7.13 Experimental Microbial Genetics

Fall 2008

Archiving the tools you make this term:

You and your fellow lab mates are doing *real* research. The constructs and strains you are making will certainly be of use to the people in Profs. Laub and Newman's labs and the better you archive your materials, the easier it will be for someone to follow up with your work and credit you for it! In addition, by now you all now know that: 1) transforming a strain with a plasmid and verifying the plasmid is a procedure that takes several days and 2) cloning a gene is a procedure that can take several weeks. If after making these strains and plasmids you do not make an archival stock, the time and money you spent performing these tasks is essentially wasted. If those justifications aren't motivation enough for you, how well you maintain your archives will also be a significant factor in your final grade! Thus, archiving the strain and plasmids you construct this term is an incredibly important part of your responsibilities as a 7.13 student.

Because this is so important, we will check your archive periodically throughout the term. These dates will be announced later, but at this time we will ask to see your strain and plasmid lists (use templates below, the first lines serve as an example of how to correctly fill out the information) and your freezer boxes that contain the items on your lists. These archive boxes should be kept entirely separate from your "everyday box" or the box that contains the things you use on a regular basis (you can get new freezer boxes in the cabinet by my office in 089).

At the end of the term you should be prepared to provide your archived strain and plasmid stocks and send her your complete strain and plasmid lists as well as copies of your plasmid maps and sequence files via e-mail. Please add your names to the file name. You can also send this info bit by bit throughout the term if you want to show off your progress or if that helps you stay on top of things! ☺ Below are some guidelines to help you with the archiving process. If anything is unclear, please ask!

Guidelines for how and what to archive:

For each plasmid you receive this term you should:

1. make a frozen bacterial stock that carries a verified form of the plasmid
2. purify the verified plasmid by mini or maxi prep depending on your needs and label the plasmid stock with the concentration
3. record the strain and plasmid in your strain and plasmid lists

For each plasmid construct you make this term you should:

1. make a frozen bacterial stock that carries a verified form of the plasmid
2. purify the verified plasmid by mini or maxi prep depending on your needs and label the plasmid stock with the concentration
3. record the strain and plasmid in your strain and plasmid lists
4. construct a map of the plasmid with the relevant restriction enzyme sites labeled and notes on how the plasmid was made
   ***__Note__: If you label a restriction site on your map, label ALL places where that site cuts (e.g. if you note a single EcoRI site on a map, then we expect that to be a unique cut!)
5. save a text file with the complete sequence of the construct, noting primer sequences used and indicating nucleotides added to maintain correct reading frame, ribosome binding sequence, stop codon, restriction sites, etc where applicable. Model your files after the example posted on the Stellar site.

_____ & _____'s Strain List

Coordinate in Box	Strain	Plasmid	Strain Number if applicable	Drug R	Comments
EXAMPLE: A1	*E .coli* DH5a	pMQ64	DKN545	gentamicin	Vector used to construct a 2-4 kb and 4-8 Kb genomic DNA library of *Pseudomonas aeruginosa* PA14 Anything else that you feel would be helpful for the next user to know

_____ & _____'s Plasmid List (every plasmid on this list should have a corresponding map and sequence file, see example posted on the Stellar website)

Position in Box	Plasmid (size)	Drug R	Parental plasmid	Details on insert and plasmid construction	Primers used	Use	Comments	Sequence File Name
EXAMPLE: A1	pT9 (7055 bp)	Gentamicin	pPL17	Nucleotides 825 to 1167 of the *Pseudomonas aeruginosa* PA14 *xxxY* gene were PCR amplified from pTF2 and cloned into EcoRI and KpnI digested pPL17 in frame with GFP.	EcoRI-*xxxY*-F (5'TGAACATGAACA) BamHI-*xxxY*-R (5'TGTTCATGTTCAC)	To investigate the localization of the *Pseudomonas aeruginosa* PA14 *xxxY* gene product	*xxxY* is fused to GFP Stock was verified to be correct using EcoRI and BamHI xxxY-GFP localizes to the outer membrane of *Pseudomonas aeruginosa* cells	pT9.doc