8/6/10
Meeting Minutes

Dr. Rao will be out of town next week.

Clustalw—Use to compare two or more sequences. We should be expecting sequencing to be pretty dead on.

Maybe try some pre-mixed mastermix to troubleshoot stuff. Invitrogen used to sell taq supermixes. Can look up components and make our own stock if we need to. It probably won't be too much more expensive to just by the mix.

The first 100 bases and last 200 bases of sequencing are usually useless.

Colony PCR—20 ul supermix, use a pipette tip to take a little bit of the colony (leave a little to streak later), stick in reaction, then just stick in thermocycler.

Restreak things out to get cleaner minipreps.

For GES, just cut out top band from initial PCR and clone, don't re-PCR and gel extract again.

PCR, PCR clean up, digest, run gel, extract, ligate. Use the intensities of the bands to judge ligation ratios.

For gel extractions, do 8 ul spin first, then can do a second 4 ul spin.

Make sure we're mixing all the reactions

Try re-transforming left over ligations the next day after letting it sit overnight.

T-4 Ligase inhibits electroporation, heat inactivate it. Can also run it through a column as well.

Hit "measurement" button, tells you time, then hit electroporate, write down the number that flashes, should be between 4.5-5.5. If you get something like 2, then the cells aren't good. It's often arcing or something. Lets you know if things are going right. Pick EC1 or EC2 based on cuvette size.

Bleach and weak base to clean out cuvettes. Probability of arcing goes up exponentially with each use. Once it arcs, throw it out.

Try chemical transformation if we can.

Miniprep our competent cells to see if there's a plasmid. Test transformed cells on different antibiotics.

Dr. Rao wants to come to the Jamboree. Would have to fly out Friday night, can work on his own tickets, will talk to us in about a week.

We all need to fill out intent to travel forms so we can start buying plane tickets soon.