

Materials List for:

Genetic Modification and Recombination of Salivary Gland Organ Cultures

Sharon J. Sequeira^{*1}, Elise M. Gervais^{*1}, Shayoni Ray¹, Melinda Larsen¹

¹Department of Biological Sciences, University at Albany, SUNY

*These authors contributed equally

Correspondence to: Melinda Larsen at mlarsen@albany.edu

URL: <https://www.jove.com/video/50060>

DOI: [doi:10.3791/50060](https://doi.org/10.3791/50060)

Materials

Name	Company	Catalog Number	Comments
DMEM/Ham's F12 Medium without phenol red	Life Technologies	21041-025	
Penicillin and Streptomycin	Life Technologies	15070-163	10X stock
Dispase	Life Technologies	17105-041	Freeze single use aliquots at -20C
BSA	Sigma	A2934-100G	Fraction V, low endotoxin
Adeno-X-GFP	BD Biosciences	8138-1	Should be high titer (1×10^{10} pfu/ml). CsCl purified viruses are more effective than column-purified viruses in this assay.
16% Paraformaldehyde	Electron Microscopy Sciences	15710	Diluted to 2% in PBS with 5% sucrose (w/v)
1X Phosphate-buffered saline (PBS)	Life Technologies	70011-044	Prepared from 10X stock
Hank's Balanced Salt Solution	Life Technologies	14175095	no Calcium, no Magnesium, no Phenol Red
Transferrin	Sigma	T8158	25 mg/ml stock solution in DMEM/F12 media. Freeze single-use aliquots at -20C
L- Ascorbic acid (Vitamin C)	Sigma	A4403	75 mg/ml stock solution in DMEM/F12 media. Freeze single-use aliquots at -20C

Table 1. List of reagents required for SMG recombination protocol.

10 cm sterile plastic dishes	Corning	430167	Non-tissue culture-treated plates can also be used.
Stereo dissecting microscope with transmitted light base	Nikon	SMZ645	Any stereo dissecting microscope can be used that has a transmitted light base.
35 mm tissue culture dishes	Falcon	353001	Non-tissue culture-treated plates can also be used.
50 mm diameter microwell dishes	MatTek Corporation	P50-G-1.5-14F	
Nuclepore Track-Etch membrane filters	Whatman	110405	13 mm diameter, 0.1 mm pore size
Widefield fluorescence microscope	Carl Zeiss, USA	Axio Observer Z1	Any fluorescence microscope (upright, inverted or stereo dissecting microscope) can be used to monitor GFP expression at low magnification with an attached digital camera.
Confocal microscope	Leica Microsystems	TCS SP5	Confocal microscopy is necessary to see detailed cell structures. Any confocal microscope can be used.

Timed-pregnant female mice, strain CD-1 or ICR	Charles River Labs		Embryos are harvested on day 13 (with day of plug discovery designated as day 0).
Scalpel blade #11	Fine Science Tools	10011-00	
Scalpel handle #3	Fine Science Tools	10003-12	
Dumont #5 forceps inox alloy, 0.05mm X 0.02mm	Fine Science Tools	11252-20	Ideal for harvesting glands from embryos
Dumont #5 forceps dumostar alloy, 0.05mm X 0.01mm	Fine Science Tools	11295-20	Fine tips are required for removing mesenchyme from epithelium. Tungsten needles can also be used.

Table 2. Equipment used in SMG recombination protocol.