AG23177*A

Certificate of Analysis

Product Description	Transgenic Murine Embryonic Stem (mES) Cell containing the transcription factor Sox9
Publication	Nishiyama et al.; PMID 19796622
Passage of mES reported at submission	26
Number of passages at Coriell	4
Freeze Passage	30
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	Pass
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 Identification	PCR	Visual Identification of PCR product using transgene specific forward primer and Flag reverse primer	Pass
Transgene Induction	Doxycycline removal	Increase in Venus expression by qPCR	37 Fold Change

Post-Thaw Viability

One vial 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	$3.8x10^6$	$7.99x10^6$

Karyotype Analysis

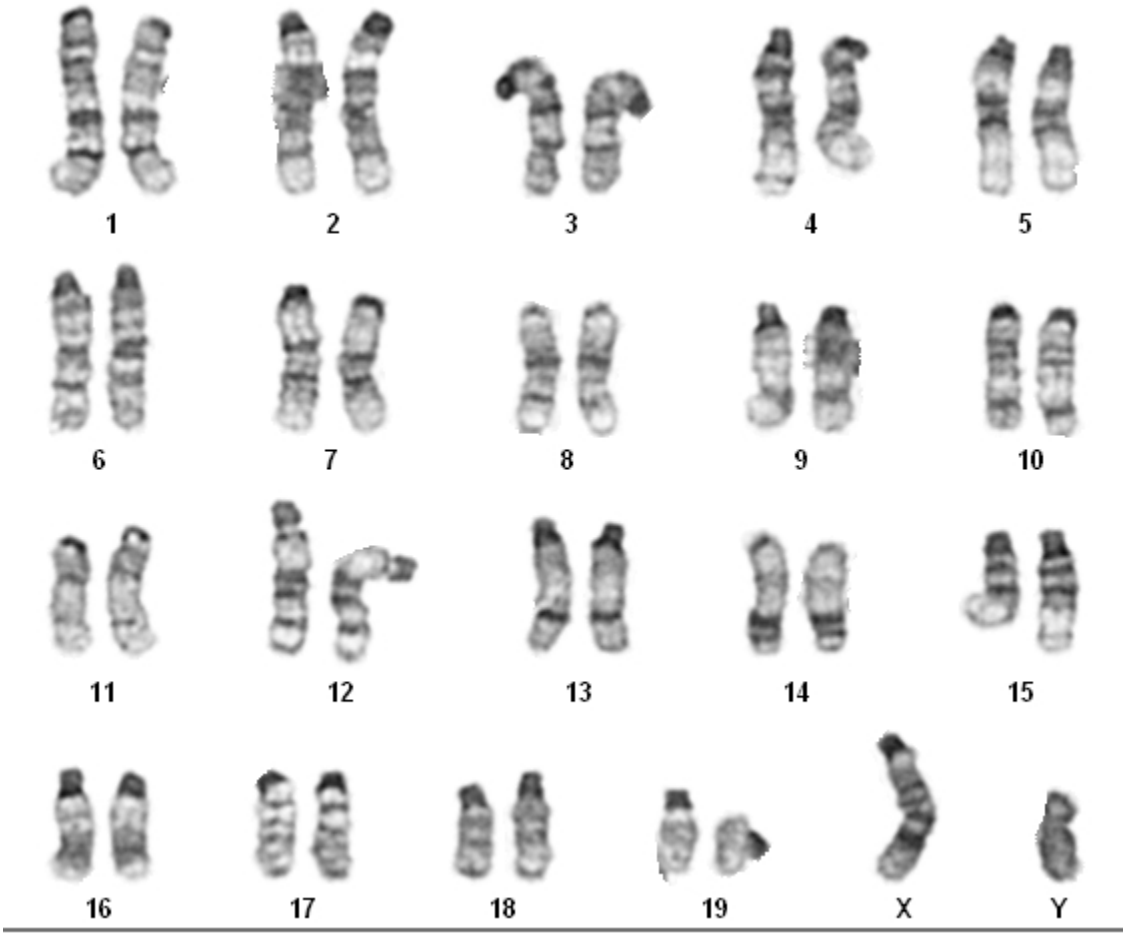

Figure 1A: Karyotype Image showing 40 XY.

Surface Antigen Expression of Stem Cell Markers

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

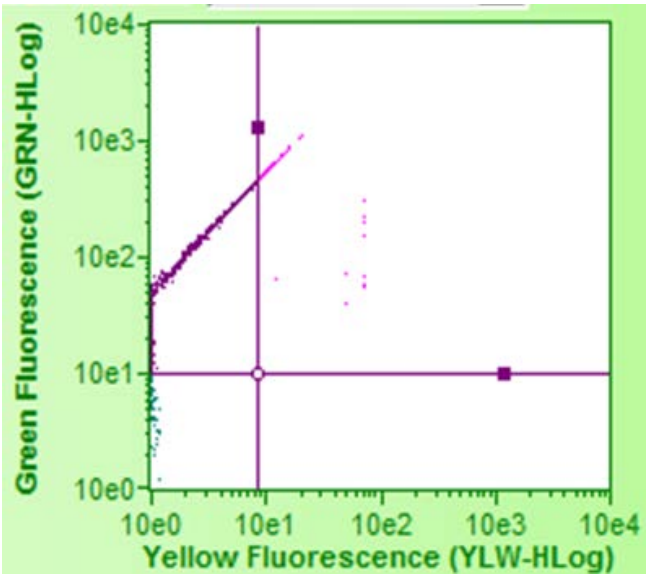

Figure 2A: Scatter plot of SSEA1 stained ES cells.

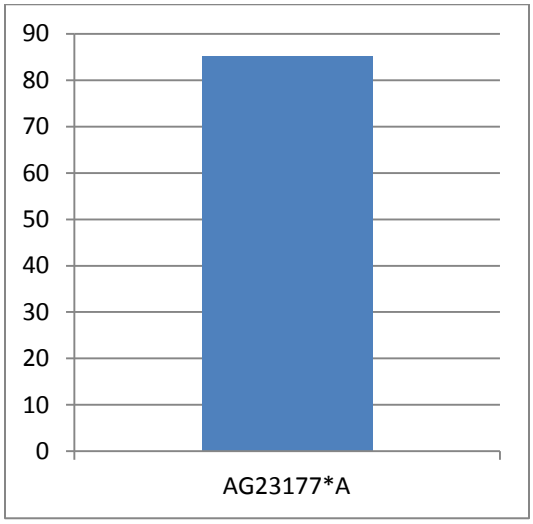

Figure 2B. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

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

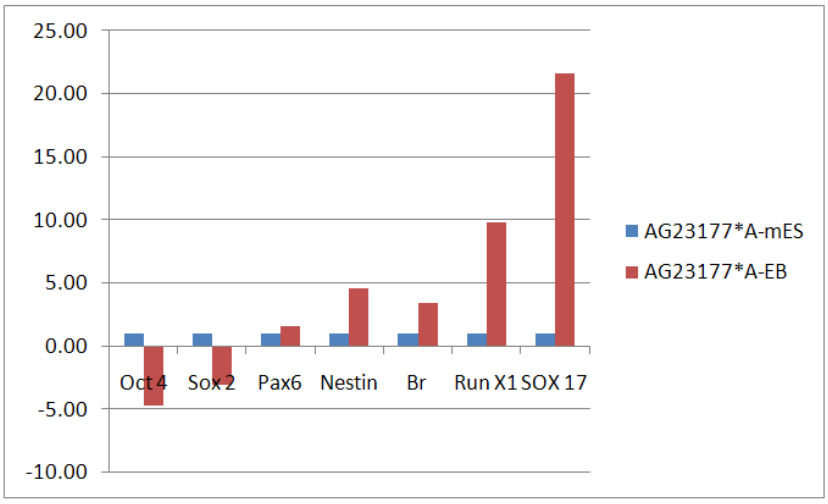

Figure 3. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated ES cell line.

	Oct 4	Sox 2	Pax6	Nestin	Br	Run X1	AFP	SOX 17
AG23177*A-mES	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AG23177*A-EB	-4.76	-3.11	1.54	4.56	3.38	9.85	59432.26	21.60

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 surrogate marker, Venus (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).

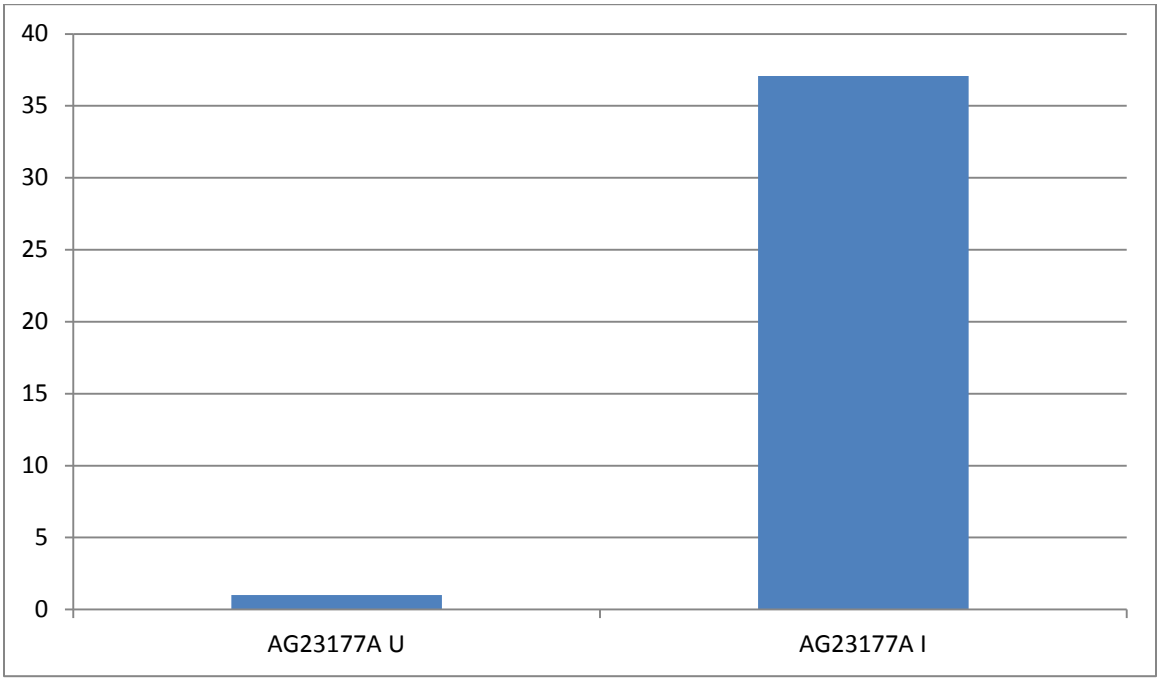

Figure 4. Gene expression following transgene induction.
Fold difference is shown relative to uninduced mES cell line.

☒ Pass
☐ Fail
☐ Other: _____

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