Genome Center

First of all, you must be aware that the Genome Center has plenty of work to do and we cannot drop everything to accommodate you immediately. **You must check with us in advance, especially if you want to use any of the equipment (cyclers, robot, hydroshear, elutrap, etc) in our lab.**
There will be sign up sheets for the cyclers as well as the robot if necessary. Check with Jen or Alexis before signing up to make sure they have time to fit you in on the equipment.
We will try to accommodate on a first come/first served basis, but we can't always promise that and many times our own work will take precedence.

When it's time for you to work with Jen and Alexis in the Genome center, there are certain things you need to be aware of.

S-GAL plates: Molly has ordered packets of S-GAL, if you need to know where they are, ask her. If the Hatfull lab is running low, inform Molly and she will take care of it. You need to pour your own plates. We add ampicillan(100mg/ml) and tetracycline(12.5mg/ml) to the media before pouring the plates.

Fast-link Ligase: Molly has also ordered this for use in the Hatfull Lab. She will tell you where to find it.
***E.coli* XL1-Blue cells:** There is a stock in the Hatfull lab. Keep an eye on it; you are responsible for making more.
LB: You need to make this in 500mL bottles. Be sure to make enough for your experiments at least 1 day in advance.
SOC: You will also be responsible for making this. See Jen/Alexis for a protocol.
T4 Polymerase/Klenow: Molly will have a stock of this for you, check with her to see where it is.

Order of Events:
When you make DNA from a large phage prep, you need to have at least 200-300uL of good quality (~1ug/uL) DNA before we will even talk to you. Run 1uL of your DNA on a gel and bring the picture to us before we discuss your sequencing options. Make sure that you have also digested your DNA with a few restriction enzymes.

Shearing DNA: Check with Jen or Alexis before beginning this procedure. Once you have done that, take a tube of your DNA and heat it in a 37C water bath for at least 30 minutes. Aliquot between 15-30uL of DNA to a clean eppendorf tube. (Jen or Alexis will give you an exact amount) Dilute the DNA with TE so the total volume equals 100uL. Put this new tube in a 37C water bath for 30 minutes and in 5 minutes intervals vortex

vigorously for several seconds. After 30 minutes of heating/vortexing, spin sample briefly and transfer contents to a clean eppendorf tube. You may now shear your DNA under the watchful eye of Jen or Alexis. Run 5uL of sheared DNA on a gel with a 1kB ladder.

DNA repair: Once sheared, your DNA now needs to be repaired. Check with Jen/Alexis for the protocol. The final volume should be resuspended in 10uL TE. This entire volume needs to be run on a 0.7% agarose gel. (It is best to tape 2 wells together) Take a photo of the gel and carefully size-fractionate between 1-3kB. **ONCE AGAIN, CHECK WITH JEN/ALEXIS.**
NEVER CUT GELS IN CASTING TRAYS!!!!!!
NEVER CUT GELS ON LIGHT BOXES!!!!!!!

Elutrap: Once you have a DNA slice, you are ready to run the elu-trap. The elutrap does not leave the genome center. Do Not take it to another lab. Jen/Alexis will help you set it up. The elutrap can run for 3 hours at 200V or overnight at 100V. Keep this time constraint in mind when it comes time for you to use the elutrap.

After extracting your sample from the elutrap, the sample needs to be phenol extracted/ EtOH precipitated and resuspended in a final volume of 10uL. If you have 2 tubes of the same sample, resuspend each tube in 5uL TE, then heat for 15 minutes at 37C and combine the tubes. The elutrap apparatus needs to be taken apart and soaked in DI water overnight. Come back the next day to dry it and put it away. Be sure to remove the membranes when taking it apart.

Ligations: You are ready to ligate. Check with Jen/Alexis for a protocol. Ligations must be stored long-term in the freezer.

Transformations: There is a stock of *E. coli* XL1-Blue cells for common use in the Hatfull Lab. It would be wise to check this stock periodically, because as a group, you are responsible for making more.
Each tube is good for 5 transformations. Always do a positive and no DNA control. See Jen/Alexis for a protocol. Transformations need to recover on the shaker in the 37C room for 1 hour in 1mL SOC broth. They can then be plated on S-GAL with Amp/Tet.

You are also responsible for making sure you have S-GAL/Amp/Tet plates. Plate 100uL of each ligation. 100uL no DNA and 1uL (in a puddle of 100uL LB) pBlue control. The plates need to grow overnight at 37C. Your plates will need to grow anywhere from 16-24 hours. Check with Jen or Alexis if you are unsure about the growth on your plates. The colonies should be well-formed and the black/white color selection should be distinguishable.

Hopefully the next day you have white and black colonies. If this is the case, pick 8-10 white colonies per ligation and grow them up overnight in test tubes with 2mL LB/AMP. See Jen/Alexis for protocol.
The next day mini-prep your colonies. Molly has qiagen mini-prep kits, check with her.

Restriction digests must be set up with EcoRI and HindIII. See Jen/Alexis for protocol. They need to digest at 37C for 3 hours. Run 5uL of the digest on a gel with a 1kB ladder. **Show picture to Jen/Alexis.**
If all is well, Jen/Alexis will tell you to pick blocks. See them for a protocol.
Blocks grow overnight and need to be spun down the next day in the Genome Center centrifuge. Check the bottom of the blocks for good size pellets before dumping out the liquid and tips. If you feel the cultures need to grow more, put them back in the 37C room. If you are unsure if your pellet size is acceptable or not, check with Jen or Alexis. If you can't deal with them right away, they can be frozen. All blocks must be frozen in the genome center –20C freezer.

Blocks need to be prepped on the biorobot in the genome center. See Jen/Alexis for instructions and check with them to find out when the robot is available for your use. **Remember to fill out the logbook.**
When finished on the robot, be sure to leave it in the same condition you found it and run the flush procedure.
All plasmid preps must be labeled according to protocol and kept in the genome center cold box. There is a diagram for proper labeling on the side of the cold box.
Sequencing reactions: See Jen/Alexis for a protocol. Also, there is a sign-up sheet for both thermocyclers in the genome center. You must first check with Jen/Alexis to see if it is ok to use the thermocyclers, then you must sign up. **DO NOT SIGN UP WITHOUT ASKING JEN/ALEXIS FIRST!!!!!**
Take 2 princeton plates out as soon as your reactions are getting cycled if you are going to clean up your reactions the same day. The plates must be warmed to room temperature before they can be used and should not be inverted.
Once the reactions are cleaned up (See Jen/ALexis for a protocol), they are ready to be sequenced. Jen/ALexis will do that.
Jen/Alexis will tell you when to expect results. Come back then to extract your data from the computer and start your project on the computer.

Do Not Touch the Sequencing Computer without Jen or Alexis present. You will have no reason to mess with this computer or the sequencer. It is a VERY expensive piece of equipment! You should not be using any equipment in the Genome Center without Jen or Alexis present and they should be made aware of when you wish to use the equipment.