First class: Basic skills in a molecular biology lab

PLAN FOR THE DAY

a) Prepare digest with BamHI or BglII, for the following DNAs: FUGW, genomic DNA, FUCEdW.
b) Prepare 1% agarose gel
c) Precipitate genomic DNA
d) Run digest
e) Take pictures

1. Preparation of the digest

Cut 3 µl of each type of DNA with BamHI or BglII.
For BamHI use buffer 2 (blue)
For BglII use buffer 3 (red tube)

Cut the following:

FUGW with BamHI
FUGW with BglI
FUGW with no enzyme
FUGW with both BamHI and BglII (in buffer 2)
FUCed with BglII
FUCed with no enzyme
Genomic DNA with BglII

(per tube)

3 µl of DNA
2 µl of the appropriate buffer (red or blue)
14 µl of water
1 µl of BSA (bovine serum albumin)
0.5 µl of enzyme (BamHI or BglII)

Mix by gently tapping.
Spin for 3 seconds
Incubate in 37C waterbath for at least 40 minutes (while waiting, start preparing the gel)

2. Preparation of the agarose gel

Pour 100 ml of TAE buffer in a 200 ml bottle

Measure 1 gram of agarose in a weigh boat
Add agarose to bottle and swirl thoroughly
Loosen cap and put in microwave for 2 minutes at low power (4)
Make sure that contents do not boil and spill over
Put bottle in water bath at 37 to cool down until you can touch it
Add 5 microliters of ethidium bromide to liquid in bottle
Swirl (without making bubbles)
Cast get with two 12 tooth combs (one on top row and one in bottom)
Add agarose into cast and let solidify
Wait 15 minutes (while you wait, precipitate the genomic DNA as explained below)
Once is solid, remove comb, turn the cast 90 degrees, and add approx. 400 ml of TAE buffer, until the liquid starts to cover the gel

3. Precipitation of DNA

Into a 1.5 ml eppendorf tube, add:

40 µl of genomic DNA
5 µl of sodium acetate 3 M
100 µl of 100% ethanol

Mix well by shaking

Spin in centrifuge at maximun speed for 5 minutes

Discard supernatant in waste bucket

Add 1 ml of 70% ethanol

Discard supernatant in waste bucket (make sure that your remove all you can by inverting tube on a piece of paper and gently tap the tube to eliminate all remaining drops)

Air dry tube for 5 minutes

Add 10 µl of water to bottom of tube

Tap bottom of tube to ensure mixing of water and pellet

Spin down for 3 seconds

4. Running of DNA digests and precipitation

Load 4 µl of 1Kb DNA ladder in first well

Mix 2 µl of loading buffer (LB tube) with each of the digests that you prepared before

Load 23 µl of each of the digest+loading buffer in each well

Add 3 µl of loading buffer to the precipitated genomic DNA, and load into a well

The loading order should be:

1kb ladder-FUGW (BaMHI)- FUGW (BglII)-FUGW (BamHI and BglII)-FUGW (no enzyme)- genomic DNA (BglII) -FUCedW (BglII)- FUCed (no enzyme)-precipitated genomic DNA (plus empty wells)

Once everything is loaded, set voltage to 150 volts and start running the gel
Run gel for 15 minutes
Stop gel and take image with UV light