High-Efficiency BioBrick Assembly Protocol

This protocol describes the construction of BioBricks. It uses much more DNA than e.g. 3A-assembly and can be used when you encounter low transformation efficiencies.

Materials needed

- DNA (Vectors, inserts)
- H_2O
- NEB Reaction buffer (1, 2, 3 or 4)
- Restriction enzymes (EcoRI-HF, XbaI, PstI, SpeI)
- BSA (Bovine Serum Albumin)

Restriction

- For restriction you need: H_2O, BSA, Restriction Buffer 1,2,3 or 4 (buffer 2 works for all combinations of EcoRI-HF, XbaI, PstI, SpeI), two restriction enzymes per sample.
- Determine which restrictions enzymes you need. Please refer to openwetware.org[1] for instructions.
- Determine the DNA-concentration of the vector and the insert.
- Prepare restriction mix for all restrictions. Add for each reaction:
    1. 12,5 µl H_2O
    2. 5,0 µl Buffer 1,2,3 or 4
    3. 0,5 µl BSA
    4. 1,0 µl restriction enzyme 1
    5. 1,0 µl restriction enzyme 2
- For each reaction:
    - Add 20 µl restriction mix to a PCR-tube.
    - Add 1 µg of DNA.
    - Add H_2O to a total volume of 50µl.
- Incubate for 2 hours at 37°C, followed by an additional 20 minutes at 80°C to inactivate the restriction enzymes.

PCR Purification

- Purify the restriction digest, e.g. using the QIAquick PCR Purification Kit. Follow the manufacturer's protocol.
- After purification, determine the concentration of the purified DNA.

Ligation

[1] http://openwetware.org/wiki/Enzyme_selection_for_BioBricks_digest

- Prepare ligation mix. Prepare two extra reactions, “No insert” and “No ligase”. These will be used in transformations later and function as negative controls.

	Per vector/insert	No insert	No ligase
Ligase	0,5 µl	0,5 µl	0 µl
Vector			
Insert		0 µl	
H_2O			
10x buffer	2,0 µl	2,0 µl	2,0 µl

- For each reaction:
    - Add 50 ng of vector-DNA.
    - Add insert in three times molar excess. Thus, add 3·50·(*length of insert* / *length of vector*) ng of insert-DNA.
    - Add H_2O to a total volume of 20 µl.
    - Incubate for 30 minutes at 16°C.
- For “No insert” omit the insert-DNA, for “No ligase” omit the ligase and replace with H_2O.

Transformation

- Transform competent E. coli with the ligation product. Make sure to also transform the two control “No insert” and “No ligase”. These can help you determine the efficiency of the restriction step. Follow the manufacturer’s protocol for transformation carefully to ensure high transformation efficiency.

- How to interpret the controls:

    - When in both cases no colonies from, this means that the restriction was perfect for both the restriction enzymes.

    - If both in both cases colonies form, then neither of the restriction enzymes worked well and most of the transformants in your actual samples will contain the vector only.

    - If the “No insert” control resulted in many colonies but “No ligase” did not, then one of the enzymes worked well and the other did not. You may find that your samples have resulted in large numbers of colonies carrying the self-ligated vector.

- Select colonies by colony PCR or visually if your construct allows you to make a distinction between colonies carrying the insert and colonies without the insert. Inoculate successfully transformed colonies in overnight culture for further use.