Colony PCR flu operon
8/4/2010

Prepare DNA Template

1. Take a stab of the frozen stock of *E. coli* K12 from the IGEM -80C frozen stock (not too much because too much template will lead to unspecific replication) and resuspend it in 50 uL of DI water
2. Mix primers to a 50 mM concentration
    a. Multiply the nmol concentration from the IDT printout by 20
    b. This is the volume of resuspension in ultrapure water in uL
        i. Forward flu primer: 24.5 nmol x 20 = 490 uL ultra pure water
        ii. Reverse flu primer 46.9 nmol x 20 = 938 uL ultra pure water
    c. Done previously
3. Turn on PCR machine to instant incubate at 98 C for the first initial denaturation step
4. Place PCR tubes for run on ice
5. PCR reaction KEEP EVERYTHING ON ICE
    a. Vortex all tubes before starting to make sure everything is well mixed
    b. For a single reaction mixture (20 uL)
        i. 14.5 uL Ultra pure water
        ii. 4 uL of 5x phusion master mix
        iii. 0.4 uL 10 mM dNTP
        iv. 0.25 uL of primer A (0.5 uM concentration from 40 uM stock)
        v. 0.25 uL of primer B (0.5 uM concentration from 40 uM stock)
        vi. 0.4 uL of DNA template (from step 1)
        vii. 0.2 uL Phusion DNA polymerase
    c. Combine all ingredients but polymerase for 5 samples in master mix (100 ul)
        i. 72.5 uL of ultra pure water (188.75 uL was added for this experiment accidentally)
        ii. 20 uL of 5x phusion master mix
        iii. 2 uL 10 mM dNTP
        iv. 1.25 uL of primer A
        v. 1.25 uL of primer B
        vi. 2 uL of DNA template (from step 1)
    d. Chill mixture for 15 minutes
    e. Add 1 uL Phusion DNA polymerase with chilled pipette tip
    f. Transfer 20 uL of sample to each PCR tube with chilled pipette tip

6. PCR cycle
    a. 95 C for 6 minutes
    b. 98 C for 30 seconds
    c. 98 C for 10 seconds
    d. 52 C to 68 C for 30 seconds
        i. Sample 1: column 2 @ 52.4C
        ii. Sample 2: column 4 @ 54.6C
        iii. Sample 3: column 5 @ 56.5C

    iv. Sample 4: column 7 @ 61.5C
    v. Sample 5: column 9 @ 65.5C
e. 72 C for 1:45 seconds (30 sec per kb)
f. Goto step b four times
g. 98 C for 10 seconds
h. 67 C for 30 seconds
i. 72 C for 1:45 seconds
j. Goto step f twenty nine times
k. 72 C for 10 minutes
l. 4C forever
m. End