

Materials List for:

### 3' End Sequencing Library Preparation with A-seq2

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#### Materials

| Name   | Company                  | Catalog Number        | Comments  |
|--|--------------------------|-----------------------|---|
| <b>Materials</b>                                     |                          |                       |   |
| Agarose, ultra pure                                  | Invitrogen               | 16500-500             |   |
| 2100 Bioanalyzer                                     | Agilent                  | G2940CA               |   |
| Cordycepin triphosphate (3' dATP)                    | SIGMA                    | C9137                 |   |
| DNA low bind vials, 1.5 ml                           | Eppendorf                | 22431021              |   |
| Dulbecco's Phosphate Buffered Saline                 | SIGMA                    | D8637                 |   |
| Dynabeads mRNA-DIRECT Kit                            | Ambion                   | AM61012               |   |
| GR-Green dye   | Excellgen                | EG-1071               | use 1:10,000 dilution   |
| HiSeq 2500 or NextSeq 500 next generation sequencers | Illumina                 | inquire with supplier |   |
| KAPA HiFi Hotstart DNA polymerase mix                | KAPA/Roche               | KK2602                |   |
| Nuclease free water                                  | Ambion                   | AM9937                |   |
| Poly(A) polymerase, yeast                            | Thermo Fisher Scientific | 74225Z25KU            |   |
| Poly(A) polymerase, E.coli                           | New England Biolabs      | M0276L                |   |
| Polynucleotide kinase                                | Thermo Fisher Scientific | EK0032                |   |
| QIAEX II Gel Extraction Kit                          | Qiagen                   | 20021                 |   |
| QIAquick PCR Purification Kit                        | Qiagen                   | 28104                 |   |
| QIAquick Gel Extraction Kit                          | Qiagen                   | 28704                 |   |
| RNA ligase 1, high concentration                     | New England Biolabs      | M0437M                | includes PEG-8000   |
| RNeasy MinElute RNA Cleanup kit                      | Qiagen                   | 74204                 |   |
| RNase H  | New England Biolabs      | M0279                 |   |
| RNasin Plus, ribonuclease inhibitor                  | Promega                  | N2618                 |   |
| Superscript IV reverse transcriptase                 | Thermo Fisher Scientific | 18090050              |   |
| Turbo DNase  | Ambion                   | AM2238                |   |
| USER enzyme mix                                      | New England Biolabs      | M5505                 |   |
| Dyna-Mag-2 magnetic rack                             | Thermo Fisher Scientific | 12321D                |   |
| Thermomixer C  | Eppendorf                | 5382000015            | Heated mixer with heated lid  |
| MicroSpin columns                                    | GE-Healthcare            | 27-5325-01            |   |
| <b>Name</b>  | <b>Company</b>           | <b>Catalog Number</b> | <b>Comments</b>   |
| <b>Buffers</b>                                       |                          |                       |   |
| Alkaline hydrolysis buffer, 1.5 x                    |                          |                       | Mix 1 part 0.1 M Na <sub>2</sub> CO <sub>3</sub> and 9 parts 0.1 M NaHCO <sub>3</sub> . Add EDTA to 1 mM. Adjust pH to 9.2. Store aliquots at -20 °C. |

| 5x poly(A) polymerase buffer   | Thermo Fisher Scientific |                | 100 mM Tris-HCl, pH 7.0, 3 mM MnCl <sub>2</sub> , 0.1 mM EDTA, 1 mM DTT, 0.5 mg/ml acetylated BSA, 50% glycerol   |
|--|--------------------------|----------------|---|
| Biotin binding buffer  |                          |                | 20 mM TrisCl pH 7.5, 2 M NaCl, 0.1% NP40  |
| TEN buffer   |                          |                | 10 mM TrisCl, pH 7.5, 1 mM EDTA, 0.02% NP40   |
| Name   | Company                  | Catalog Number | Sequence  |
| <b>Oligonucleotides according to Illumina TruSeq Small RNA Sample Prep Kits, for GA-IIx and HiSeq2000/2500 sequencers</b>        |                          |                |   |
| revRA3 (RNA)   | Microsynth               |                | 5' amino-CCUUGGCACCCGAGAAUCCA-3'  |
| revDA5   | Microsynth               |                | 5' amino-GTTCAGAGTTCTACAGTCCGACGATCNNNN-3'  |
| Bio-dU-dT25, RT primer   | Microsynth               |                | 5' Biotin-TTTTTTTTTTTTTTTTTTTTTTTT-dU-TTTVN 3' (V = G, A or C)  |
| PCR primer forward, RP1  | Microsynth               |                | 5' AATGATACGGCGACCACCGAGA TCTACACGTTCTACAGTTCTACAG TCCGA 3'   |
| PCR primer reverse, RPI1, barcode in bold  | Microsynth               |                | 5' CAAGCAGAAGACGGCATAACGAG AT <b>CGTGAT</b> GTGACTGGAGTTCCCT TGGCACCCGAGAATTCCA 3'  |
| Name   | Company                  | Catalog Number | Comments  |
| <b>Oligonucleotides according to Illumina TruSeq HT-Small RNA Sample Prep Kits, for HiSeq2000/2500 and NextSeq500 sequencers</b> |                          |                |   |
| HT-rev3A (DNA/RNA)   | Microsynth               |                | 5'-amino-GTGACTGGAGTTCAGACGTGTGCTCTTCCrGrAUrC-3'  |
| HT-rev5A   | Microsynth               |                | 5' amino-ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNN 3'  |
| Bio-dU-dT25, RT primer   | Microsynth               |                | 5' Biotin-TTTTTTTTTTTTTTTTTTTTTTTT-dU-TTTVN 3'  |
| PCR primers forward (D501-506)   | Microsynth or Illumina   |                | 5'-AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTCCCTACACGACGCTCTTCCGATCT -3'  |
| PCR primers reverse (D701-D712)  | Microsynth or Illumina   |                | 5'-CAAGCAGAAGACGGCATAACGAG A[i7]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-3'  |
| Documentation for Illumina multiplexing:   | Illumina                 |                | <a href="https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences_100000002694-01.pdf">https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences_100000002694-01.pdf</a> |