

Materials List for:

Genotypic Inference of HIV-1 Tropism Using Population-based Sequencing of V3

Rachel A. McGovern¹, P. Richard Harrigan¹, Luke C. Swenson¹

¹Laboratory Program, BC Centre for Excellence in HIV/AIDS

Correspondence to: P. Richard Harrigan at prharrigan@cfenet.ubc.ca

URL: <https://www.jove.com/video/2531>

DOI: [doi:10.3791/2531](https://doi.org/10.3791/2531)

Materials

Name	Company	Catalog Number	Comments
RNA extraction using NucliSens easyMAG version 1.0			
NucliSens easyMAG Magnetic Silica	bioMerieux	cat. # 280133	(48 x 0.6 ml)
NucliSens easyMAG Lysis Buffer	bioMerieux	cat. # 280134	(4 x 1000 ml)
NucliSens easyMAG Extraction Buffer 1	bioMerieux	cat. # 280130	(4 x 1000 ml)
NucliSens easyMAG Extraction Buffer 2	bioMerieux	cat. # 280131	(4 x 1000 ml)
NucliSens easyMAG Extraction Buffer 3	bioMerieux	cat. # 280132	(4 x 1000 ml)
NucliSens easyMAG version 1.0	bioMerieux		
Class II A2 biological safety cabinet			
One-Step Reverse Transcriptase PCR and Second Round PCR			
DEPC-treated water	Ambion	cat. # 9922	
SuperScrip III One-Step RT-PCR System with Platinum Taq High Fidelity	Invitrogen	cat#12574-035	Kit components used: - SuperScript III RT/ Platinum Taq High Fidelity Enzyme Mix - 2X Reaction Mix (a buffer containing 0.4mM of each dNTP, 2.4mM MgSO ₄)
Expand High Fidelity PCR System	Roche Group	cat. # 1 759 078	Kit components used:- Expand High Fidelity Buffer 10X conc. with 15 mM MgCl ₂ (lids labelled 2)- Expand HF PCR Enzyme Mix (3.5U/μl)- MgCl ₂ Stock Solution (25mM) (lids labelled 4)
dNTPs: dATP, dGTP, dCTP, dTTP	Roche Group	cat. # 11969064001	(100 mM)
Sucrose	Sigma-Aldrich	cat. # S0389-500G	
Bromophenol blue sodium salt	Sigma-Aldrich	cat.# B5525	
Cresol Red	Sigma-Aldrich	cat.# 114472-5G	indicator grade
Thermocycler			
Gel Electrophoresis			
50X TAE buffer	Invitrogen	cat. # 24710030	(1000 ml)
SYBR Safe DNA gel stain	Invitrogen	cat. # S33102	
Agarose (ultra pure)	Invitrogen	cat. # 15510-027	
E-Gel Low Range Quantitative DNA Ladder	Invitrogen	cat. # 12373-031	

Reagent Grade Type II water			
Gel apparatus			
Sequencing			
ABI PRISM BigDye Terminator Cycle Sequencing Kit	Applied Biosciences	cat. # 4337458	(25000 reactions)
Hi-Di Formamide	Applied Biosciences	cat. # 4311320	
Sodium Acetate (NaOAc)	Sigma-Aldrich	cat. # S-2889	
EDTA	Sigma-Aldrich	Cat.# 03690-100ML	0.5M, pH=8.0
TRIZMA Hydrochloride Buffer Solution	Sigma-Aldrich	cat. # T-2819	1 M, pH 9.0
Magnesium Chloride (MgCl ₂)	Sigma-Aldrich	cat. # M-1028	1 M
95% Ethanol			
Running Buffer (10X) with EDTA	Applied Biosystems	cat. # 4335613	500 mL
Polymer Pop-7 (25mL) Or Polymer Pop-7 (10mL)	Applied Biosystems	cat. # 44363929 or cat. # 4352759	
3700/3730 BigDye Terminator v3.1 Sequencing Standard Kit	Applied Biosciences	Cat# 4336943	
Thermocycler			
Centrifuge with plate holders			
3730xl DNA Analyzer	Applied Biosciences		