Advancing Wet and Dry Lab Tools to Accelerate the BioFoundry Design-Build Process

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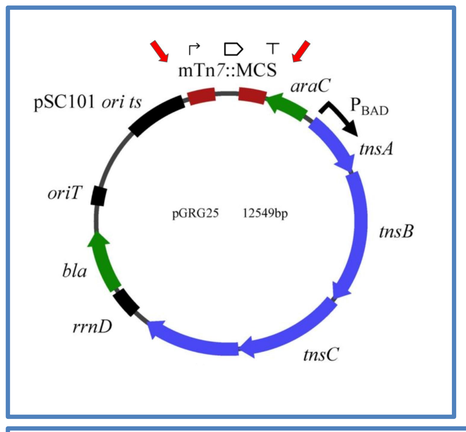
Abstract

Reducing the number of constituent steps in the Design-Build-Test engineering process is necessary to facilitate high-throughput organism assembly, allowing the loop to be more conducive to automation and a higher-level approach to engineering biology. One major bottleneck in this pipeline is the post-assembly transformation and replication of large (>10kb) constructs for downstream application. Single-low copy backbones require volumes of culture that are unsuitable for high throughput processes, while high copy vectors yield unstable and mutated inserts. Current commercial solutions are costly, proprietary and difficult to integrate into custom biofoundry workflows. Engineering a microbial strain that incorporates the desired characteristics from low and high copy vectors, allowing for both stability of cloned insert and high vector yield on demand, would streamline the Design-Build pipeline and provide a standardized platform for downstream application. The proposed TOP10CC *E. coli* will leverage cloning capabilities found in TOP10 with a inducible operon driving *trfA*, the plasmid replication initiator protein required by OriV, which then allow for copy-control. TOP10CC will maintain construct stability until a medium-high copy number is induced when desired. In addition, we applied a differentiated script to sequencing output files in order to leverage the screening and identification of positive clones from large batches of constructs.

Objective



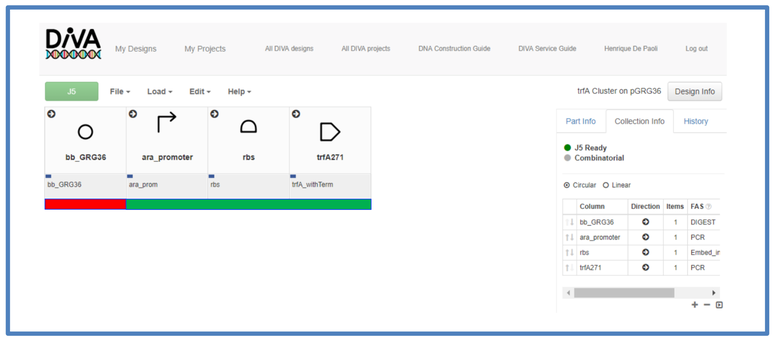
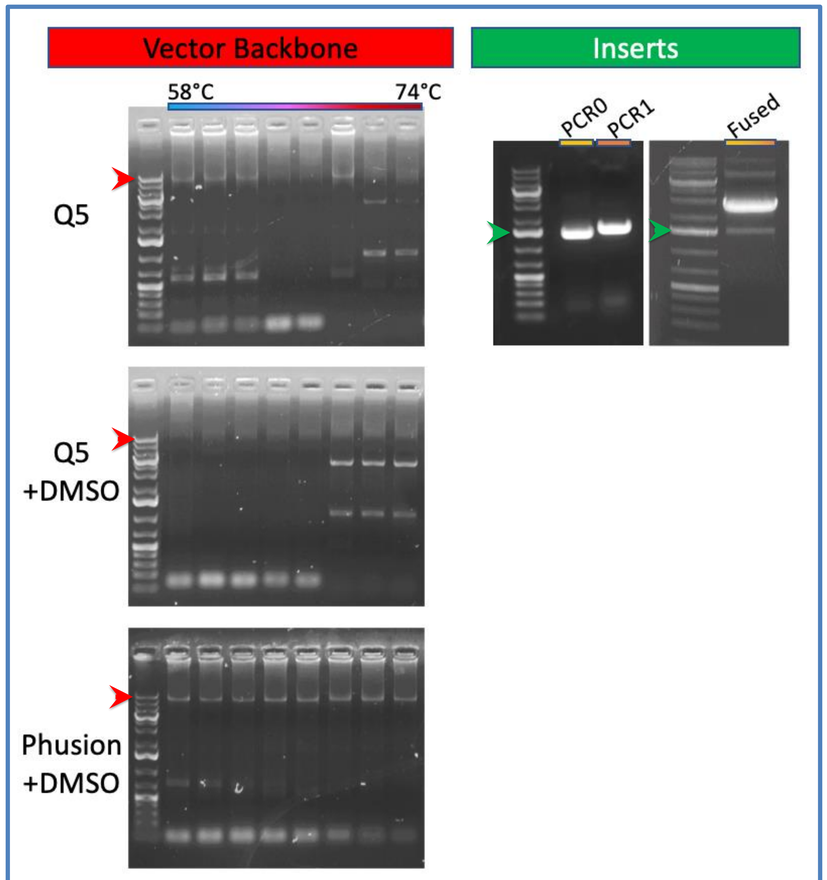
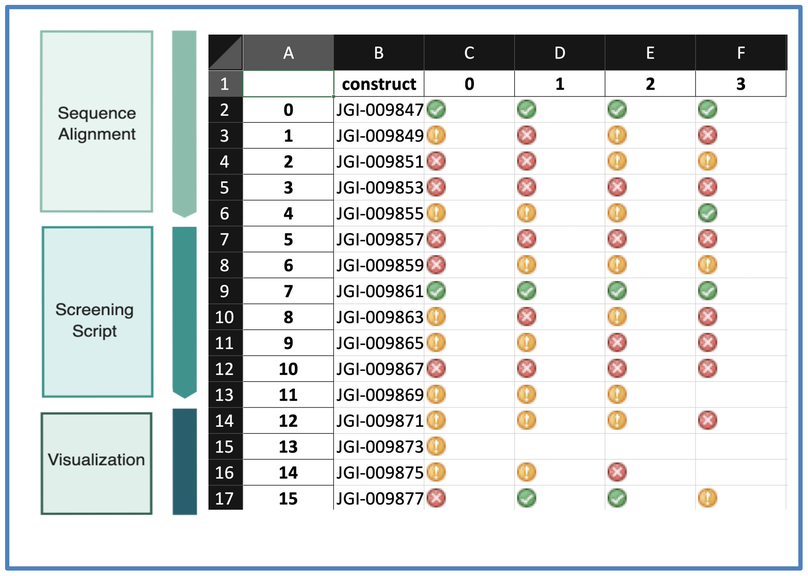


Figure 1 - (**Left top**) Map of delivery vector showing arabinose induced trfA operon to be cloned between chromosomal arms (red arrows) and the transposon elements (blue). (**Left bottom**) Genetic components as represented in Device Editor (color coding matches gel)

Results





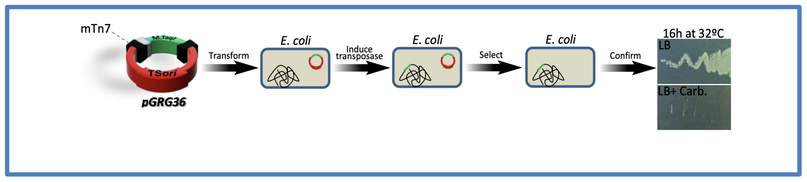


Figure 2 - (**Left)** 1% Agarose gel showing PCR generated backbone (12.5kb) under various conditions and both inserts (1247bp and 1393bp) before assembly; Phusion+DMSO at TM72˚C was the most optimal reaction condition for amplifying the backbone. Ladder: Red arrow (20kb, right above 10kb) and Green arrow (1.5kb, right below 2kb). (**Right, top**) Sequence alignment produces .bam/.vcf files; colonies that meet certain variant/coverage threshold are validated, ignored, or tabled for refined analysis using bcftools, python, and bash scripting. (**Right, bottom**) Graphical representation of operon insertion into TOP10 E. coli strain at the mTn7 locus for generating TOP10CC.

Conclusion

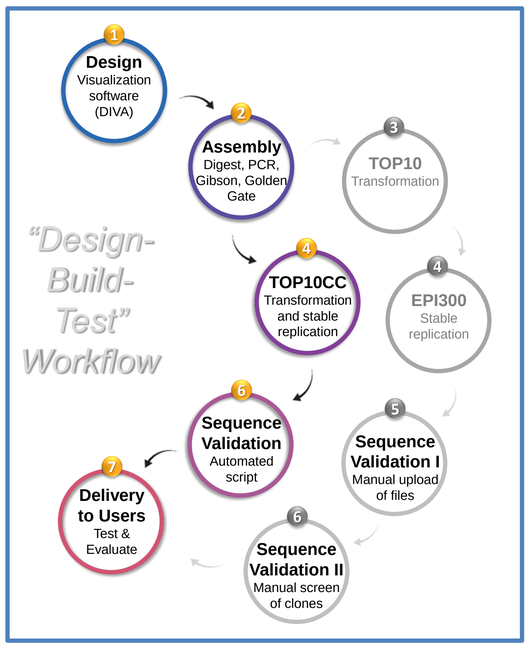


Figure 3 - Integration of Top10CC and sequence validation script with the high level "Design, Build, Test" workflow. Pipeline is faster and components are more amenable to high-throughput (colored) when compared to previous pipeline (gray).

Acknowledgements

This work was supported by the U.S. Department of Energy's (DOE's) Bioenergy Technologies Office (BETO) grant awarded to the Agile BioFoundry (ABF) consortium. We would like to thank the U.S. Department of Energy's Office of Biological and Environmental Research, which supports the Joint BioEnergy Institute, and Jennifer Chiniquy, Mark Kulawik, and Xiaoxian Liu for enriching our discussion of the validation pipeline.