

UMMS TRANSGENIC ANIMAL MODELING CORE (TAMC)

iPSC INDUCTION (Reprogramming) - PLUS INDUCTION OF PLURIPOTENCY IN HUMAN PRIMARY CELLS

The Facility will perform:

1. Thawing and expansion of primary cells.
2. Mycoplasma testing.
3. Transduction of cells in 6-well plate format with non-integrating Sendai virus to transduce the four Yamanaka factors (Klf4, Oct4, Sox2, and cMyc).
4. Splitting transduced cells onto 10 cm dishes with inactivated feeder cell layers or feeder-free extra-cellular matrices. Once iPS colonies are formed, up to 20 colonies displaying the most ES cell-like morphology will be picked individually and expanded.
5. Based on morphology 5 to 6 clones will be selected and further propagated as individual clones.
6. After 6 passages loss of the virus will be tested by immunostaining against viral antigens. Retention of pluripotency in the viral-negative lines will be confirmed by immunostaining.
7. For clones still containing virus in the fraction of population, passaging will continue and tests for virus and pluripotency will be repeated.
8. Up to 5 individual iPS lines derived from a given primary sample will be provided to the PI as frozen cells and/or in culture. Sister-vial of frozen cells will be retained by the TAMC if the Core is to provide further services.
9. If required, the TAMC will also provide frozen cell pellets to facilitate subsequent nucleic acid analysis.

The time required to generate iPS lines from a given primary sample will vary, but is estimated to require 8-12 weeks. Although the pluripotency of generated lines (as determined by *in vivo* assays) cannot be guaranteed for any sample, the TAMC will do all it can to ensure successful induction, and repeat certain steps if the initial performance was judged to be sub-optimal.

Charges: \$4,500.00 per cell line

If performed by Core, services listed under 1, 2 and 7, are part of "iPS Induction – Plus" service. Please see the description of "iPS induction" service.

PI Name _____

Department _____

Account Number _____

Number of Lines _____

Date Received _____

Total Charges \$ _____

Notes:

X _____

X _____

UMMS Investigator

Date

UMMS TAMC

Date