Hydrate - $MgH_2O$

$C_1$ = Cholera 1
$C_2$ = Cholera 2
$C_3$ = Cholera 3

FF = Forward Primer
FR = Reverse Primer
VF = Vector Forward Primer
VR = Vector Reverse Primer

$C_1S$ = 25 ml
$C_1T$ = 25 ml

$FFC_1S$ = 46 ml
$FRC_1S$ = 191 ml
$VFC_1S$ = 194 ml
$VRC_1S$ = 201 ml

$FFC_1T$ = 148 ml
$FRC_1T$ = 220 ml
$VFC_1T$ = 179 ml
$VRC_1T$ = 187 ml

$C_2S$ = 25 ml
$C_2T$ = 25 ml

$FFC_2S$ = 174 ml
$FRC_2S$ = 295 ml
$VRC_2S$ = 219 ml
$VRC_2S$ = 217 ml

$FFC_2T$ = 293 ml
$FRC_2T$ = 184 ml
$VFC_2T$ = 221 ml
$VRC_2T$ = 242 ml

$C_3S$ = 25 ml
$C_3T$ = 25 ml

$FFC_3S$ = 264 ml
$FRC_3S$ = 213 ml
$VFC_3S$ = 218 ml
$VRC_3S$ = 187 ml

$FFC_3T$ = 235 ml
$FRC_3T$ = 246 ml
$VFC_3T$ = 167 ml
$VRC_3T$ = 162 ml

Primer Dilutions: 10 mM
1ml primer (+) 45 ml $H_2O$

# PCR

50 µl Reaction

- 5x Q5 Reaction Buffer : 10 µl
- DNTPs : 1 µl
- Forward Primer : 2.5 µl
- Reverse Primer : 2.5 µl
- Template DNA (1 ng/µl) → $C_1S$, $C_1T$, $C_2S$, $C_2T$, $C_3S$, $C_3T$
- Q5 polymerase : 0.5 µl
- MQ $H_2O$ : up to 50 µl

↳ Annealing Temp : 68°C

50 µl Reaction → Vector

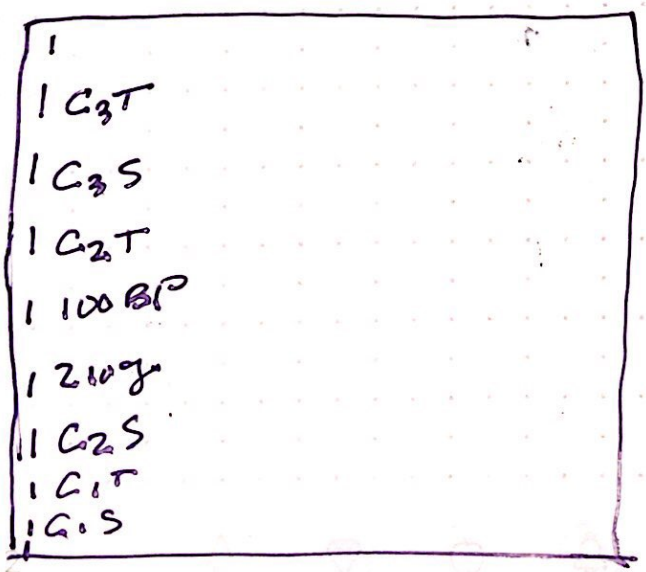
- 5x Q5 Reaction Buffer : 10 µl
- dNTPs : 1 µl
- VF Primer : 2.5 µl
- VR Primer : 2.5 µl
- template DNA (Tochold-Sturdy)
- Q5 Polymerase : 0.5 µl
- MQ $H_2O$ : up to 50 µl

↳ Annealing Temp : 56°C

## Gel Electrophoresis

- 5 µl DNA + 1 µl loading dye
- 5 µl 100BP ladder & 2log ladder ...

* Diagram




* All parts are here.

## PCR Purification

### Reliaprep DNA-clean up

- PCR Volume — Membrane Binding Soln
  45 µl — 45 µl
  ↳ 1.5 mL microcentrifuge tube and vortex

1. Load sample into minicolumn and centrifuge for 30 secs.
2. 200 µl of Column Wash Solution
   300 µl of Buffer B (x2)
   15 µl of Nuclease Free Water
3. Centrifuge

Purified

| | | |
|---|---|---|
| $C_1S$ | $C_2S$ | $C_2S$ |
| $C_1T$ | $C_2T$ | $C_2T$ |
| $VC_1S$ | $VC_2S$ | $VC_2S$ |
| $VC_1T$ | $VC_2T$ | $VC_2T$ |

### Gibson Assembly Ligation

- Gibson MasterMix 15 µl
- 1.5 µl insert ($C_1S$, $C_1T$, $C_2S$, $C_2T$, $C_2S$, $C_2T$)
- 3.5 vector ($VC_1S$, $VC_1T$, $VC_2S$, $VC_2T$, $VC_2S$, $VC_2T$

20 µl Total

Run for 15 mins at 50°C

## Transformation

- NEB DH10 Alpha E.coli
  - ↳ 50 µl per Reaction
- 10 µl of Gibson Assembly
  - ↳ Keep on Ice for 30 mins
  - add 300 µl of LB or SOC Media
  - Shake in incubator for 1 hour
  - Plate on correct antibiotic
    - ↳ 14-16 hr in incubator

Inoculate Plates
- ↳ 5 mL of LB + 5 µl of Antibiotic
  - Shake for 24 hours

## MiniPrep

CFS : 188.5 ng/µl 1.79

C1T : 43.4 ng/µl 1.92

C2S : 213.4 ng/µl 1.85

C2T : 57.1 ng/µl 1.82

C3S : 398 ng/µl 1.64

C3T : 45.0 ng/µl 1.78

↳ Sent for Sequencing

Backbone SWITCH
- ↳ All Triggers & Switches in pSB1C3
  - ↳ Sent to iGEM Headquarters

# Dual Plasmid Transformation

- $C_1S$ + $C_1T$ : Carb + CAM + XGAL
- $C_1S$ : CAM + XGAL
- $C_2S$ + $C_2T$ : Carb + CAM + XGAL
- $C_2S$ : CAM + XGAL
- $C_3S$ + $C_3T$ : Carb + CAM + XGAL
- $C_3S$ : CAM + XGAL

## Electroporation

- 50 µl of electrocompetent cells (ΔT) BL21 Δ7
- 10 ng of Plasmid
  * Made dilutions

1 µl of Plasmid + 50 µl of cells

1. transfer to chilled electroporation cuvette.

Electroporate
Values (time constant)

$C_1$ = 1.79, 3.00

$C_2$ = 1.82, 2.70

$C_3$ = 1.80, 2.90

Add 1 mL of prewarmed LB to cuvette
Transfer to microcentrifuge tube
Shake in incubator for 1.5 hrs.

10/12/18 Sequencing

$C_1S$ = 5.3 ml DNA, 0.8 ml $VF_2$, 5.9 ml $H_2O$

$C_2S$ = 4.7 ml DNA, 0.8 ml $VF_2$, 6.5 ml $H_2O$

$C_{2a}S$ = 2.5 ml DNA, 0.8 ml $VF_2$, 8.7 ml

$C_1T$ = 11.2 ml DNA, 0.8 ml $VF_2$

$C_2T$ = 7 ml DNA, 0.8 ml $VF_2$, 4.2 $H_2O$

$C_3T$ = 11.2 ml DNA, 0.8 ml $VF_2$

Dual Plasmid Transformation

$C_1S$ = 0.27 ml
$C_1T$ = 1.15 ml
] 50 ng + 50 ng of DNA

$C_2S$ = 0.23 ml
$C_2T$ = 0.88 ml
]

$C_3S$ = 0.13 ml
$C_3T$ = 1.11 ml
]

$C_1S$ = 0.53 ml  100 ng of DNA

$C_2S$ = 0.47 ml  100 ng of DNA

$C_3S$ = 0.25 ml  100 ng of DNA