Laboratory project PART II

Day 5 (Lecture #7): DNA restriction digest
Agarose gel electrophoresis
Hand-out of the sequencing results

Your job: **A)** Find as much information as possible about your clones. Remember that the clones stem from an animal model. Why is the chicken an appropriate model for inner ear gene discovery? What are disadvantages of the chicken model?

Pick one or two clones that may have significance for inner ear function. What is known about these genes?

B) Document your experiments as detailed as possible starting with day1. A written lab report including a discussion of the results has to be submitted on or before December 18.

DNA restriction digest

Each plasmid contains a cDNA insert that was cloned via EcoRI and XhoI. Consequently, a double-digest with the restriction endonucleases EcoRI and XhoI should release the insert from the plasmid.

To make pipetting easier, we will use an enzyme Master Mix that already contains the appropriate buffers and the two restriction enzymes.

1. Aliquot 10µl of the master mix for each DNA to be analyzed into a reaction tube. Label the tubes.
2. Add 10µl of each DNA solution to the Master Mix.
3. Collect the 20µl of DNA and enzyme mixture on the bottom of the tube by brief centrifugation.
4. Incubate at 37°C for 45-90 min.
5. Add 7µl DNA loading buffer and analyze by Gel electrophoresis and comparison with Marker DNA fragments of known size.

Master Mix (2x concentrated):

280µl	sterile MilliQ water
80µl	10x buffer (New England Biolabs)
20µl	EcoRI enzyme (10-20U/µl)
20µl	XhoI enzyme (10-20U/µl)

Agarose Gel electrophoresis:

1. Wear gloves because the electrophoresis plasticware could be contaminated with the carcinogen Ethidiumbromide, that is used to visualize DNA.
2. Boil 1g Agarose in 100ml Gel running buffer (microwave: 45sec, swirl, 20sec, swirl, 20sec). Let cool for 10min.
3. Add 3µl of Ethidiumbromide and pour into assembled gel tray.
4. Let gel solidify (15min).
5. Load DNA samples (15-20µl/well).
6. Run at 100V for 30-45min and visualize DNA fragments with UV light (wear eye protection!).
7. Get a printout of your gel and determine the inserts' sizes by comparison with the markers.