

### **Guideline For Getting ES clones from repositories**

If you plan to order targeted ES cells from either KOMP or EUCOMM to make mice, you need to order more than 2 ES cell clones per gene. Below is why you need to order multiple clones.

1. The percentage of targeted ES cell clones contributing to the germline is about 60% for KOMP and 50% for EUCOMM as they have declared. These repositories strongly recommend injecting at least 2-3 clones to ensure germline transmission.
2. Before making mice, you need to confirm correct targeting of your ES cells by Southern analysis. Some of their ES cells are not validated for both arms. In addition, Southern is not performed. However, EUCOMM declared that, in a random sampling of the resource, about 15% of the targeted ES cell clones revealed some issues. Extra random integration in another genomic location might be the most common issue.

KOMP and EUCOMM are mainly using C57BL/6-derived ES cells called JM8A3 for their targeting and there have been a lot of concerns about these lines being unstable and not contributing to germline. We strongly advise you to check chromosomal abnormalities in your ES cells before making mice. KOMP recommends injecting the clones that are identified as being higher than 70% euploid. We provide chromosome counting and this should be done before microinjection.

### **Submitting Expansion service request**

You can bring us ES cell vials on dry ice and submit a completed service request form. Unless we receive all the information required including fund/dpa and your PI's signature, your project will not be scheduled. Once initiated, shown below are the steps you have to follow to generate mice.

1. We will expand your ES clones in order to freeze down for future injection and to validate correct targeting and normal karyotyping.
2. Validation of targeting by Southern analysis by you
3. Karyotyping (chromosome counts) by the core
4. Once identify the best clone , schedule injection date with Gladstone injection core (Junli Zhang, [junli.zhang@gladstone.ucsf.edu](mailto:junli.zhang@gladstone.ucsf.edu)).
5. We will revive and prepare the cells for the injection.
6. 5~6 weeks after the injection, chimeras will be transferred from the Gladstone to your mouse room. Once male chimeras are about 5~6 weeks old, set up 4~6 mating cages with wild type females. Depending on ES cell types, you

## ES Cell Targeting Core

- need to genotype pups with a specific coat color or all of them regardless of coat color. Please make sure you understand the mating scheme.
7. If your male chimera doesn't plug females for more than a month or doesn't produce any germline pups from more than 6 full size litters, this is time to switch chimeras.