BIO/CHEM 475   Molecular Biology Laboratory    Spring 2005
Biol/Chem 475   Part 2 of Cloning Lab

Week 6 (5/5 & 5/6) Analysis of pGEM recombinant clones
Week 7 (5/9) Midterm Quiz: Practice problems will be posted
Week 7 (5/12 & 5/13) Finish analysis of clones
Week 8 (5/16) Summaries of clone analyses due to CT (see pg. 3)

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**Week 6  5/5 & 5/6   PRELAB ASSIGNMENT #1**

1. Count colonies on your positive (pGEM) control plate and calculate the number of transformants per ng of pGEM DNA. Submit your raw data (including % white colonies) and calculations (in some sort of readable form). Circle your answer.
2. Select one of your plates with colonies transformed with the ligation mix and do the same as indicated in #1.
3. In lab notebook, write out protocol for TNE (total nucleic acid check) and plasmid preps. Photocopy protocol for CT

**PRELAB ASSIGNMENT #2**

*Before you come into lab on Week 6* you must have a written plan/diagram for analyzing your clones (see pag. Your plan should include the specific restriction digestions that you will do and the predicted results.  

**NOTE:** There is more than one reasonable way to analyze your clones. Don’t feel like you need to do the same experiment as your neighbor.

**NOTE:** I want to see a plan of action. You don’t need to have recipes written out for the digests or the gels.
PRELAB LAB ACTIVITIES

At least two days before your next scheduled lab:
Each student should streak out four white colonies on L + amp plates. Add IPTG and Xgal to the plates before you streak. Divide each plate in half and streak two colonies per plate. See Appendix 4 of previous handout for instructions. If you haven’t done this before, get a lesson in microbiological streaking from CT or SS or a knowledgeable fellow student.

To do the afternoon or evening before your next scheduled lab
• Each student should set up overnight cultures of four colonies. Each colony should be inoculated into 5 ml of L broth + 50 µg/ml ampicillin in a 15 ml screw-capped plastic tube. Shake at 37°C overnight. (The tube should be horizontal.)
• Control cultures (blue colonies and untransformed XL1 blues) for the TNE portion of the lab will be set up for you.

WEEK 6 lab protocols:
Analysis of Recombinant Clones: Viewing total nucleic acids (Appendix 1)
Analyze total nucleic acids from each of your cultures and two control cultures: untransformed XL1Blues and blue colony. Examine migration of plasmid DNA and confirm that each white colony has an insert (or not).

Plasmid preps: Each student should prepare plasmid from two clones (with confirmed insert).

Run a minigel to estimate the concentration of plasmid in the eluate from the Qiagen column. Run undiluted and a couple dilutions of your plasmid prep. We will provide a reference sample of plasmid of known concentration (determined by an A260 reading) to run on the minigel.
Week 6 & 7 (5/12 & 5/13)
Analysis of subclones by restriction enzyme digests
• Each student will analyze two clones.

NOTE: There is more than one reasonable way to analyze your clones. Don’t feel like you need to do the same experiment as your neighbor.
• Examine the restriction maps of the insert and vector DNAs.
• Draw maps of the 4 possible clones that you generated assuming that your recombinant molecules consist of one insert fragment and one vector molecule.

Using restriction enzyme digests, you will analyze the clones that you picked to determine:
• which BamHI-Bgl-II fragment you cloned
• the orientation of the insert DNA in the recombinant clone

The following restriction enzymes are available to you:
Sal I, Pst I, Xho I, Eco RI, Hind III, Bgl II, Bam HI, HaeIII, Dra I and Ssp I.
• What single and/or double digests would be useful for the analysis?
• For each digest, diagram the predicted results and determine if it will give you the information you need.

When you run your gels, be sure to run uncut controls and size standards that cover the full range of fragment sizes that you will see.

Monday May 16 at 1pm:
Workup of data for each subclone due to CT
(i) Name your subclones [Plasmid names always start with a lower case p, usually followed by uppercase letters (somebody’s initials or something clever) and a number.]
(ii) Write out a summary of what you know about your subclone
(iii) Draw a map of the insert and vector, indicating the orientation of the insert. Be sure to include a scale and relevant restriction sites.
(iv) Attach a labelled photo of your gel
Appendix 1: Viewing total nucleic acids

Quick method to confirm that your transformed culture contains a recombinant plasmid

1. Place 50\(\mu\)l of phenol chloroform (1:1) and 10 \(\mu\)l of loading dye [0.25% bromophenol blue (BPB) and 40% glycerol] in a microcentrifuge tube.
2. Add 100 \(\mu\)l of overnight culture and vortex the mixture for 10 seconds.
3. Centrifuge for 3 min.
4. Load 20-30 \(\mu\)l of supernatant into a gel slot that is 5 mm long X 1.5 mm wide. The gel should be about 6 mm thick and 0.8% agarose cast in 1X TBE with 0.5 \(\mu\)g/ml of ethidium bromide. (Note: These specifications for the gel are from the original protocol.)
5. Be sure to run control samples: \textit{E. coli} strain with no pGEM plasmid and \textit{E. coli} with pGEM only (no insert fragment). The control cultures will be provided for you.
6. Run gel at 50-80 volts. Check gel after BPB has run about half-way down the gel bed.
7. Note: when you examine your gels, you should see total nucleic acids: RNA and DNA. Can you identify bands corresponding to rRNA, tRNA, mRNA?

Appendix 2: Preparation of plasmid DNA using a Qiagen Kit

Qiagen Hype: Qiagen miniprep columns contain a unique silica-gel membrane that binds up to 20 \(\mu\)g DNA in the presence of a high concentration of chaotropic salt, and allows elution in a small volume of low-salt buffer. RNA, proteins and metabolites are not retained on the membrane. QIaprep membrane technology eliminates time-consuming phenol–chloroform extraction and alcohol precipitation, as well as the problems and inconvenience associated with loose resins and slurries. High-purity plasmid DNA eluted from QIaprep modules is immediately ready to use — there is no need to precipitate, concentrate, or desalt. See additional handout for info on using kit