

Transcription in a **SCRaMbLEd** genome

Aaron Brooks /  @scalefreegan

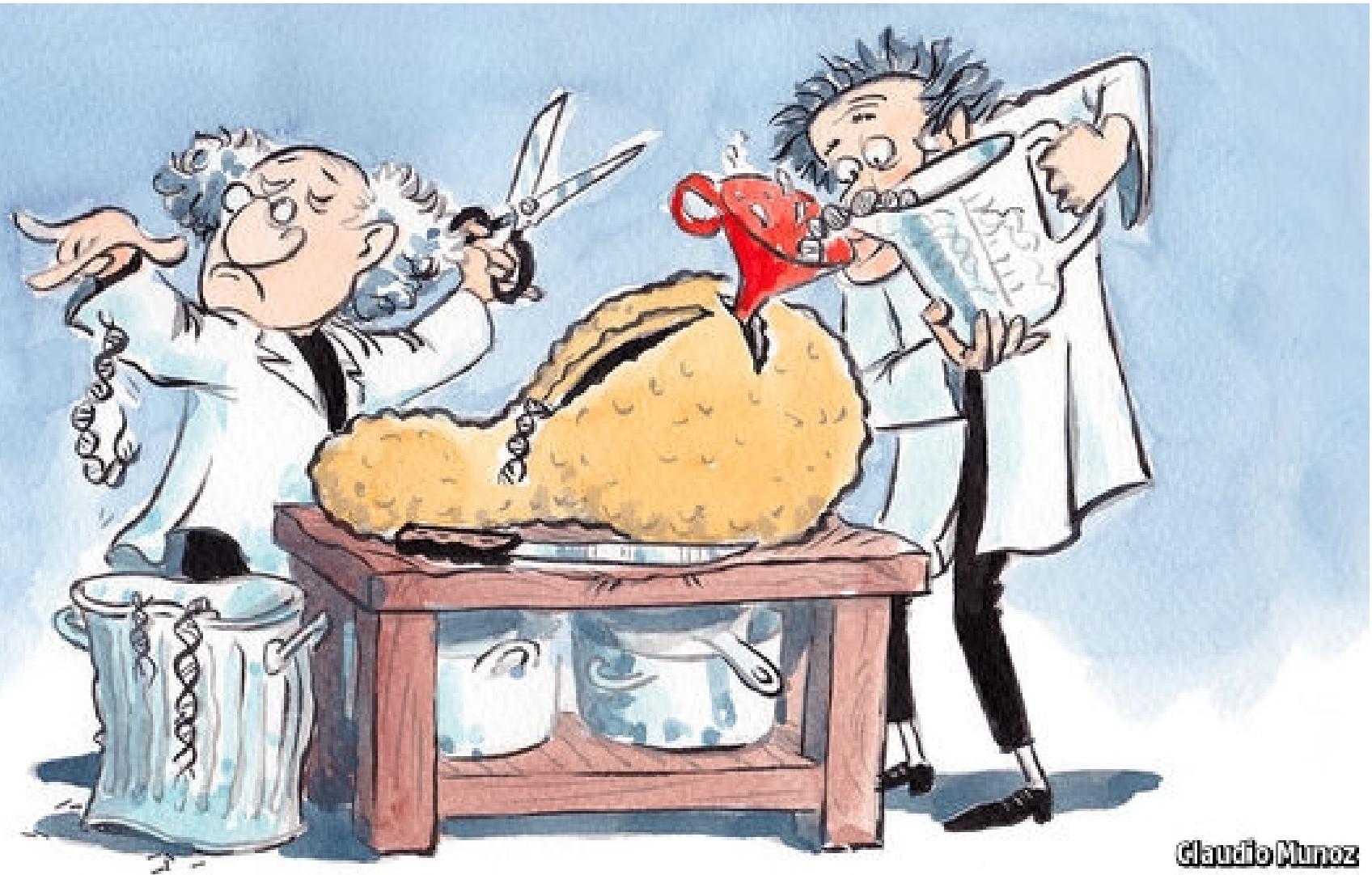
Steinmetz and Stegle Groups

Follow along on 
bit.ly/2fKpAbO



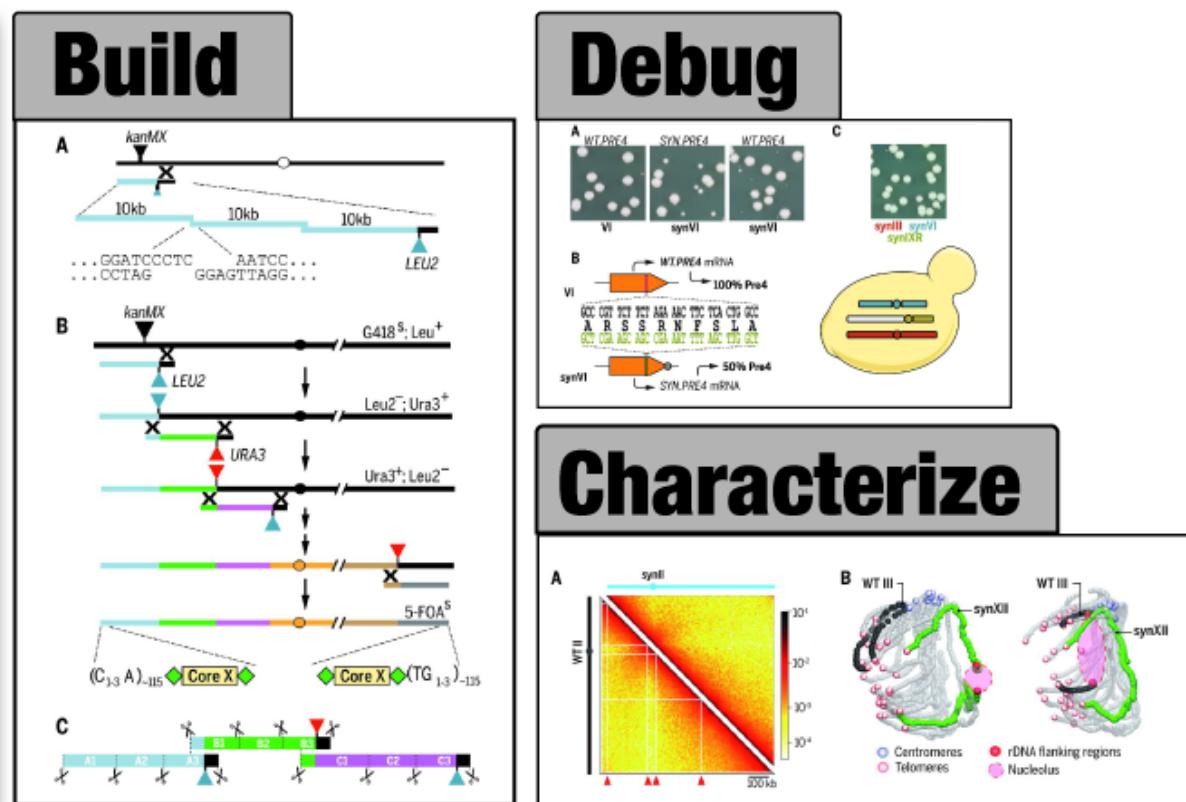
Why are genomes organized as we observe them?

"A big step towards an artificial yeast genome"



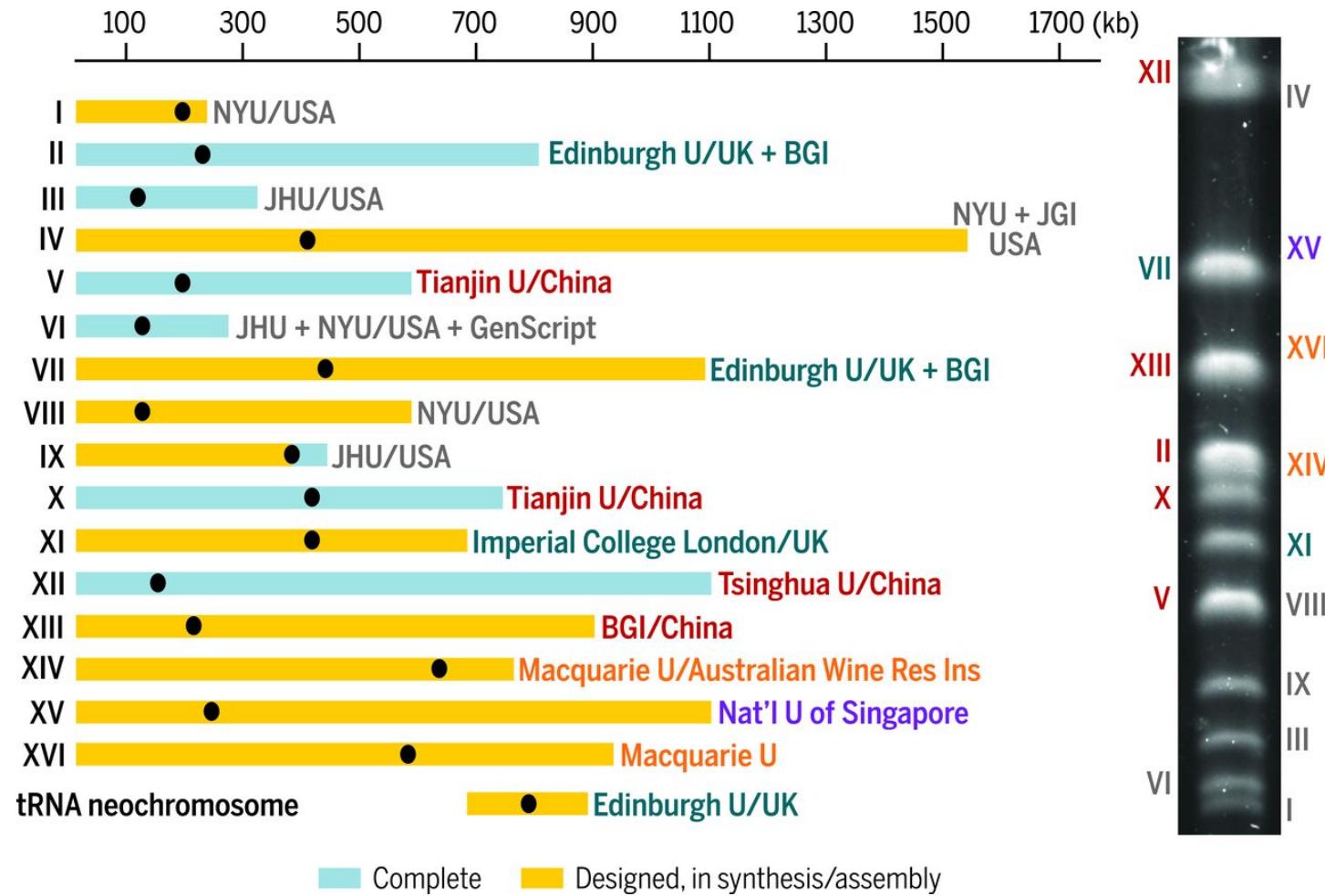
Economist, Mar 11th 2017

"Remodeling the yeast genome piece by piece"



Synthetic Yeast Genome Project (Sc2.0) Science Special Issue

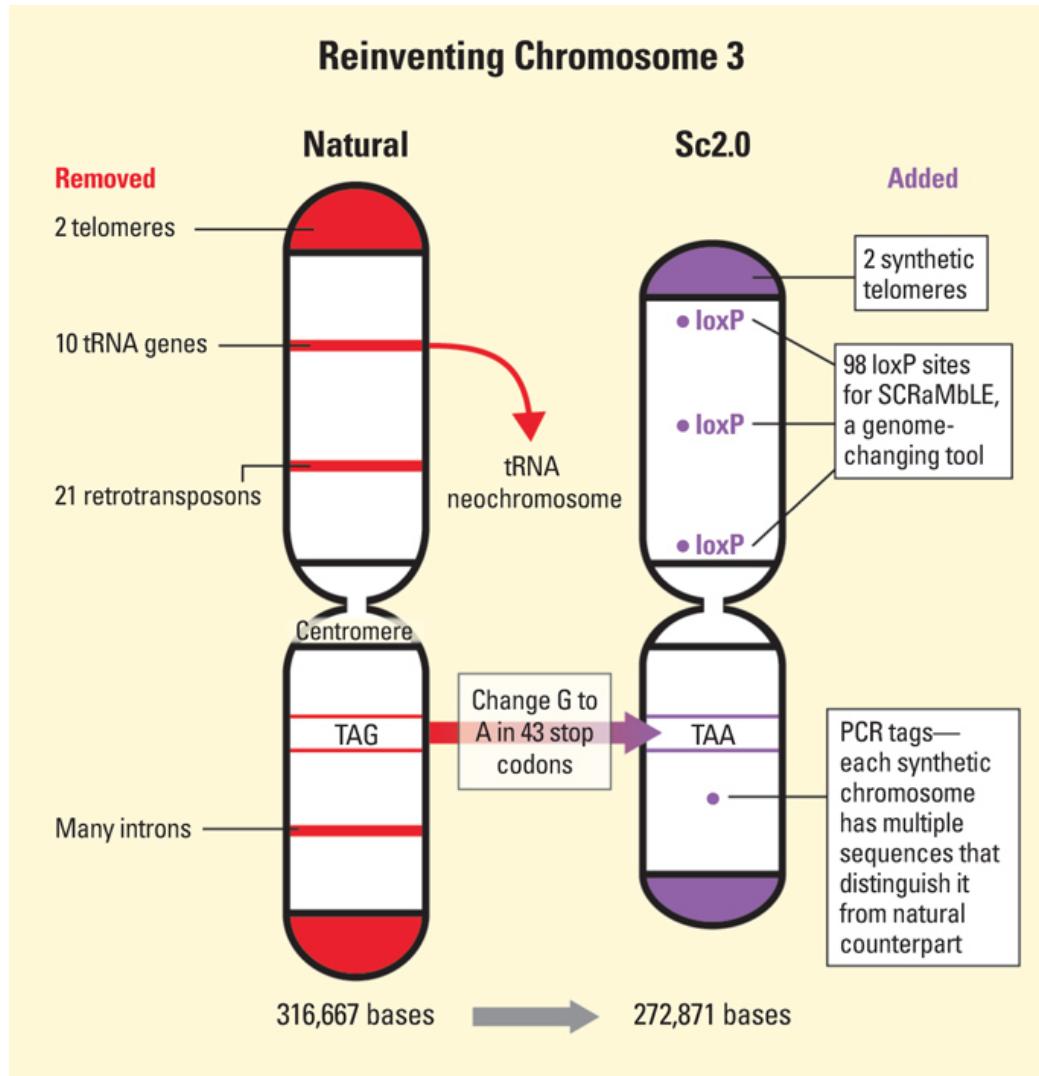
International consortium for design and synthesis



...synthesis is ongoing...

Richardson et al. 2017

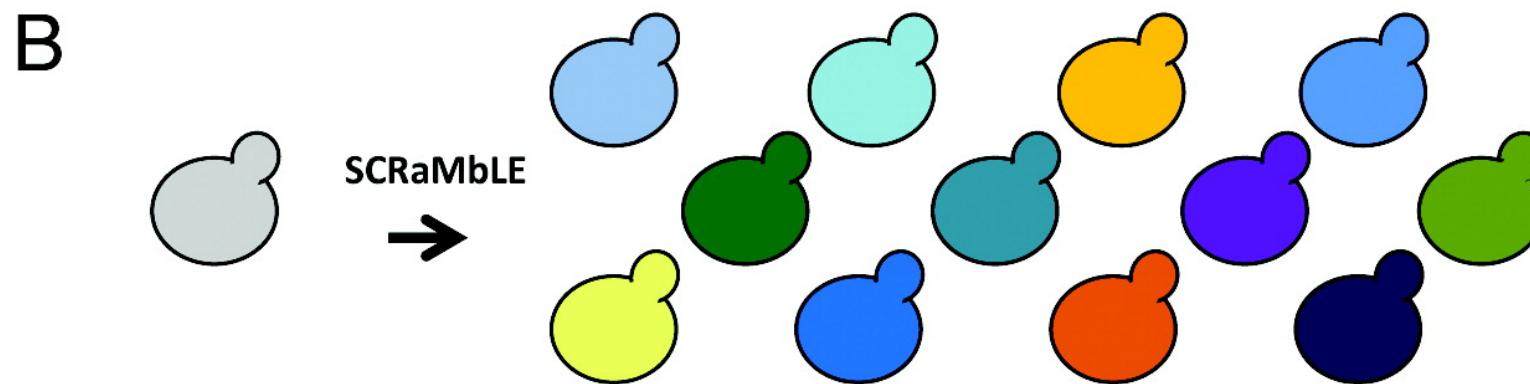
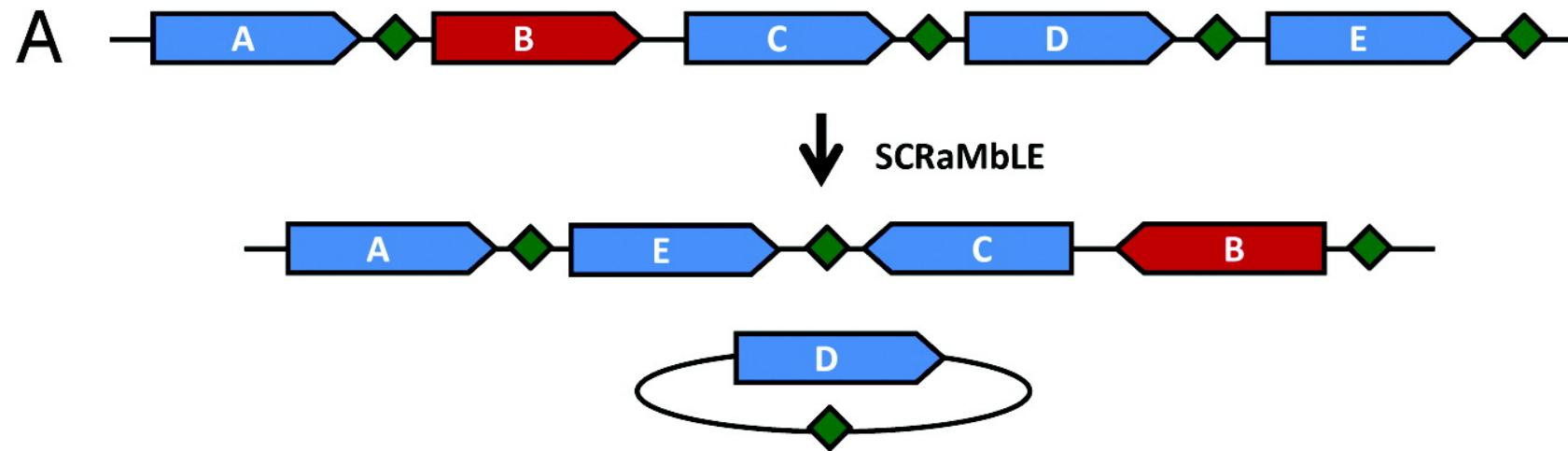
Encode sequence diversity by design: Sc2.0



Pennisi et al. 2014

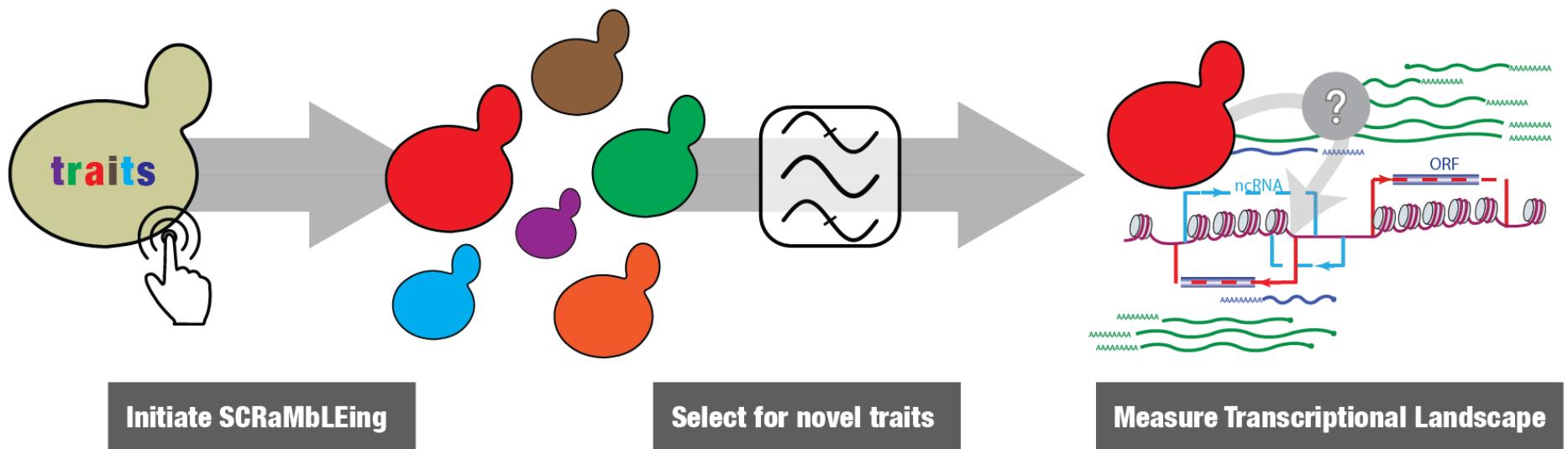
Gene shuffling with SCRaMbLE

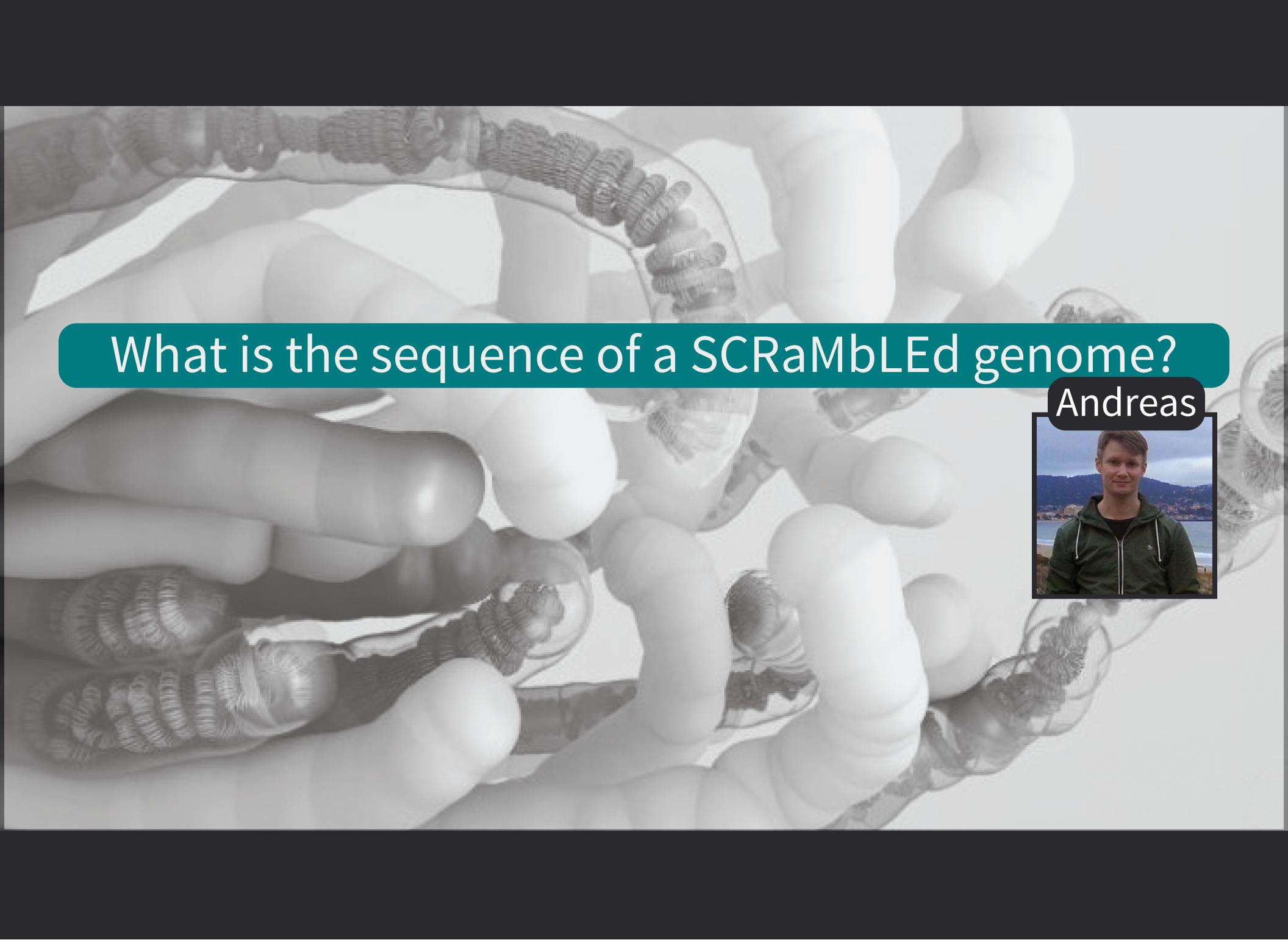
Synthetic Chromosome Recombination and Modification by LoxP-mediated Evolution



Dymond and Boeke 2012

How is the transcriptional landscape modified by genome SCRaMbLEing?



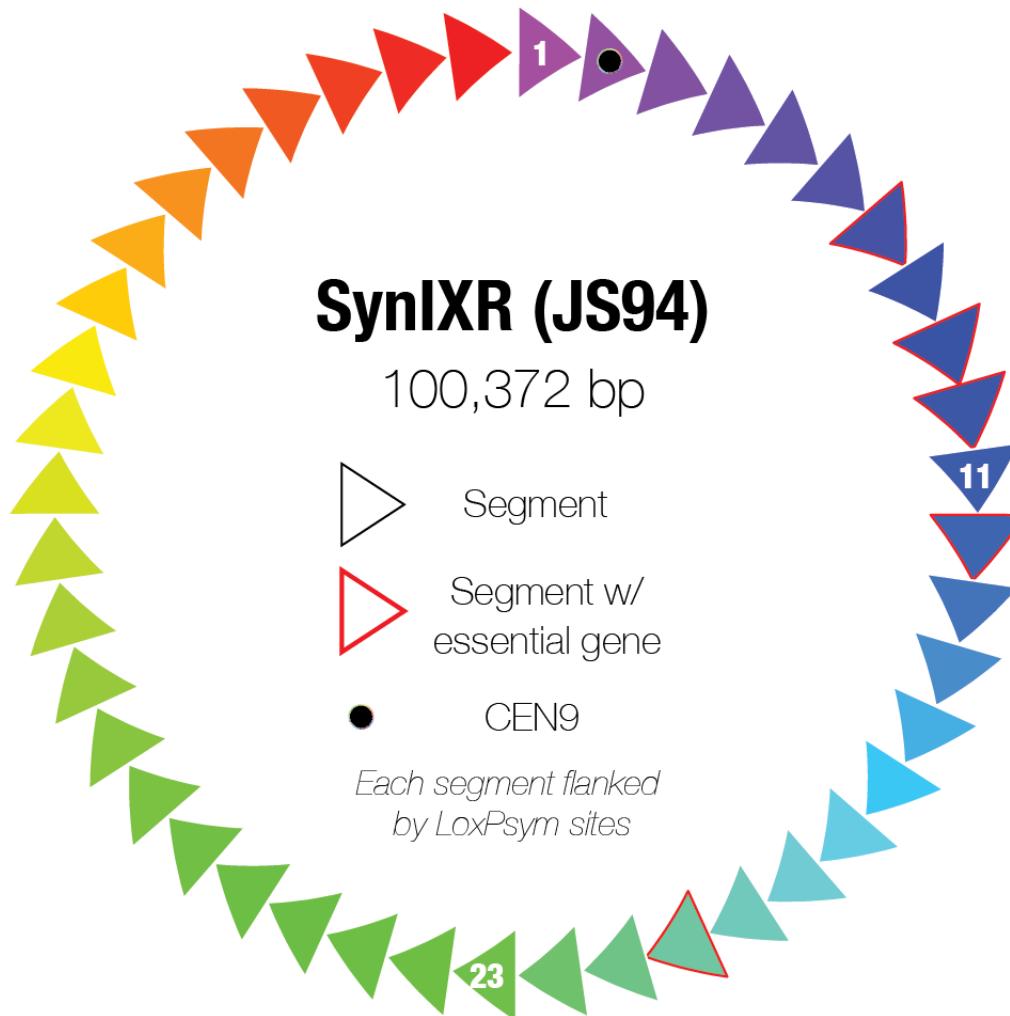


What is the sequence of a SCRaMbLED genome?

Andreas

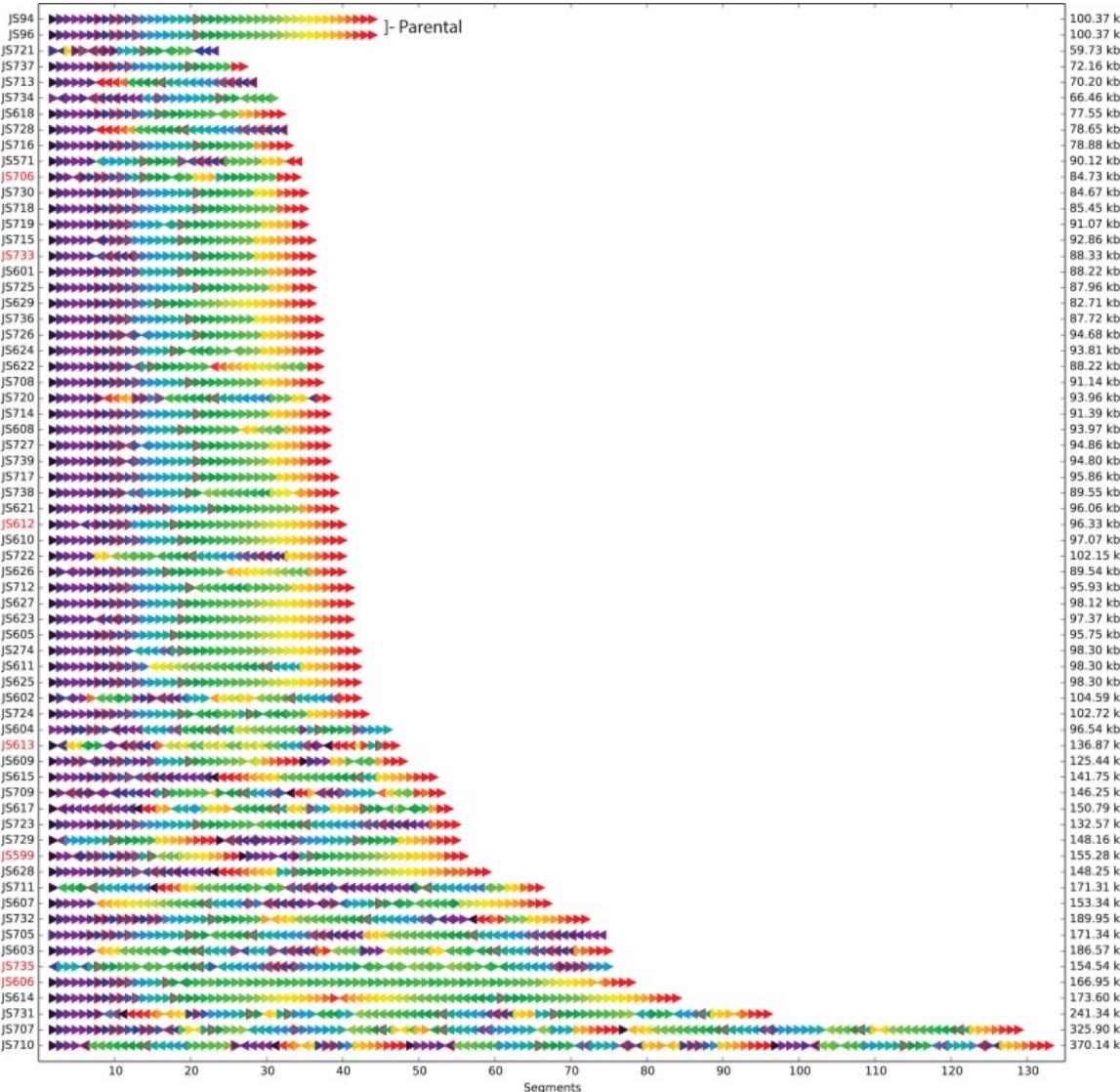


64 strains derived from SCRaMbLE of SynIXR



Each genome segment identified by color and number. ~1 gene per segment. [SCRaMbLEgram](#)

Extensive heterogeneity among SCRaMbLE strains



Shen et al. 2016

Many genomes cannot be assembled fully with short reads

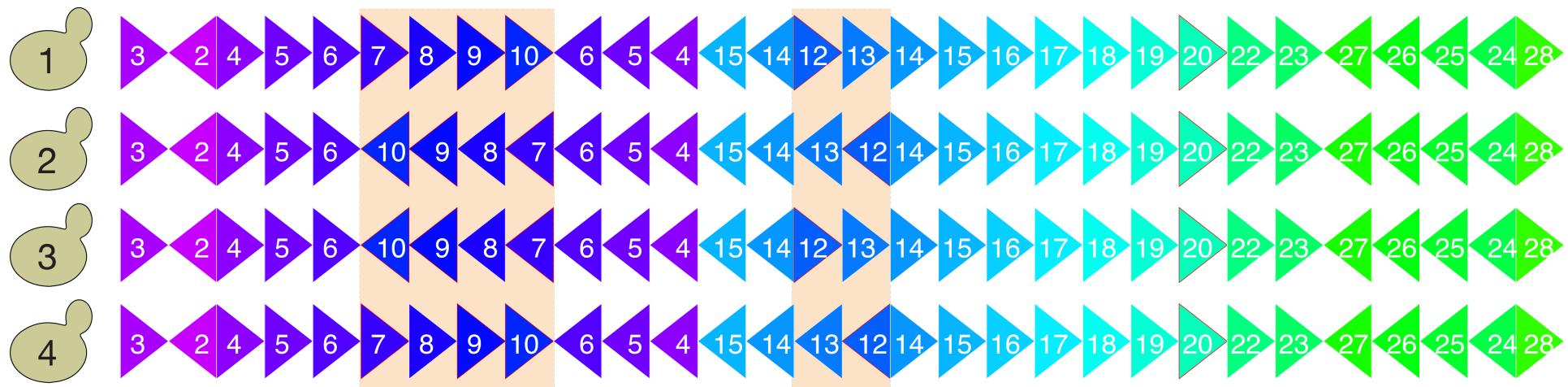
JS734 has four potential solutions



Shen et al 2016

Short-read sequencing was able to determine unique sequence reconstructions for 39 out of the 64 synIXR SCRaMbLE strains.

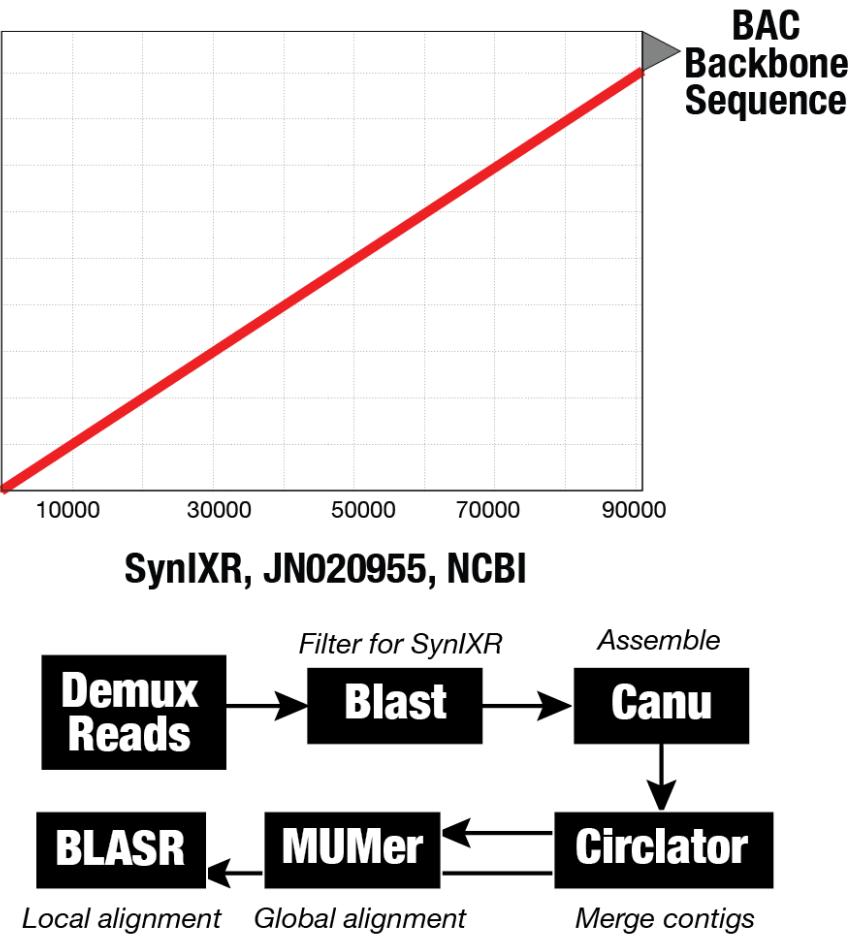
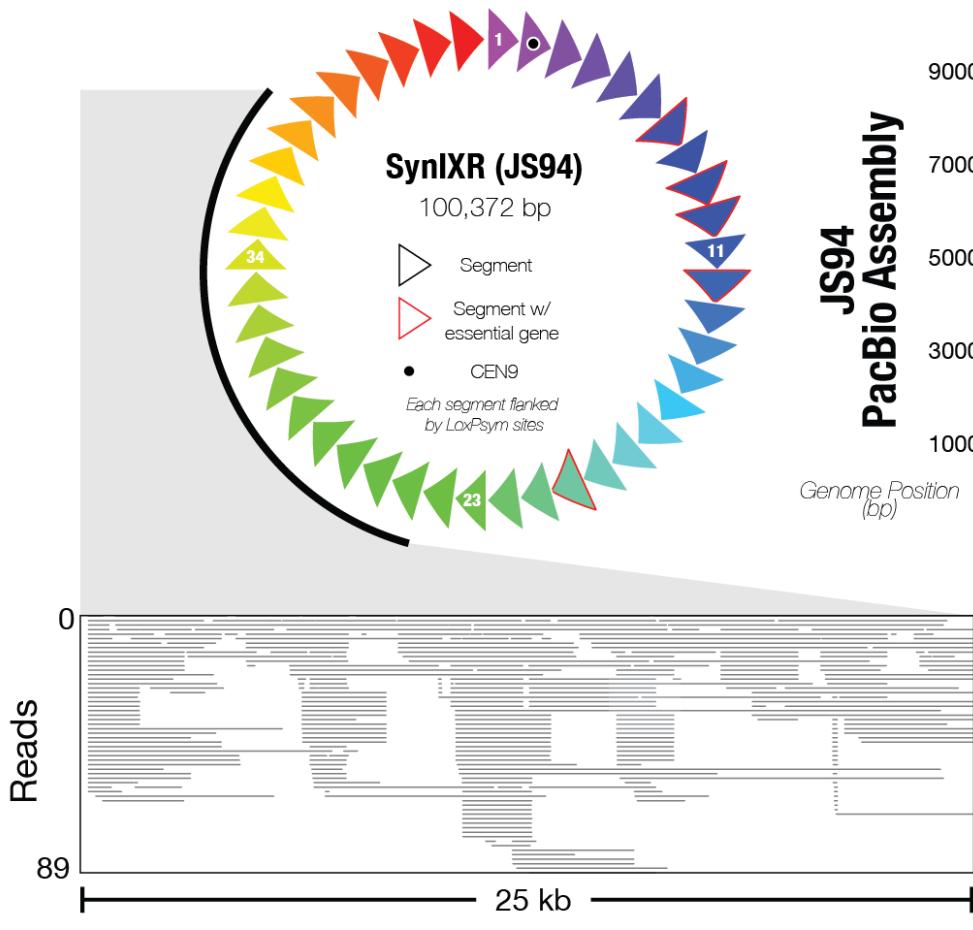
JS734 Potential Solutions



Shen et al. 2016

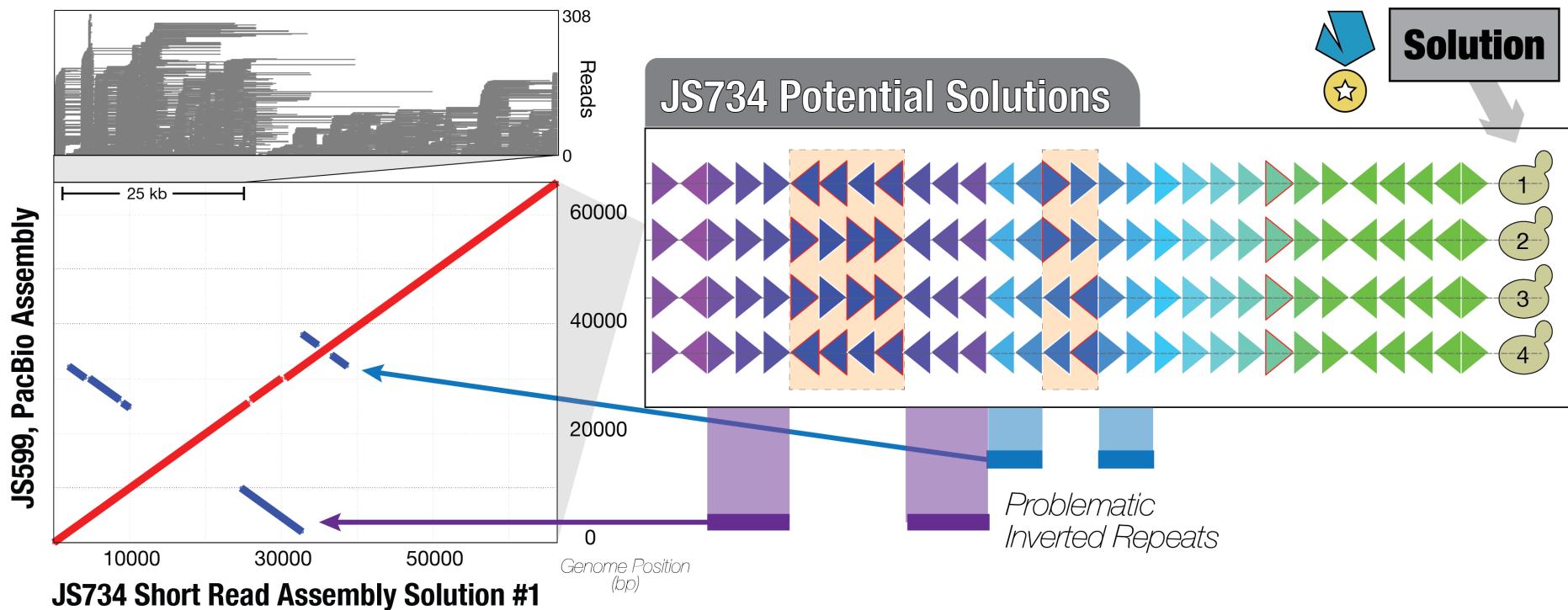
Workflow for *de novo* assembly with long-reads

Parental strain (JS94) can be solved with single SMRT cell



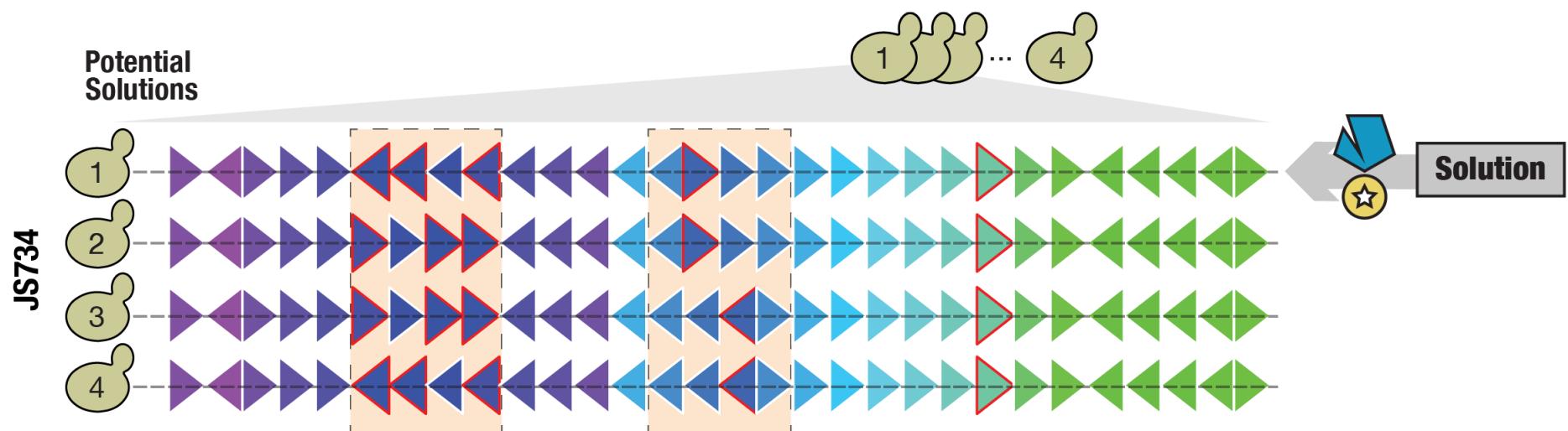
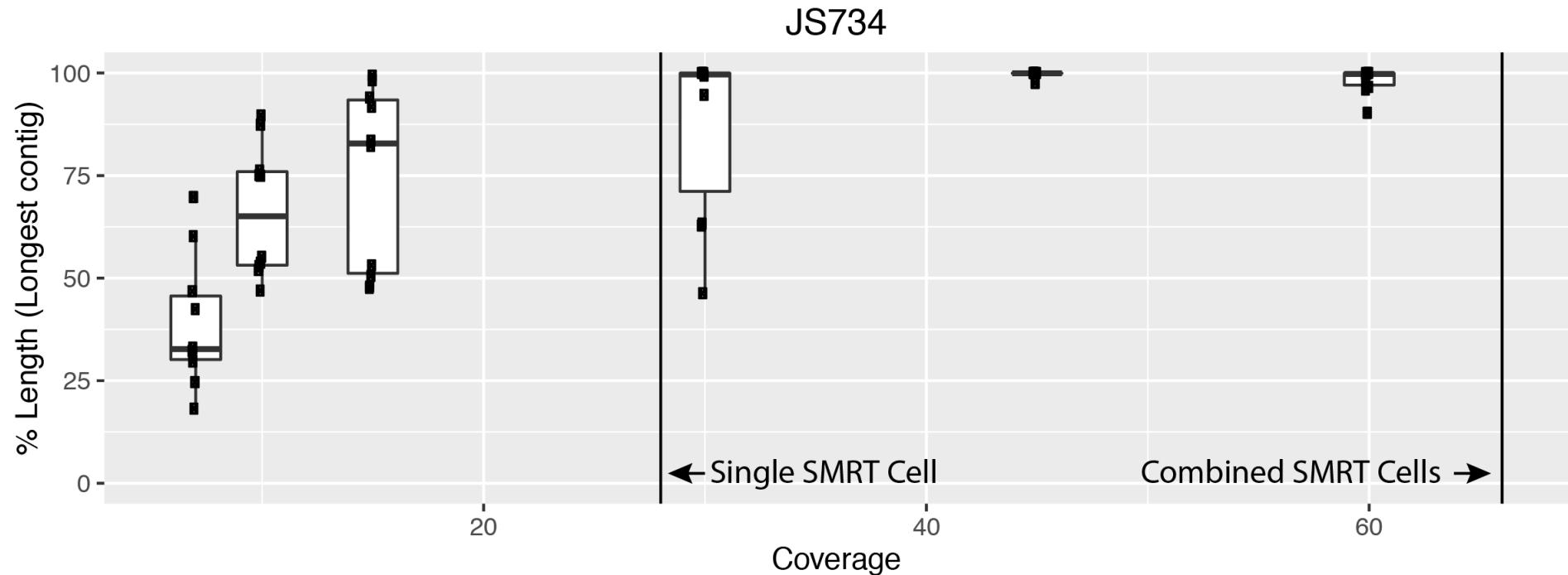
Near complete assembly of SCRaMbLE genomes with long-reads

JS734 could only be solved by aggregating data from several SMRT cells



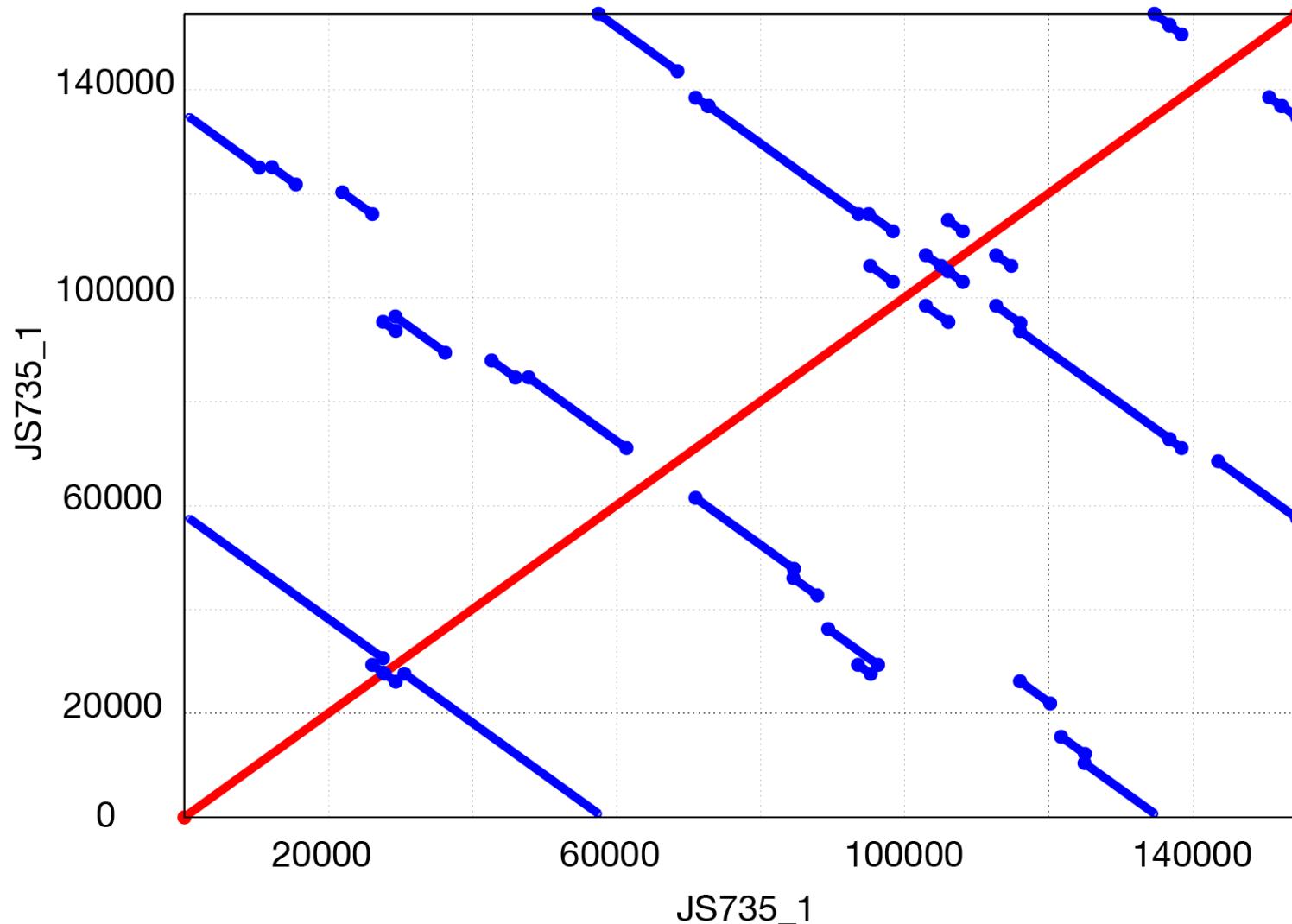
1. Is it possible to solve every SCRaMbLE genome?
2. If so, under what conditions (e.g. coverage, read-length)?

Complete assembly achieved frequently at high coverage for JS734

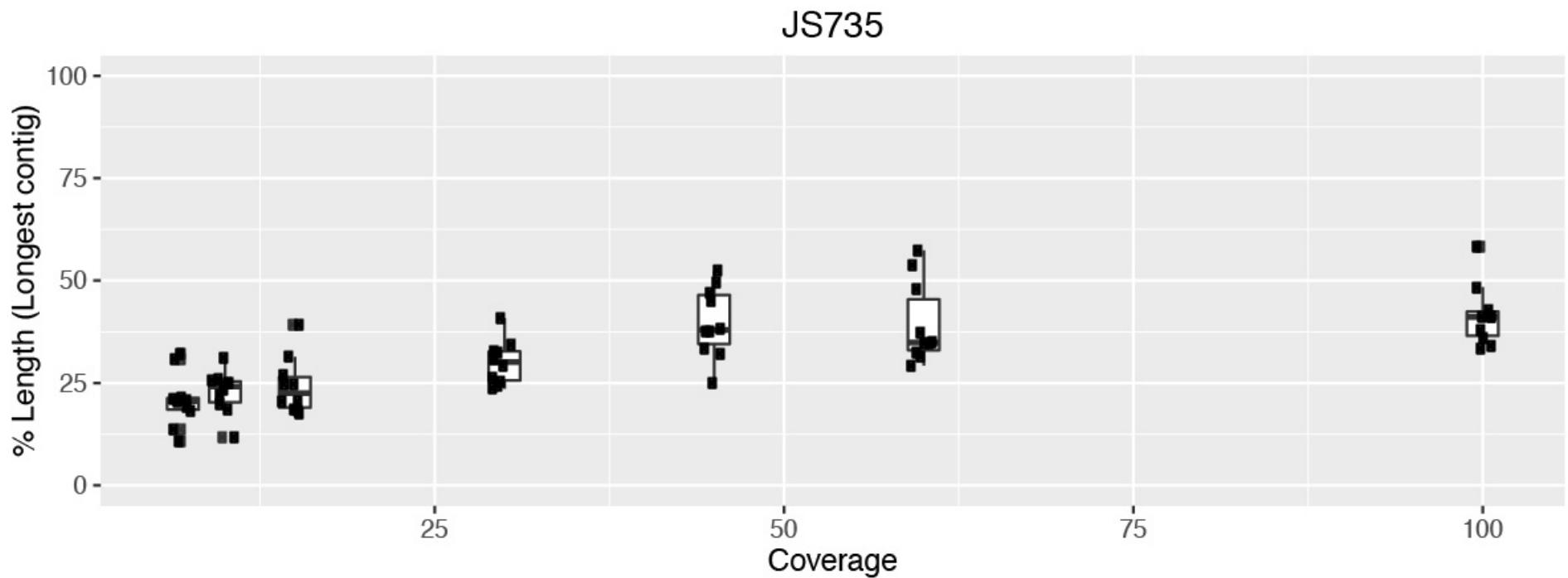


Some SCRaMbLE genomes are full of inverted repeats

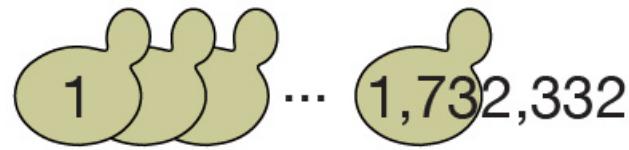
Will increased coverage still lead to complete assembly?



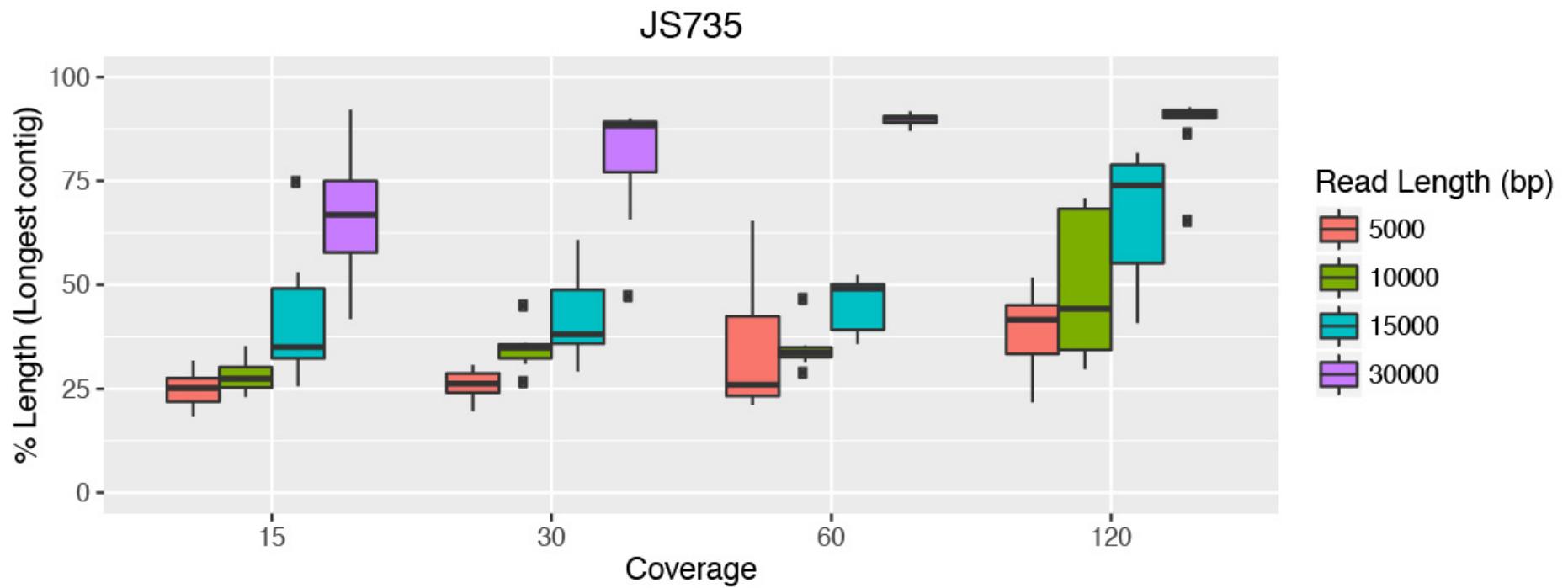
Increased coverage doesn't guarantee resolution of more complicated genomes



Potential
Solutions



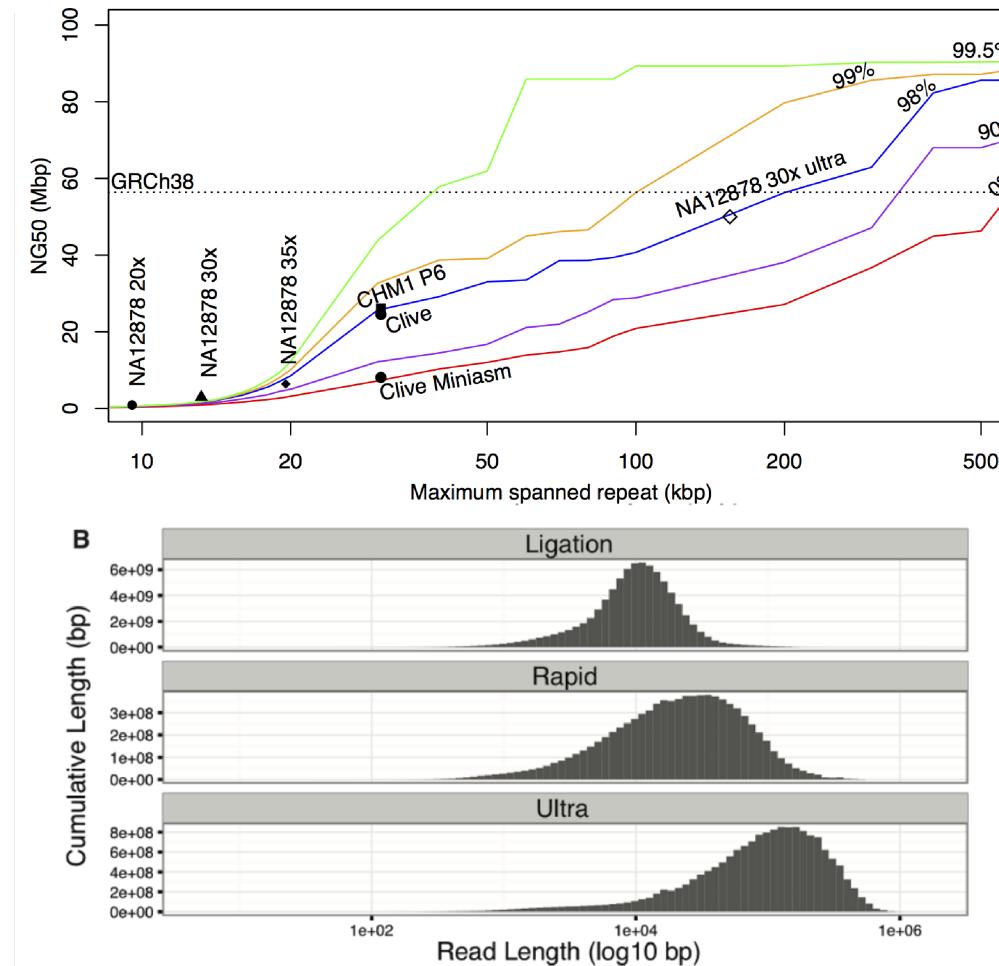
Some strains require a combination of depth and increased read length



Potential
Solutions

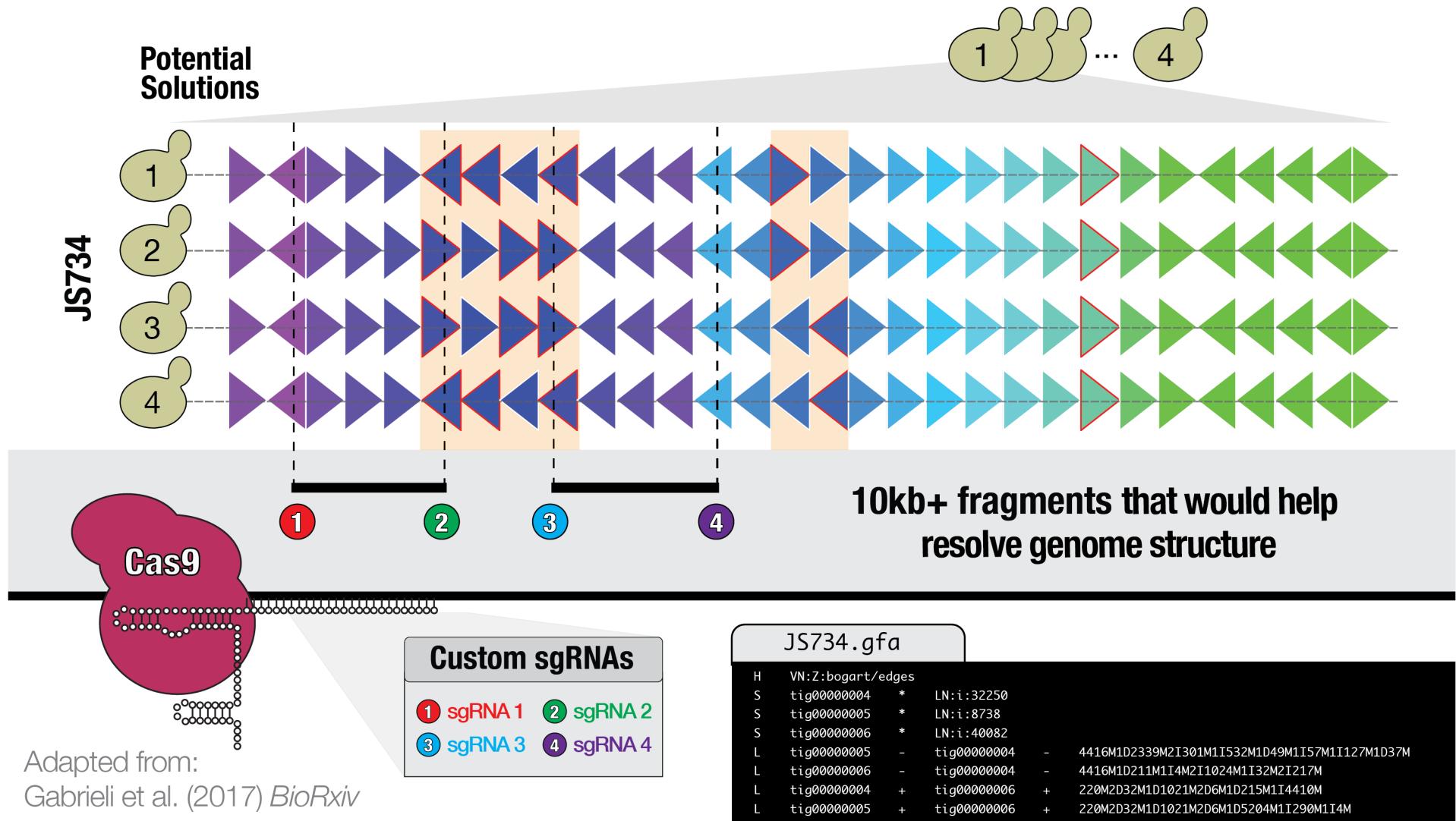
1 ... 1,732,332

Nanopore sequencing + ultralong reads = reference quality assembly?



Jain et al. 2017 *bioRxiv* and Blog post: "Assembling the Cliveome"

CATCH-seq: Cas9-Assisted Targeting of CHromosome segments



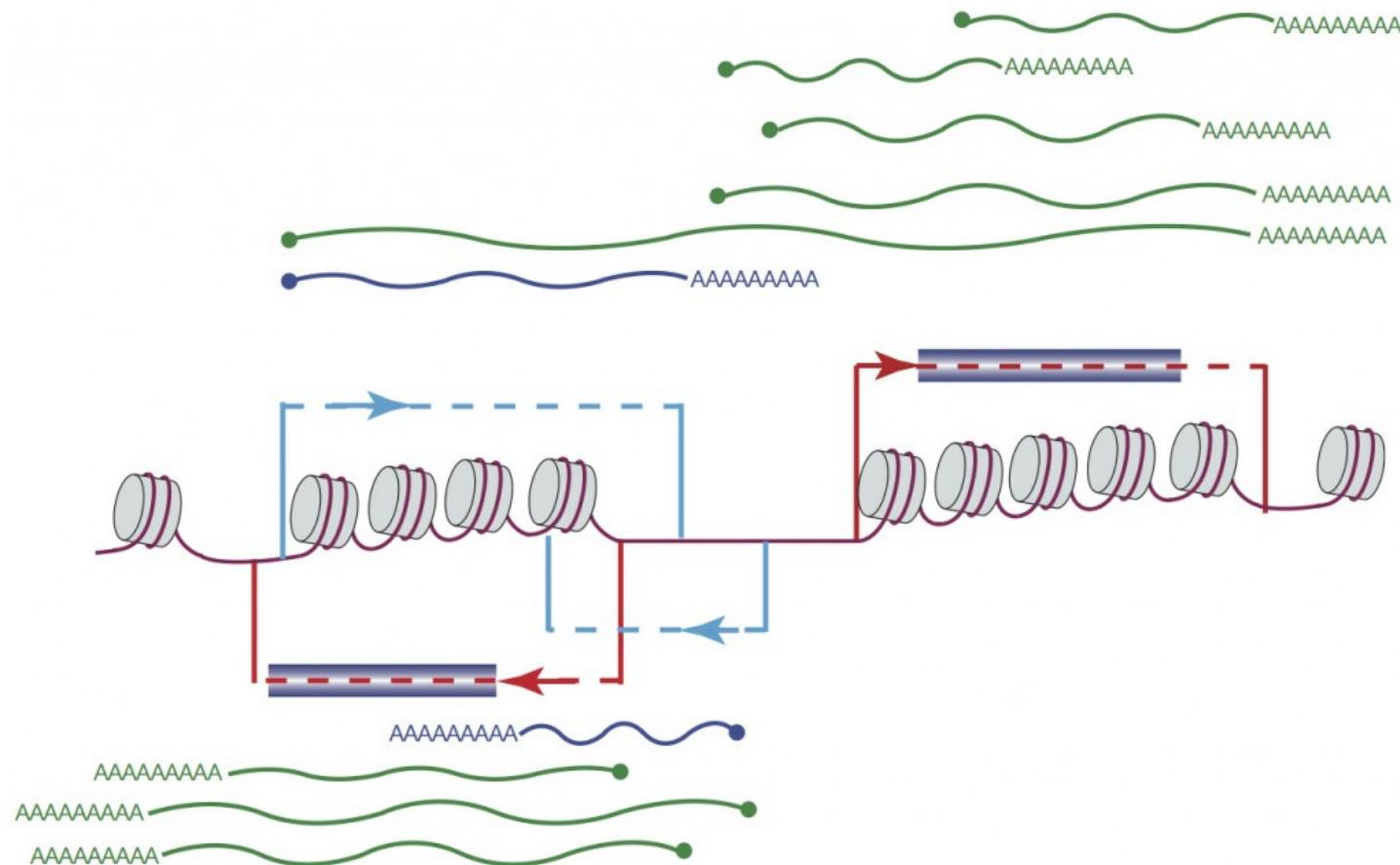
Gabrieli et al. 2017 *bioRxiv*

How is a SCRaMbLED genome transcribed?

Andreas

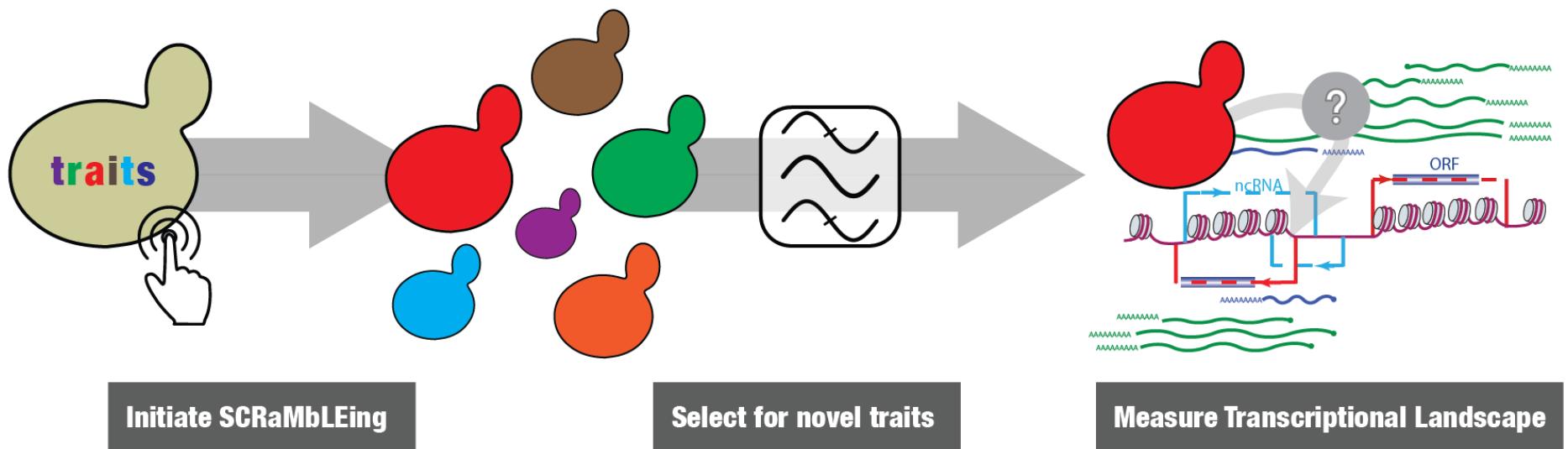


Transcription is linked to genome architecture

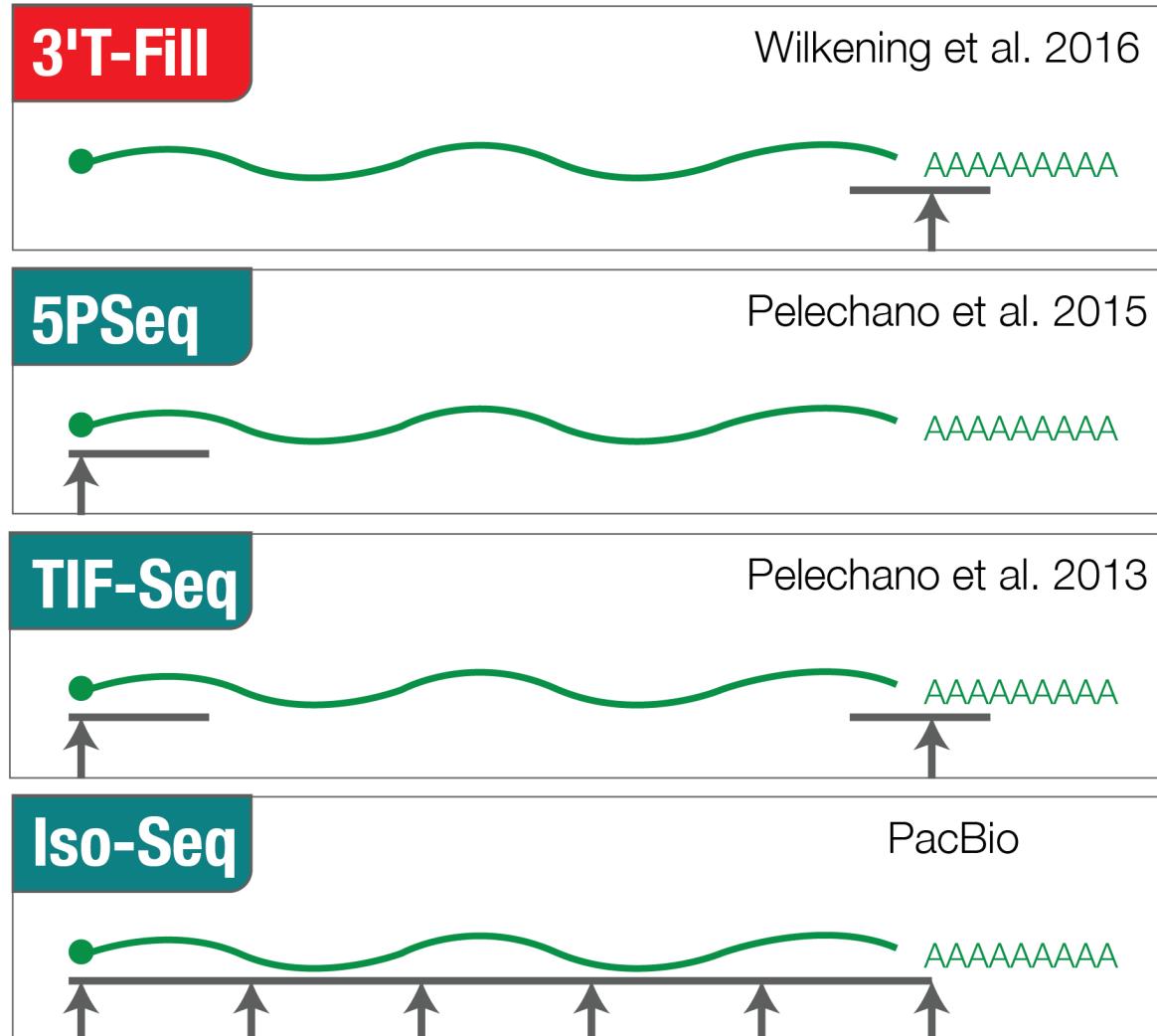


Pelechano Lab

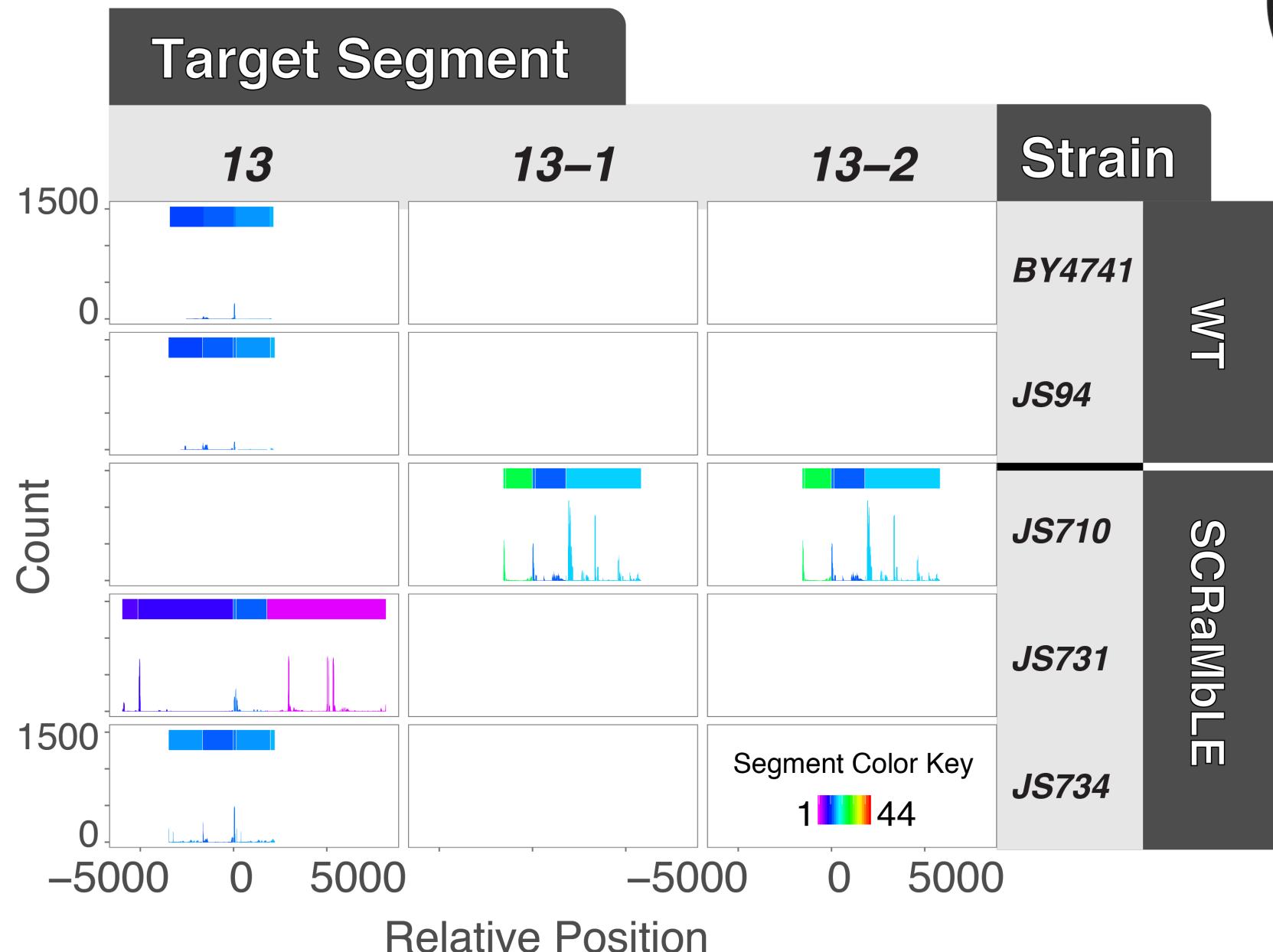
How is the transcriptional landscape modified by genome SCRaMbLEing?



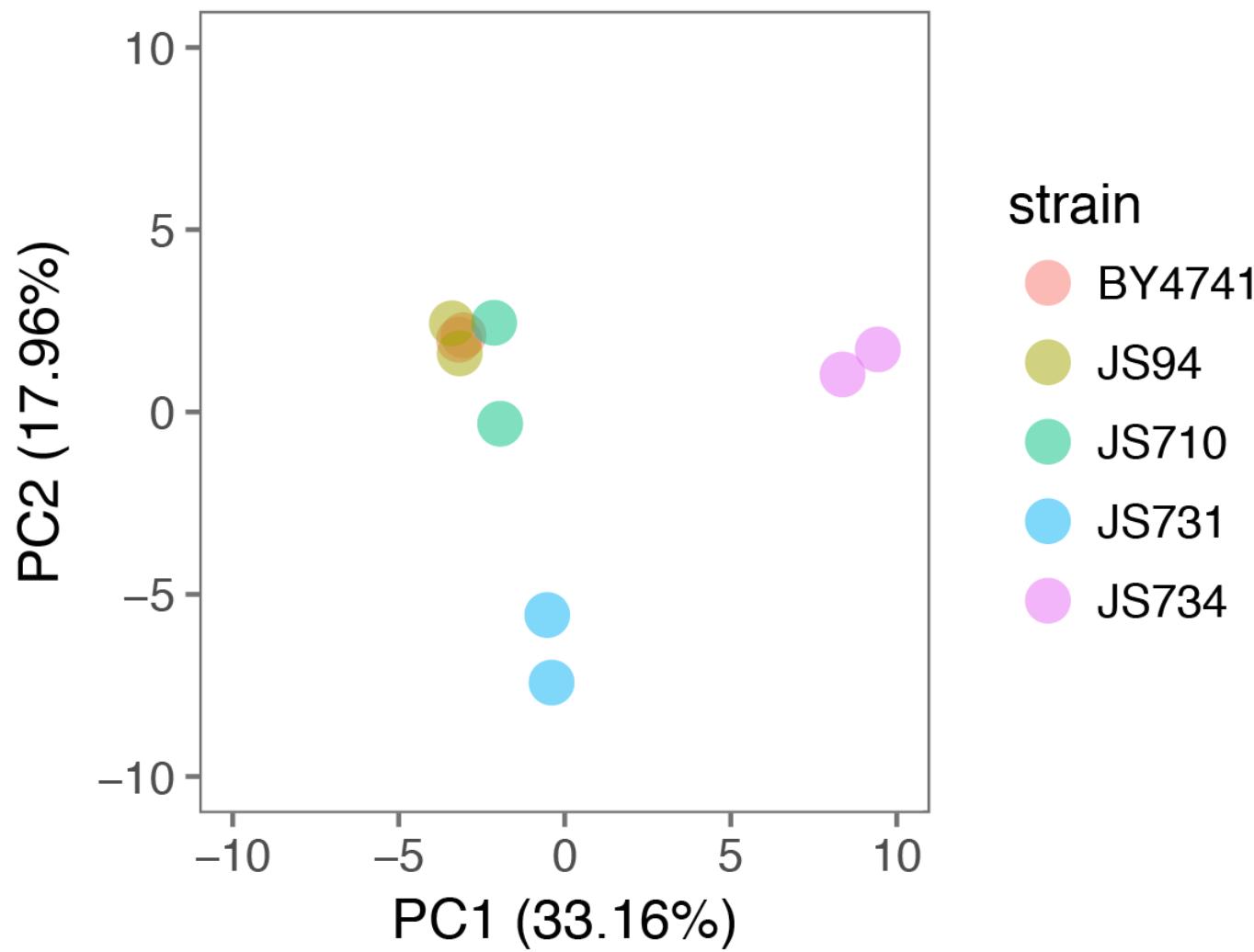
Sequencing methods to measure changes in transcriptome



3'-end profiles change after SCRaMbLE



Genome-wide 3'-end variation?

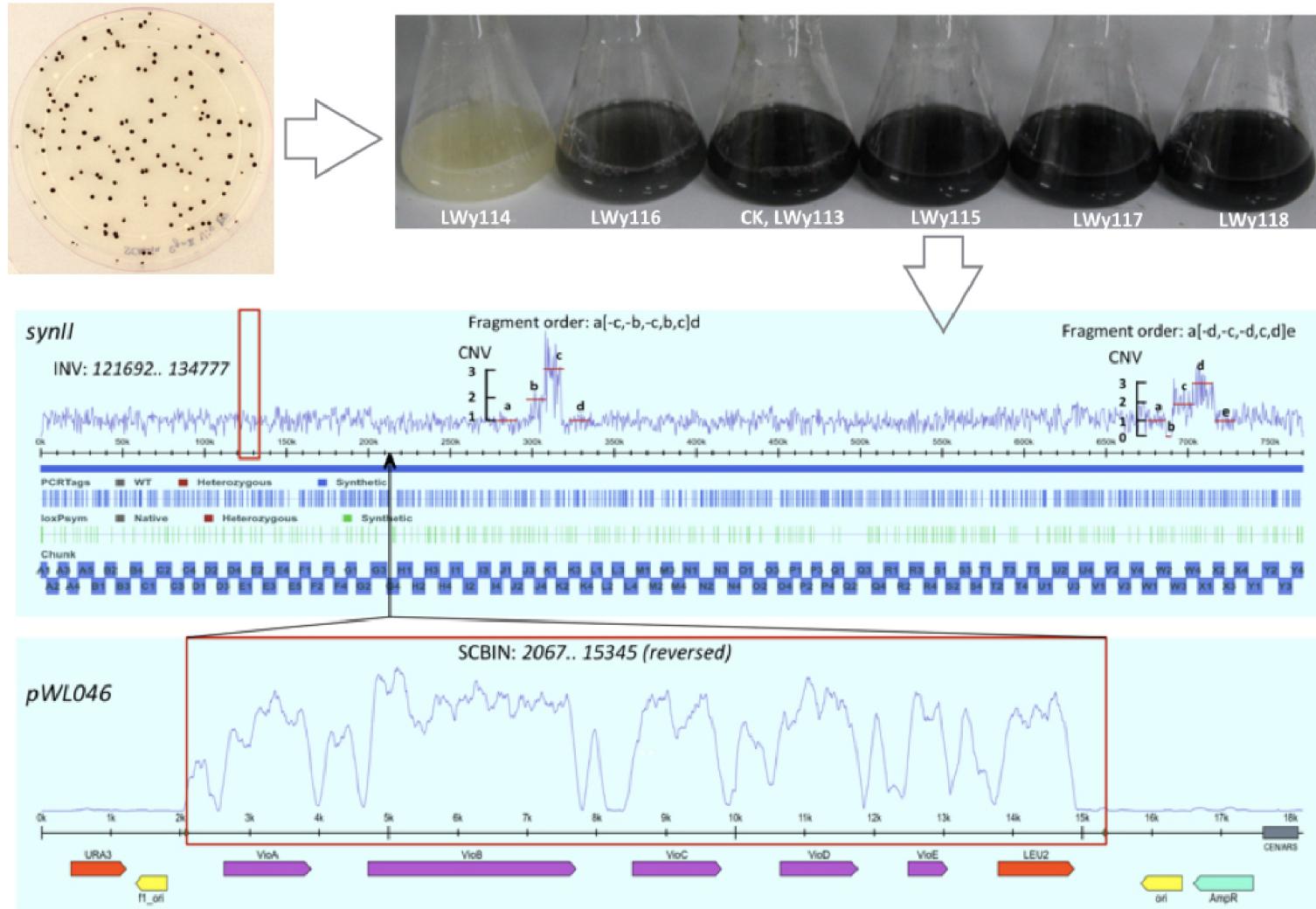


Kernel PCA (Cosine kernel) from 22 segments represented in all strains



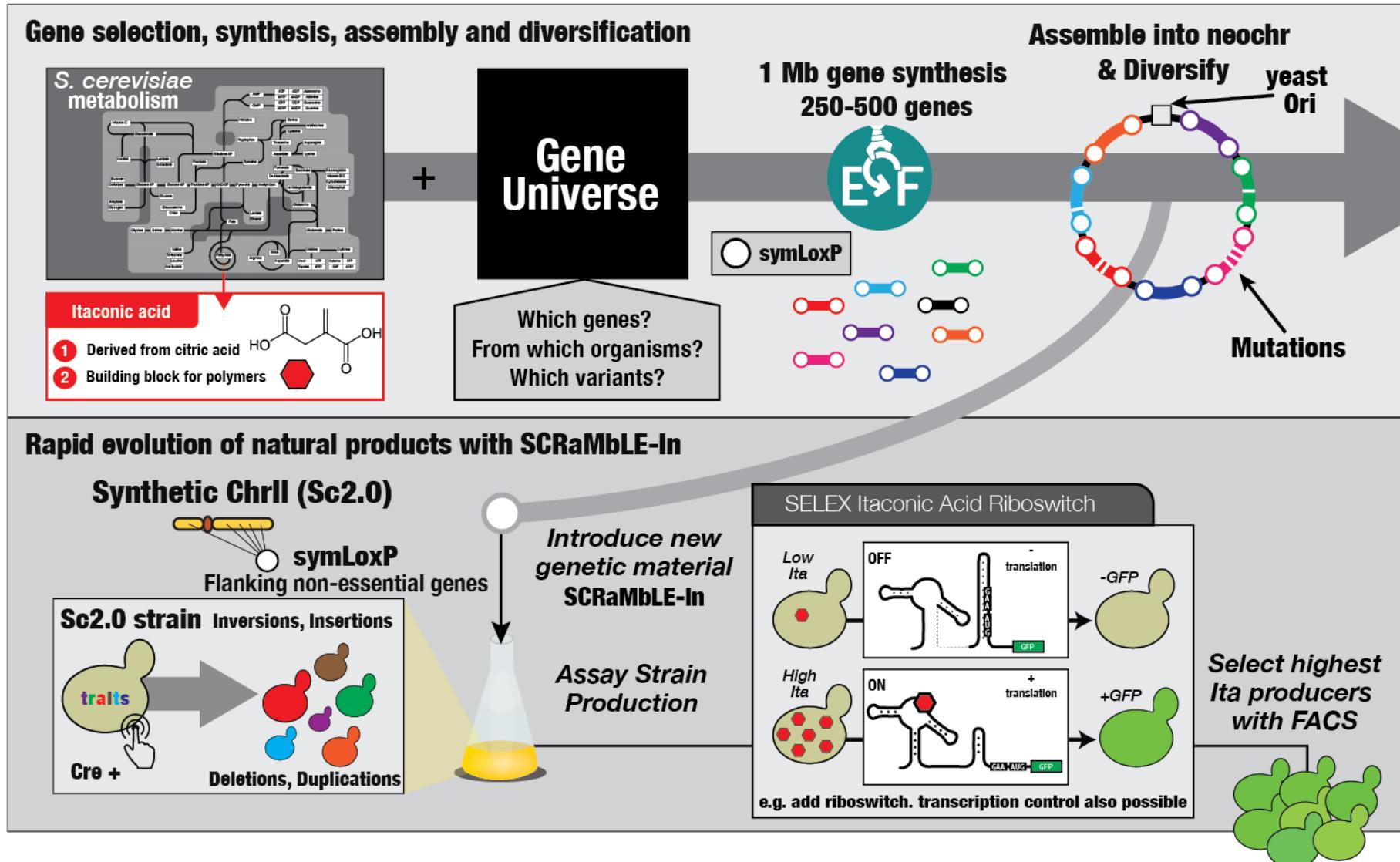
Can we SCRaMBLE-**in** new features?

SCRaMbLE-in of violacein biosynthetic pathway



Cai Lab, University of Edinburgh

HERMES: Hasenting Evolution with Recurrent Multiplexed Engineering with SCRaMbLE-in



Acknowledgements: Steinmetz Lab



Lars Steinmetz group: Raeka Aiyar, Chiara Bae, Francesco Biganti, Aaron Brooks, Sandra Clauder-Münster, Gozde Durmus, Lin Gen, Andreas Gschwind, Bianca Hahn, Saiful Islam, Petra Jakob, Cosimo Jann, Andreas Johansson, Allan Jones, Bastian Lindner, Will Mueller, Mariona Nadal Ribelles, Jen Millbank, Michelle Nguyen, Ragini Phansalkar, Kevin Roy, Federica Sartori, Daniel Schraivogel, Michael Sikora, Ben Story, Han Sun, Chelsea Szu-Tu, Karen Tessmer, Lars Velten, Sibylle Vonesch, Wu Wei, Kristen Wells, Jingyan Wu, Chenchen Zhu

Collaborators: Utkan Demirci, Maureen Hillenmeyer, Ron Davis, Wolfgang Huber, Andreas Trumpp