The Fleischman Lab

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Title: Viral Production – X-tremeGENE 9 Method

Introduction

This protocol describes the use of 239T cells to produce retrovirus. In our hands, the best transfection rates are obtained by using the X-tremeGENE 9 method. Cells are transfected with a retroviral vector expressing the gene of interest along with a helper plasmid called EcoPak. We generally utilize the MSCV-IRES-GFP, or MIG, vector in our experiments. Infected cells are easily distinguished by expression of GFP on a flow cytometer.

Materials

1. 293T cells
2. Roche X-tremeGENE 9
3. Opti-MEM
4. D10 (DMEM + 10% FBS)
5. Plasmid(s) of interest (15-30µg per transfection)
   * Plasmid should be very high quality with a concentration ≥1mg/ml
6. EcoPak plasmid (5µg per transfection)
7. 10cm tissue culture plates
8. 1.5ml Eppendorf tubes
9. 35ml syringes
10. 0.45µm syringe filters
11. 15ml and 50ml conical tubes
12. Serological pipettes and pipet-aid
13. Micropipettes and tips
14. 37°C 5% CO_2 incubator with ≥95% humidity
15. Swinging bucket centrifuge with plate carriers.

Protocol	A. Prepare 239T cells	*Notes*
1.	Thaw 293T cells into warm DMEM or D10.	*It is important to use low passage 293T cells.*
2.	Centrifuge at 1200 rpm for 10 minutes. Discard supernatant.	
3.	Resuspend cells in D10 and plate onto 10cm TC dishes.	
4.	Expand cells at 37°C and 5% CO_2 until you have enough cells for one 10cm dish per transfection.	
5.	Seed cells into 10cm dishes (1 per transfection) the evening before transfection.	*At time of transfection 293T cells should be about 50% confluent so seed cells onto dish accordingly.*
	B. Transfect Cells	
1.	Prior to transfection, replace 293T cell media with 10ml fresh D10. Gently add media by pipetting slowly along the side of the plate to avoid detaching cells.	*If cells look too crowded do not use, split, replate, and try again the next day.*
2.	Label tubes for transfections. Each 10cm dish of 239T cells is one transfection.	

3.	To each tube, add 740µl of Opti-MEM and 60µl of XtremeGENE 9.	
4.	To each tube, add 5µg of EcoPak and 15-30µg of plasmid.	*Add 15µg of empty MIG plasmid. Add 30µg of all other plasmids.*
5.	Incubate at room temperature for 15 minutes.	
6.	Add mixture to 239T cell plates by slowly pipetting along the side of the plate to avoid detaching the cells.	
7.	Gently tilt the plate back and forth in both directions several times to mix.	
8.	Return plates to incubator overnight.	
9.	Change media the following day. Gently add media by pipetting slowly along the side of the plate to avoid detaching cells.	
	C. Harvest Virus	
1.	72 hours after transfection, collect media from plates into 50ml conical tubes.	
2.	Centrifuge at 1500 rpm for 5 minutes.	
3.	Using a 0.45µm syringe filter and a 35ml syringe, filter the supernatant to remove any contaminating 239T cells.	
4.	Aliquot viral supernatant into several 15ml conical tubes. Label with the virus name and the date.	*Make aliquots of 2-12ml each (make various volumes so can have as single use vials for various experiments)*
5.	Flash-freeze in liquid nitrogen and store at -80°C.	