# AG23192*A

## Certificate of Analysis

| | |
|---|---|
| Product Description | Transgenic Murine Embryonic Stem (mES) Cell containing the transcription factor Tcf3 |
| Publication | Nishiyama et al.; PMID 19796622 |
| Passage of mES reported at submission | 26 |
| Number of passages at Coriell | 6 |
| Freeze Passage | 32 |
| Media | DMEM + 20% ES cell FBS + puromycin + doxycycline + LIF |
| Feeder | DR4 MEFs on 0.1% gelatin |
| Passage method | Accutase |
| Split ratio | Seed at 1.2 x $10^6$ cells per 1 well of 6 well plate (1.0 x $10^5$ cells/$cm^2$) split at 80% confluence (2-3 days) |

**The following testing specifications have been met for the specified product lot:**

| Test Description | Test Method | Test Specification | Result |
|---|---|---|---|
| **Viability** | Cell Count Post Thaw of Cryopreserved Cells | Cells double within 3 days after recovery | Pass |
| **Sterility** | Growth on agar | Negative | Pass |
| **Mycoplasma** | PCR | Negative | Pass |
| **Karyotype** | G-banding | At least 60% normal cells | 94% 40 XY |
| **Identity** | Nucleoside Phosphorylase Isoenzyme Electrophoresis | Murine | Pass |
| **Surface Antigen Expression** | Immunostaining | > 80% expression of SSEA1 | Pass |
| **Pluripotency** | Embryoid Body Formation | Morphology and expression of lineage-specific genes | Pass |
| **Transgene Induction** | Doxycycline removal | Increase in transgene expression by qPCR | 14.5 Fold Change |

## Post-Thaw Viability

One vial of was thawed after cryopreservation. Cells are counted following recovery and plated in one well of a 6 well plate. Cultures are observed daily and passaged when cells are approximately 80% confluent. Following dissociation with accutase, cells are counted and viable cell number is determined. The viable cell number must double within 3 days following recovery.

| Days from Recovery to First Passage | Viable Cell Number at Thaw | Viable Cell Number at First Passage |
|---|---|---|
| 2 | $4.49 \times 10^6$ | $7.9 \times 10^6$ |

## Karyotype Analysis

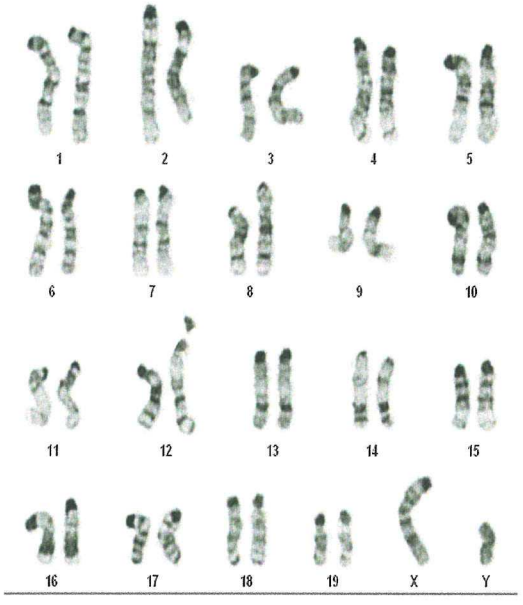


Figure 1A: Karyotype Image showing 40XY.

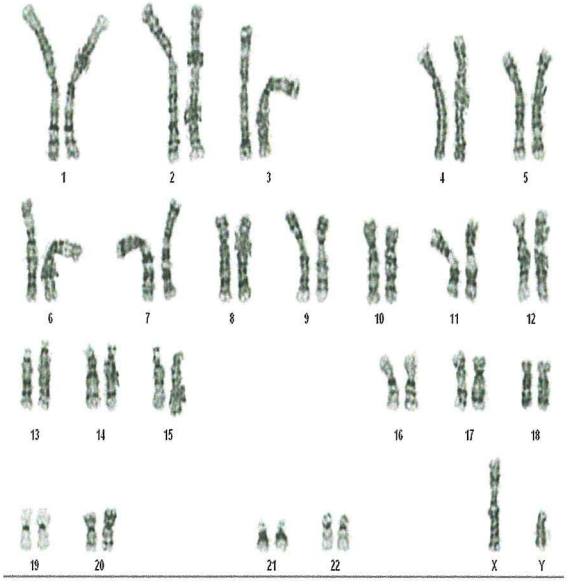


Figure 1B: Karyotype Image showing 40XY.

# Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for the surface antigens, SSEA1. SSEA1 (stage specific embryonic antigen 1) is expressed on undifferentiated murine stem cells.

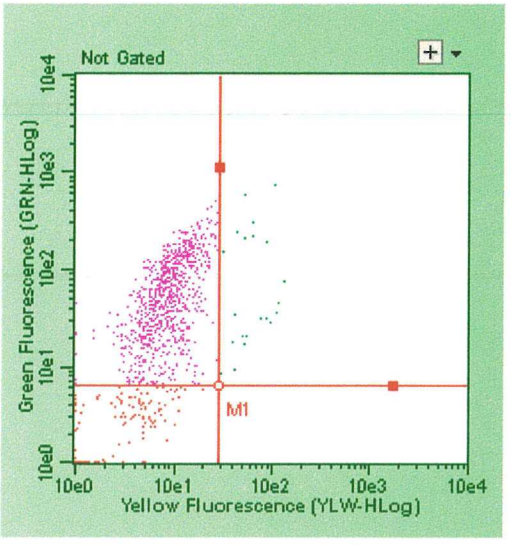


Figure 2A: Scatter plot of SSEA1 stained iPS cells.

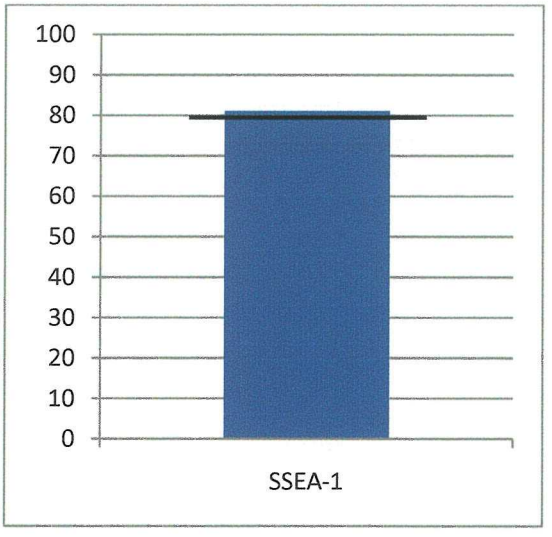


Figure 2B. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

## Assessment of Pluripotency of a Cell Line

Cells are subjected to direct differentiation to assess the pluripotency of the cell line. RNA is harvested and gene expression is analyzed by quantitative real-time PCR. Ct values are adjusted for loading using a housekeeping gene. Gene expression is shown as fold difference to undifferentiated cells.

## Embryoid Body (EB) Formation Assay

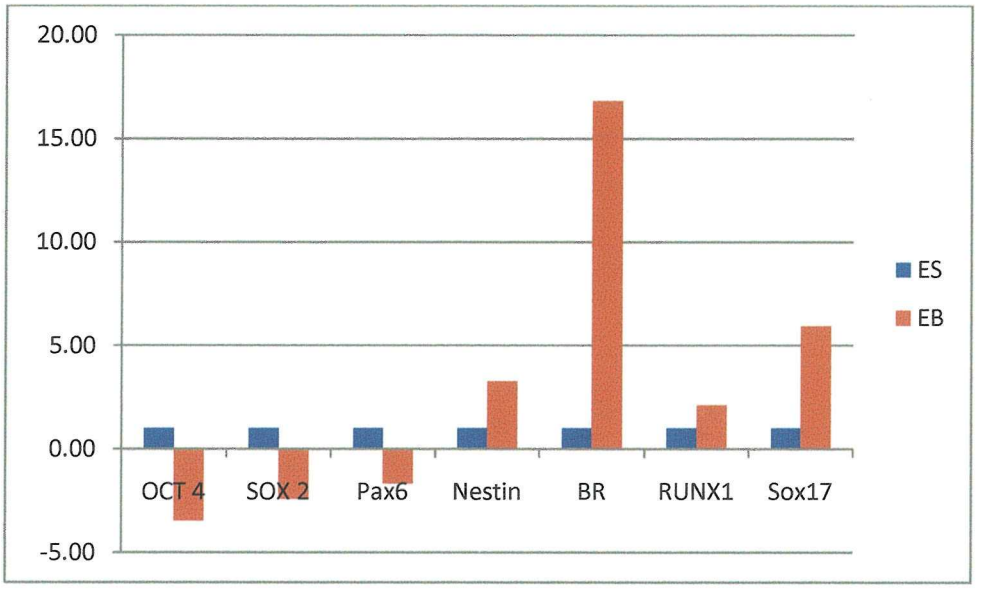


Figure 3. Gene expression following EB differentiation. Fold difference is shown realitve to undifferentiated iPS cell line.

| | OCT 4 | SOX 2 | Pax6 | Nestin | BR | RUNX1 | AFP | Sox17 |
|---|---|---|---|---|---|---|---|---|
| ES | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| EB | -3.46 | -2.39 | -1.65 | 3.27 | 16.82 | 2.12 | 7560.01 | 5.94 |

Table 1. Fold difference values of gene expression of EB. Fold difference is shown as fold difference to undifferentiated cells.

# Transgene Induction

Briefly, cells are plated on a gelatinized 6-well plate at low density for 3 days and maintained in medium containing both puromycin and doxycycline. On day 3, transgene expression is induced by withdrawal of doxycycline. After 48 hours, the cells are harvested for RNA extraction, followed by quantitative PCR using specific primers targeting the tansgene (SYBR green PCR Master Mix, ABI) Amplification results are normalized to the histone H2A transcript and analyzed using the delta-delta Ct method to approximate fold change in gene expression (induced to uninduced control vector).

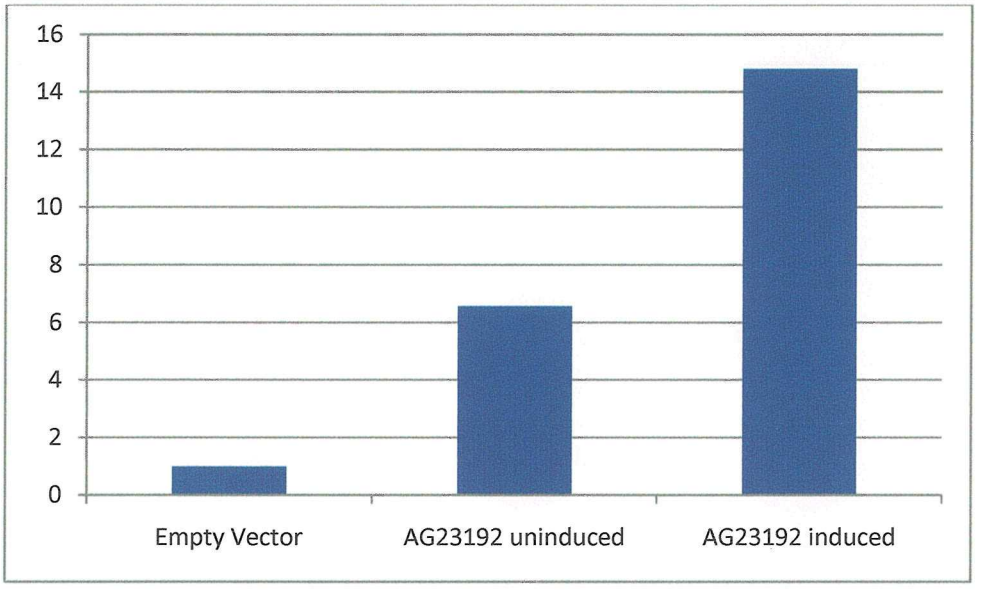


Figure 4. Gene expression following transgene induction. Fold difference is shown relative to uninduced mES cell line containing empty vector.

☒ Pass
☐ Fail
☐ Other: _____

Chi Tarn, PhD
Director, Stem Cell Biobank
July 7, 2011