

Materials List for:

## Amplification, Next-generation Sequencing, and Genomic DNA Mapping of Retroviral Integration Sites

Erik Serrao<sup>1</sup>, Peter Cherepanov<sup>2</sup>, Alan N. Engelman<sup>1</sup>

<sup>1</sup>Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute

Correspondence to: Alan N. Engelman at alan\_engelman@dfci.harvard.edu

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## **Materials**

| Name                            | Company                   | Catalog Number | Comments   |
|---------------------------------|---------------------------|----------------|--|
| DMEM                            | Gibco                     | 11965-084      | Standard cell culture medium, compatible with HEK293T cells  |
| Fetal Bovine Serum              | Thermo Scientific         | SH 30088.03    | Different lots of serum may need to be pre-screened for optimal viral production                                     |
| Penicillin/Streptomycin         | Corning                   | 30-002-CI      | Antibiotics to be added to DMEM  |
| Phosphate-Buffered saline       | Mediatech                 | 21-040-CV      | Used to wash cells   |
| Trypsin EDTA                    | Corning                   | 25-053-CI      | Used to detach adherent cells from tissue culture plates   |
| PolyJet                         | SignaGen Laboratories     | SL100688       | DNA transfection reagent   |
| 0.45 μm Filters                 | Thermo Scientific         | 09-740-35B     | Used to filter virus particle-<br>containing cell culture media  |
| Turbo DNase                     | Ambion                    | AM2239         | Used to degrade carryover plasmid DNA from virus stocks  |
| HIV-1 p24 Antigen Capture Assay | ABL Inc.                  | 5447           | Used to quantify yield of virus production   |
| DNeasy Blood & Tissue Kit       | Qiagen                    | 69506          | Used to purify genomic DNA from cells  |
| Sonicator                       | Covaris                   | S2             | With this model of sonicator perform two rounds of duty cycle, 5%; intensity, 3; cycles per burst, 200; time, 80 sec |
| Nuclease-Free Water             | GeneMate                  | G-3250-125     | Commercially-available water is recommended to reduce the possibility of sample cross-contamination                  |
| QIAQuick PCR Purification Kit   | Qiagen                    | 28106          | Used to purify DNA during library construction   |
| End-It DNA End-Repair Kit       | Epicentre                 | ER81050        | Used to repair DNA ends of sonicated DNA samples   |
| Klenow Fragment (3'-5' exo-)    | New England Biolabs (NEB) | M0212S         | Used with dATP to A-tail repaired DNA fragments  |
| dATP                            | Thermo Scientific         | R0141          | Deoxyadenosine triphosphate  |
| Msel                            | NEB                       | R0525L         | Restriction endonuclease for genomic DNA cleavage  |
| BgIII                           | NEB                       | R0144L         | Restriction endonuclease to suppress amplification of upstream HIV-1 U5 sequence                                     |
| T4 DNA Ligase                   | NEB                       | M0202L/6218    | Enzyme for covalent joining of compatible DNA ends   |

<sup>&</sup>lt;sup>2</sup>Chromatin Structure and Mobile DNA, The Francis Crick Institute

| DNA Oligonucleotides       | Integrated DNA Technologies | custom        | Have the company purify the oligos. HPLC purification suffices for DNAs <30 nucleotides; PAGE purify longer DNAs          |
|----------------------------|-----------------------------|---------------|---|
| Advantage 2 Polymerase Mix | Clontech                    | 639202        | Commercial mix containing DNA polymerase for PCR  |
| dNTPs (100 mM solutions)   | Thermo Scientific           | R0181         | Dilute the four chemicals on ice with sterile water to reach the intermediate worrking concentrations of 2.5 mM each dNTP |
| NanoDrop                   | Thermo Scientific           | NanoDrop 2000 | Spectrophotometer for determination of DNA concentration  |
| Qubit Fluorimeter          | Life Technologies           | Qubit® 3.0    | Fluorometer used to confirm integration site library DNA concentration  |
| 2200 TapeStation System    | Agilent                     | G2964AA       | Tape-based assay to confirm integration site library DNA size distribution  |
| MiSeq                      | Illumina                    | SY-410-1003   | Used for NGS  |