Colony PCR flu operon
8/5/2010
Modifications from 8/4/2010 are highlighted in red

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 250 uL of DI water (Unspecific amplification so template concentration is too high)
    a. Add 200 uL of ultra pure water to DNA template already prepared
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 (50 uL)
        i. 36.275 uL Ultra pure water
        ii. 10 uL of 5x phusion master mix
        iii. 1 uL 10 mM dNTP
        iv. 0.625 uL of primer A (0.5 uM concentration from 40 uM stock)
        v. 0.625 uL of primer B (0.5 uM concentration from 40 uM stock)
        vi. 1 uL of DNA template (from step 1)
        vii. 0.5 uL Phusion DNA polymerase
    c. Combine all ingredients but polymerase for 3 samples in master mix (150 ul)
        i. 108.825 uL of ultra pure water
        ii. 30 uL of 5x phusion master mix
        iii. 3 uL 10 mM dNTP
        iv. 1.875 uL of primer A
        v. 1.875 uL of primer B
        vi. 3 uL of DNA template (from step 1)
    d. Chill mixture for 15 minutes
    e. Add 1.5 uL Phusion DNA polymerase with chilled pipette tip
    f. Transfer 50 uL of sample to each PCR tube with chilled pipette tip

6. PCR cycle
    a. 98 C for 30 seconds
    b. 98 C for 10 seconds
    c. 65 C to 68 C for 30 seconds
        i. Sample 6: column 6 @ 64.8C
        ii. Sample 7: column 7 @ 66C
        iii. Sample 8: column 9 @ 67.4C

d. 72 C for 1:45 seconds (30 sec per kb)
e. Goto step b four times
f. 98 C for 10 seconds
g. 67 C for 30 seconds
h. 72 C for 1:45 seconds
i. Goto step f twenty nine times
j. 72 C for 10 minutes
k. 4C forever
l. End