Saturday 7/25/09

Measure concentration of DNA with the Nano Drop

1. Log into Gulari computer
    a. User: gulariad
    b. Password: mufasa
2. Start Nanodrop 3.0.1
3. Rinse with 2 uL of DI water twice (clean off top and bottom with chem wipe afterward each sample)
4. Blank with 2 uL of buffer (whatever DNA was eluded with in the miniprep)
5. Run 2 uL of each sample
    a. After each sample rinse with 2 uL of buffer
6. Clean with 2 uL of DI water twice after use
7. MAKE SURE TO PLACE COVER BACK ON!!!!!!

Digest DNA overnight

From openwetware: Knight:Restriction Digest

Materials

Restriction enzymes (EcoR I, Spe I, Xba I or Pst I) from NEB

RBS: cut with EcoRI and XbaI

Bacteriophage (Brown team) lysis: cut with EcoRI and XbaI

NEB2 buffer

BSA

Deionized, sterile H_2O

Digest Mix

Example - 50 µL reaction. 100 µL reactions are also common especially if your DNA to be cut is dilute.

5 µL NEB2 buffer (for all digests with BioBricks enzymes, we use NEB2 buffer. It keeps things simple and seems to work).

X µL DNA (usually ~500 ng depending on downstream uses).

0.5 µL 100X BSA (added to all digests because BSA never hurts a restriction digest)

1 µL BioBricks enzyme 1 (EcoRI) (regardless of the volume of the reaction, 1 µL enzyme is used because generally this represents a 10-25 fold excess of enzyme and is therefore sufficient for most digests. Also, it can be difficult to accurately pipet less than 1 µL of enzyme since it is sticky due to the glycerol content.)

1 µL BioBricks enzyme 2 (XbaI)

(42.5 - X) µL deionized, sterile H_2O

Because DNA concentrations are all approximately 50 ng/uL, 10 uL of DNA will be added for each digest

Procedure

Add appropriate amount of deionized H_2O to sterile 0.6 mL tube

Add restriction enzyme buffer to the tube.

Vortex buffer before pipetting to ensure that it is well-mixed.

Add BSA to the tube.

Vortex BSA before pipetting to ensure that it is well-mixed.

Add appropriate amount of DNA to be cut to the tube.

Vortex DNA before pipetting to ensure that it is well-mixed.

Add 1 µL of each enzyme (this digestion we are using XbaI and SpeI).

Vortex enzyme before pipetting to ensure that it is well-mixed.

Also, the enzyme is in some percentage of glycerol which tends to stick to the sides of your tip. To ensure you add only 1 µL, just touch your tip to the surface of the liquid when pipetting.

Place in thermal cycler and run digest protocol.

Creating a Thermalcycler program

- Select Enter from main menu then hit proceed
- Name program ANNDIGEST
    1. Scroll through the alphabet with the up and down arrows
    2. Press proceed to select the letters
    3. Press proceed twice after last letter
- Next select block temperature control and hit proceed
- Next enter program steps
    1. Select temp and hit proceed
        - Use the keyboard to enter 37C and press proceed
        - Use the keyboard to enter 14 hours and press proceed
            - 4-6 hours should be fine, but since enzymes are old incubating overnight
    2. Select temp and hit proceed
        - Use the keyboard to enter 80C and press proceed
        - Use the keyboard to enter 20 min and press proceed
    3. Select temp and hit proceed

- Use the keyboard to enter 4C and press proceed
- Use the keyboard to enter 0 sec (to hold indefinitely) and press proceed

Generally, use some method of DNA purification to eliminate enzymes and salt from the reaction.

Results

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Concentration of DNA from the miniprep

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Sample ID	ng/uL	A260	260/280	260/230	Constant
lambda 1	86.13	1.723	1.93	2.10	50
lambda 2	104.18	2.084	1.81	1.37	50
lambda 3	82.54	1.651	1.88	1.93	50
T4 1	138.76	2.775	1.86	2.17	50
T4 2	58.78	1.176	1.92	1.96	50
T4 3	62.53	1.251	1.92	1.97	50
RBS 1	53.54	1.071	2.04	2.33	50
RBS 2	57.17	1.143	1.96	2.16	50
RBS 3	56.25	1.125	2.00	2.13	50
B 1	48.64	0.973	1.96	2.06	50
B 2	56.89	1.138	1.75	1.02	50
B 2	35.81	0.716	1.93	1.82	50
B 3	89.99	1.800	1.80	1.23	50
B 3	65.94	1.319	1.93	2.07	50