Alma iGEM 2020 Protocols

Here are some brief details of the protocols we used, especially for those that are unique or unusual.

Standard / Kit Protocols

For details on these steps, please follow manufacturer instructions

Mini-prep isolation of plasmid DNA was accomplished with the QIAgen kit.

Gibson Assembly and Golden Gate Cloning was accomplished with the kits from New England Biolabs

Transformations into E. coli were accomplished with New England Biolabs NEB Stable Strain, or NEB-5-alpha. Transformation into E. coli DH5a or HL2483 were accomplished using the iGEM competent cell protocol (https://parts.igem.org/Help:Protocols/Competent_Cells) or the TSS transformation protocol (https://openwetware.org/wiki/Preparing_chemically_competent_cells).

Routine PCR was accomplished with Taq polymerase or Q5 polymerase (New England Biolabs) according to manufacturer.

Measuring Fluorescence

LB Miller plates with Chloramphenicol (final concentration of 30ug/mL) were prepared through creation of a 2X LB liquid media and 3% agar – these are then mixed in equal portions before pouring plates. Plates were used in transformations or streaked with the indicated strains and grown overnight at 37^{o}C.

For measuring fluorescence of liquid cultures, the initial steps of the 2018 Interlab protocol were followed: the strains were grown overnight in LB media. The following day, the OD600 was measured, and the cultures were diluted into fresh media with antibiotic (and, in some cases, IPTG or aTc) to a final OD600 level of 0.02. 100uL of this culture was then added to a 96 well black, clear bottom plate and incubated at 37C for several hours. During this time, fluorescence of RFP was measured (excitation at 585nm, emission at 615nm) and cell growth was monitored via OD600 in a SpectraMax M3 plate reader.

Electroporation of Vibrio Natriegens was performed as described in Weinstock et al, 2016 (https://pubmed.ncbi.nlm.nih.gov/27571549/)

Measuring Plasmid Copy Number by qPCR

Liquid cultures of the strains used were grown (either LB Miller for *E. coli* or BHI-V2 for *Vibrio*) overnight. The following day, OD600 was measured and the equivalent of 1mL of culture at an OD600 of 1.0 was pelleted by centrifugation. The supernatant was removed, and 1mL of dH20 was added and cells resuspended. The cells were then boiled for 10 minutes, and then frozen at -20c for 10 minutes. Following this, 1uL was used as a template in a LunaDye qPCR reaction (New England Biolabs) as described by the manufacturer.

Sanger Sequencing

Sanger Sequencing was performed by the MSU genomics core facility. As per their instructions, we provided them 96 well plates containing, in each well, 9uL of plasmid or fragment DNA and 3uL of a single primer at 10uM concentration.

Results were aligned using the tools embedded in Benchling and the NCBI global align tool.

Generating Circuit Diagrams with Pigeon

Used to create diagrams for the Engineering success page

Diagrams were coded and then represented with the Pigeon tool: (http://pigeon.synbiotools.org/)

The codes used were as follows – with numbers referring to the order of images on the Engineering Success page on the Wiki.

(1) *Initial Design:*

```
o A 11
p Tet 10
c RFP 6
t
o B 11
p Lac 2
? hERE 2
c TetR 9
o C 11
p Con 8
c hERa 1
o D 11
# Arcs
TetR rep Tet
hERa rep Lac
DDT ind hERa-Lac
```

(2) *Semi-Quantitative*

```
o A 11
p cI 8
c GFP 4
t
o B 11
p Tet 10
c RFP 6
t
o C 11
p Lac 2
? hERE 2
? hERE 2
c TetR 9
o D 11
p Lac 2
? hERE 2
c cI 7
o E 11
p Con 13
c hERa 1
o F 11
# Arcs
cI rep cI
TetR rep Tet
hERa rep Lac
DDT ind hERa-Lac
```

(3) *Discriminating*

```
o A 11
p Tet 10
? hERE 10
c RFP 6
t
o B 11
p Lac 2
? rtERE 2
c hERa 5
c TetR 9
o C 11
p Con 8
c rtER 1
o D 11
# Arcs
rtER rep Lac
TetR rep Tet
hERa rep Tet
Estradiol ind hERa-Tet
DDT ind rtER-Lac
```