7/23/13

- Plasmid Prep: K081012 (Single Colonies 1,2,3)
  I721002 (Single Colonies 1,2,3)
  ~~* these were mutagenic samples~~
- Streaked and made liquid cultures of:
  K174015 - Trans. 1 (1,2,3,4,5)
  - Trans. 2 (1,2,3,4,5)
  * These were mutagenic samples
- Prepared Competent Cells using DH5α
  ~~1) place 1mL of culture in 2mL tubes~~
  2) spin 2 min
  3) resuspend with pipet in 600λ
  ~~4)~~

1) Get pre-chilled MCF tubes from fridge
2) Prepare ice water bucket
3) Evenly transfer DH5α cells into MCF tubes
4) Centrifuge for 10 min @ 6000 rpm
   - (use smaller clinical centrifuge ~~Rob~~)
5) Prepare bleach container and pour supernatant in
6) Add 400λ of ice-cold ccMB80 buffer
   - ~~resuspend~~ mix using pipet until solution is consistent
   - do 5 cycles (plung in and out)
7) Add 400λ of buffer and shake gently by inversion
8) Place in ice bucket for 20 min.
9) Centrifuge again for 10 min
10) Decant supernatant into bleach
11) Resuspend in 175λ of buffer
12) Place in ice bucket for 20 min.
13) Label floats and tubes
14) Continue with transformation

- Started transformations using DH5α competent cells and K174015 (Kan, mutagenic), K824008 A (Chlor, mutagenic), K824008 B (Chlor, mutagenic), K824012 (Chlor, mutagenic)

1) place SOC medium (from fridge door) in incubator
2) also place 4 plates in the incubator (each with appropriate antibiotic resistance
3) Get an ice water bath
4) Get 4 microcentrifuge tubes (MCF tubes) and a float
5) Add 50 λ of competent cells
6) Add 2 λ of DNA
7) Place in water bath for 2 min
8) While waiting
   - obtain 400 mL beaker, fill with hot water, adjust water to 42°C (at least)
9) Place tubes in 42° bath for 30 sec.
10) Another 2 min on ice
11) Add 1 mL of warm SOC medium (put SOC back in fridge)
12) Place each tube in incubator (37°C) for 1 hour
13) Carefully label each plate
14) pour/spread appropriate tube onto each plate using the bar or disposable spreaders
15) place the plates back in the incubator overnight

placed 1:25 pm

Start here

Trans. 1	= K174015
Trans. 2	= K824008 A
Trans. 3	= K824008 B
Trans. 4	= K824012

- Began ligation of digest from 7/18 (Steven Notebook P. 36)
  1) – K174015 + Kan backbone
  2) – K824008 A + Chlor
  3) – K824008 B + Chlor
  4) – K824012 + chlor

1)

✓ Promoter/Construct	2λ
✓ Backbone	2λ
✓ 10× Buffer	2λ
Ligase	1λ
✓ dH_2O	13λ
	20λ

2) ~~Leave at room temp.~~ for 10 min

3) Put in ~~hot~~ water bath for 20 min. at 80°C

↑ No, this is correct.