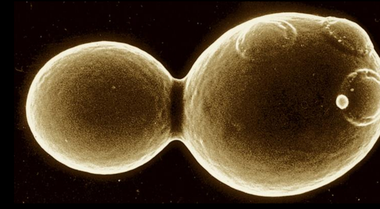
The construction of transcription factor and promoter libraries in *Saccharomyces cerevisiae* for simplifying the characterization of synthetic gene regulatory networks.

Yara Abou-Hamde*, Ian Roney and Mads Kaern, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa.



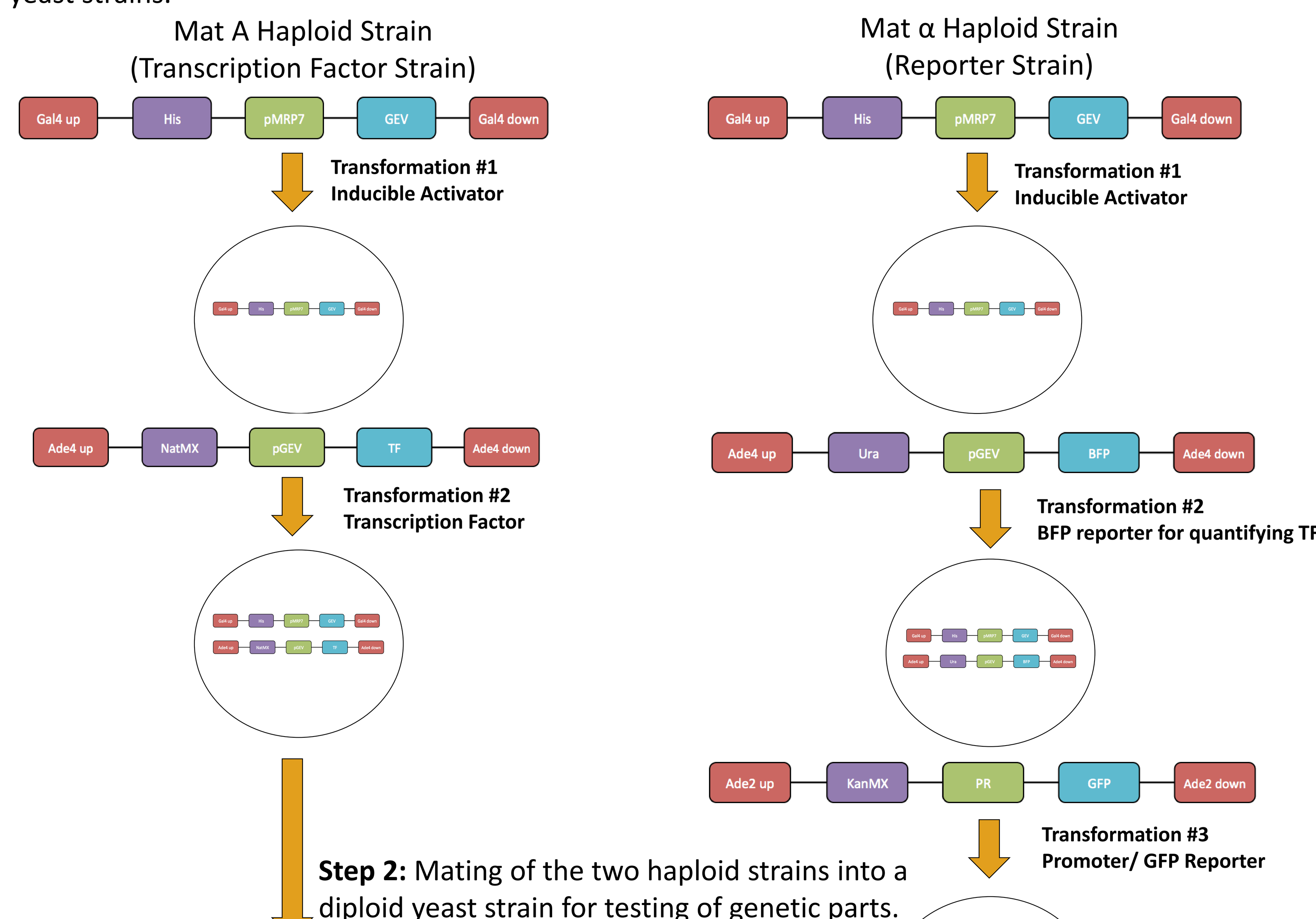
Introduction

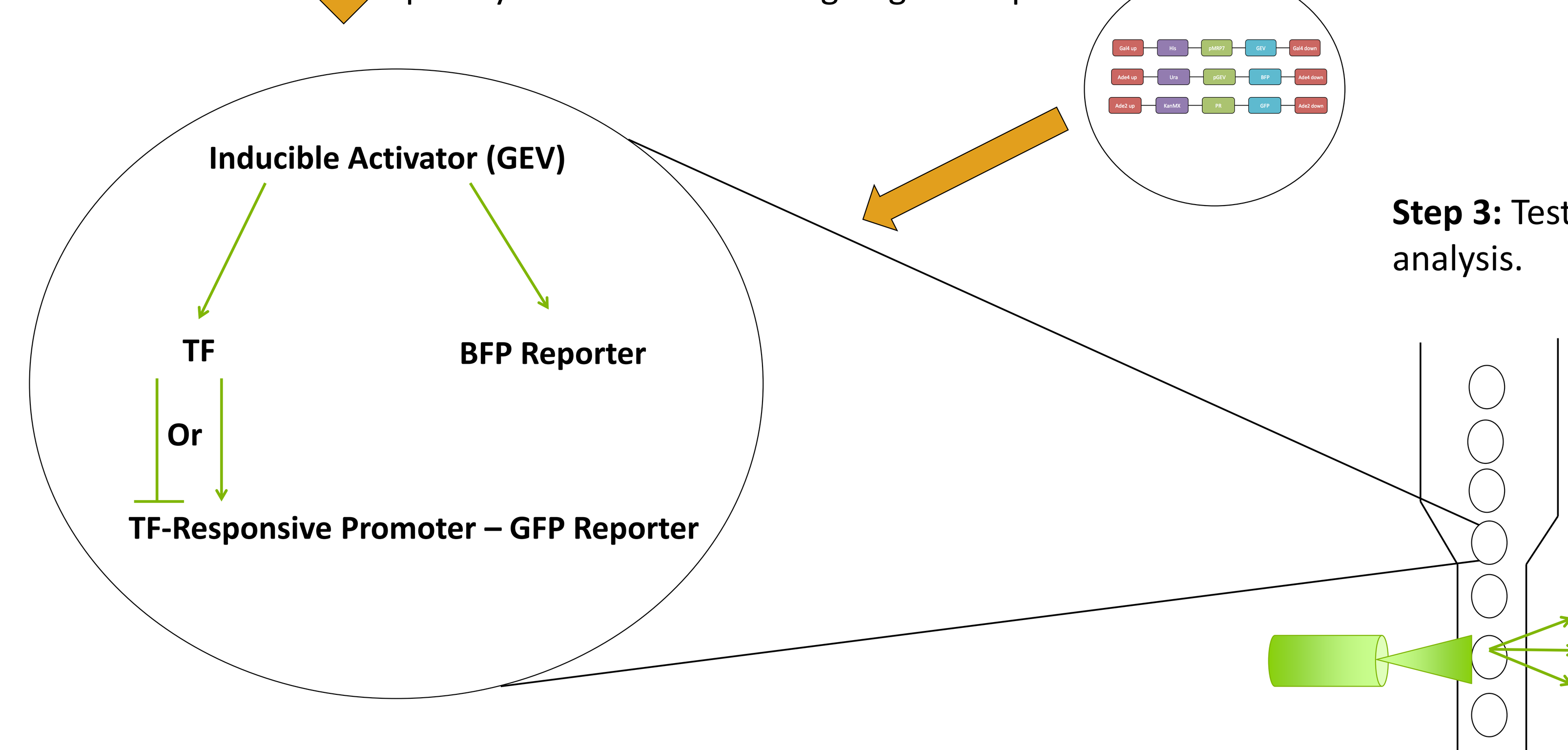
The emerging field of synthetic biology attempts to use engineering principles to modify organisms' genetic material so that they can serve new, useful functions. Currently, the field is hindered by a lack of well-characterized genetic components that could be used to rationally design synthetic gene networks.

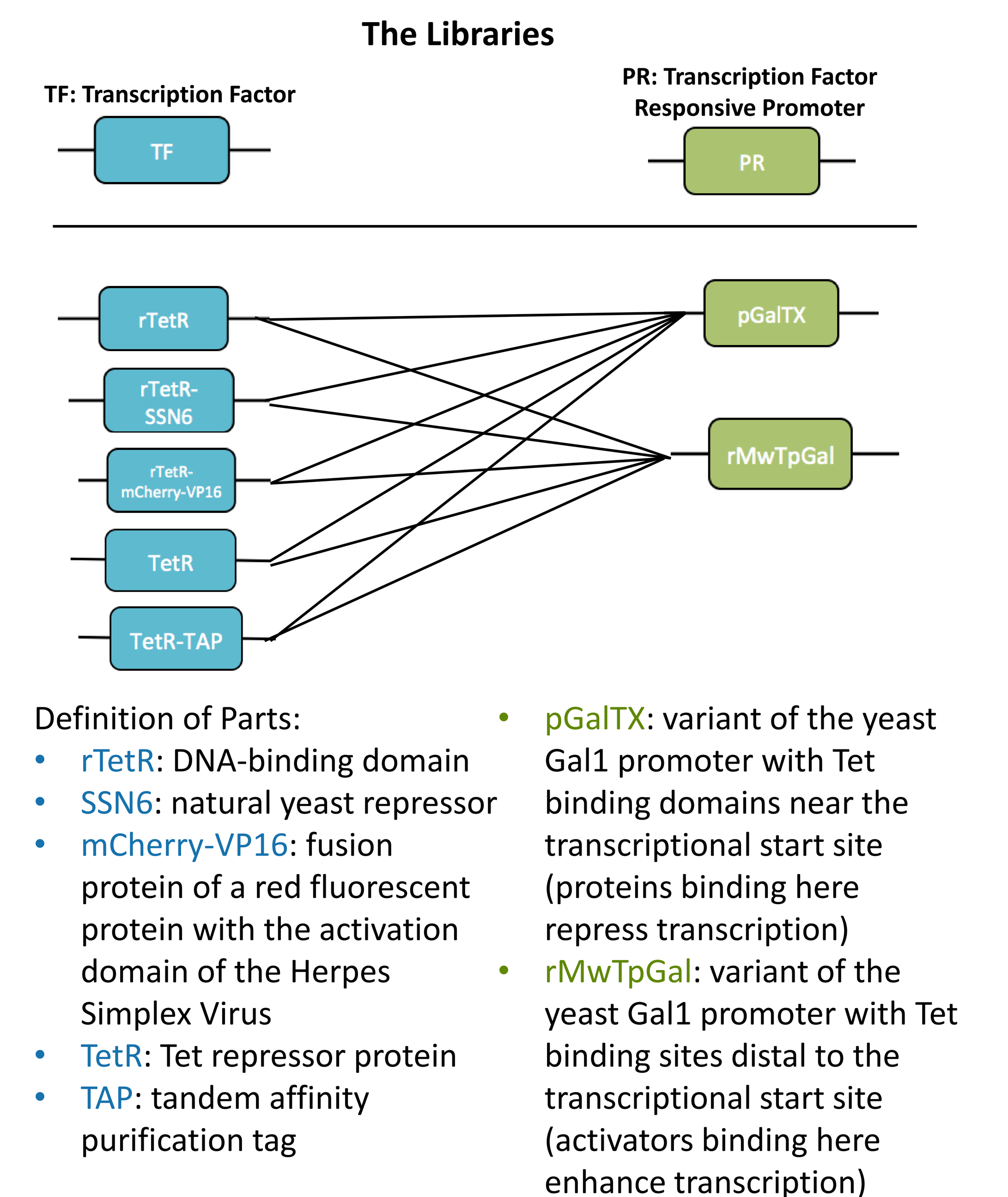
This project aims to develop a standard for characterizing transcription factors and transcription factor-responsive promoters. By creating libraries of the respective genetic parts in yeast strains of opposite mating type, the parts can be tested combinatorially with robust mating protocols. In this manner, the action of the same transcription factor can be efficiently tested on a range of promoters by simply mating the appropriate strains. Each promoter will be driving the expression of a fluorescent protein marker and gene expression can be quantified using fluorescent microscopy or flow cytometry. As a proof of concept, several yeast strains will be mated and expression levels of the fluorescent proteins will be tested via flow cytometry.

Methodology

Step 1: Transformation of genetic constructs into haploid yeast strains.

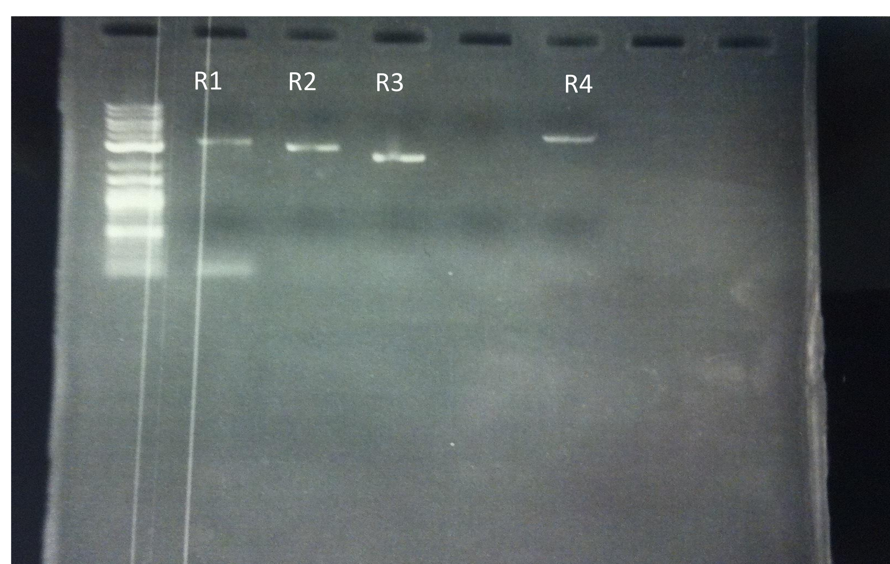






Results

PCR amplification of the pertinent genetic constructs and transformation of inducible activator construct.



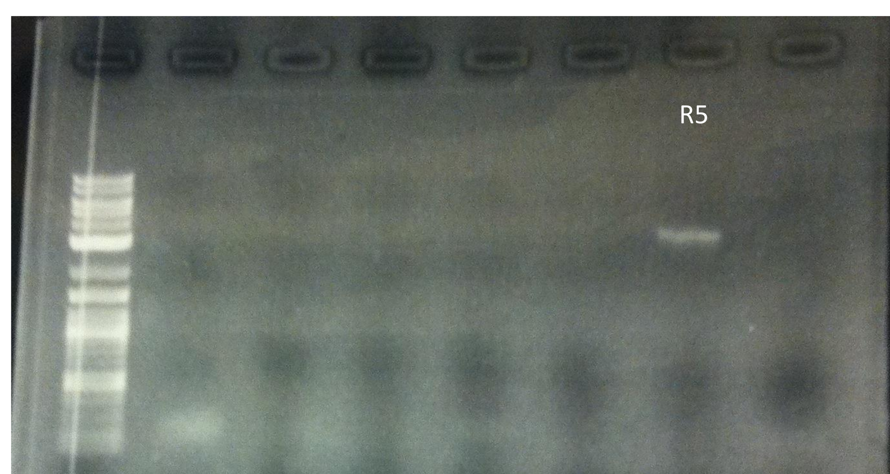


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Figure 1. Multiple PCR reactions were run to isolate the desired genetic constructs from various pre-existing yeast strains as the first step in building the libraries. Some constructs had to be amplified in two fragments due to difficulties encountered in amplifying the construct in its entirety. R1 (Gal4::His-pMRP7-GEV); R2 (Ade4up-Nat-pGEV-rTetR); R3 (rTetR-mCherry-VP16-Ade4down); R4 (rTetR-SSN6-Ade4down); R5 (Ade2::Kan-rMwTpGal-GFP); R6 (Ade2::Kan-pGalTX-GFP).

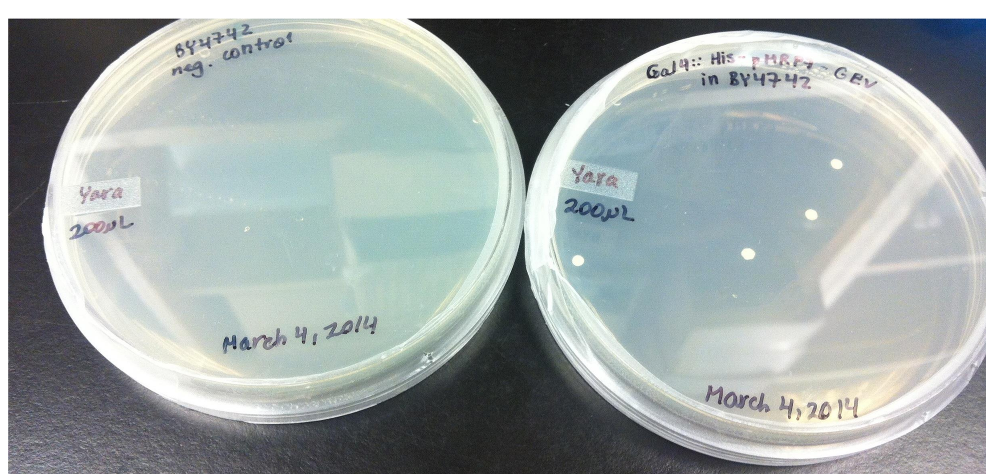

Figure 2. The first transformation was done to insert the inducible activator construct in both haploid strains. Colonies were observed for only one of the two strains (right). The negative control plate did not contain any colonies (left). The presence of the desired Gal4 construct in these colonies must be confirmed using genomic extraction.

Conclusion

The building of the libraries described in this research project is underway but has not yet been completed. Next steps include a) the verification of the Gal4 knockout in the haploid strain via genomic extraction followed by PCR, b) the subsequent transformations of the genetic constructs into the two haploid yeast strains as described in the Methodology section and c) mating of any two haploid strains and testing the expression of GFP and BFP via flow cytometry.

The concept of library construction described here can also be expanded to any other project requiring the construction of a genetic network and/or the characterization of novel transcription factors and promoters.

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*Contact Information:
Email: yabou101@uottawa.ca