Dear Investigators,

Most of you are already aware of the cells produced through the International Knockout Mouse Consortium—IKMC-- (commonly known as KOMP and EUCOMM cells). These cells are a source of frustration for many (most) transgenic facilities, as they are fragile and often, not very good. It is extremely difficult for most of us to obtain mice from them. In fact, there are a number of facilities who now request their PI's to obtain the construct for electroporation rather than using the cells generated through this effort (we suggest this as well, as an alternative in case the ES cells do not generate the germline mice you desire).

In May of 2012, I attended a conference in Spain hosted by EMMA, the European Mouse Mutant Archive. They are responsible both for cryopreservation of unique models as well as participating in the EUCOMM project. Thus, while there, I had an opportunity to discuss our work with these cells first-hand with the leaders of EUCOMM (the European distributor), NORCOMM (the Canadian Distributor) and KOMP (the US distributor).

The KOMP director told me that anyone ordering the IKMC cells should always order in 3 different clones, and that all clones should be tested cytogenetically to insure integrity. He also stated that we should only inject a maximum of 50 blasts for any given clone; if no pups or chimeras are produced, we should stop the injections and go to the next clone. That is their in-house procedure.

The NORCOMM director suggested that we do the same. She also indicated that these cells should only be passaged 3 times prior to stopping the experiment, as the C57BL/6N cells used are prone to accumulating cytogenetic abnormalities. This falls in line with what we are doing here, but we will take extra care to note how many passages we use for each clone.

There are some facilities that report success using these cells. Typically, those reports include the Bay Genomics and TIGM cells that were used during the early days of this project. We also have produced many germline-transmitting chimeras from Bay Genomics and TIGM cells. Other facilities have reported that using naturally mated blasts/morula for the KOMP/EUCOMM cells works better. We have attempted this, but the use of naturally mated embryos requires many more animals that we presently have, so we need to cryopreserve any embryos we obtain until we have enough for a complete injection, which adds much more time to complete the experiment.

Given what I have learned, I have instituted the following ideas for our injections of KOMP/EUCOMM clones:

1) I will expect the PI to order in at least 2 and preferably 3 clones of their gene.
2) The Quality Control on these Clones should preferentially be done at KOMP, EUCOMM or other. We've done it here, which is fine, but Southern analysis is not done to confirm mutations unless requested at KOMP/EUCOMM, so if you do not get QC Southern done, then we could end up screening wildtype clones here.
3) We will inject up to 3 injections of embryos/clone and then stop injections of that clone.

PLEASE NOTE THAT WE WILL STOP OUR ATTEMPTS AFTER 3 injection days, WHETHER OR NOT WE OBTAIN PUPS, AND THE PI WILL BE CHARGED FOR THE SERVICE!!!

It is unfortunate that these cells are not better, but we are trying to do our best with them to get what you need.

Sincerely,
Aimee Stablewski