6/05/17

Monday, June 5, 2017 9:05 AM

Who was in lab today: Jeff, Ayesha, Qingxi, Ana, Salma, Martin

Picked some plasmids to use in future Gibson Assembly to ligate our genes into

- pSB3T5 (TetR, p15A ori) http://beta.labgeni.us/registries/parts_registry/?part=pSB1C3
- pSBK3 (KanR, pMB1 ori) http://beta.labgeni.us/registries/parts_registry/?part=pSB1C3

Plasmids picked have different antibiotic resistances and origins of replications so that the E. Coli will not be forced to uptake/choose only one

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Ligation of Insert and Vector:

20uL total
12uL PET28
5uL cj blue
2uL buffer
1uL DNA Ligase
**ADD THE LIGASE LAST!!!

Making the agarose gel for gel electrophoresis:

1 % agarose gel (essentially 1g/ 100 mL) is the base range for separating DNA.

0.8% agarose is used for separating plasmids.

O.6% -2% agarose gel is used for separating DNA base pairs.

<0.6% too floppy

We used 500mg agarose in 50mL TAE buffer to create 1% agarose buffer.

Protocol:

1. Microwave for 130 seconds at a time.
2. Add 5 uL of Ethidium Bromide to heated mixture.
3. Add 10 uL of 6X loading dye to DNA digest samples.
4. Run Gel at 100mA.
   - Rule of Thumb: 10 mA per inch of gel you run.

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Gel Extraction/Purification Protocol

1. Excise the DNA fragment from the agarose gel with a clean, sharp razor blade.
2. Weigh the gel slice in a colorless tube. Add 3 volumes Solubilizing Buffer to 1 volume gel (100 mg gel ~ 100 µl).
3. Incubate on 50ºC water bath for 10 minutes until gel is completely dissolved in buffer (option to vortex every 2 minutes to mix)
4. Pipet 800 uL of the gel/buffer mixture into a spin column, centrifuge for 1 min @ 13000 rcf, discard the flow through from the collection tube, repeat until all of the liquid has been spun down
5. Pipet 500 uL solubilizing buffer to the white membrane of the spin column
6. Add 720 PE uL to the white membrane of the spin column, let sit for 5 minutes
7. Centrifuge for 1 min @ 13000 rfu, discard flow through
8. Centrifuge for 2 min @ 13000 rfu, discard flow through
9. Add 50 uL EB buffer to spin column in clean eppendorf tube, then spin down