Date: 2017/10/19
Operators: Gabriel

DNA digestion

Aim: Digest DNA using restriction enzymes.

Equipment:

- Restriction enzymes stored at –20°C (NEB : New England Biolabs)
- Plasmid to digest stored at –20°C
- 10X NEB buffer CutSmart stored at –20°C
- De-ionized water
- Water-bath at 37°C
- Heater block for deactivation at 65°C
- Timer
- Pipette p10, p20, p200 & associated cones
- Gel loading dye 6X

Name of DNA to be digested:
- pSB1C3.E1_1
- pSB1C3.E1_2
- pSB1C3.E2
- pSB1C3.E3
- pSB1C3.E4
- pSB1C3.S1

Restriction enzymes:
-EcoRI-HF
-PstI-HF

Protocol:

Global solution for all the six plasmids: Sol0

Restriction enzyme: EcoRI	4.1 µl
Restriction enzyme: PstI	4.1 µl
10X NEB buffer	44 µl
Sterile water	76.6 µl
Total	128.8 µl

Mix:

	Volume of plasmid to digest (µl)	Volume of Sol0 (see above) (µl)	Deionized water (µl)	Reaction total (µl)
pSB1C3.E1_1 A	8.8	11.7	19.5	40
pSB1C3.E1_1 B	6.0	11.7	22.3	40
pSB1C3.E1_2 A	/	/	/	/
pSB1C3.E1_2 B	17.2	11.7	11.1	40
pSB1C3.E2 A	5.4	11.7	22.9	40
pSB1C3.E2 B	16.7	11.7	11.6	40

pSB1C3.E3 A	28.3	11.7	0	40
pSB1C3.E3 B	21.5	11.7	6.8	40
pSB1C3.E4 A	12.9	11.7	15.4	40
pSB1C3.E4 B	16.8	11.7	11.5	40
pSB1C3.S1 A	16.5	11.7	11.8	40
pSB1C3.S1 B	13.7	11.7	14.6	40

1. Mix gently by pipetting up and down 4-6 times
2. Microcentrifuge briefly 3 sec
3. Incubate at 37°C for 15 min
4. Stop reaction by heat inactivation: incubate at 65°C for 20 min. This step is only for specific restriction enzymes (XbaI)
5. Stop reaction by adding 10 µl of 6X gel loading dye to the 50 µl reaction.
6. Load digested DNA on an agarose gel. (see Gel Electrophoresis Protocol)

Results

All the digestions have successfully shown the presence of the insert in the plasmid, except for the colony of cells transformed by pSB1C3.E4 B, which had become to take a yellow taint in the petri dish.
So now, the BioBricks are ready for the sequencing and then to be sent to iGEM HQ.