Date: 2017-10-07
Operators: Juliette

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
Restriction enzymes:
- EcoRI
- PstI

Protocol:
DNA plasmid to digest concentration:

192 ng	1 µl
1000 ng	5.0 µl
500 ng	2.5 µl
50 ng	0.25 µl

For 1000 ng of DNA sequence to digest: volume = 5.0 µl

Volume of restriction enzyme needed:

Quantity of DNA to digest	Volume of restriction enzyme
1 µg	1 µl

Mix for a total volume of 50 µl

DNA: pSB1C3 : 1000 ng	5.0 µl
Restriction enzyme: EcoR1	1 µl
Restriction enzyme: Pst1	1 µl
10X NEB buffer	5.0 µl
deionized water	38 µl
Total reaction volume	50 µl

Mix for a total volume of 50 µl of negative control

DNA: pSB1C3, 50 ng	0.25 µl
10X NEB buffer	5.0 µl
deionized water	44.75 µl
Total reaction volume	50 µl

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)

Before loading the DNA on an agarose gel, the digested pSB1C3 is **dephosphorylated**, so that the backbone collected after gel extraction is immediately usable in a ligation:

Equipment:

- rSAP enzyme (stored at -20°C) (rSAP : recombinant Shrimp Alkaline Phosphatase)
- Dephosphorylation buffer 10X (stored at -20°C)

Add:

Dephosphorylation buffer 10X	2 µl
rSAP (add last)	1 µl

1. Mix gently by pipetting up and down.
2. Micro-centrifuge briefly.
3. Incubate at 37°C for 30 min.
4. Stop reaction by heat inactivation at 65°C for 5 min.
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)

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DNA Electrophoresis & Gel Extraction

Aim: Separate DNA on an agarose gel.

Equipment:

- Electrophoresis tank
- Power supply
- TAE 50X
- DNA ladder
- Scalpel
- UV table + Gel Doc (Biorad)
- Eppendorf tubes
- Pipette, cones
- EB (Ethidium bromide)
- De-ionized water

Protocol:

1. Prepare an electrophoresis gel (see Agarose Gel Preparation Protocol).
2. Pour 500 ml of TAE 1X in the electrophoresis tank.
3. Remove comb delicately.
4. Load the wells with the digested DNA solutions, undigested DNA as negative control (30-45 µl per well) and the ladder.

Gel 1 lay out: 2017/10/07

Lane number	1	2	3	4	5	6	7	8	9	10
Volume (µl)	50	5	…….	…….	…….	…….	…….	…….	…….	…….
Sample Name	pSB1C3	1 Kb Ladder								

5. At the start, set the voltage at 75 V for 10 min
6. Afterwards, set the voltage at 150 V (80 mA) for 1h30 approximately
7. Place gel in EB* bath (de-ionized H_2O + 3 drops of EB) for 15 min
8. Wash gel in de-ionized water bath for 5 min
9. Check by UV imaging the presence of expected DNA fragments. And check that the size of the DNA sequence to extract is correct. Save image.
10. Weigh microcentrifuge tubes.
11. Cut gel with clean scalpel to extract DNA fragment and place it in microcentrifuge tube previously weighed.
12. Check with UV imaging that the gel was correctly cut. Save image.
13. Weigh microcentrifuge tubes containing the gel.

Date: 07-10-2017
Operators: Deshmukh and Juliette

Liquid culture of transformed bacteria and induction of protein expression

Aim: Induce the synthesis of a protein in transformed bacteria

Equipment:

- Antibiotics: Carbenicillin 50 mg/ml (CARB 50 mg/ml stored at -20°C)
- Sterile LB broth
- IPTG (0.5 M)
- Sterile Erlenmeyer of 2 liter
- Pipet p100, p200, and p10 and corresponding cones
- Plastic graduated pipette (10 ml or 20 ml)
- Electric propipet
- Incubator 37°C with integrated shaker (150 rpm)
- Pre-Growth liquid culture of transformed bacteria: BL21DE3
- Lysis buffer (Tris-Cl 50 mM, pH7.4, NaCl 100 mM, Glycerol 5% v/v, PMSF 15 µM)
- 1-4 1 liter Beckman centrifuge flasks
- XJ-26 Beckman centrifuge

Transformed Bacteria:

- BL21DE3 transformed by pET32a.E1_1, E1_2, E2.

Protocol:

1) Retrieve pre-grown O/N cultures of BL21De3 from incubator
2) Centrifuge for 5 minutes at 3 000 x g
3) Remove supernatant and re-suspend pellet in 5 ml of LB
4) Repeat steps 2 & 3
5) Centrifuge again at 3 000 x g for 5 minutes
6) Remove supernatant completely (use a p1000, and then a p200 for the last drops of LB)
7) Add 5 ml of LB and re-suspend pellet.
8) Prepare 800 ml of LB broth and add 800 µl of carbenicillin (50 mg/ml)
9) Add 20 ml of this pre-growth solution to the 800 ml LB/CARB broth prepared in step 8
10) Incubate at 37°C and set shaker (Infors minitron) at 150 rpm
11) Measure OD_{600nm} every hour until OD_{600nm} = 0.3-0.4
12) Then measure OD_{600nm} every 20 min until OD_{600nm} = 0.7
13) When OD_{600nm} = 0.7: sample 1 ml of the liquid culture, centrifuge the 1 ml sample at 3 000 x g for 5 min, remove supernatant, and store the pellet T=0 at -20°C
14) After sampling 1 ml of the liquid culture of BL21De3, add IPTG (0.5 M) to the rest of the liquid culture of BL21De3, as to have a final concentration of 0.5 mM of IPTG
15) Measure OD_{600nm}, and sample 1 ml of the liquid culture that you treat like in step 13, every hour.
16) Spin 800 ml of culture in a 1 liter centrifuge flask for the JLA-8.1000 rotor in a Beckman XJ-26 centrifuge at 4500 x g for 25 mins.

17) Resuspend the pellet in 10 ml lysis solution supplemented with 15 µM PMSF
18) Store pellet in a labeled 50 ml Falcon tube at -80°C

Time before IPTG induction	OD_{600nm} of liquid culture of transformed bacteria: BL21De3 transformed by pET32a.E1_1
10h20	Culture launching
13h45	0.416
14h30	0.601
15h45	0.690

Time before IPTG induction	OD_{600nm} of liquid culture of transformed bacteria: BL21De3 transformed by pET32a.E1_2
10h20	Culture launching
13h45	0.925

Time before IPTG induction	OD_{600nm} of liquid culture of transformed bacteria: BL21De3 transformed by pET32a/E2
10h20	Culture launching
13h45	0.995