RT-PCR cloning of expressed VSGs

Harvesting cells

Starting material: 5–10x10^7 cells (BF)
Centrifuge cells at 4°C at 2,800 rpm and discard medium
Resuspend in 1ml of STAT-60
Freeze at -70°C (can be stored for at least 1 month).

RNA extraction

STAT-60 according to manufacturer's instructions.
Resuspend RNA in 50 µl of DEPC-treated water.
Measuring the A_{260} will suggest a yield of ~ 100 µg RNA from 10^8 cells. This measurement is probably inaccurate, but it is the basis for the amounts indicated below, in the next step.

cDNA synthesis

Stratagene kit: StrataScript™ First Strand Synthesis System (Cat# 200420)

	V / uL
RNA (5-10ug)	x
H20 - DEPC	38-x
PolyT primer	3
65C, 5 minutes	
Cool to RT for 10min	
10x First strand buffer	5
RNase Inhibitor	1
dNTPs 100mM	2
Reverse Transcriptase	1
42C, 1hr	
90C, 5min	

PCR amplification (The products will be 1.5–2.0 kb, depending upon the VSG)

	1	12
Template (cDNA)	2	-
Oligo 54 (Splice Leader)	1	12
Oligo 42 (VSG all)	1	12
dNTP 10mM	2.5	30
10x Buffer HF+MgCL2	2.5	30
H2O	15.25	183
Taq (rTAQ)	0.75	9

Use 'Expand High Fidelity PCR System' (Roche # 1-732-650) Taq polymerase.

PROGRAM

1	94C, 5'	1x
2	94C, 30" / 40C, 30" / 72C, 2'00"	30x
3	72C, 10'	1x

Oligo 54 (spliced leader) 5´-gactAGTTTCTGTACTAT-3´ (*Spe*I site)
Oligo 42 (VSG all) 5´-ccgggtaccGTGTTAAAATATATC-3´ (*Kpn*I site)
Clone into a T/A PCR-product-cloning vector.