3A ASSEMBLY AND GEL ANALYSIS PROTOCOL

Introduction

The starting point of every 3A assembly is plasmid DNA preps of three different BioBricksTM. The endpoint is, hopefully, colored bacterial colonies.

Materials

- DNA plasmids
- ddH2O
- 10x reaction buffer for restriction enzymes provided by manufacturer
- EcoRI restriction endonuclease, heat-inactivatable
- XbaI restriction endonuclease, heat-inactivatable
- SpeI restriction endonuclease, heat-inactivatable
- PstI restriction endonuclease, heat-inactivatable
- T4 DNA ligase
- 10x reaction buffer for T4 DNA ligase provided by manufacturer
- Papers of every different color, as well as black, grey and white

Procedure

Digestion

1. Make three mixes: each contains 500 ng of one of the three plasmids and ddH2O to 43 µL.

2. To each mix, add 5 µL of 10x reaction buffer for restriction enzymes.

3. Add 1 µL each of the appropriate endonucleases (two per tube) according to Fig. 25 to give a final volume of 50 µL.

4. Tap on the tubes to mix. If necessary, centrifuge for a few seconds to spin down the liquid.

5. Incubate at 37°C for 30 min.

6. Heat-inactivate the enzymes by incubating at 80°C for 20 min.

Gel analysis of digested parts

7. Run 20 µl of each digestion mixture (200 ng) on a 1% agarose gel **(Protocol 4)** to measure the extent of digestion. Also run the three uncut plasmids (negative controls) directly beside their cut versions, and a DNA ladder marker should be loaded in a middle lane.

8. After the run, there should be one or more DNA bands visible under UV light in each lane. The marker will help indicate the size of the linear (cut) fragments. The lanes with cut plasmid should contain two bands: The slower one is the vector and the faster one is the insert. If the insert is very small (e.g. some promoter parts), it may be invisible due to a low amount of stainable DNA and low resolution or due to running off the gel. The uncut plasmid should be one or two bands: a supercoiled version and a nicked form (one or both of the circular strands of DNA has a break in the phosphodiester chain, thereby allowing relaxation of the coil). Note that the different topologies of linears, nicked circles and supercoils cause each to migrate at a different rate.

Ligation

9. Add 2 µL (20 ng) of each of the three digestion mixtures to 11 µL of water.

10. Add 2 µL 10x reaction buffer for T4 DNA ligase.

11. Add 1 µL of T4 DNA ligase to give a final volume of 20 µL.

12. Incubate at room temperature (~22°C) for 30 min.

13. Heat-inactivate the enzymes by heating at 80°C for 20 min.