

Materials List for:

Transcription Start Site Mapping Using Super-low Input Carrier-CAGE

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Materials

| Name | Company | Catalog Number | Comments |
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| 2-propanol, Bioultra, for molecular biology, ≥99.5% | Sigma-Aldrich | 59304-100ML-F | Used in RNAClean XP purification. |
| 3' linkers | | | Sequences are described in Murata et al. 2014 and Supplementary Table 1 of this manuscript. Annealing of strands to produce 3'linkers is described in the supplementary of this protocol. |
| 5' linkers | | | Sequences are described in Murata et al. 2014 and Supplementary Table 1 of this manuscript. Annealing of strands to produce 5'linkers is described in the supplementary of this protocol. |
| Agencourt AMPure XP, 60 mL | Beckman Coulter | A63881 | Purification of DNA |
| Agencourt RNAClean XP Kit | Beckman Coulter | A63987 | Purification of RNA and RNA:cDNA hybrids in CAGE steps. |
| Axygen 0.2 mL Polypropylene PCR Tube Strips and Domed Cap Strips | Axygen (available through Corning) | PCR-0208-CP-C | Or any 8-tube PCR strips (used only for water and mixes). |
| Axygen 1 x 8 strip domed PCR caps | Axygen (available through Corning) | PCR-02CP-C | Caps for PCR plates. |
| Axygen 1.5 mL Maxymum Recovery Snaplock Microcentrifuge Tube | Axygen (available through Corning) | MCT-150-L-C | Low-binding 1.5 mL tubes, used for enzyme mixes or sample concentration. |
| Axygen 96 well no skirt PCR microplate | Axygen (available through Corning) | PCR-96-C | Low-binding PCR plates - have to be used for all steps in the protocol. Note that plates should be cut to contain 2 x 8 wells for easier visibility of the samples |
| Bioanalyzer (or Tapestation): RNA nano and HS DNA kits | Agilent | | To determine quality of RNA, efficient size selection and final quality of the library (Tapestation can also be used) |
| Biotin (Long Arm) Hydrazide | Vector laboratories | SP-1100 | Biotinylation/tagging |
| Cutsmart buffer | NEB | | Restriction enzyme buffer |
| Deep Vent (exo-) DNA Polymerase | NEB | M0259S | Second strand synthesis |
| DNA Ligation Kit, Mighty Mix | Takara | 6023 | Used for 5' and 3'-linker ligation |
| dNTP mix (10 mM each) | ThermoFisher Scientific | 18427013 | dNTP mix for production of carrier templates (or any dNTPs suitable for PCR) |
| Dynabeads M-270 Streptavidin | Invitrogen | 65305 | Cap-trapping. Do not use other beads as these are optimised with the buffers used. |

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| DynaMag-2 Magnet | ThermoFisher Scientific | 12321D | Magnetic stand for 1.5 mL tubes - used to prepare Streptavidin beads. |
| DynaMag-96 Side Skirted Magnet | ThermoFisher Scientific | 12027 | Magnetic stand for PCR plates (96 well-plates) - used with cut plates to contain 2 x 8 wells. |
| Ethanol, BioUltra, for molecular biology, ≥99.8% | Sigma-Aldrich | 51976-500ML-F | Used in AMPure washes. Any molecular biology suitable ethanol can be used. |
| Exonuclease I (<i>E. coli</i>) | NEB | M0293S | Leftover primer degradation |
| Gel Loading Dye, Purple (6x), no SDS | NEB | B7025S | agarose gel loading dye |
| HiScribe T7 High Yield RNA Synthesis Kit | New England Biolabs | E2040S | Kit for carrier in vitro transcription |
| Horizontal electrophoresis apparatus | | | purification of carrier DNA templates from agarose gels |
| I-Ceu | NEB | R0699S | Homing endonuclease used for carrier degradation. |
| I-SceI | NEB | R0694S | Homing endonuclease used for carrier degradation. |
| KAPA HiFi HS ReadyMix (2x) | Kapa Biosystems (Supplied by Roche) | KK2601 | PCR mix for target library amplification |
| KAPA SYBR FAST qPCR kit (Universal) 2x | Kapa Biosystems (Supplied by Roche) | KK4600 | qPCR mix to assess degradation efficiency and required number of PCR amplification cycles |
| Micropipettes and multichannel micropipettes (0.1-10 µL, 1-20 µL, 20-200 µL) | Gilson | | Use of Gilson with the low-binding Sorenson tips is recommended. Other micropipettes might not be compatible. Different brand low-binding tips may not be of equal quality and may increase sample loss. |
| Microplate reader | | | For Picogreen concentration measurement of the final library. Microplates are used to allow small volume measurement and reduce sample waste. |
| nuclease free water | ThermoFisher Scientific | AM9937 | Or any nuclease (DNase and RNase) free water |
| PCR thermal cycler | | | incubation steps and PCR amplification |
| Phusion High-Fidelity DNA Polymerase | ThermoFisher Scientific | F530S | DNA polymerase for amplification of carrier templates (or any high fidelity polymerase) |
| QIAquick Gel Extraction Kit (50) | Qiagen | 28704 | Purification of carrier PCR templates from agarose gels. |
| qPCR machine | | | determining PCR amplification cycle number and degree of carrier degradation |
| Quant-iT PicoGreen dsDNA Reagent | ThermoFisher Scientific | P11495 | Used to measure final library concentration - recommended as, in our hands, it is more accurate and reproducible than Qubit. |
| Quick-Load Purple 100 bp DNA Ladder | NEB | N0551S | DNA ladder |
| Quick-Load Purple 1 kb Plus DNA Ladder | NEB | N0550S | DNA ladder |

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| Ribonuclease H | Takara | 2150A | Digestion of RNA after cap-trapping. |
| RNase ONE Ribonuclease | Promega | M4261 | Degradation of single stranded RNA not protected by cDNA. |
| RNase-Free DNase Set | Qiagen | 79254 | Removal of carrier DNA templates after in vitro transcription. |
| RNeasy Mini Kit | Qiagen | 74104 | For cleanup of carrier RNA from in vitro transcription or capping |
| Sodium acetate, 1 M, aq.soln, pH 4.5 RNase free | VWR | AAJ63669-AK | Or any nuclease (DNase and RNase) free solution |
| Sodium acetate, 1 M, aq.soln, pH 6.0 RNase free | | | Or any nuclease (DNase and RNase) free solution |
| Sodium periodate | Sigma-Aldrich | 311448-100G | Oxidation of vicinal diols |
| Sorenson low binding aerosol barrier tips, MicroReach Guard, volume range 10 µL, Graduated | Sorenson (available through SIGMA-ALDRICH) | Z719390-960EA | Low-binding tips - recommended use throughout the protocol to minimise sample loss. |
| Sorenson low binding aerosol barrier tips, MultiGuard, volume range 1,000 µL, Graduated | Sorenson (available through SIGMA-ALDRICH) | Z719463-1000EA | Low-binding tips - recommended use throughout the protocol to minimise sample loss. |
| Sorenson low binding aerosol barrier tips, MultiGuard, volume range 20 µL, Graduated | Sorenson (available through SIGMA-ALDRICH) | Z719412-960EA | Low-binding tips - recommended use throughout the protocol to minimise sample loss. |
| Sorenson low binding aerosol barrier tips, MultiGuard, volume range 200 µL, Graduated | Sorenson (available through SIGMA-ALDRICH) | Z719447-960EA | Low-binding tips - recommended use throughout the protocol to minimise sample loss. |
| SpeedVac Vacuum Concentrator | | | concentrating samples in various steps to lower volume |
| SuperScript III Reverse Transcriptase | ThermoFisher Scientific | 18080044 | Used for reverse transcription (1st CAGE step) |
| Trehalose/sorbitol solution | | | Preparation is described in Murata et al. 2014. |
| Tris-HCl, 1 M aq.soln, pH 8.5 | | | 1 M solution, DNase and RNase free |
| tRNA (20 mg/mL) | | | tRNA solution. Preparation is described in Murata et al. 2014. |
| UltraPure Low Melting Point Agarose | ThermoFisher Scientific | 16520050 | Or any suitable pure low-melt agarose. |
| USB Shrimp Alkaline Phosphatase (SAP) | Applied Biosystems (Provided by ThermoFisher Scientific) | 78390500UN | |
| USER Enzyme | NEB | M5505S | Degradation of 3'linker's upper strand, Uracil Specific Excision Reagent/Enzyme |
| Vaccinia Capping System | NEB | M2080S | Enzymatic kit for in vitro capping of carrier molecules |
| Wash buffer A | | | Cap trapping washes. Preparation is described in Murata et al. 2014. |
| Wash buffer B | | | Cap trapping washes. Preparation is described in Murata et al. 2014. |
| Wash buffer C | | | Cap trapping washes. Preparation is described in Murata et al. 2014. |