# Thaw and Culture Details

| | |
|---|---|
| Cell Line Name | SA02 |
| WiCell Lot Number | SA02-DL-01 |
| Parent Material | SA02-MCB-01 |
| Provider | Cellartis |
| Banked By | WiCell |
| Thaw and Culture Recommendations | WiCell recommends thawing 1 vial into 1 well of a 6 well plate. |
| Culture Platform | Feeder Dependent |
| | Medium: hESC Medium (KOSR) |
| | Matrix: MEF |
| Protocol | WiCell Feeder Dependent Protocol |
| Passage Number | p35<br>These cells were cultured for 34 passages prior to freeze. WiCell adds +1 to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw. |
| Date Vialed | 08-May-2009 |
| Vial Label | SA02-DL-01<br>P35 DF<br>08 MAY 2009<br>SOPCC035D |
| Biosafety and Use Information | Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells.<br>Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans. |

# Testing Performed by WiCell

| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| Karyotype by G-banding | WiCell | SOP-CH-003 | Expected karyotype | Pass |
| | *Result from report: This is an abnormal karyotype, with trisomy 13 as the only clonal aberration detected. Trisomy 13 was found in all cells examined. The finding of trisomy 13 in this culture is consistent with previous reports of inherent trisomy 13 in this cell line.* | | | |
| Post-Thaw Viable Cell Recovery | WiCell | SOP-CH-305 | ≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation | Pass |
| Identity by STR | UW Molecular Diagnostics Laboratory | PowerPlex 1.2 System by Promega | Match | Pass |
| Sterility - Direct transfer method | Apptec | 30744 | No contamination detected | Pass |
| Mycoplasma | Bionique | M250 | No contamination detected | Pass |
| Flow Cytometry for ESC Marker Expression | UW Flow Cytometry Laboratory | SOP-CH-101<br>SOP-CH-102<br>SOP-CH-103<br>SOP-CH-105 | Report - no specification | See report |

| Approval Date | Quality Assurance Approval |
|---|---|
| 08-January-2010 | 8/9/2017<br>X AMK<br>AMK<br>Quality Assurance<br>Signed by: Klade, Anjelica |

# WiCell Cytogenetics Report: 001135-060309
## NSCB 7755

**Report Date:** June 10, 2009

## Case Details:

***Cell Line:*** *SA02-DL-1 (7755)*

***Passage #:*** *38*

***Date Completed:*** *6/10/2009*

***Cell Line Gender:*** *Female*

***Investigator:*** *National Stem Cell Bank*

***Specimen:*** *hESC on MEF feeder*

***Date of Sample:*** *6/3/2009*

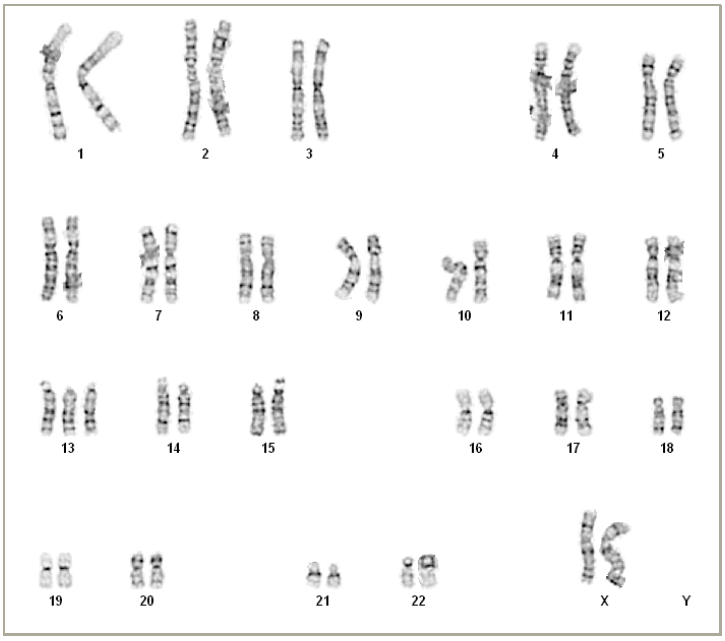
***Tests,Reason for:*** *CH 2-1-3*

***Results:*** *47,XX,+13*

*Completed by , MS, CLSp(CG), on 6/9/2009*

*Reviewed and interpreted by , PhD, FACMG, on 6/10/2009*

***Interpretation:*** *This is an abnormal karyotype, with trisomy 13 as the only clonal aberration detected. Trisomy 13 was found in all cells examined. The finding of trisomy 13 in this culture is consistent with previous reports of inherent trisomy 13 in this cell line.*




***Cell:*** *S01-01*

***Slide:*** *A*

***Slide Type:*** *Karyotyping*

***Cell Results:*** *Karyotype: 47,XX,+13*

***# of Cells Counted:*** *20*

***# of Cells Karyotyped:*** *4*

***# of Cells Analyzed:*** *8*

***Band Level:*** *425-550*

**Results Transmitted by Fax / Email / Post** **Date:**_____
**Sent By:**_____ **Sent To:**_____
**QC Review By:** _____ **Results Recorded:** _____

UW Health

University of Wisconsin
Hospital and Clinics

**Histocompatibility/Molecular Diagnostics Laboratory**

# Short Tandem Repeat Analysis*

**Sample Report: 7755-STR** UW HLA#: 61154

Sample Date: 06/18/09
Received Date: 06/18/09

Requestor: WiCell Research Institute

Test Date: 06/23/09 File Name: 090624

Report Date: 06/26/09
Amended Report: 07/24/09

**Sample Name:** (label on tube)
**7755-STR**

**Description:** DNA Extracted by WiCell

258.36 ug/mL; 260/280 = 1.86

| Locus | Repeat # | STR Genotype |
|---|---|---|
| D16S539 | 5, 8-15 | 12,14 |
| D7S820 | 6-14 | 10,12 |
| D13S317 | 7-15 | 9,11,14 |
| D5S818 | 7-15 | 12,12 |
| CSF1PO | 6-15 | 11,12 |
| TPOX | 6-13 | 8,9 |
| Amelogenin | NA | X,X |
| TH01 | 5-11 | 9,9.3 |
| vWA | 11, 13-21 | 14,16 |

**Comments: Based on the 7755-STR DNA submitted by WI Cell dated 06/18/09 and received on 06/18/09, this sample (UW HLA# 61154) matches the STR profile of the human stem cell line SA02 comprising 16 allelic polymorphisms across the 8 STR loci analyzed (Josephson, R. et al., BMC Biol. 2006 Aug 18;4:28). Consistent with published results on the human embryonic stem cell line SA02 (Josephson, R. et al., BMC Biol. 2006 Aug 18;4:28), the 7755-STR DNA sample displays the tri-allelic genotype (9,11,14) at the D13S317 loci with each allele having approximately equal amplification strengths. No STR polymorphisms other than those corresponding to the human SA02 stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 7755-STR DNA sample submitted corresponds to the SA02 stem cell line and it does not appear to be contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.**

7-22-09

Manager Date
HLA/Molecular Diagnostics Laboratory

07/24/09

PhD, Director Date
HLA/Molecular Diagnostics Laboratory

* Testing to assess engraftment following bone marrow transplantation was accomplished by analysis of human genetic polymorphisms at STR loci. This methodology has not yet been approved by the FDA and is for investigational use only.

File: Final STR Report

WuXi AppTec



WiCell Research Institute

June 04, 2009
P.O. #:

# STERILITY TEST REPORT

**Sample Information:** hES Cells
3: SA02-DL-1 #6700

**Date Received:** May 19, 2009
**Date in Test:** May 20, 2009
**Date Completed:** June 03, 2009

**Test Information:** Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.201

| TEST PARAMETERS | PRODUCT | |
|---|---|---|
| **Approximate Volume Tested** | 0.5 mL | 0.5 mL |
| **Number Tested** | 2 | 2 |
| **Type of Media** | SCD | FTM |
| **Media Volume** | 400 mL | 400 mL |
| **Incubation Period** | 14 Days | 14 Days |
| **Incubation Temperature** | 20 °C to 25 °C | 30 °C to 35 °C |
| **RESULTS** | 2 NEGATIVE | 2 NEGATIVE |

Page 1 Signed
**QA Reviewer** **Date**

Page 1 Signed
**Technical Reviewer** **Date**

*Testing conducted in accordance with current Good Manufacturing Practices.*

Mycoplasma Testing Services

BIONIQUE TESTING LABORATORIES, INC.



Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: **M-250 FINAL REPORT SHEET**

## M-250 FINAL REPORT

Direct Specimen Culture
Procedure 3008, 3011, 3013

**TO: Wicell QA**

BTL SAMPLE ID#: **57733** P.O.#: DATE REC'D: **06/16/2009**

TEST/CONTROL ARTICLE:

**SA02-DL-01-I #7755**

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **06/17/2009**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

| | | | DATE |
|---|---|---|---|
| THIOGLYCOLLATE BROTH | DAY 7 | + ⊖ | 06/24/2009 |
| | DAY 28 | + ⊖ | 07/15/2009 |
| BROTH-FORTIFIED COMMERCIAL | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 06/24/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 07/15/2009 |
| BROTH-MODIFIED HAYFLICK | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 06/24/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 07/15/2009 |
| BROTH-HEART INFUSION | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 06/24/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 07/15/2009 |

(See Reverse)

Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: M-250 FINAL REPORT SHEET

| SAMPLE ID#: 57733 | | AEROBIC | MICROAEROPHILIC | DATE |
|---|---|---|---|---|
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + (−) | + (−) | 06/24/2009 |
| | DAY 14 | + (−) | + (−) | 07/01/2009 |
| | DAY 21 | + (−) | + (−) | 07/08/2009 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + (−) | + (−) | 06/24/2009 |
| | DAY 14 | + (−) | + (−) | 07/01/2009 |
| | DAY 21 | + (−) | + (−) | 07/08/2009 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + (−) | + (−) | 06/24/2009 |
| | DAY 14 | + (−) | + (−) | 07/01/2009 |
| | DAY 21 | + (−) | + (−) | 07/08/2009 |
| **BROTH SUBCULTURES (DAY 7)** | | DATE: 06/24/2009 | | |
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + (−) | + (−) | 07/01/2009 |
| | DAY 14 | + (−) | + (−) | 07/08/2009 |
| | DAY 21 | + (−) | + (−) | 07/15/2009 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + (−) | + (−) | 07/01/2009 |
| | DAY 14 | + (−) | + (−) | 07/08/2009 |
| | DAY 21 | + (−) | + (−) | 07/15/2009 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + (−) | + (−) | 07/01/2009 |
| | DAY 14 | + (−) | + (−) | 07/08/2009 |
| | DAY 21 | + (−) | + (−) | 07/15/2009 |

RESULTS: No detectable mycoplasmal contamination

7.15.09
Date

Laboratory Director
Ph.D.

M-250 Procedural Summary: The objective of this test is to ascertain whether or not detectable mycoplasmas are present in an in vitro cell culture sample, be it a primary culture, hybridoma, master seed stock or cell line. This procedure combines an indirect DNA staining approach to detect non-cultivable mycoplasmas with a direct culture methodology utilizing three different mycoplasmal media formulations. The indirect approach involves the inoculation of the sample into a mycoplasma-free VERO (ATCC) indicator cell line and performing a DNA fluorochrome assay after 72-120 hours of incubation. The direct culture aspect of the test utilizes three different mycoplasmal media including both broth and agar formulations. The sample is inoculated into each of the 3 broth formulations and also onto duplicate plates (0.1 mL/plate) for each of the 3 agar formulations. Subculture from broth to fresh agar plates is carried out after 7 days incubation. Agar plates are incubated aerobically and microaerophillically in order to detect any colony forming units morphologically indicative of mycoplasmal contamination. Issuance of the final report with signature of the Laboratory Director signifies that the required controls were performed concurrently with the test sample(s) as detailed in the referenced SOPs and that all test conditions have been found to meet the required acceptance criteria for a valid test, including the appropriate results for the positive and negative controls.

Mycoplasma Testing Services

MYCOPLASMA TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC

APPENDIX I

| | |
|---|---|
| Document #: | DCF3008A |
| Edition #: | 06 |
| Effective date: | 9/17/2003 |
| Title: | DNA FLUOROCHROME ASSAY RESULTS |

## DNA-FLUOROCHROME ASSAY RESULTS

Procedures 3008, 3009, 3011

Sample ID # **57733** **M-250** Date Rec'd: **06/16/2009** P.O. #

Indicator Cells Inoculated: Date/Initials: 6/18/09 / HS

Fixation: Date/Initials: 6/22/09 / KG

Staining: Date/Initials: 6/22/09 / KG

TEST/CONTROL ARTICLE:

**SA02-DL-01-I #7755**

LOT# **NA**

**Wicell OA**

**DNA FLUOROCHROME ASSAY RESULTS:**

X **NEGATIVE:** A reaction with staining limited to the nuclear region, which indicates no mycoplasmal contamination.

____ **POSITIVE:** A significant amount of extranuclear staining which strongly suggests mycoplasmal contamination.

____ **INCONCLUSIVE:**

____ A significant amount of extranuclear staining consistent with low - level mycoplasmal contamination or nuclear degeneration.

____ A significant amount of extranuclear staining consistent with bacterial, fungal or other microbial contaminant or viral CPE. Morphology not consistent for mycoplasmal contamination.

COMMENTS: _____

Date: 6/22/09 Results Read by: KG Date of Review: 6/22/09 Reviewed by: LC

**Procedures performed:**
SOP-CH-101 SOP-CH-102
SOP-CH-103 SOP-CH-105

**Cell Line: SA02-DL-01**
**Passage**
**Sample ID: 4633-FAC**

**Date of**: *(mm/dd/yy)*
acquisition: 11/23/09
file creation: 11/23/09
file submission: 11/24/09

| antigen2: | SSEA4 - antigen2 + | SSEA4 + antigen2 + | SSEA4 + antigen2 - | SSEA4 - antigen2 - | ALL SSEA4 + | ALL antigen2 + |
|---|---|---|---|---|---|---|
| SSEA3 | 0.86 | 97.30 | 1.18 | 0.62 | 98.48 | 98.16 |
| TRA1-60 | 0.25 | 93.80 | 5.06 | 0.91 | 98.86 | 94.05 |
| TRA1-81 | 0.27 | 93.50 | 5.23 | 1.02 | 98.73 | 93.77 |
| Oct-4 | 0.87 | 91.30 | 6.59 | 1.24 | 97.89 | 92.17 |
| SSEA1 | 0.11 | 6.26 | 92.70 | 0.89 | 98.96 | 6.37 |