

Materials List for

Establishing a High Throughput Epidermal Spheroid Culture System to Model Keratinocyte Stem Cell Plasticity

Yvon Woappi^{1,2}, Geraldine Ezeka³, Justin Vercellino⁴, Sean M. Bloos⁵, Kim E. Creek⁶, Lucia Pirisi¹

¹Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine ²Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School ³Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine Baltimore

⁴Department of Pathology, Albert Einstein College of Medicine ⁵Department of Pediatrics, Vanderbilt University Medical Center ⁶Department of Drug Discovery and Biomedical Sciences, College of Pharmacy, University of South Carolina

Corresponding Author

Yvon Woappi

YWOAPPI@gmail.com

Citation

Woappi, Y., Ezeka, G., Vercellino, J., Bloos, S.M., Creek, K.E., Pirisi, L. Establishing a High Throughput Epidermal Spheroid Culture System to Model Keratinocyte Stem Cell Plasticity. *J. Vis. Exp.* (), e62182, doi:10.3791/62182 (2021).

Date Published

January 30, 2021

DOI

10.3791/62182

URL

[jove.com/video/62182](https://doi.org/10.3791/62182)

Materials

Name	Company	Catalog Number	Comments
Affymetrix platform	Affymetrix		For microarray experiments
Affymetrix's HuGene-2_0-st library file	Affymetrix		Process
Agilent 2100 Bioanalyzer	Agilent		For microarray experiments
All Prep DNA/RNA Mini Kit	Qiagen	80204	Used for RNA isolation
Analysis Console Software version 3.0.0.466			analyze cell type specific transcriptional responses using one-way between-subject analysis of variance
BD FACSAria II flow cytometer	Beckman		For flow cytometry
Console Software version 3.0.0.466/ Expression console Software	Affymetrix/Thermo Fisher Scientific		For confirming data quality
Cytokeratin 14	Santa Cruz Biotechnology	sc-53253	1:200 dilution
Dispase	Sigma-Aldrich	D4818	For cell media
FITC-conjugated anti-integrin α 6	Abcam	ab30496	For FACS analysis
GeneChip Command Console 4.0 software	Affymetrix/Thermo Fisher Scientific		For confirming data quality
GeneChip Fluidics Stations 450 (Affymetrix/Thermo Fisher Scientific)	Affymetrix/Thermo Fisher Scientific		For washing and staining of hybridized arrays
GeneChip HuGene 2.0 ST Arrays	Affymetrix/Thermo Fisher Scientific		For hybridization and amplification of total RNA
GeneChip Hybridization Oven 640	Thermo Fisher Scientific		For hybridization and amplification of total RNA Amplify labeled samples
GeneChip Hybridization Wash, and Stain Kit (Affymetrix/Thermo Fisher Scientific)	Affymetrix/Thermo Fisher Scientific		For washing and staining of hybridized arrays
GeneChip Scanner 3000 7G system	Affymetrix/Thermo Fisher Scientific		Scanning hybridized arrays
GeneChip WT PLUS Reagent Kit	Affymetrix/Thermo Fisher Scientific		For amplification of biotinyling total RNA

Human Basic Fibroblast Growth Factor (hFGF basic/FGF2)	Cell Signaling Technology	8910	For cell media
Human Epidermal Growth Factor (hEGF)	Cell Signaling Technology	8916	For cell media
Human Insulin	Millipore Sigma	9011-M	For cell media
iQ SYBR Green Supermix (Bio-Rad)	Bio-Rad	1708880	Used for RT-qPCR
iScript cDNA Synthesis Kit	Bio-Rad	1708890	Used for RT-qPCR
KSFM	ThermoFisher Scientific	17005041	Supplemented with 1% Penicillin/Streptomycin, 20 ng/ml EGF, 10 ng/ml basic fibroblast growth factor, 0.4% bovine serum albumin (BSA), and 4 µg/ml insulin
KSFM-scm	ThermoFisher Scientific	17005042	Supplemented with 1% Penicillin/Streptomycin, 20 ng/ml EGF, 10 ng/ml basic fibroblast growth factor, 0.4% bovine serum albumin (BSA), and 4 µg/ml insulin
MCDB 153-LB basal medium	Sigma-Aldrich	M7403	MCDB 153-LB basal media w/ HEPES buffer
NEST Scientific 1-Well Cell Culture Chamber Slide, BLACK Walls on Glass Slide, 6/PK, 12/CS	Stellar Scientific	NST230111	For immunostaining
P63	Thermo Scientific	703809	1:200 dilution
PE-conjugated anti-EGFR (San Jose, CA; catalog number)	BD Pharmingen	555997	For FACS analysis
pMSCV-IRES-EGFP plasmid vector	Addgene	20672	For transfection
Promega TransFast kit	Promega	E2431	For transfection
Qiagen RNeasy Plus Micro Kit	Qiagen		For microarray experiments
Thermo Scientific™ Sterile Single Use Vacuum Filter Units	Thermo Scientific	09-740-63D	For cell media
Zeiss Axiovert 135 fluorescence microscope	Zeiss		Use with Axiovision Rel. 4.5 software