Centre for Stem Cell Biology

Stem Cell Derivation Facility (SCDF)
FQC.00.B Test Report

The University Of Sheffield.

Section (i) Test Details

Test Material:

| ICB | | preMCB | | MCB | | WCB | GC |

| Pre-Freeze | | Viability | | Level II | | Other | GC |

SCDF ID/ Description: MShef 7 QC4/5 Nutristem

Test Scheduled, include WI reference: Immunohistochemistry: Cell Surface Markers

Sample Received and Test Initiation: Sign [signature] Print G. CLEAVER Date 5 Jun 13

Section (ii) Results Summary

Comments / Results:

Complete as a summary if results are attached as separate pages, detail electronic location of supporting documents, changes in test procedure, deviations etc.

Internal Job/ Sample Ref. No. NA

SSEA1 was expressed only around the edge of the colony on many of the colonies. However several colonies showed some SSEA1 expression throughout the colony. These tended to be smaller colonies.
There was good SSEA3 expression, it was clearly visible throughout the majority of colonies.
Tra-1-81 expression was good, it was clearly expressed throughout the majority of colonies.

No. of attached pages: 4

Events associated to the test

dHACC PE_33 restricted release of primary antibodies.

Analysed By: [signature] **Date** 18 Jun 13

Job Title: Quality Assistant.

Note: On completion the original of this form along with any attachments **must** be returned to QC.

Section (iii) To be completed by QC

OUTCOME: (Within/ Outside Specification or For Information Only) For Information only

Comments or Further Actions: (Note: if outside specification refer to event number.)

Consistent with expected characteristics of undifferentiated hESCs.

Reviewed By: [signature] Zoe Newitt **Date** 16-JAN-2014.

Note: On completion of this report the results from multiple tests must be compiled by the Quality Manager onto an FQC.00.C style form as appropriate.

Sample Description

Sample Reference/ ID Attach separate sheet if required	Culture Vessel Details: include passage number, date passaged and initials
Test Cells	ushef 7 QC 4/S Nutristem undifferentiated. P+6 1:4 H Mor 13 fixed 3 days after passage
Control Cells	N/A

Reagent Details

Reagent	Details: include, if appropriate, lot numbers, source, expiry, date aliquoted, species raised against	Antibody Concentration	QC released
Fixative or TrypLE select (if applicable)	R777 PFA prep 1feb13 exp feb14 alq 7 & 8 of 8	N/A	GC
Primary Antibodies			
SSEA1	480-1-1 lot: 00008-220208 tube008	1:10	29 Apr 13
SSEA3	R876 ⎱ PE-33	1:10	6 Apr 13
Tra-1-81	R879 ⎰	1:50	6 Apr 13
Secondary Antibodies	R896 a Rat AF 594	1:50	Y
	R897 x mouse AF 488	1:200	Y
Nuclear Stain (if applicable)	Hoescht RS33	1:5000	Y
Cell Viability Stain (if applicable)	N/A		
Samples fixed by: (Signature)	[signature]	Date Fixed:	22 Mar 13
Samples prepared by: (Signature)	[signature]	Date Prepared:	5/6 Jun13

| Author: | Heather Da Costa | Doc Ref: | FQC.03.C | Issue Date: | 20-Mar-2012 |
| Approver: | Zoe Hewitt | Version: | 2.0 | Effective Date: | 20-Apr-2012 |

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Additional Details (observations of cells before harvest that could impact on quality, sticky cells, support reagents used – blocking buffer, cell counts if performed, comments)

PQC0011   Blocking buffer exp 29 Jun13

PQC 0010   Antibody buffer exp 9 Jun13

R837   PBS(-) washes

Staining Map (Use this to record any shorthand used to label your samples, further comments, or for notes made during the sample run)

1 SSEA-1 AF488	2 SSEA-3 AF594	3 Tra-1-81 AF488
4 2° only AF488	5 2° only AF594	6

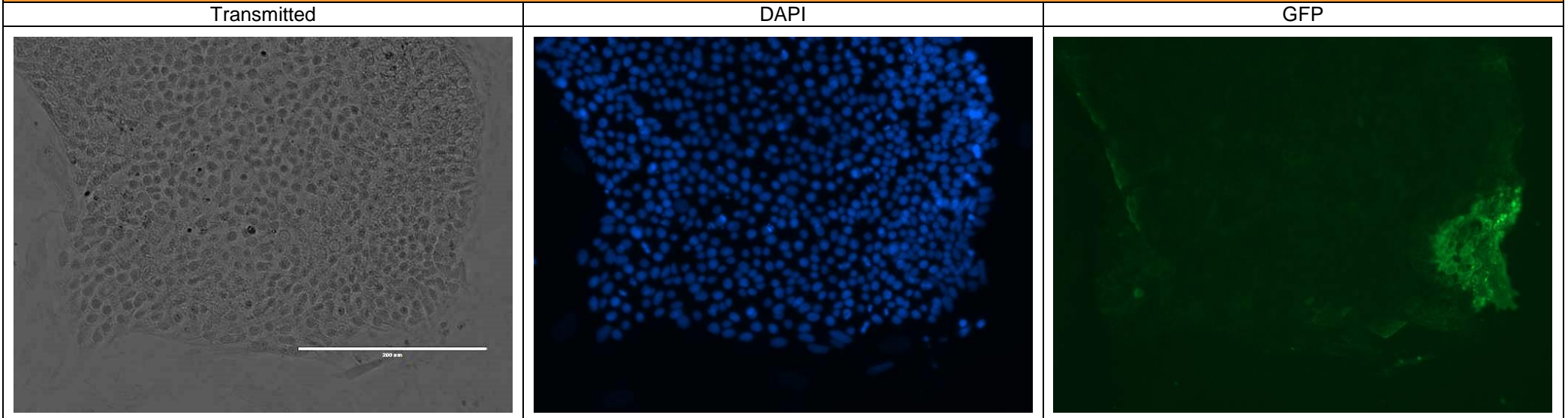| Name & Serial No of Machine used:	GJOS	Alignment Confirmation Date: (please attach alignment report if appropriate)	NA
Samples Processed By: (Signature)	GJ	Date Samples Processed:	17 Jun13

MShef7 QC4/5 Nutristem: SSEA1		
Transmitted	DAPI	GFP

MShef7 QC 4/5 Nutristem: SSEA3		
Transmitted	DAPI	RFP

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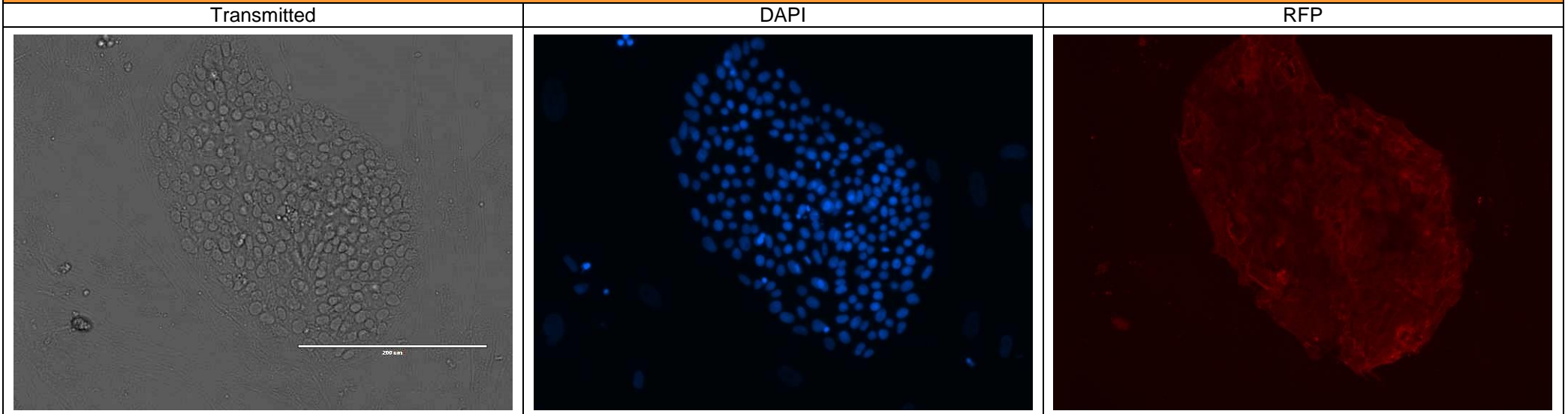
MShef7 QC4/5 Nutristem Tra-1-81		
Transmitted	DAPI	GFP
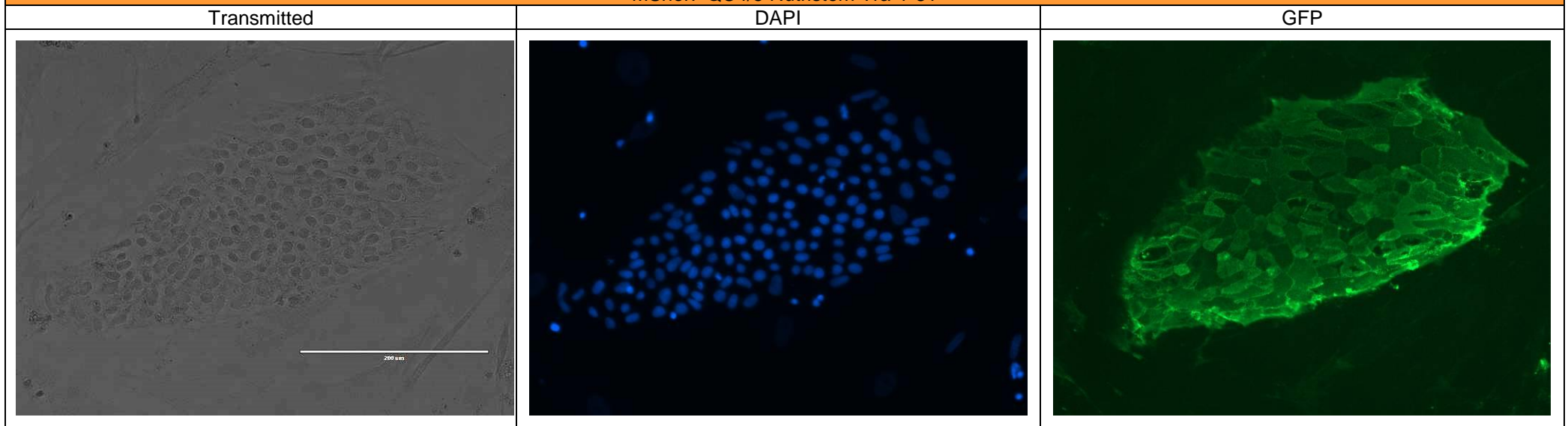
Image Location (Folder)	Comments	2° Only AF488	2° Only AF594
V:\SCDF\Quality Control Data\QC - Product Test Data\Immuno Images\MShef7\Nutristem\139617 MSHef7 Cell surface markers	All photos taken on the following settings. DAPI: 30ms 50% GFP: 60ms 100% RFP: 500ms 100% All photos have the following image settings Brightness 50%, Contrast 50%		