Gibson-Assembly

Name Date:

Continue from Experiment
(Date).

(Name)

Project Name:

1. Prepare 5X ISO buffer. Six ml of this buffer can be prepared by combining the following:

3 ml of 1 M Tris-HCl pH 7.5
150 µl of 2 M $MgCl_2$
60 µl of 100 mM dGTP
60 µl of 100 mM dATP
60 µl of 100 mM dTTP
60 µl of 100 mM dCTP
300 µl of 1 M DTT
1.5 g PEG-8000
300 µl of 100 mM NAD
Add water to 6 ml
Aliquot 100 µl and store at -20 °C

2. Prepare an assembly master mixture. This can be prepared by combining the following:

320 µl 5X ISO buffer
0.64 µl of 10 U/ µl T5 exo
20 µl of 2 U/µl Phusion pol
160 µl of 40 U/µl Taq lig
Add water to 1.2 ml

Aliquot 15 µl and store at -20 °C. This assembly mixture can be stored at -20 °C for at least one year. The enzymes remain active following at least 10 freeze-thaw cycles.
This is ideal for the assembly of DNA molecules with 20-150 bp

overlaps. For DNA molecules overlapping by larger than 150 bp, prepare the assembly mixture by using 3.2 µl of 10 U/ µl T5 exo.

3. Thaw a 15 µl assembly mixture aliquot and keep on ice until ready to be used.

4. Add 5 µl of DNA to be assembled to the master mixture. The DNA should be in equimolar amounts. Use 10-100 ng of each ~6 kb DNA fragment. For larger DNA segments, increasingly proportionate amounts of DNA should be added (e.g. 250 ng of each 150 kb DNA segment).

5. Incubate at 50 °C for 15 to 60 min (60 min is optimal).

6. If cloning is desired, electroporate 1 µl of the assembly reaction into 30 µl electrocompetent *E. coli*.

Documentation:

Why are you doing this experiment? Name the parts for the Gibson-Assembly.

Describe your results and mistakes. Did you digest it? Results?

How did you label your samples and where are they stored?