

Materials List for:

An Efficient *In Vitro* Transposition Method by a Transcriptionally Regulated Sleeping Beauty System Packaged into an Integration Defective Lentiviral Vector

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Materials

| Name | Company | Catalog Number | Comments |
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| Dulbecco's modified Eagle's medium with 4.5g/L glucose w/o L-Glutamine | Lonza | BE12-614F | Cell culture medium. |
| HyClone Fetal Bovine Serum (U.S.), Characterized | GE Healthcare Life Sciences | SH30071.03 | Serum used HEK293T cell culture for lentiviral particle production. |
| Penicillin 10.000 UI/ml Streptomycin 10.000 UI/ml | Lonza | DE17-602E | Reagents for cell culture medium. |
| L-Glutamine 200mM | Lonza | BE17-605E | Reagent for cell culture medium. |
| Sodium Chloride (NaCl) | Sigma-Aldrich | S7653 | Reagent for HBS 2X preparation. |
| HEPES Buffer, 1M Stock in normal saline | Lonza | BE17-737E | Reagent for HBS 2X preparation. |
| Disodium hydrogen phosphate dihydrate (Na ₂ HPO ₄ ·2H ₂ O), 99.5% | Merck | 106580 | Reagent for HBS 2X preparation. |
| Hydrochloric acid - ACS reagent 37% (HCl) | Sigma-Aldrich | 320331 | Reagent for HBS 2X preparation. |
| Sterile water for injection | Fresenius Kabi | - | Reagent for HBS 2X preparation. |
| 0.45 µm PESS filter | Whatman | 10462100 | Filters used to remove cell debris from viral supernatant. |
| Polyallomer Beckman tubes | Beckman Coulter | 326823 | Tubes for concentrating IDLV vectors by ultracentrifugation. |
| PBS-1X w/o Ca, Mg | Lonza | BE17-516F | Buffer for cell wash and lentiviral particle resuspension. |
| Bovine Serum Albumin (BSA) | Sigma-Aldrich | A2153 | (Optional) Buffer for lentiviral particle resuspension. |
| HIV-1 Gag p24 immunocapture kit | Perkin Elmer | NEK50001KT | Kit for IDLV particle titration. |
| Polybrene | Sigma-Aldrich | 107689 | Reagent to enhance transduction efficiency. |
| Trypsin (10X) 2.5% in HBSS w/o Ca, Mg | GIBCO | BE17-160E | Reagent to detach HeLa cells (stock solution, to be diluted). |
| Ethylenediaminetetraacetic acid disodium salt (EDTA) | 100938B | AnalaR BDH | Reagent for trypsin working solution preparation. |
| 0.5% Trypsin-EDTA, no phenol red (10X) | GIBCO | 15400-054 | Reagent to detach keratinocytes (stock solution, to be diluted). |
| Donor Bovine Serum, New Zealand Origin | GIBCO | 16030-074 | Serum for Swiss mouse 3T3-J2 cell culture (feeder layer). |
| Ham's F12 media | GIBCO | 21765 | Medium for keratinocytes. |
| Fetal bovine serum | Lonza | DE14-801F | Serum for HeLa cell culture medium. |

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| Fetal Bovine Serum, qualified, Australia Origin | GIBCO | 10099-141 | Serum for human primary keratinocyte culture medium. |
| Insulin from bovine pancreas | Sigma-Aldrich | I5500 | Reagents for keratinocyte growth. |
| Adenine | VWR | 1152-25 | Reagents for keratinocyte growth. |
| Hydrocortisone | VWR | 3867-1 | Reagents for keratinocyte growth. |
| Cholera Toxin from Vibrio cholerae | Sigma-Aldrich | C8052 | Reagents for keratinocyte growth. |
| Triiodothyronine | Sigma-Aldrich | T5516 | Reagents for keratinocyte growth. |
| Human EGF | Austral Biological | GF-010-9 | Reagents for keratinocyte growth. |
| Mouse monoclonal APC-conjugated Anti-Feeder antibody | Miltenyi Biotech | 130-096-099 | To label feeder layer. |
| RNeasy Plus Mini Kit | Qiagen | 74134 | RNA purification kit. |
| SuperScript III Reverse Transcriptase | Life Technologies | 18080051 | Reverse transcriptase kit. |
| Deoxynucleotide Mix, 10 mM (dNTP) | Sigma-Aldrich | D7295 | Reagent for semi-quantitative PCR. |
| UltraPure DNase/RNase-Free Distilled Water | (any) | - | Reagent for semi-quantitative PCR and for qRT-PCR. |
| GoTaq G2 Hot Start Polymerase | Promega | M740B | Taq polymerase. |
| Agarose, for molecular biology | Sigma-Aldrich | A9539 | Reagent for agarose gel electrophoresis. |
| UltraPure TBE Buffer, 10X | Invitrogen | 15581028 | Reagent for agarose gel electrophoresis, to be dilute (1X) in ddH ₂ O. |
| Ethidium bromide | Sigma-Aldrich | E1510 | Reagent for agarose gel electrophoresis. |
| Doxycycline | Sigma-Aldrich | D-9891 | drug to induce transposase expression in HeLa cells and in keratinocytes |
| TaqMan Universal PCR Master Mix | Applied Biosystem | 4304437 | TaqMan Universal PCR Master Mix including primers and probe. |
| 15mL High Clarity polypropylene Centrifuge Tube, Conical Bottom, with Dome Seal Screw Cap, Sterile | Falcon Corning | 352096 | Tubes for cell collection and vector dilution preparation. |
| 150 mm TC-Treated Cell Culture Dish with 20 mm Grid, Sterile | Falcon Corning | 353025 | Cell culture dish for viral production. |
| 6 Well Clear Flat Bottom TC-Treated Multiwell Cell Culture Plate, with Lid, Individually Wrapped, Sterile | Falcon Corning | 353046 | Cell culture plate for HeLa and human primary keratinocyte transduction. |
| Safe-Lock microcentrifuge tubes, volume 1.5 mL | Eppendorf | 0030 120.086 | Tubes for dilution preparation. |
| Safe-Lock microcentrifuge tubes, volume 2 mL | Eppendorf | 0030 120.094 | Tubes for dilution preparation. |
| 0.2ml PCR Tubes with flat caps, RNase, DNase, DNA and PCR Inhibitor free | (any) | - | Tubes for semi-quantitative PCR. |
| MicroAmp Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL | Applied Biosystem | 4346906 | 96-well for qRT-PCR. |
| 5mL Round Bottom Polystyrene Tube, without Cap | BD Falcon | 352052 | Tubes for flow cytometry acquisition. |
| Disposable Serological Pipets, Polystyrene, Individually Wrapped, Sterile (different volumes) | (any) | - | Pipettes for sterile tissue culture applications. |

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| Filter Tips, Sterile, and RNase, DNase, DNA and Pyrogen free (different volumes) | (any) | - | Filter tips for sterile tissue culture and molecular biology applications. |
| pH meter | (any) | - | Equipment for measurement of HBS 2X pH. |
| PCR Thermal cycler | (any) | - | Equipment for semi-quantitative PCR. |
| Agarose gel electrophoresis equipment | (any) | - | Equipment for agarose gel electrophoresis of semi-quantitative PCR. |
| BD FACS Canto II 2LSR 4/2 | BD | - | Flow cytometer. |
| 7900HT Fast Real-Time PCR system | Applied Biosystem | 7900HT | Equipment for qRT-PCR. |
| Benchtop centrifuges for efficient sample processing in cell culture applications, refrigerated, with microplate buckets. | (any) | - | Centrifuge for cell culture processing and spinoculation. |
| Optima L-90K Ultracentrifuge equipped with SW32TI rotor | Beckman Coulter | - | Ultracentrifuge for lentiviral particle production. |
| Equipment for cell culture and molecular biology laboratory (e.g., Class II laminar flow hood designed for work involving BSL2 materials, cell culture incubator, refrigerators and freezers (+4 °C, -20 °C, -80 °C), micropipettes and pipet-aid). | | | |
| Protective laboratory coats, gloves, face protection in accordance with Institute rules and regulations, in particular for lentiviral particle production and transduction. | | | |