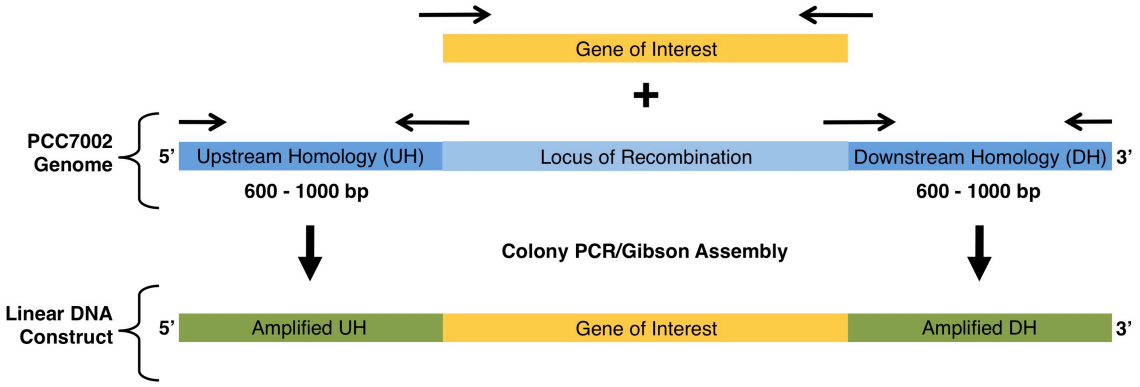
Transformation of Linear DNA Fragments into *Synechococcus* sp. PCC 7002 for Recombination

Adapted from Frigaard et al. 2004

- The protocol that follows was designed to screen for recombination events, so it is useful for gene knockouts (i.e. FLP-FRT knockout and recombination).
- The linear construct to be transformed must have 600 to 1000 bp homology arms both up- and downstream of the DNA fragment of interest in order to successfully recombine with the PCC7002 genome. Yale iGEM tested 1000 bp fragments, but other literature protocols report successful recombinations with homology arms as short as 600 bp (Ruffing 2014). The homology arms should be amplified by colony PCR and assembled into the full linear construct by Gibson assembly.



Amplification and assembly of linear DNA construct for natural transformation and recombination. Horizontal arrows represent primers.

- The protocol calls for A+ medium containing 1.0 g/L of $NaNO_3$ (See Growth protocols).
- When attempting mutagenesis of genes encoding proteins involved in photosynthesis, glycerol is included in the A+ medium to provide an alternative carbon source to CO_2. Currently, most mutagenesis on Synechococcus sp. PCC 7002 involves destroying elements of the photosynthetic pathway, therefore the protocol given here includes 10 mM glycerol in the A+ medium.
- Synechococcus sp. PCC 7002 grows optimally at 38°C; however, plates with solid medium are most conveniently incubated at, or slightly above, room temperature (typically around 30°C). Aerosol-barrier pipette tips should be used when handling liquid cultures, sterile media, and gas mixtures to prevent contamination.

Protocol:

1. Prepare an exponentially growing culture of Synechococcus sp. PCC 7002 (OD730 of about 0.5) in liquid A+ medium. Adding 10 mM glycerol and bubbling samples with sterile air supplemented with 1% CO_2 can increase the growth rate. About 4 ml of this culture will be needed per experimental sample.

2. Harvest the cells and resuspend them in fresh medium to an OD730 of 2 to 3 by centrifuging at 2750 x g for 10 min. Centrifuging 10 ml of culture in 15 ml falcon tubes works well.

3. Mix 1 to 5 µg of DNA and 0.8 mL of culture. Keep the volume of the added DNA solution less than one-tenth of the culture volume

4. Incubate samples (with shaking) under strong illumination (about 250 µE/m2 s) at 38°C for 5 hours to overnight, bubbling with sterile air supplemented with 1% CO_2.

5. Spread the cell suspension over a plate with solid A+ medium containing 10 mM glycerol and allow the cells to grow under moderate illumination (about 150 µE/m2 s) at 30°C for about 3 days until a thin lawn of growth is visible.

6. Overlay the plate with sterile, melted 0.8% (w/v) agar in water containing antibiotic (not warmer than about 50°C) over the cells. Use about 3 mL per plate (diameter 9 cm) containing 40 to 50 mL of solid medium. Adjust the amount of antibiotic added such that the final concentration is calculated for the entire volume of solid medium in the plate.

7. Incubate the plate under the same conditions as in step 3 until single colonies appear, at least 2 to 3 weeks.

8. Transfer several colonies on fresh solid, A+ medium containing 10 mM glycerol (if the mutant cells have an impaired photosynthesis pathway) and the appropriate antibiotic and incubate again. Restreaking should be repeated until a homozygous isolate is obtained (two to three restreakings from a single colony is usually sufficient unless the gene product is required for viability or strongly selected for under the growth conditions employed).

9. Homozygous cell colonies should be identified by a colony PCR assay.

References:

Frigaard NU, Sakuragi Y, and Bryant DA. "Gene Inactivation in the Cyanobacterium *Synechococcus* sp. PCC 7002 and the Green Sulfur Bacterium *Chlorobium tepidum* Using In Vitro-Made DNA Constructs and Natural Transformation." In *Photosynthesis Research Protocols*. Methods in Molecular Biology: Humana Press **2004** 274, 325-340.

Ruffing, A. "Improved Free Fatty Acid Production in Cyanobacteria with Synechococcus sp. PCC 7002 as Host." *Front. Bioeng. Biotechnol.* **2014** 2.