Third class (PCR and digestion, 2006)

PLAN FOR THE DAY

a) Set up PCR reaction of GFP and potassium channel
b) Check the size of expected PCR product by computer
c) Check PCR product on gel and take picture
d) Ethanol precipitate DNA
e) Set up digestion of PCR product and vector.

1. PCR reaction

Because PCR takes some time, we will start the reaction first. As you come in, please set up PCR reaction.

Mix the following solution in a thin-wall PCR tube (200 µl).

82 µl of water
10 µl of buffer [for taq Polymerase]
2 µl of CaMKII 5' primer (50 pmol/µl)
2 µl of CaMKII 3' primer (50 pmol/µl)
2 µl of dNTP (10 mM each)
1 µl of Taq polymerase
1 µl of CaMKII plasmid DNA (100 ng/µl)

Mix by gently tapping.
Spin for 3 seconds in small centrifuge

Split the contents of the tube into 3 thin-walled tubes with 33µl each.

Start PCR reaction at the following condition (the machine will be preset to this)

1. 94 °C, 1 min
2. 55 °C, 0.5 min
3. 72 °C, 1 min

Repeat the same reaction for 20 times

2. Estimate the size of PCR product

The primer sequences are as follows;

5' GAT CCT CGA GCT ATG GCT ACC ATC ACC TGC ACC
3' GAT CG AAT TCG CAT GCC CTG GCC GTT GCC TTC AAT G

We are amplifying rat CaMKII alpha conding region..

Using these hints and the DNA database, estimate the size of expected PCR product.

3. Run PCR product on agarose gel

(prepare an agarose gel as soon as you are done with the PCR)

Take 5 µl from the tube, mix with 1 µl of DNA loading dye (Xc for xylene cyanol) on parafilm, and load on to a gel.
Load 4 µl of 1 kb DNA ladder

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

4. Ethanol precipitate PCR product

You can do this while running the gel.

To the rest (95 µl) of PCR product, add the following
10 µl of sodium acetate 3 M
250 µl of 100% ethanol
Mix well by shaking
Spin in big centrifuge at maximun speed (14000 rpm) for 5 minutes
Discard supernatant in waste bucket
Add 1 ml of 70% ethanol
Discard supernatant in waste bucket
Air dry tube for 5 minutes
Resuspend in 54 µl of water

5. Set up thedigestion of vector and insert

For the vector: (Note: make sure that you take 1 µl or less of enzyme!)

6 µl of pEGFP-C1
6µl of EcoRI buffer
46 µl of water
3 µl of BSA
3 µl of EcoRI
3 µl of XhoI
1 µl of shrimp alkaline phosphatase

Finger-mix, spin for 5 seconds in small centrifuge.

For the PCR insert:

Take 54 µl of the PCR product
Add 6 µl of EcoRI buffer
3 µl of EcoRI
3 µl of XhoI

Finger-mix, spin for 5 seconds.

Incubate both reactions with insert and vector at 37C for at least three hours.

We will continue next week.