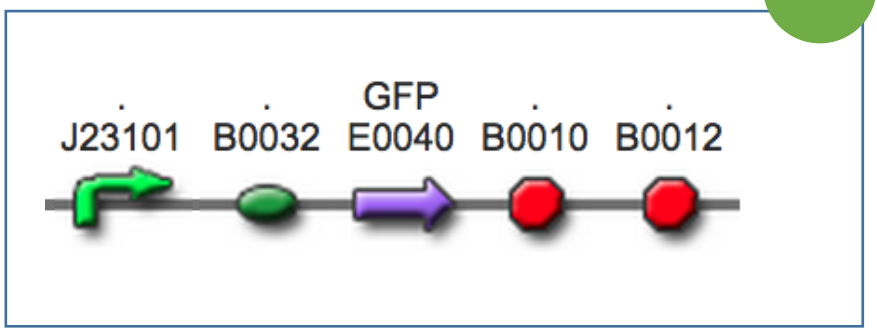
BBa_I20260



Day 1

Transformation

Organism: *Escherichia coli* DH5-α

Plasmid resistence: Kanamycin

Materials

- 1,5ml tube
- Styrofoam box with ice
- Agar plate with antibiotic
- Competent cells
- Plasmidial DNA
- Centrifuge
- Water bath at 42°C
- Liquid LB medium
- Shaker

Method

- Ressuspend the biobrick in 10ul of nuclease-free water. Wait for 10 minutes.
- Briefly spin the competent cells and put then on ice
- Add 3 ul of the ressuspended DNA in a 1,5ml tube
- Add the 50ul of competent cell in the same tube
- Keep the tube on ice for 25min
- Put the tube in a 42° water bath for 3 min
- Put the tube on ice for 5 min
- Add 200ul of liquid LB
- Incubate at 250rpm/37°C/1 hour
- Plate the the solution in a Agar plate with the appropriate antibiotic
- Incubate the plate at 37°C overnight

Day 2

- Inoculate overnight/37°C/250rpm 3 – 4 colonies in a 6 ml LB with the same antibiotic used in the transformation protocol.

Day 3

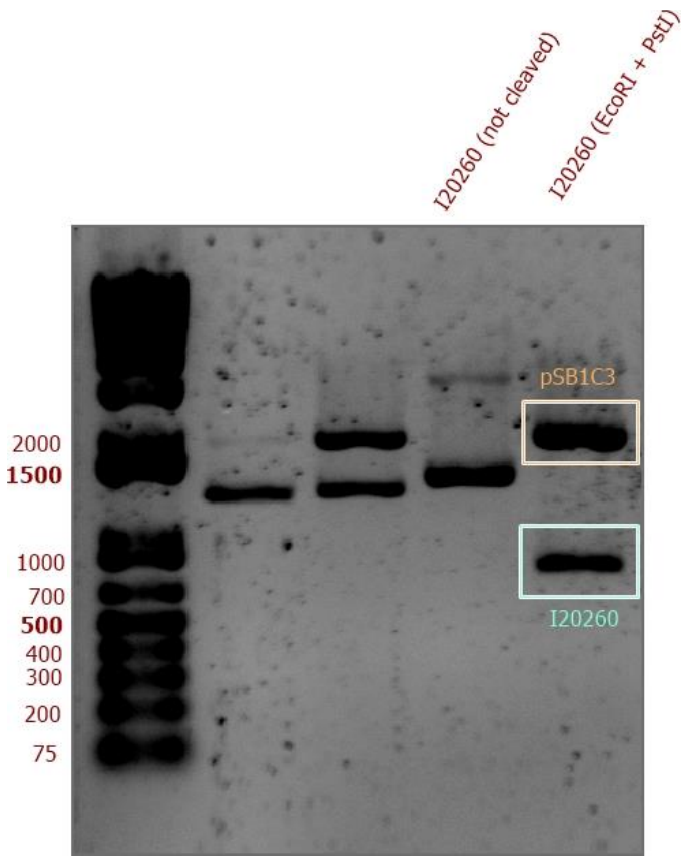
Miniprep

- Prepare a **glycerol stock** of the inoculum (500ul glycerol 40% + 500ul of grown inoculum)
- Extract plasmidial DNA (we used the extraction kit PureLink Invitrogen without the vacuum manifold)
    - http://tools.lifetechnologies.com/content/sfs/manuals/purelink_quick_plasmid_qrc.pdf

Restriction analysis

- EP Digestion

Biobrick	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H_2O to 10ul (ul)
BBa_I20260	3	1	0,5	0,5	5



Size expected	Size in gel
919 bp	~ 1000 bp