Two Part BioBrick Assembly

Aim

The aim is to assemble two BioBricks with a high rate of success. The method differs from 3A assembly since only two parts are assembled instead of three (and only one backbone is involved), but the result is similar. No linearized plasmid backbone is needed, since the vector of one of the BioBricks are used as the final vector. One of the BioBricks, e.g. a promoter, needs to be in a plasmid (e.g. pSB1C3). The second BioBrick needs to be PCR amplified to remove its plasmid backbone. These BioBricks are assembled by cutting with SpeI and PstI in the BioBrick suffix of the plasmid containing the first BioBrick. The second BioBrick is cut with XbaI and PstI in the BioBrick prefix and suffix respectively. The two BioBricks are then ligated and a circular plasmid is formed.

Materials

- 10X FastDigest Buffer
- FastDigest enzymes: BcuI (SpeI), PstI, XbaI
- 10X T4 DNA Ligase Buffer
- T4 DNA Ligase (5 U/µl)
- Nuclease-free water

Procedure

Digestion

1. Prepare the following mix for digestion of the **vector**, a plasmid containing a BioBrick:

Component	Amount
Nuclease-free water	to a final volume of 20 µl
10X FastDigest Buffer	2 µl
Plasmid with BioBrick 1	up to 1 µg
FastDigest BcuI (SpeI)	1 µl
FastDigest PstI	1 µl
Total volume	**20 µl**

Two Part BioBrick Assembly

2. Prepare the following mix for digestion of the **insert**, a PCR amplified BioBrick:

Component	Amount
Nuclease-free water	to a final volume of 20 µl
10X FastDigest Buffer	2 µl
PCR amplified BioBrick 2	up to 1 µg
FastDigest XbaI	1 µl
FastDigest PstI	1 µl
Total volume	**20 µl**

3. Incubate the digestion mixes at 37°C (in a heat block) for 15 min.
4. **PCR purification:** Use a PCR purification kit to purify the digestion mixes from restriction enzymes etc.

Ligation

1. Prepare the following mix for ligation of the insert into the vector:

Component	Amount
Digested vector DNA (containing promoter)	25 -100 ng
Digested insert DNA	3:1 to 5:1 molar ratio over vector
10x T4 DNA Ligase buffer	2 µl
T4 DNA Ligase (5 U/µl)	1 µl
Nuclease-free water	to 20 µl
Total volume	**20 µl**

2. Incubate the ligation mix at room temperature overnight.
3. Incubate at 65°C for 10 min to inactivate the ligase.
4. **For transformation:** Use 5 µl of the ligation mix to 50 µl chemically competent cells.

Two Part BioBrick Assembly

Notes

Always add enzymes last!

The digestion mixes can be scaled up linearly if more than 1 µg DNA needs to be digested.

The original protocol from Thermo Fisher states a reaction time of 10 min to 1 hour for the ligation. However, this reaction time was extended overnight to ensure successful ligation.

References

The digestion procedure is a modified version of the protocol Fast Digestion of DNA from Thermo Fisher Scientific:
assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012413_Fast_Digestion_DNA_UG.pdf and the product information of the
It has been modified based on the product information of the restriction enzymes PstI, BcuI (SpeI) and XbaI.

The ligation procedure is a modified version of the original Sticky-end Ligation protocol provided by Thermo Fisher Scientific:
assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0011906_DNAsert_Ligation_Vector_DNA_UG.pdf