

Thaw and Culture Details

Cell Line Name	BG03
WiCell Lot Number	BG03-DL-02
Parent Material	BG03-MCB-01
Provider	Novocell
Banked By	WiCell
Thaw and Culture	WiCell recommends thawing 1 vial into 1 well of a 6 well plate.
	Feeder Devendent
Culture Platform	
	Medium: Sem Cell Culture Medium
	Matrix: MEF
Protocol	WiCell Feeder Dependent Protocol
Passage Number	p29
	These cells were cultured for 28 passages prior to freeze. WiCell adds +1 to the passage number at
	freeze to best represent the overall passage number of the cells at thaw. Plated cells at thaw should
	be labeled passage 29.
Date Vialed	03-September-2008
Vial Label	BG03-DL-2
	p29 LK
	3SEPT2008
	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.
	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	See Report
Post-Thaw Viable Cell Recovery	WiCell	≥ 15 Undifferentiated Colonies prior to passage,SOP-CH-305≤ 30% Differentiation prior to passage, and recoverable attachment after passage		Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Consistent with STR profile of deposited cell line	Pass
Sterility - Direct transfer method	Apptec	30744 No contamination detected		Pass
Mycoplasma	Apptec	30055	No contamination detected	Pass
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105 Report - no specification		See report

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The material provided under this certificate has been subjected to the tests specified and the results and data described herein are accurate based on WiCell's reasonable knowledge and belief. Appropriate Biosafety Level practices and universal precautions should always be used with this material. For clarity, the foregoing is governed solely by WiCell's Terms and Conditions of Service, which can be found at http://www.wicell.org/privacyandterms.



Approval Date	Quality Assurance Approval	
16-March-2009	7/9/2020 X_AA Quality Assurance Signed by: Arritz, Andy	

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Histocompatibility/Molecular Diagnostics Laboratory

University of Wisconsin Hospital and Clinics

Short Tandem Repeat Analysis*

Sample Report:	8059-STR
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UW HLA#: 60043

Sample Date: 12/18/08 Received Date: 12/18/08

Requestor: WiCell Research Institute

Test Date: 12/21/08, 12/28/08

File Name: 081222, 081229 Report Date: 12/31/08

Sample Name: (label on tube)

8059-STR

Description: DNA Extracted by WiCell

262.50 ug/mL; 260/280 = 1.85

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

Comments: Based on the 8059-STR DNA dated 12/18/08 and received on 12/18/08 from WI Cell, this sample (UW HLA# 60043) matches exactly the STR profile of the human stem cell line BG03 comprising 11 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human BG03 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 8059-STR DNA sample submitted corresponds to the BG03 stem cell line and it was not 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%. A preliminary copy of this report was issued via electronic mail to the Cytogenetics <u>of WI Cell Research Institute on Wednesday, December 31, 2008.</u>

Manager Date HLA/Molecular Diagnostics Laboratory

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.

Test Facility:

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.

WuXi AppTec

Report Number 788629 Page 1 of 6

October 07, 2008 P.O. #:

WiCell Research Institute

STERILITY TEST REPORT

Sample Information:	hES 1:	Cells / iPS Cells WA13C-DL-2
	2:	BG03-DL-2
	3:	ES02-DL-3
	4:	iPS(IMR90)-4-DL-1
	5:	WA07-DL-2
Date Received: Date in Test: Date Completed:	Sep Sep Oct	otember 17, 2008 otember 22, 2008 ober 06, 2008
Test Information:	Tes Imr Pro	t Codes: 30744, 30744A nersion, USP / 21 CFR 610.12 cedure #: BS210WCR.201

QA Reviewed:

Reviewed:

Testing conducted in accordance with current Good Manufacturing Practices.

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.

WuXi AppTec

Report Number 788629 Page 3 of 6

October 07, 2008 P.O. #:

WiCell Research Institute

STERILITY TEST REPORT

Sample Information:	hES Cells / iPS Cells	
	2: BG03-DL-2	
Date Received:	September 17, 2008	

Date Received:	September 17, 2008
Date in Test:	September 22, 2008
Date Completed:	October 06, 2008

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	

QA Reviewed:

Page 1 Signed

Reviewed:

Page 1 Signed

Testing conducted in accordance with current Good Manufacturing Practices.

WuXi AppTec

FINAL STUDY REPORT

STUDY TITLE:

MYCOPLASMA DETECTION: "Points to Consider"

PROTOCOL NUMBER:

30055G

BG03-DL-2

TEST ARTICLE IDENTIFICATION:

SPONSOR:

WiCell Research Institute

PERFORMING LABORATORY:

WuXi AppTec, Inc.

STUDY NUMBER:

113761

RESULT SUMMARY:

Considered **negative** for mycoplasma contamination

Reference PO #

 Study Number: 113761 Protocol Number: 30055G

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QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider"

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

Critical PhaseDateStaining of Cover Slips11/24/08Final Report12/26/08

Study Director 11/24/08 12/26/08

Management 12/29/08 12/29/08

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor:

Date: 12/29/08

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:

Date: 12-29-08

Professional Personnel Involved:

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1.0 PURPOSE

To demonstrate that a test article consisting of a cell bank, production or seed lots, or raw materials is free of mycoplasmal contamination, according to "Points to Consider" criteria.

- 2.0 SPONSOR: WiCell Research Institute
- 3.0 TEST FACILITY: WuXi AppTec. Inc.

4.0 SCHEDULING

DATE SAMPLE RECEIVED:	11/18/08
STUDY INITIATION DATE:	11/20/08
STUDY COMPLETION DATE:	12/29/08

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute BG03-DL-2

6.0 SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing. Unused test samples remained in the storage area until the study was completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

7.0 TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

The Vero cells were maintained by WuXi AppTec's Cell Production Laboratory.

8.0 EXPERIMENTAL DESIGN

8.1 Overview

Whereas no single test is capable of detecting all mycoplasmal strains, freedom from mycoplasmal contamination may be demonstrated by the use of both an indirect and direct procedure.

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8.2 Justification for Selection of the Test System

Contamination of cell cultures by mycoplasma is a common occurrence and is capable of altering normal cell structure and function. Among other things, mycoplasma may affect cell antigenicity, interfere with virus replication, and mimic viral actions. Testing for the presence of mycoplasma for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under "Points to Consider."

9.0 EXPERIMENTAL SUMMARY

The indirect method of detection allows visualization of mycoplasma, particularly noncultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Poor cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* was used to ensure cyto-adsorption. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay was performed with both negative and positive controls. A fermentative mycoplasma (*M. pneumoniae*) and a non-fermentative mycoplasma (*M. orale*) were used as positive controls. The procedure employed in this study is based on the protocol described in the 1993 Attachment # 2 to the "Points To Consider" document, as recommended by the FDA, Center for Biologics Evaluation and Research (CBER).

10.0 TEST MATERIAL PREPARATION

10.1 Test Article Identification:

Test Article Name:	BG03-DL-2
Lot/Batch #:	Not Applicable
General Description:	hES cells
Number of Aliquots used:	1 x 15 mL
Species of Origin:	Human
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (< -60°C)
Safety Precautions:	BSL-1
Intended Use/Application:	Not Given

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10.2 Test Sample Preparation

The test article was thawed in a water bath at 37 \pm 2 °C and 1:5 and 1:10 dilutions were prepared in sterile phosphate buffered saline (PBS). 1.0 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated onto each of two (2) coverslips (per sample/dilution) containing Vero cells. The coverslips were incubated in incubator E770 for 1-2 hours at 36 \pm 1 °C / 5 \pm 2% CO₂ and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 36 \pm 1 °C / 5 \pm 2% CO₂. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of two SP-4 agar plates, and 10.0 mL was inoculated into a 75 cm² flask containing 50 mL of SP-4 broth. The plates were incubated anaerobically at 36 \pm 1 °C for a minimum of 14 days.

The broth flask was incubated aerobically at 36 ± 1 °C, and subcultured onto each of two SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were incubated anaerobically at 36 ± 1 °C for a minimum of 14 days. The broth flask was read each working day for 14 days. The SP-4 agar plates (Day 0) were read after 14 days of incubation. The SP-4 broth subculture plates (Days 3, 7, and 14) were read after 14 days incubation.

10.3 Controls and Reference Materials

10.3.1 Sterile SP-4 broth served as the negative control for both the direct and indirect assays.

10.3.2 Positive Controls

a. Indirect Assay

- **a.1** Strongly cyto-adsorbing species *M. hyorhinis* GDL (ATCC #23839) at 100 or fewer colony forming units (CFU) per inoculum.
- **a.2** Poorly cyto-adsorbing species *M. orale* (ATCC #23714) at 100 or fewer CFU and at approximately 100 ID₅₀ per inoculum

b. Direct Assay

- **b.1** Nonfermentative mycoplasma species *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.
- **b.2** Fermentative mycoplasma species *M. pneumoniae* FH (ATCC #15531) at 100 or fewer CFU per inoculum.

Study Number: 113761 Protocol Number: 30055G

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10.3.3 Control Preparation

a. Negative Controls

- a.1 1.0 mL of sterile SP-4 broth was inoculated onto each of two
 (2) coverslips containing Vero cells to serve as the negative control in the indirect assay.
- a.2 0.2 mL of SP-4 broth was inoculated onto each of two (2) SP-4 agar plates to serve as the negative control in the direct assay. 10.0 mL of SP-4 broth was inoculated into a 75 cm² flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

b. Positive Controls

- b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinis* and *M. orale* at less than 100 CFU/mL was inoculated onto each of two (2) coverslips containing Vero cells. 1.0 mL of *M. orale* at 100 ID₅₀ CFU per inoculum was also inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.
- **b.2** The coverslips were incubated in incubator E950 for 1-2 hours at $36 \pm 1 \degree C / 5 \pm 2\% \degree CO_2$ and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E950 at $36 \pm 1 \degree C / 5 \pm 2\% \degree CO_2$. After three days of incubation, the cell cultures were fixed, stained, and then read using an epifluorescent microscope.
- **b.3** 0.2 mL of *M. orale* and *M. pneumoniae* at less than 100 CFU/plate was inoculated onto each of two (2) SP-4 agar plates. 10.0 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL (≤100 CFU/inoculum) were each inoculated into a 75 cm² flask containing 50 mL of sterile SP-4 broth.
- **b.4** The agar plates were placed in an anaerobic chamber and incubated at 36 ± 1 °C for 14 days. The broth cultures were incubated aerobically at 36 ± 1 °C for a minimum of 14 days and were read each working day for 14 days. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two (2) SP-4 agar plates. These subculture plates were placed in an anaerobic chamber and incubated at 36 ± 1 °C. The subculture plates were observed microscopically for the presence of mycoplasma colonies after a minimum of 14 days incubation.
- c.

See Section 15.0, Results, for the results of these controls.

11.0 DATA ANALYSIS

The results of this study were based on visual observations, therefore, no data analysis was required.

12.0 STATISTICAL METHODS

The results of this study were qualitative, therefore, no statistical analysis was required.

13.0 EVALUATION CRITERIA

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

13.1 Indirect Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

MYCOPLASMA FLUORESCENCE OBSERVED (AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
+
+/_*
+

*Mycoplasma must be observed for at least one dilution of the poorly cyto-adsorbing mycoplasma species M. orale.

13.2 Direct Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	M. PNEUMONIAE	M. ORALE
Broth (Color change or turbidity change)	-	+/-	+ / -
Agar Day 0 (at least one plate)	-	+	.,-
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

14.0 TEST EVALUATION

14.1 Indirect Assay

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20 μ m in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3 μ m in diameter.

14.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from the carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth to turn red. In general, growth of mycoplasma can cause the broth to become turbid. These changes must be confirmed by agar plate subculture or DNA-staining since changes in the appearance of the broth culture can also be caused by the properties of the inoculum.

Mycoplasma colonies grow down into the agar causing the center of the colony to appear opaque and the peripheral surface growth to appear translucent. These "fried-egg" colonies vary in size, 10-500 μ m, and can be readily observed unstained using a light microscope.

14.3 Indirect Assay and Direct Assay Results Interpretation

IF:	TEST ARTICLE				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
THEN: OVERALL FINAL RESULT	-	+	+	+	-

*A change in the appearance of a broth culture must be confirmed by positive subculture plate(s).

14.4 Positive Results

The test article is considered positive if the direct assay (agar or broth media procedure) or the indirect assay (indicator cell culture procedure) show evidence of mycoplasma contamination and resemble the positive controls for the procedure.

14.5 Negative Results

The test article is considered as negative if both the direct assay (agar and broth media procedure) and the indirect assay (indicator cell culture procedure) show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

15.0 RESULTS

Indirect Assay and Direct Assay Results

	The Rouger of State	[
	INDIRECT	Day 0 Agar Plates	BROTH FLASKS AND ASSOCIATED SUBCULTURE PLATES	OVERALL	
Test Article: BG03-DL-2	Negative	Negative	Negative	Negative	
Negative Control	Negative	Negative	Negative	Negative	
M. hyorhinis	Positive			Positive	
M. orale	Positive	Positive	Positive	Positive	
M. pneumoniae		Positive	Positive	Positive	

For the indirect assay, the coverslips for the undiluted test article were read and determined negative.



Report Date: November 29, 2008

Case Details:

Cell Line: BG03-DL-2 (8059) Passage #: 36 **Date Completed:** 11/29/2008 Cell Line Gender: Female **Investigator:** National Stem Cell Bank **Specimen:** hESC on MEF feeder **Date of Sample:** 11/21/2008 Tests, Reason for: NSCB DL testing **Results:** 46.XX *Completed by* , CLSp(CG), on 11/26/2008 *Reviewed and interpreted by* PhD, FACMG, on 11/29/2008 Interpretation: No clonal abnormalities were detected at the stated band level of resolution.

State Band Pluggine. COMPACT. SUCCERCION OF £, 12 In the second 98 88 22 Y

Cell: S01-02 Slide: A Slide Type: Karyotyping Cell Results: Karyotype: 46,XX

of Cells Counted: 40
of Cells Karyotyped: 4
of Cells Analyzed: 8
Band Level: 500-550

Results Transmitted by Fax / Email / Post Sent By:

Date:____ Sent To:



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

Cell Line: BG03-DL-2 Passage p33 Sample ID: 8059-FAC **Date of**: (*mm/dd/yy*) acquisition: 11/04/08 file creation: 11/12/08 file submission: 11/12/08

antigen2: SSEA3 TRA1-60 TRA1-81 Oct-4 SSEA1	SSEA4 - <u>antigen2 +</u> 0.11 0.19 1.21 1.82 0.27	SSEA4 + <u>antigen2 +</u> 89.20 97.70 94.10 84.00 4.86	SSEA4 + <u>antigen2 -</u> 10.30 1.74 4.26 12.60 92.50	SSEA4 - <u>antigen2 -</u> 0.44 0.32 0.48 1.58 2.40	ALL <u>SSEA4 +</u> 99.50 99.44 98.36 96.60 97.36	ALL <u>antigen2 +</u> 89.31 97.89 95.31 85.82 5.13
					SSEA	4 +
100					antig	en2 +
100						
90						
	0.00 -					
60	0.00 -					
SC SC	0.00 -					
40 Hage	0.00 -					
10 30	0.00 -					
م 20	0.00 -					
10	0.00 -					
C	0.00			81 Oct 4	SSEA1	 _
	SSEAS	TKAI-0	U IRAI-	81 001-4	SSEAL	
10^4 1.8	32		84			
	-					
⊄ □10 ³		-				
4 PE			1.2.1			
$\frac{10}{0}$ 10 ²	• •		N.			
A: BC						
ப் 1 ய 10 ¹ –						
			hESO			
$10^0 \frac{1.5}{1.5}$	58		12.6	t Count: 11927		
10 ⁰	10 ¹ AP	10 ² 10 ³ C C-A	10 ⁴			

*PE-conjugated Oct-3/4 from BD Biosciences was used (cat #560186).