9/8/09

Ligation Protocol

From the Ginko Biowork BioBrick Assembly Manual

1. Remove the 10X T4 DNA Ligase Reaction Buffer* from the freezer to thaw. You can also remove the T4 DNA Ligase enzyme from the freezer at this point but leave the ligase in a cold box to keep it close to -20C. Thawing is fast if the buffer tube is immersed in room temperature water. Once thawed, agitate the 10X T4 DNA Ligase Reaction Buffer until all precipitate goes into solution.
2. Mix reagents in the following order in a 200 uL PCR tube
    1. 11uL of ultra pure water
    2. 2 uL of leu landing pad digest, Pu promoter digest and gfp digest,
        a. All of the digests are unpurified
    3. Add 2 µl of 10X T4 DNA Ligase Reaction Buffer to the tube
    4. Add 1 µl of the T4 DNA Ligase to the tube.
3. The total volume in each tube should now be 20 µl. Ensure the ligation is well-mixed by flicking the tube. You can spin the tube in a microcentrifuge for a few seconds to collect the liquid in the bottom of the tube again.
4. Incubate with the following PCR program (Incubation time from Jeremy's ligation protocol)
    1. 37 C for 30 seconds
    2. 16 C for 16 hours
    3. 65 C for 10 minutes
    4. 4 C forever
5. Store in the -20 C freezer in the IGEM box until transformation

Adapted from Jeremy's DNA ligation Protocol

1. Remove buffer from freezer and thaw. Vortex buffer and [lightly] DNA to mix and touch centrifuge. Keep restriction enzyme on ice or in cold box prior to use, flick a few times to mix and then touch centrifuge prior to use
2. Calculate the amount of DNA in ng to be added to the ligation for a 1:3 and 1:6 molar excess of vector to insert using the following equation from openwetware DNA ligation:

$$\text{Insert mass in ng} = (\text{excess amount}) \text{ x } \frac{\text{insert length in bp}}{\text{vector length in bp}} \text{ x (vector mass in ng)}$$

    a. Where the vector mass will be 10 ng/10uL of solution
    b. Digested DNA lengths
        i. Pu promoter: 480 bp

ii. GFP generator: 904 bp
iii. Leu Landing Pad: 5809 bp

c. For 1:3 excess for a 60 uL solution

$$\text{Pu mass in ng} = 3 \text{ x } \frac{480 \text{ bp}}{5809 \text{ bp}} \text{ x } 60 \text{ ng} = 14.87 \text{ ng}$$

$$\text{GFP mass in ng} = 3 \text{ x } \frac{904 \text{ bp}}{5809 \text{ bp}} \text{ x } 60 \text{ ng} = 28.01 \text{ ng}$$

d. For 1:6 excess for a 60 uL solution

$$\text{Pu mass in ng} = 6 \text{ x } \frac{480 \text{ bp}}{5809 \text{ bp}} \text{ x } 60 \text{ ng} = 29.75 \text{ ng}$$

$$\text{GFP mass in ng} = 6 \text{ x } \frac{904 \text{ bp}}{5809 \text{ bp}} \text{ x } 60 \text{ ng} = 56.02 \text{ ng}$$

3. Set up the reactions by pipeting the reagents in the following order
   a. DNA concentrations from digestion
      i. Pu prom 3.3: 10 ng/ul
      ii. GFP gen 3: 12 ng/ul
      iii. leu landing pad: 10 ng/ul

Component	**1:3** **Volume (60 μL total)**	**1:6** **Volume (60 μL total)**	**1:1 Ginko** **Volume (20 μL total)**
Ultrapure dH2O	41.18 μL	37.35 uL	11 uL
Leu landing pad	6 uL	6 uL	2 uL
Pu promoter 3.1	1.487 uL	2.98 uL	2 uL
GFP gen 3	2.334 uL	4.67 uL	2 uL
10x Ligase Buffer	6 μL	6 μL	2 uL
T4 DNA ligase	3 μL	3 μL	1 uL

4. Run Controls for transformation

Component	**Vector Only (10 μL total)**	**Insert Only (10 μL total)**
Ultrapure dH2O	3.5 μL	3.5 μL
DNA	5 μL	5 μL
10x Ligase Buffer	1 μL	1 μL
T4 DNA ligase	0.5 μL	0.5 μL

5. Incubate under the same conditions for the above protocol

Results

Ligation of leu Landing pad, Pu promoter and GFP generator

Ligations according to both protocols were performed to determine which concentration of insert to vector would ligate.