

Materials List for

# AQRNA-seq for Quantifying Small RNAs

Ruixi Chen<sup>1</sup>, Daniel Yim<sup>2</sup>, Peter C. Dedon<sup>1,2</sup>

<sup>1</sup>Department of Biological Engineering, Massachusetts Institute of Technology <sup>2</sup>Singapore-MIT Alliance for Research and Technology Antimicrobial Resistance Interdisciplinary Research Group

## Corresponding Author

Peter C. Dedon  
pcdedon@mit.edu

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## Materials

| Name                                  | Company                 | Catalog Number                   | Comments                                                                                                                                                                      |
|---------------------------------------|-------------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2-ketoglutarate                       | Sigma-Aldrich           | 75890                            | Prepare a working solution (1 M) and store it at -20°C                                                                                                                        |
| 2100 Bioanalyzer Instrument           | Agilent                 | G2938C                           |                                                                                                                                                                               |
| Adenosine 5'-Triphosphate (ATP)       | New England Biolabs     | M0437M (component #: N0437AVIAL) | NEB M0437M contains T4 RNA Ligase 1 (30 U/μL), T4 RNA Ligase Reaction Buffer (10X), PEG 8000 (1X), and ATP (100 mM); prepare a working solution (10 mM) and store it at -20°C |
| AGAROSE GPG/LE                        | AmericanBio             | AB00972-00500                    | Store at ambient temperature                                                                                                                                                  |
| Ammonium iron(II) sulfate hexahydrate | Sigma-Aldrich           | F2262                            | Prepare a working solution (0.25 M) and store it at -20 °C                                                                                                                    |
| Bioanalyzer Small RNA Analysis        | Agilent                 | 5067-1548                        | The Small RNA Analysis is used for checking the quality of input RNAs and the efficiency of enzymatic reactions (e.g., Linker 1 ligation)                                     |
| Bovine Serum Albumin (BSA; 10 mg/mL)  | New England Biolabs     | B9000                            | This product was discontinued on 12/15/2022 and is replaced with Recombinant Albumin, Molecular Biology Grade (NEB B9200).                                                    |
| Chloroform                            | Macron Fine Chemicals   | 4441-10                          |                                                                                                                                                                               |
| Demethylase                           | ArrayStar               | AS-FS-004                        | Demethylase comes with the rtStar tRNA Pretreatment & First-Strand cDNA Synthesis Kit (AS-FS-004)                                                                             |
| Deoxynucleotide (dNTP) Solution Mix   | New England Biolabs     | N0447L (component #: N0447LVIAL) | This dNTP Solution Mix contains equimolar concentrations of dATP, dCTP, dGTP and dTTP (10 mM each)                                                                            |
| Digital Dual Heat Block               | VWR Scientific Products | 13259-052                        | Heating block is used with the QIAquick Gel Extraction Kit                                                                                                                    |
| DyeEx 2.0 Spin Kit                    | Qiagen                  | 63204                            | Effective at removing short remnants (e.g., oligos less than 10 bp in length)                                                                                                 |
| Electrophoresis Power Supply          | Bio-Rad Laboratories    | PowerPac 300                     |                                                                                                                                                                               |
| Eppendorf PCR Tubes (0.5 mL)          | Eppendorf               | 0030124537                       |                                                                                                                                                                               |
| Eppendorf Safe-Lock Tubes (0.5 mL)    | Eppendorf               | 022363611                        |                                                                                                                                                                               |
| Eppendorf Safe-Lock Tubes (1.5 mL)    | Eppendorf               | 022363204                        |                                                                                                                                                                               |

|                                                    |                          |                                  |                                                                                                                                                                               |
|----------------------------------------------------|--------------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Eppendorf Safe-Lock Tubes (2 mL)                   | Eppendorf                | 022363352                        |                                                                                                                                                                               |
| Ethyl alcohol (Ethanol), Pure                      | Sigma-Aldrich            | E7023                            | The pure ethanol is used with the Oligo Clean and Concentrator Kit from Zymo Research                                                                                         |
| Gel Imaging System                                 | Alpha Innotech           | FluorChem 8900                   |                                                                                                                                                                               |
| Gel Loading Dye, Purple (6X), no SDS               | New England Biolabs      | N0556S (component #: B7025SVIAL) | NEB N0556S contains Quick-Load Purple 50 bp DNA Ladder and Gel Loading Dye, Purple (6X), no SDS                                                                               |
| GENESYS 180 UV-Vis Spectrophotometer               | Thermo Fisher Scientific | 840-309000                       | The spectrophotometer is used for measuring the oligo concentrations using the Beer's law                                                                                     |
| HEPES                                              | Sigma-Aldrich            | H4034                            | Prepare a working solution (1 M; pH = 8 with NaOH) and store it at -20 °C                                                                                                     |
| Hydrochloric acid (HCl)                            | VWR Scientific Products  | BDH3028                          | Prepare a working solution (5 M) and store it at ambient temperature                                                                                                          |
| Isopropyl Alcohol (Isopropanol), Pure              | Macron Fine Chemicals    | 3032-16                          | Isopropanol is used with the QIAquick Gel Extraction Kit                                                                                                                      |
| L-Ascorbic acid                                    | Sigma-Aldrich            | A5960                            | Prepare a working solution (0.5 M) and store it at -20°C                                                                                                                      |
| Microcentrifuge                                    | Eppendorf                | 5415D                            |                                                                                                                                                                               |
| NanoDrop 2000 Spectrophotometer                    | Thermo Fisher Scientific | ND-2000                          |                                                                                                                                                                               |
| NEBuffer 2 (10X)                                   | New England Biolabs      | M0264L (component #: B7002SVIAL) | NEB M0264L contains RecJf (30 U/ µL) and NEBuffer 2 (10X); store at -20 °C                                                                                                    |
| Nuclease-Free Water (not DEPC-Treated)             | Thermo Fisher Scientific | AM9938                           |                                                                                                                                                                               |
| Oligo Clean & Concentrator Kit                     | Zymo Research            | D4061                            | Store at ambient temperature                                                                                                                                                  |
| PEG 8000 (50% solution)                            | New England Biolabs      | M0437M (component #: B1004SVIAL) | NEB M0437M contains T4 RNA Ligase 1 (30 U/µL), T4 RNA Ligase Reaction Buffer (10X), PEG 8000 (1X), and ATP (100 mM); prepare a working solution (10 mM) and store it at -20°C |
| Peltier Thermal Cycler                             | MJ Research              | PTC-200                          |                                                                                                                                                                               |
| Phenol:chloroform:isoamyl alcohol 25:24:1 pH = 5.2 | Thermo Fisher Scientific | J62336                           |                                                                                                                                                                               |
| PrimeScript Buffer (5X)                            | TaKaRa                   | 2680A                            |                                                                                                                                                                               |
| PrimeScript Reverse Transcriptase                  | TaKaRa                   | 2680A                            |                                                                                                                                                                               |
| QIAquick Gel Extraction Kit                        | Qiagen                   | 28704                            | This kit requires a heating block and isopropanol to work with                                                                                                                |
| Quick-Load Purple 100 bp DNA Ladder                | New England Biolabs      | N0551S (component #: N0551SVIAL) |                                                                                                                                                                               |
| Quick-Load Purple 50 bp DNA Ladder                 | New England Biolabs      | N0556S (component #: N0556SVIAL) | NEB N0556S contains Quick-Load Purple 50 bp DNA Ladder and Gel Loading Dye, Purple (6X), no SDS                                                                               |
| RecJf (30 U/µL)                                    | New England Biolabs      | M0264L (component #: M0264LVIAL) | NEB M0264L contains RecJf (30 U/ µL) and NEBuffer 2 (10X); store at -20 °C                                                                                                    |
| RNase Inhibitor (murine; 40 U/µL)                  | New England Biolabs      | M0314L (component #: M0314LVIAL) | Store at -20 °C                                                                                                                                                               |
| SeqAMP DNA Polymerase                              | TaKaRa                   | 638509                           | TaKaRa 638509 contains SeqAMP DNA Polymerase and SeqAMP PCR Buffer (2X)                                                                                                       |
| SeqAMP PCR Buffer (2X)                             | TaKaRa                   | 638509                           | TaKaRa 638509 contains SeqAMP DNA Polymerase and SeqAMP PCR Buffer (2X)                                                                                                       |
| Shrimp Alkaline Phosphatase (1 U/ µL)              | New England Biolabs      | M0371L (component #: M0371LVIAL) |                                                                                                                                                                               |

|                                     |                     |                                  |                                                                                                                     |
|-------------------------------------|---------------------|----------------------------------|---------------------------------------------------------------------------------------------------------------------|
| Sodium hydroxide (NaOH)             | Sigma-Aldrich       | S5881                            | Prepare a working solution (5 M) and store it at ambient temperature                                                |
| T4 DNA Ligase (400 U/μL)            | New England Biolabs | M0202L (component #: M0202LVIAL) | NEB M0202L contains T4 DNA Ligase (400 U/μL) and T4 DNA Ligase Reaction Buffer (10X)                                |
| T4 DNA Ligase Reaction Buffer (10X) | New England Biolabs | M0202L (component #: B0202SVIAL) | NEB M0202L contains T4 DNA Ligase (400 U/μL) and T4 DNA Ligase Reaction Buffer (10X)                                |
| T4 RNA Ligase 1 (30 U/μL)           | New England Biolabs | M0437M (component #: M0437MVIAL) | NEB M0437M contains T4 RNA Ligase 1 (30 U/μL), T4 RNA Ligase Reaction Buffer (10X), PEG 8000 (1X), and ATP (100 mM) |
| T4 RNA Ligase Reaction Buffer (10X) | New England Biolabs | M0437M (component #: B0216SVIAL) | NEB M0437M contains T4 RNA Ligase 1 (30 U/μL), T4 RNA Ligase Reaction Buffer (10X), PEG 8000 (1X), and ATP (100 mM) |