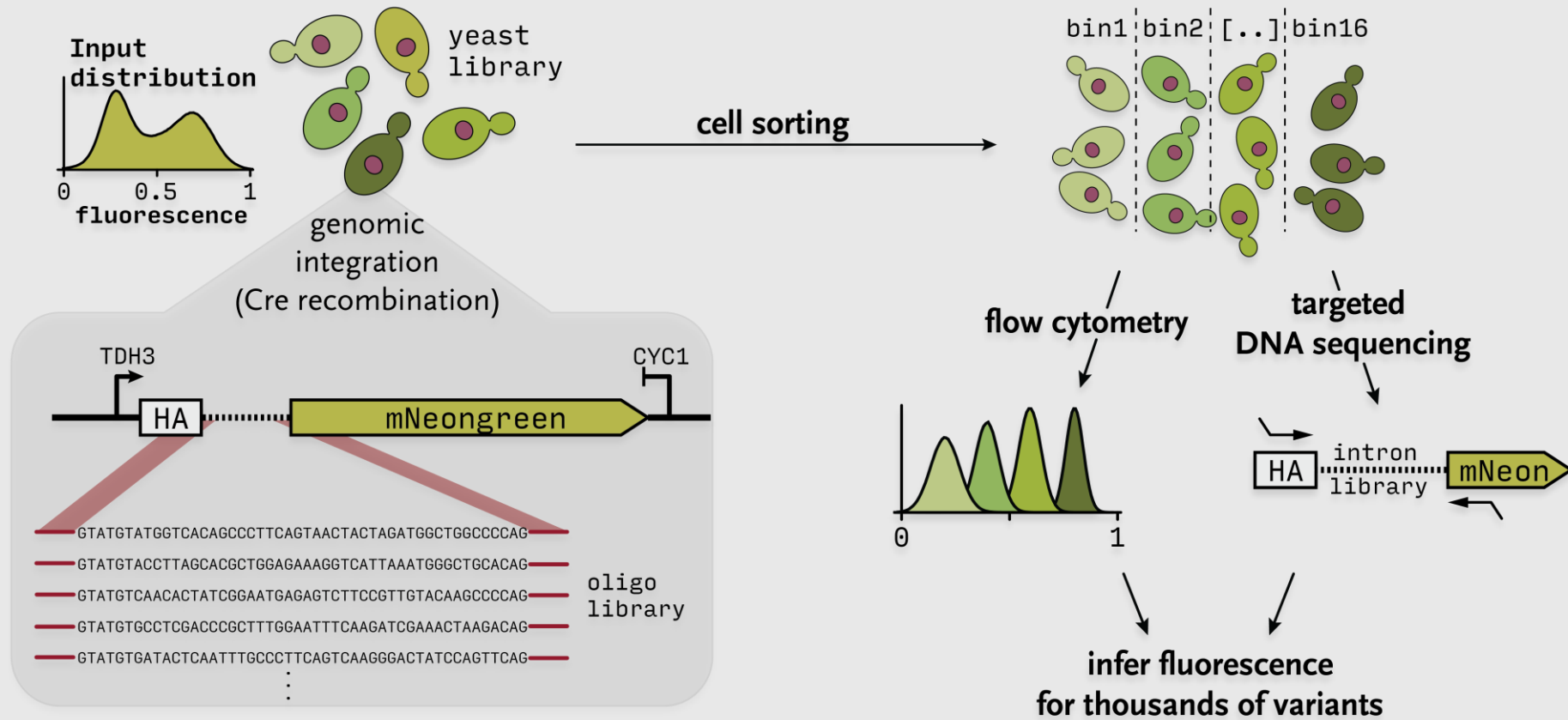
To what degree is RNA processing regulated by structure formation?



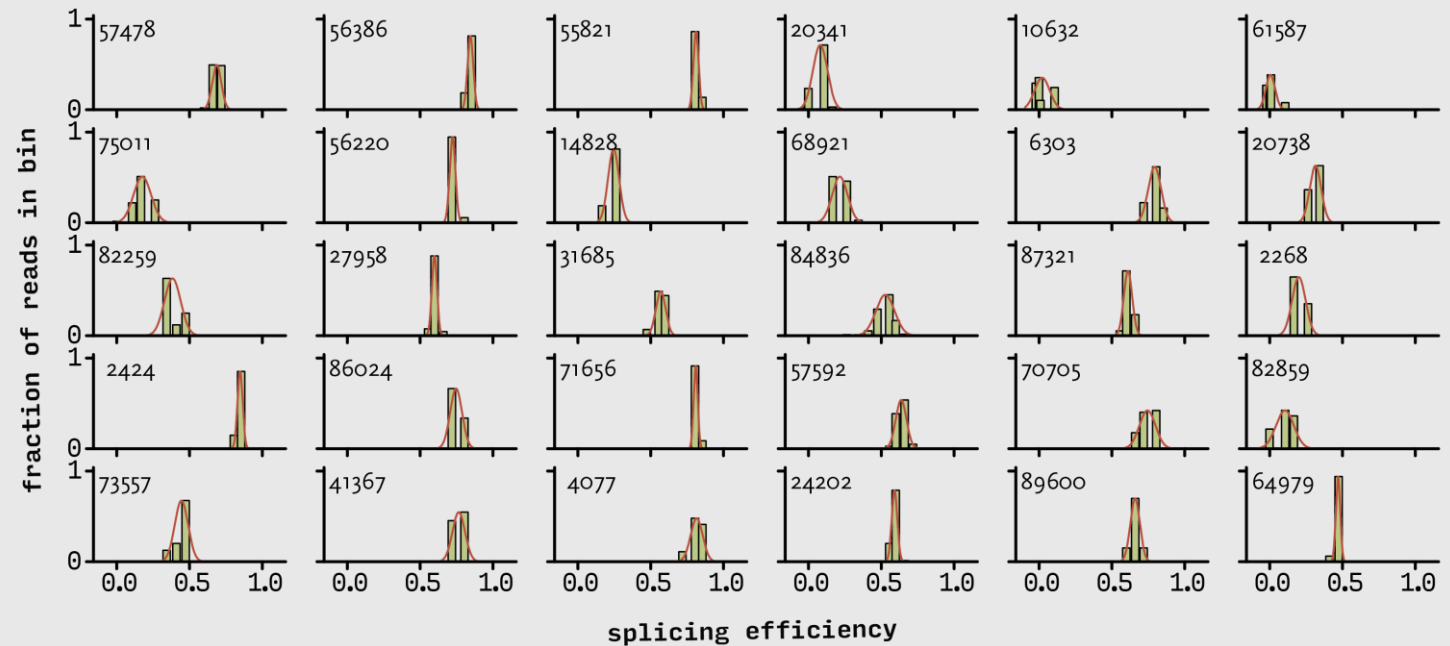
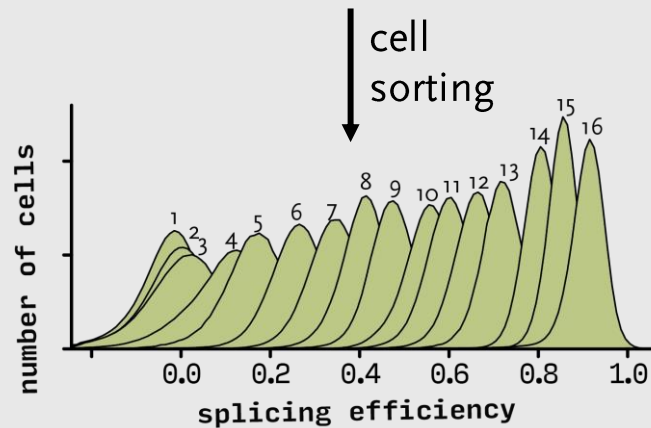
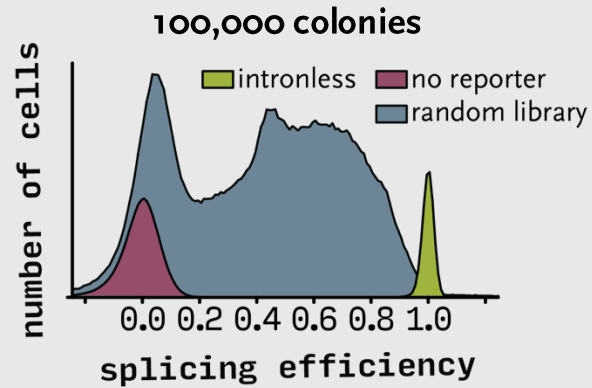
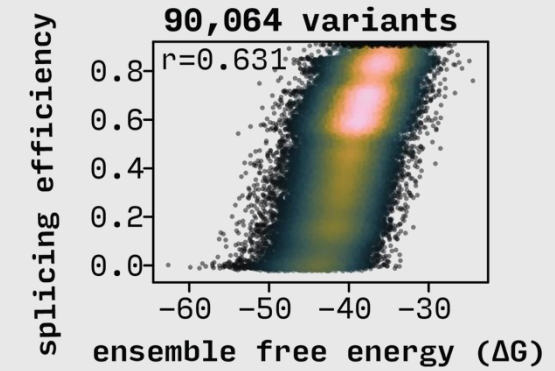
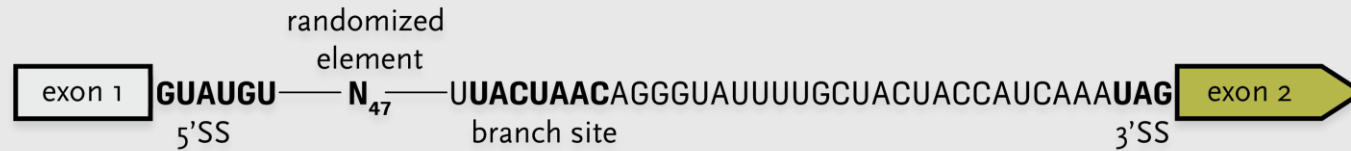
A flow cytometry experiment that reports on splicing efficiency



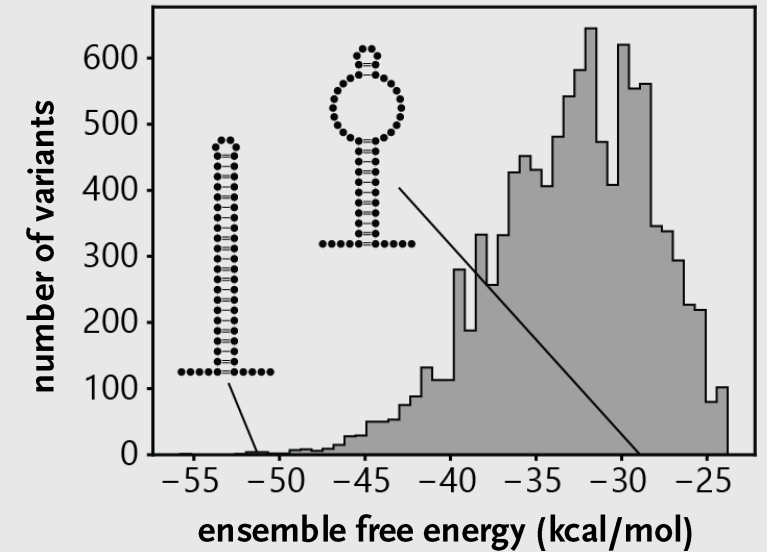
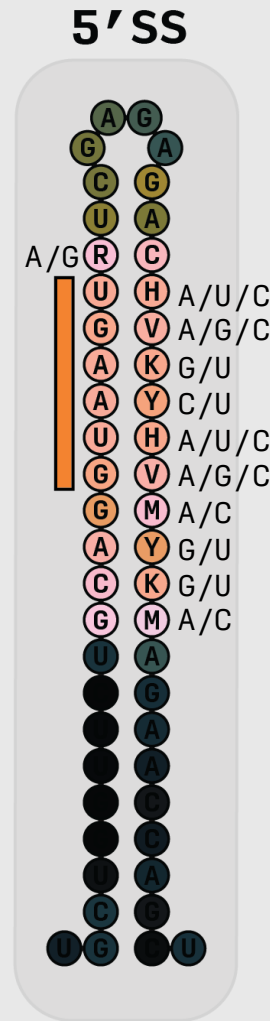
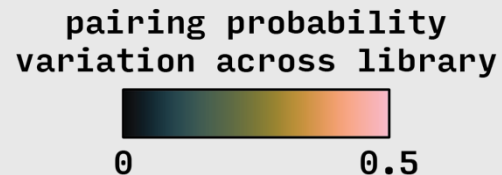
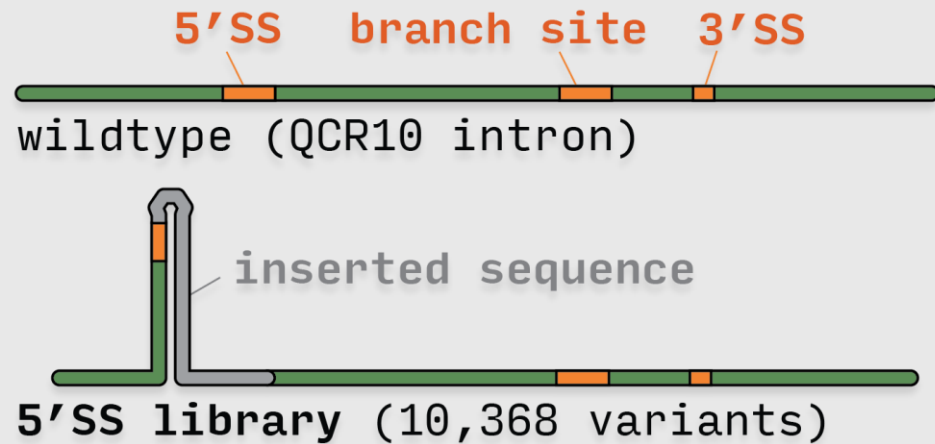
Measuring fluorescence for thousands of reporters with Sort-seq

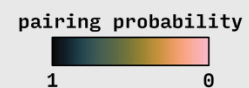
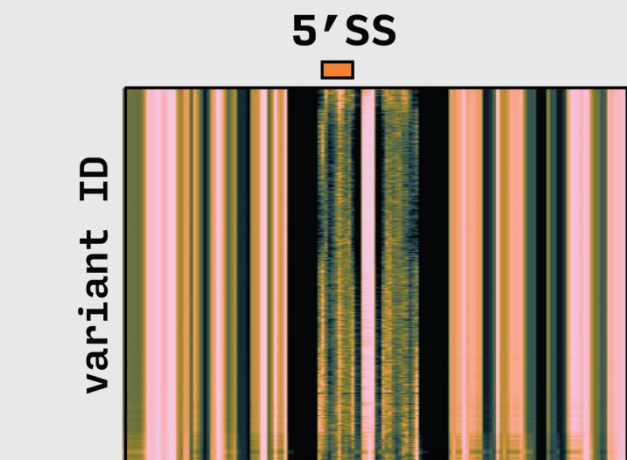


Splicing depends on intron RNA structure

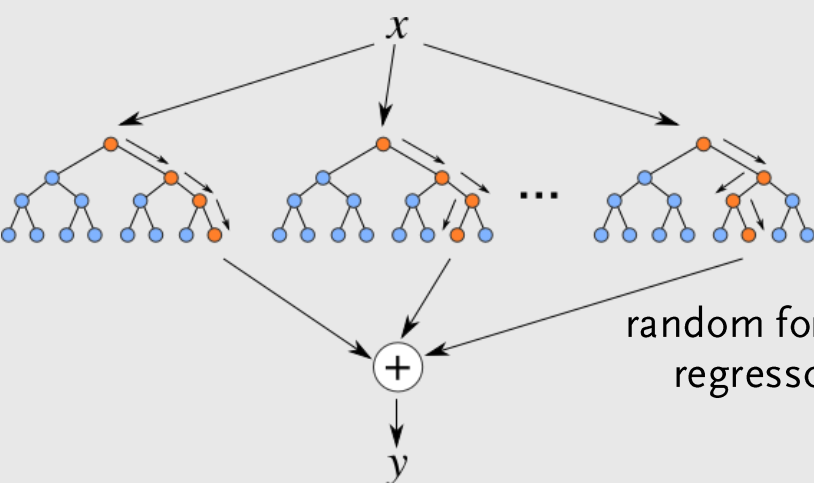


A combinatorial library generates variants with paired 5' SS

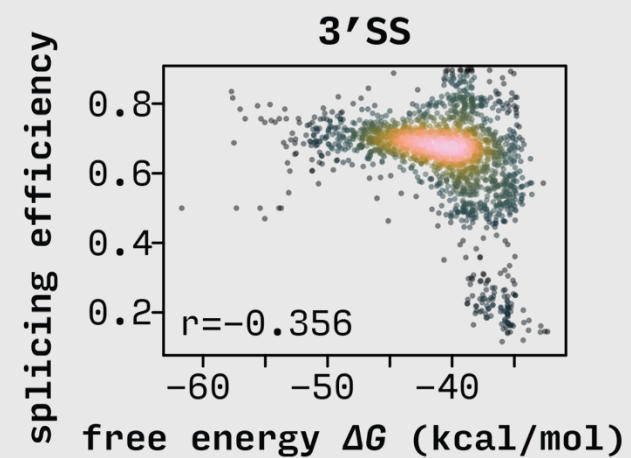
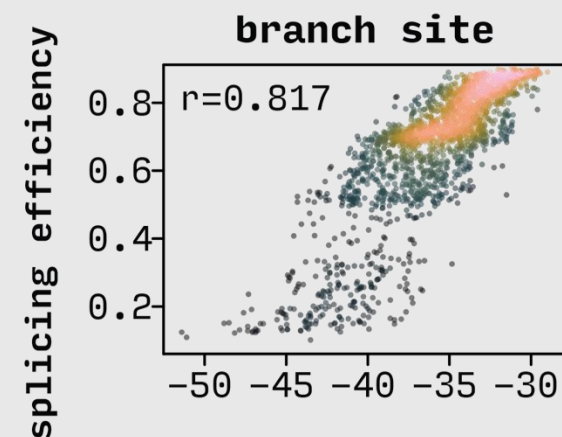
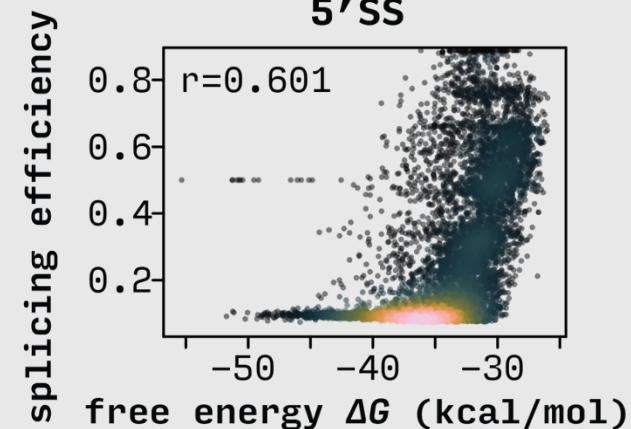
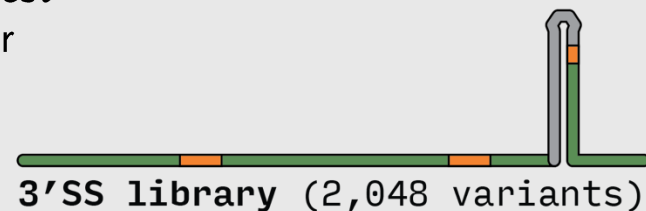
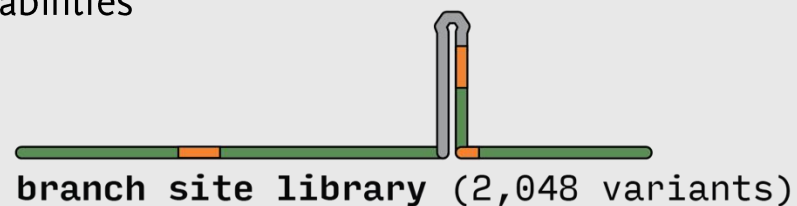


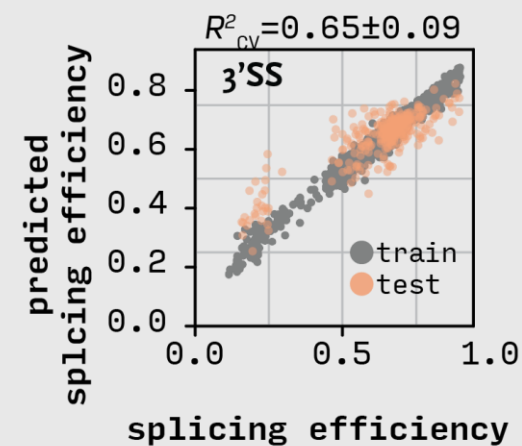
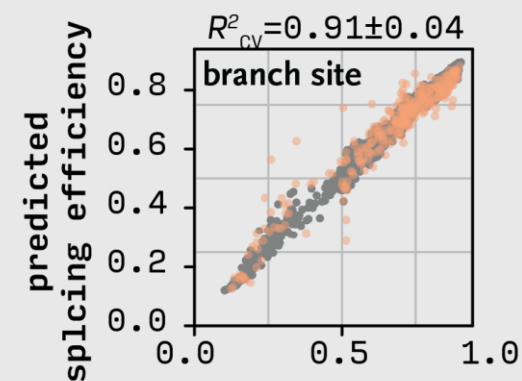
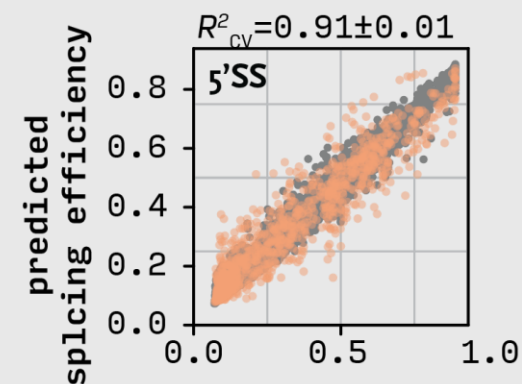
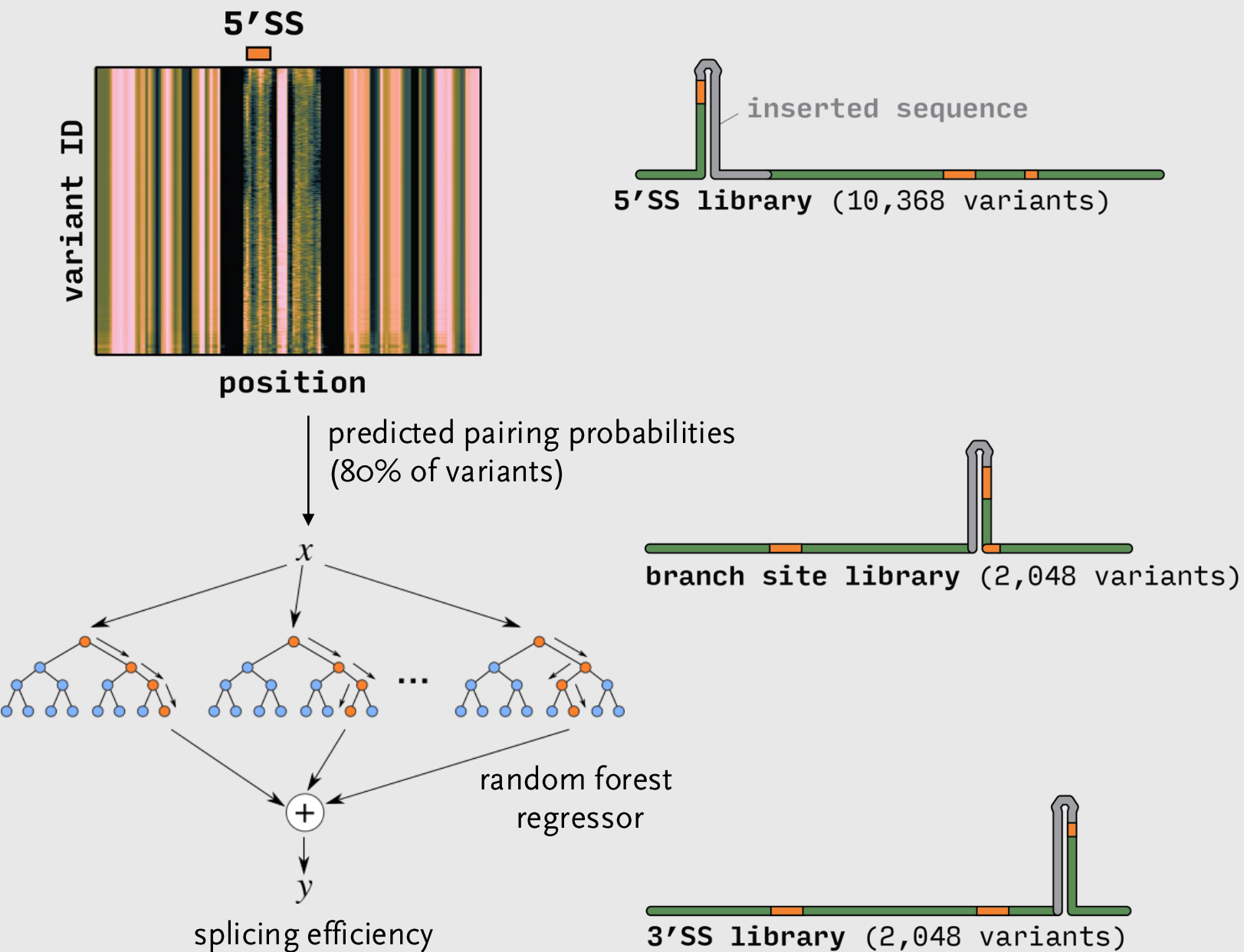


predicted pairing probabilities
(80% of variants)



fluorescence





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