

UMMS TRANSGENIC ANIMAL MODELING CORE
Gene Targeting & Stem Cell Facility - Transgenic Animal Facility

Orthotopic Tumor or Teratoma Assay

If the TAMC performs Stage 1 (iPS or ES expansion & testing):

1. Expansion of human iPS cell or ES cell clone(s) on an inactivated feeder cell layer from either frozen cells or a live colony.
2. Generation of a frozen cell pellet, which is sent to IDEXX Laboratory for pathogen screen (Impact III panel).
3. Subsequent expansion of clone to sufficient cell numbers ($\sim 1.8 \times 10^7$ cells in clusters) to perform the teratoma assay (Stage 2).

The minimum time for Stage 1 is 4 weeks (including testing). Cost of Stage 1 service = \$350 (plus \$375 for IDEXX pathogen screen).

If the TAMC performs Stage 2 (teratoma or tumor assay):

1. Suspension of human iPS cell or ES cell (1.8×10^7 cells) or mouse cells provided by on ice according to TAMC protocol. Cells will be mixed with matrigel by the Core and immediately injected in nude-SCID gamma (NSG) mice or nude-Swiss mice at **two** sub-cutaneous sites per mouse. **Three** mice will be used to assay each clone. Alternatively, NSG mice will be injected into mammary fat pad of four mice.
2. Mice will be monitored every other day for signs of tumor growth.
3. Teratomas or tumors will be harvested from these mice when they reach 3-4cm² in size, or between 4-6 weeks, or if mass begin to ulcerate (whichever come first). Each teratoma or tumor will be dissected and a representative portion snap-frozen for RNA analysis. The remainder will be fixed in phosphate-buffered formalin for IHC.
4. Fixed and frozen samples of each teratoma or tumor will be provided to the Investigator for subsequent analysis.

The minimum time for Stage 2 is 4-6 weeks. All murine cells, or mouse or human iPS or ES cell clones used in a teratoma or tumor assay MUST first be tested for pathogens as per UMMS IACUC regulations. Although teratoma or tumor formation in mice cannot be guaranteed for any clone, the TAMC will do all it can to ensure successful induction, including the repeat of certain (or all) steps if the initial performance was judged to be sub-optimal.

X _____
UMMS INVESTIGATOR

UMMS GENE TARGETING CORE

P.I. Name _____

Date Received _____

Department _____

Number of starting samples _____

Account Number _____

Charge for Stage 1: \$ _____
(Number of clones x \$350, plus \$375 pathogen test cost)

Charge for Stage 2: \$ _____
(Number of clones x \$1,000)

dated 7/1/14