Identifying Ribosomal Protein Interacting Partners

By Akhilesh Kesavan

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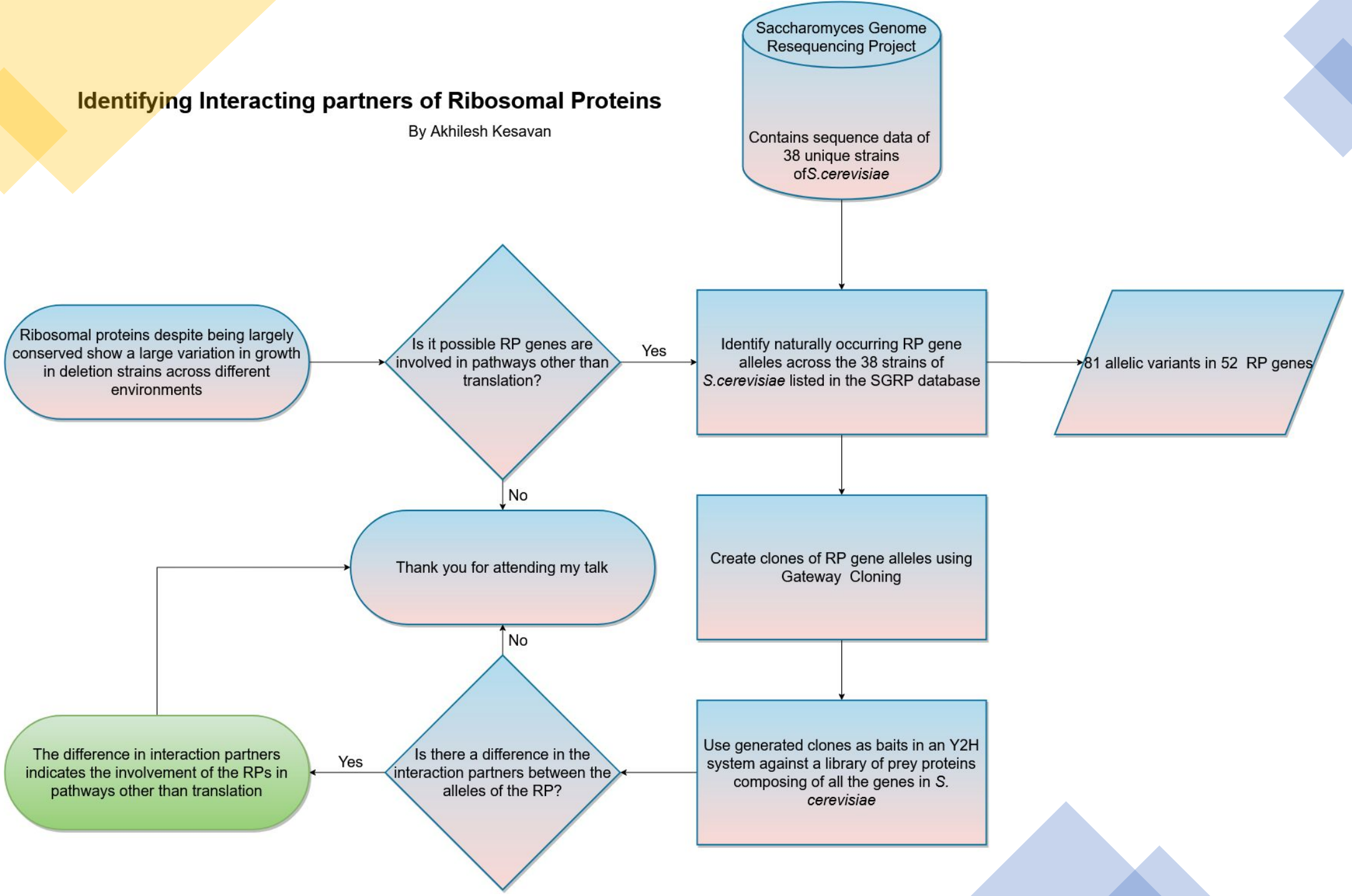
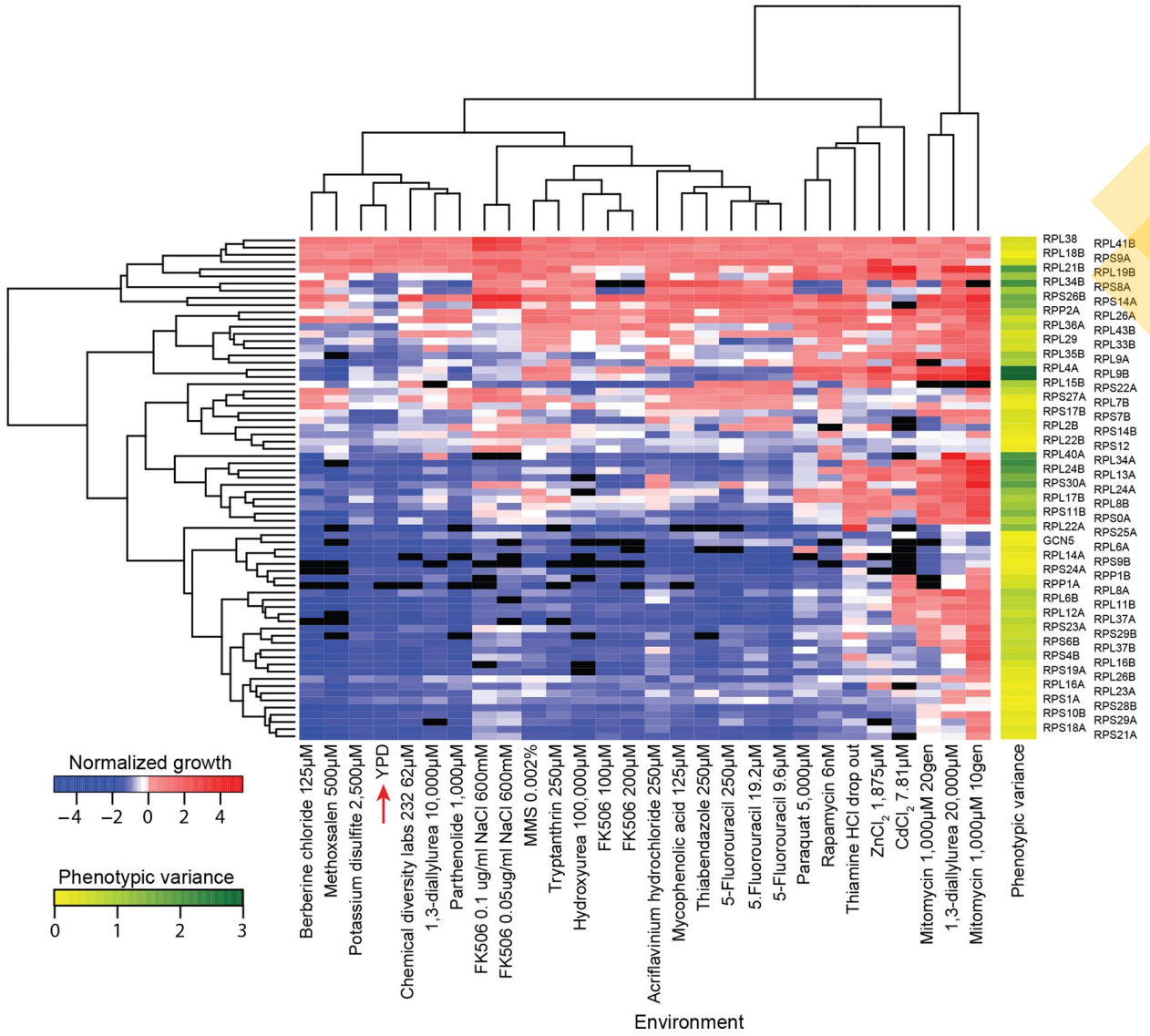


Introduction

- Ribosomes are the cellular machinery that translate mRNA to synthesise proteins
- Made of 2 subunits; A small subunit with about 32 different ribosomal proteins(RPS) and a large subunit with about 43 different ribosomal proteins(RPL)
- These proteins are primarily involved in translation
- However recent studies suggest that they may have interactions in other pathways
- The aim of the study is to identify the interacting partners of the certain RPs that have polymorphisms across different strains

Phenotypic Variability

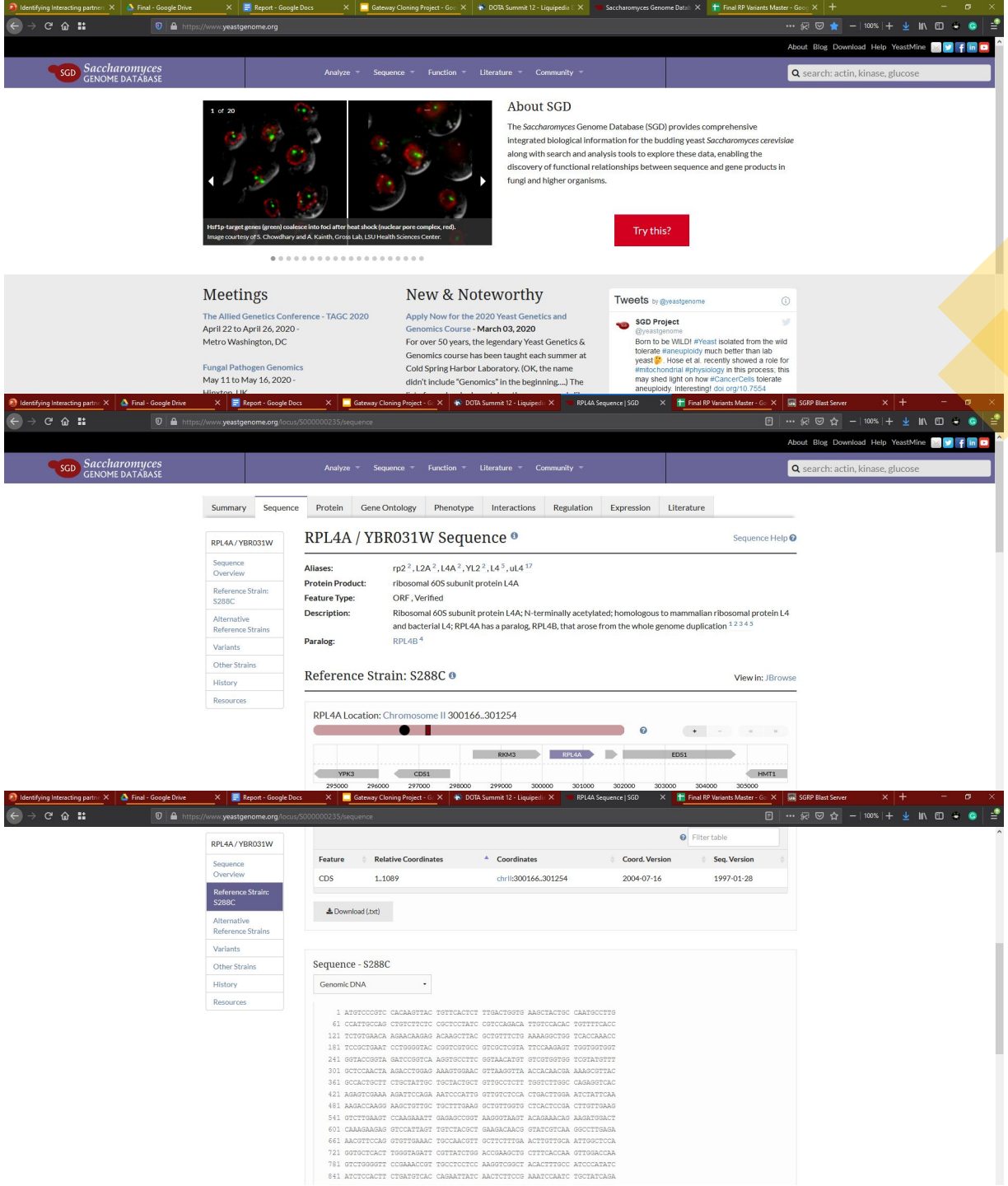
- 68 single deletions in 26 environments
- RPs show higher phenotypic variance



Source: Yadav *et. al.*

Identification of RP Variants

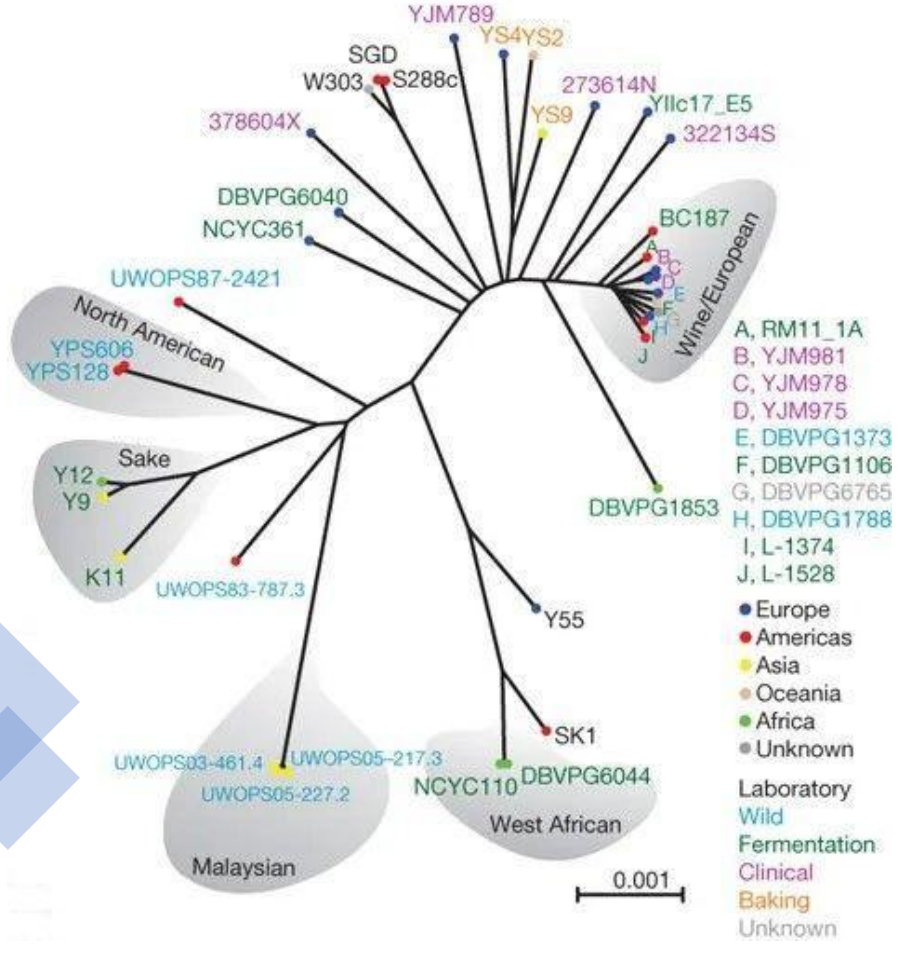
Obtain sequence from SGD

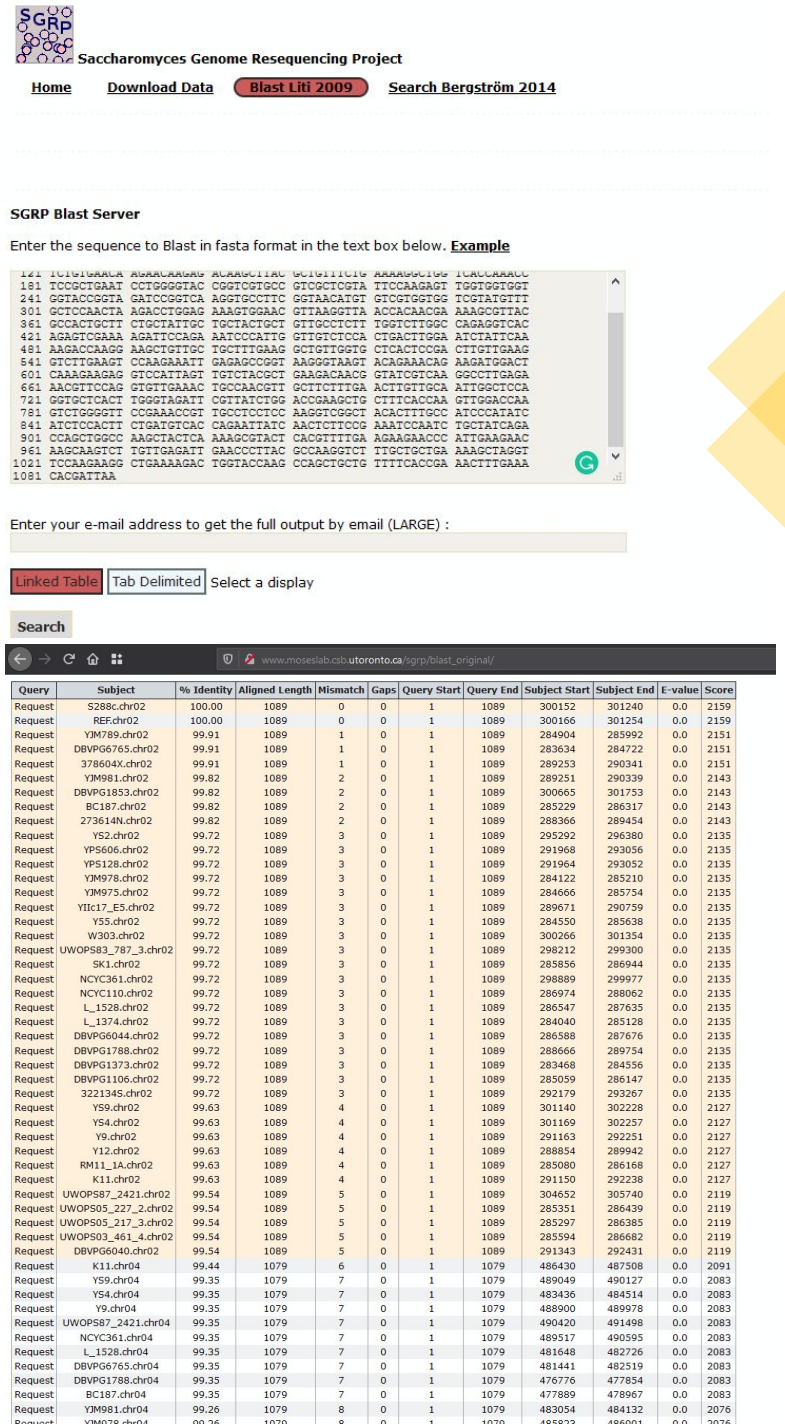


Identification of RP Variants

Obtain sequence from SGD

Source: Litti *et. al.*





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Request	YJM981.chr04	99.26	1079	8	0	1	1079	483054	484132	0.0	2076

Identification of RP Variants

- Align protein sequences to identify non-synonymous variants

Gene	Strain 1	Variant1	Strain 2	Variant2
RPL04A	YS4	168S (502T)	S288c	168A (502G)
RPL04A	RM11_1A	256A (766G)	S288c	256T (766A)

So what can do we do?

SELECT RPS WITH HIGH VARIANCE, GENERATE ALLELE SPECIFIC POPULATIONS AND STUDY GROWTH IN DIFFERENT ENVIRONMENTS

PROVIDES IN DEPTH ANALYSIS FOR SPECIFIC **RP ALLELE**

NOT VIABLE FOR LARGE SCALE ANALYSIS OF ALL VARIANTS

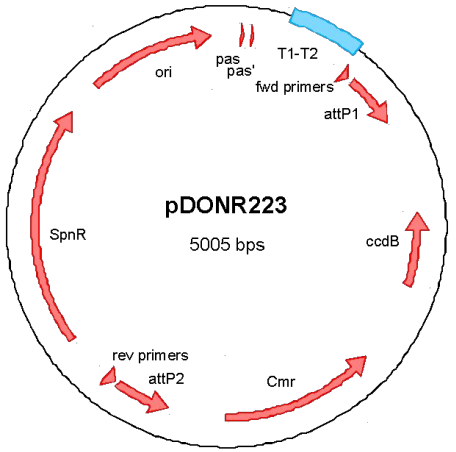
Now what?

PCR amplify the RP of interest

Clone into plasmid of interest

pDONR223

- AttB sequence + Specific Sequence
- Forward: GGGGACAACTTTGTACAAAAAAGTTGGCACC ATGTCCCGTCCACAAGTTAC
- Reverse: GGGGACAACTTTGTACAAGAAAGTTGGCAA TTAATCGTGTTTCAAAGTTTCGGT



Gateway Cloning

- Inspired by lambda phage's mechanism of inserting it's genetic material into host by homologous recombination
- BP reaction mediated by integration host factor and intergrase
- LR reaction mediated by integrase, integration host factor and excisionase

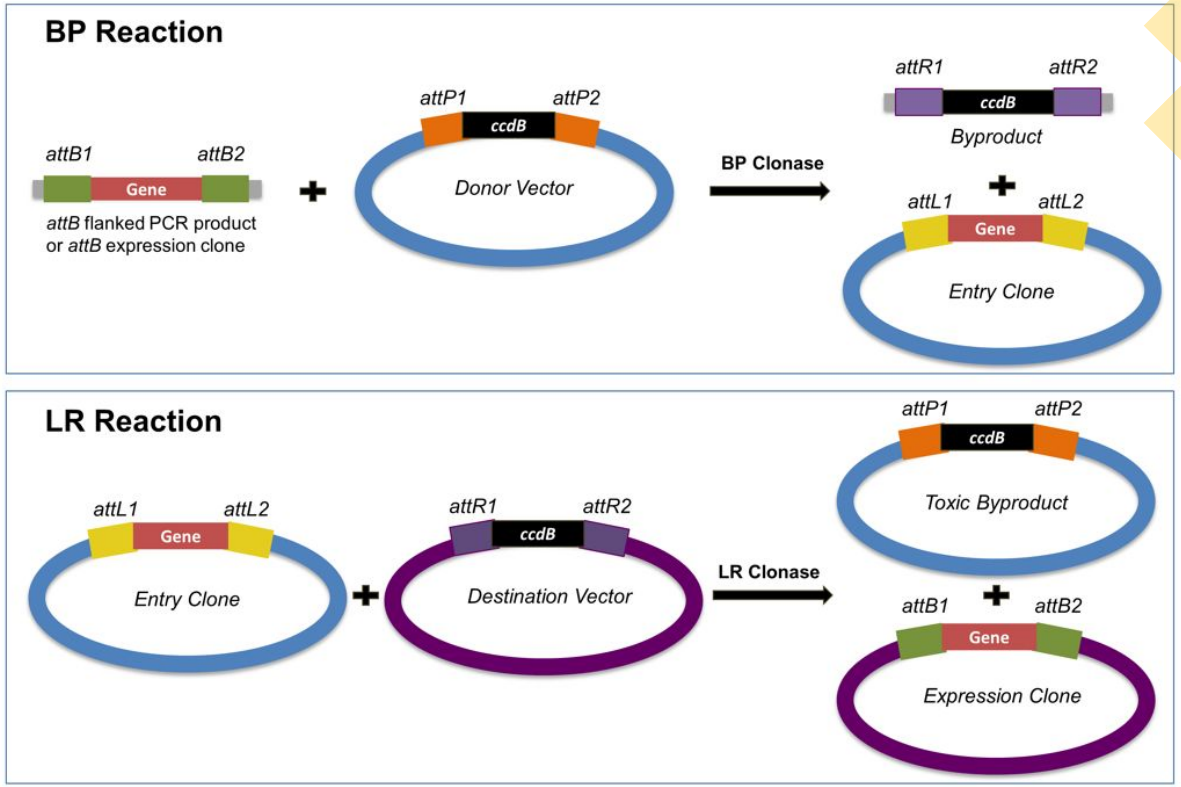
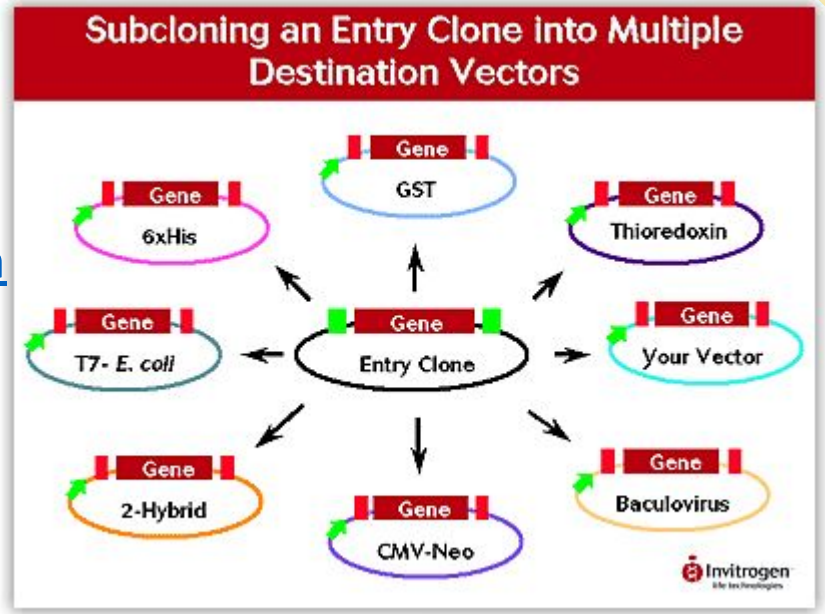


Figure 2: The Gateway system adopts phage integration into the BP and LR reactions. The BP reaction creates an attL-flanked entry clone. The LR reaction creates an expression clone with all of the components necessary for gene expression.

Source: https://blog.addgene.org/plasmids-101-gateway-cloning

Advantages

- Fast reactions—1 hour room-temperature cloning reactions
- Accurate results—cloning reactions achieve >95% efficiency to deliver the clone you need
- Versatile technology—easily shuttle DNA material/ insert from vector to vector
- Source: https://www.thermofisher.com/in/en/home/life-science/cloning/gateway-cloning/protocols.html



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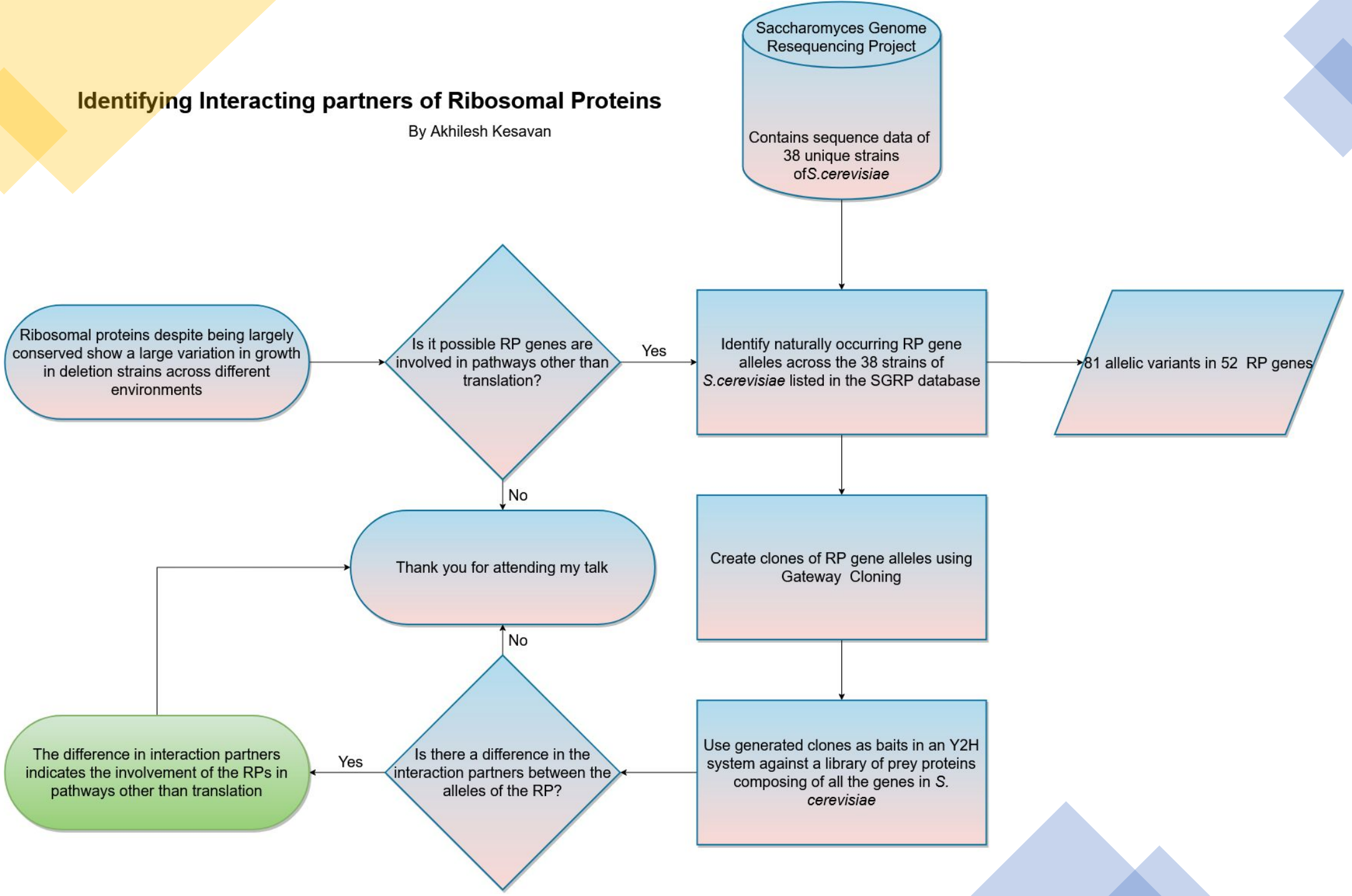


What Next?

- Naive GAL4 (transcription factor) contains a DNA binding domain and an activating domain
- The GAL4 protein is modified as following:
  - Binding domain fused to protein X (Bait)
  - Activating domain fused to protein Y (Prey)
- Only when the bait and prey interact, transcription machinery is recruited and the cell shows β-Galactosidase activity

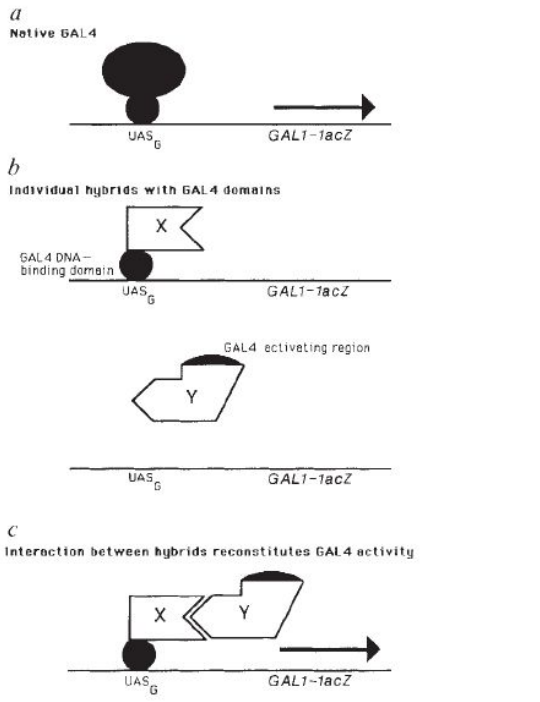
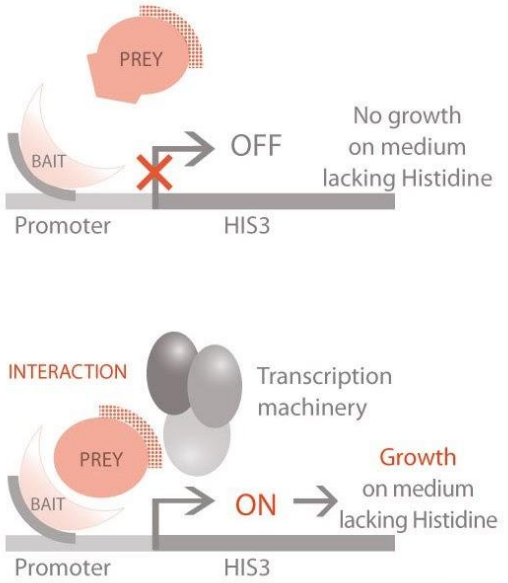


FIG. 1 Model of transcriptional activation by reconstitution of GAL4 activity. *a*, The native GAL4 protein contains both DNA-binding and activating regions and induces *GAL1–lacZ* transcription. *b*, Hybrids containing either the DNA-binding domain (upper) or activating region (lower) are incapable of inducing transcription. *c*, A protein–protein interaction between proteins X and Y brings the GAL4 domains into close proximity and results in transcriptional activity.

Source: Fields, S. & Song, O., 1989. A novel genetic system to detect protein-protein interactions. Nature, 340(6230), pp.245–246



Source: https://www.hybrigenics-services.com/contents/resources/yeast-two-hybrid-principle

Why Y2H?

Flexible and less time consuming compared to biochemical methods

In-vivo technique using a eukaryotic model system giving more accurate results compared to in-vitro or bacterial systems

No need of high quality purified proteins and antibodies

Capable of screening large scale libraries more quickly and efficiently