

Appendix 1

Retroviral Vector Production and Qualification

All the retroviral vectors used on this study will be manufactured using a transient transfection procedure whereby three plasmids will be cotransfected into 293T cells. The three plasmids used for transfection will include: 1) a plasmid encoding the ecotropic Moloney murine leukemia virus (MoMLV) envelope (allows for efficient infection of mouse cells), 2) a plasmid encoding the MoMLV gagpol (expresses the core structural and enzymatic proteins required to make the nucleocapsid and matrix of the retroviral vector), and 3) a plasmid encoding the vector genome. Empty vector particles will be generated in a manner analogous to above with the exception of omitting the vector genome-encoding plasmid.

The contract laboratory will use a qualified Master Cell Bank (MCB) for the source of 293T cells. The MCB will be tested and will need to pass pre-set specifications for the following:

- Absence of Aerobic and anerobic bacteria and fungi
- Absence of Mycoplasma (culture and vero indicator cells)
- In vitro adventitious virus testing (NIH 3T3, MRC-5, and Vero cells with hemagglutination and hemadsorption on red blood cells from three species)
- In vivo adventitious virus testing
- Bovine and porcine viral testing
- Transmission EM (lack of virus particles)
- Absence of RCR for ecotropic and other murine retroviruses (NIH 3T3 marker rescue and S+L-/PG-4)
- ADA isoenzyme to confirm human origin
- Absence of Human viral contaminants (PCR for HTLV I/II, HIV 1/2, Hepatitis B and C, HHV-6, 7, and 8, CMV, EBV)
- SV40 T antigen
- E1A

In addition, a set of qualification tests will be performed on each vector to certify them as suitable (both from a point of safety and transduction efficiency) for the NTP study (Table 2).

Table 2. Qualification testing of retroviral vectors and “empty vector particles”

Test or specification	Test done on Retroviral Vectors (LTR and SIN)	Test done on Empty Vector
Absence of aerobic and anaerobic bacteria and fungi	Yes	Yes
Absence of Mycoplasma (culture assay and vero indicator cells)	Yes	Yes
In vitro adventitious virus testing	Yes	Yes
Endotoxin, ≤ 0.12 EU/ml	Yes	Yes
Replication competent retrovirus testing on End of Production cells and on supernatant (NIH 3T3 marker rescue)	Yes	Yes
General Safety	Yes	Yes
Residual DNA packaging cell and plasmid DNA sequences	Yes	Yes
Infectious Titer by FACS	By ds-Red expression	Vector titer determined by infection inhibition assay
Vector DNA sequence in transduced target cells	Yes	No

The hematopoietic stem cells will also be subject to limited testing prior to release for use in the animal studies, with further characterization tests post-release to determine the transduction efficiency:

For Release:

Stat gram stain (negative)

Endotoxin ≤ 0.12 EU/ml

Post-release:

Negative for mycoplasma by PCR

Absence of aerobic and anaerobic bacteria and fungi

Transduction efficiency (ds-Red vector titer)

Analysis of CFU from transduced target cells

Cryopreserve 2% of transduced cells for archive

