

# Collection, Expansion, and Differentiation of Primary Human Nasal Epithelial Cell Models for Quantification of Cilia Beat Frequency

Katelin M. Allan<sup>1,2</sup>, Sharon L. Wong<sup>1,2</sup>, Laura K. Fawcett<sup>1,2,3</sup>, Alexander Capraro<sup>1,2</sup>, Adam Jaffe<sup>1,2,3</sup>, Cristan Herbert<sup>4</sup>, Elvis Pandzic<sup>5</sup>, Shafagh A. Waters<sup>1,2,3</sup>

<sup>1</sup>School of Women's and Children's Health, Faculty of Medicine and Health, University of New South Wales <sup>2</sup>Molecular and Integrative Cystic Fibrosis Research Centre (miCF RC), Faculty of Medicine and Health, University of New South Wales <sup>3</sup>Department of Respiratory Medicine, Sydney Children's Hospital <sup>4</sup>Department of Pathology, School of Medical Sciences, University of New South Wales Australia <sup>5</sup>Katharina Gaus Light Microscopy Facility, Mark Wainwright Analytical Centre, University of New South Wales

## Corresponding Authors

Elvis Pandzic  
e.pandzic@unsw.edu.au

Shafagh A. Waters  
shafagh.waters@unsw.edu.au

## Citation

Allan, K.M., Wong, S.L., Fawcett, L.K., Capraro, A., Jaffe, A., Herbert, C., Pandzic, E., Waters, S.A. Collection, Expansion, and Differentiation of Primary Human Nasal Epithelial Cell Models for Quantification of Cilia Beat Frequency. *J. Vis. Exp.* (177), e63090, doi:10.3791/63090 (2021).

## Date Published

November 10, 2021

## DOI

10.3791/63090

## URL

jove.com/video/63090

## Materials

Name	Company	Catalog Number	Comments
Adenine	Sigma-Aldrich	A2786	10 mg/mL
Advanced DMEM/F-12	Thermo Fisher Scientific	12634-010	
Alanyl-glutamine	Sigma-Aldrich	G8541	200 mM
Andor Zyla 4.2 sCMOS	Oxford Instruments		Fast frame rate (>100 Hz) scientific camera
Bottle-top vacuum filter system	Sigma-Aldrich	CLS431098	
Ceftazidime hydrate	Sigma-Aldrich	A6987	50 mg/mL
Cell Culture Microscope	Olympus	CKX53	
CFI S Plan Fluor ELWD 20XC	Nikon Instruments Inc.	MRH08230	Long working distance objective lens. NA0.45 WD 8.2-6.9
Cholera toxin	Sigma-Aldrich	C8052-1MG	200 µg/mL
Corning Gel Strainer 40 UM	Sigma-Aldrich	CLS431750	Pore size 40 µm
Corning Matrigel Matrix (Phenol red-free)	Corning	356231	Extracellular matrix (ECM)
Corning bottle-top vacuum filter system	Sigma-Aldrich	CLS431098	
Corning CoolCell LX Cell Freezing Container	Sigma-Aldrich	CLS432002	
Corning Transwell polyester membrane cell culture inserts	Sigma-Aldrich	CLS3470	Permeable support inserts. 6.5 mm Transwell with 0.4 µm pore polyester membrane insert.
Countess Cell Counting Chamber Slides	Thermo Fisher Scientific	C10228	
Countess II Automated Cell Counter	ThermoFisher Scientific	AMQAX1000	Automated cell counter

Cytology brushes	McFarlane Medical	33009	
DMEM/F12-Ham	Thermo Fisher Scientific	11330032	
DMEM/F12-Ham	Thermo Fisher Scientific	11330032	
DMEM-High Glucose	Thermo Fisher Scientific	11965-092	
Dulbecco's Phosphate Buffered Saline (PBS)	Sigma-Aldrich	D8537	
Eclipse Ti2-E	Nikon		Live-cell imaging microscope.
Fetal Bovine Serum, certified, heat inactivated, United States	Thermo Fisher Scientific	10082147	
Fungizone (Amphotericin B)	Thermo Fisher Scientific	15290018	250 µg/mL
Gentamicin solution	Sigma-Aldrich	G1397	50 mg/mL
Graphpad Prism	Graphpad		Scientific analysis software
Greiner Cryo.s vials	Sigma-Aldrich	V3135	Cryogenic vials
HEPES solution	Sigma-Aldrich	H0887	1 M
HI-FBS	Thermo Fisher Scientific	10082-147	
Hydrocortisone	Sigma-Aldrich	H0888	3.6 mg/mL
Incubator NL Ti2 BLACK 2000	PeCon		Microscope environmental chamber. Allows warm air incubation and local CO2 and O2 gassing
Insulin	Sigma-Aldrich	I2643	2 mg/mL
Lab Armor 74220 706 Waterless Bead Bath 6L	John Morris Group	74220 706	Bead bath
Lab Armor Beads	Thermo Fisher Scientific	A1254302	Thermal beads
MATLAB	MathWorks		Computing software
Microsoft Excel	Microsoft		Spreadsheet software
NIH/3T3	American Type Culture Collection	CRL-1658	Irradiated NIH-3T3 mouse embryonic feeder cells
NIS-Elements AR	Nikon Instruments Inc.		Image acquisition software
Penicillin-Streptomycin	Sigma-Aldrich	P4333	10,000 units penicillin and 10 mg streptomycin/mL
Dulbecco's Phosphate Buffered Saline (PBS)	Sigma-Aldrich	D8537	
PneumaCult Airway Organoid Kit	StemCell Technologies	5060	Airway Organoid Kit
PneumaCult-ALI Medium	StemCell Technologies	5001	
PneumaCult-Ex Plus Medium	StemCell Technologies	5040	
PureCol-S	Advanced BioMatrix	5015	Type I Collagen solution
ReagentPack Subculture Reagents	Lonza	CC-5034	
rhEGF (Epidermal Growth Factor, human)	Sigma-Aldrich	E9644	25 µg/mL
Y-27632 2HCl (ROCK inhibitor)	Selleckchem	S1049	10 mM
Tobramycin	Sigma-Aldrich	T4014	100 mg/mL
Trypan blue solution	Sigma-Aldrich	T8154	0.4%
UNO Stage Top Incubator	Okolab		Microscope incubator. Allows temperature, humidity and CO2 conditioning