

Materials List for:

Production of Replication-Defective Retrovirus by Transient Transfection of 293T cells

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URL: <https://www.jove.com/video/550>

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Materials

Name	Type	Company	Catalog Number	Comments
293T cells	cell-line			Split 1:3 to 1:4 every 3 days, otherwise they will tend to clump with replating and will not transfect as well.
293T cell medium				DMEM/hi-glu + 10 % FBS + 1% Pen/Strep + 1% L-Glutamine (1% NEAA, optional). We obtain our tissue culture slutions from CellGro.
coding DNA plasmid				Twice purified by CsCl. MSCV backbone, encoding oncogene and marker of choice (Neo, GFP, etc.)
EcoPak				also called pMCV-Ecopac: ecotropic packaging plasmid, encoding gag-pol-env
2x HBS				For 500 ml: 8.0 g NaCl + 0.37 g KCl + 106.5 mg Na2HPO4 (anhydrous; 201.1 mg if 7xH2O) + 1.0 g dextrose (D-glucose) + 5.0 g HEPES powder. Dissolve in 450 ml dH2O (milli-Q), adjust pH to 7.05 with NaOH, then complete to 500 ml with dH2O. Sterile filter thru 0.45 µ filter. Store at room temperature, with the cap on tight.
2M CaCl2		Sigma-Aldrich		
milliQ H2O				sterile-filtered
Chloroquine				1000x stock is 25 mM in PBS- (w/o Ca2+/Mg2+), stored at -20C. Add fresh to the medium when needed.
6 cm plates				for tissue culture
Incubator				for tissue culture. Set at 37C, 10% CO2.
10 cc sterile syringes				Sterile. One per virus type.
18G needles				single use, one per virus type.
45 um syringe filters				

5 ml plastic tubes				Sterile, to mix transfection solution, for up to 2 plates at a time (2 ml). We use Falcon tubes.
50 ml conical tubes				
cryovials				2 and 4 ml, for virus aliquots.
All reagents used in the transfection must be sterile-filtered (CaCl ₂ , 2x HBS, miliQ H ₂ O) and kept sterile.				