

Consumables and Equipment

Check to make sure that you have all of the necessary user-supplied consumables and equipment before starting the TruSeq RNA Access Library Prep protocol.



NOTE

The TruSeq RNA Access Library Prep protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Table 6 User-Supplied Consumables

Consumable	Supplier
1.5 ml RNase/DNase-free non-sticky tubes	Life Technologies, part # AM12450
1.7 ml microcentrifuge tubes	General lab supplier
10 μ l barrier pipette tips	General lab supplier
10 μ l multichannel pipettes	General lab supplier
10 μ l single channel pipettes	General lab supplier
20 μ l barrier pipette tips	General lab supplier
20 μ l multichannel pipettes	General lab supplier
20 μ l single channel pipettes	General lab supplier
200 μ l barrier pipette tips	General lab supplier
200 μ l multichannel pipettes	General lab supplier
200 μ l single channel pipettes	General lab supplier
1000 μ l barrier pipette tips	General lab supplier
1000 μ l multichannel pipettes	General lab supplier
1000 μ l single channel pipettes	General lab supplier

Consumable	Supplier
96-well flat clear bottom black microplates Note: Used when quantifying samples with a SpectraMax M5 spectrofluorometer.	Corning, part # 3904
96-well storage plates, round well, 0.8 ml ("MIDI" plate)	Fisher Scientific, part # AB-0859
Adhesive seal roller	General lab supplier
Agencourt AMPure XP, 60 ml kit	Beckman Coulter, part # A63881/A63880
Aluminum foil	General lab supplier
Conical centrifuge tubes (15 ml or 50 ml)	General lab supplier
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
Hard-Shell 96-well PCR Plates ("HSP" plate)	Bio-Rad, part # HSP-9601
Microseal 'A' film	Bio-Rad, part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
Nuclease-free ultra pure water	General lab supplier
One of the following (for library quality control): <ul style="list-style-type: none"> • Standard Sensitivity NGS Fragment Analysis Kit, 1–6000 bp (500 samples) • DNA 1000 Kit 	<ul style="list-style-type: none"> • Advanced Analytical Technologies, part # DNF-473-0500 • Agilent Technologies, part # 5067-1504
RNase/DNase-free eight-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
RNaseZap (to decontaminate surfaces)	General lab supplier

Consumable	Supplier
SuperScript II Reverse Transcriptase	Invitrogen, part # 18064-014
Tris-HCl 10 mM, pH8.5	General lab supplier
Tween 20	Sigma, part # P7949
[Optional] 96-well 2 ml deep well plates (to aliquot reagents)	Thomson Instrument Company, part # 951652
[Optional] Amicon Ultra-0.5 centrifugal filter unit (0.5 ml, 30 kDa) Note: Used to concentrate a pooled library. Another option is to use a vacuum concentrator.	Millipore, part # UFC503008
[Optional] One of the following (for library quality assessment): <ul style="list-style-type: none"> • Standard Sensitivity NGS Fragment Analysis Kit, 1–6000 bp (500 samples) • High Sensitivity DNA Kit 	<ul style="list-style-type: none"> • Advanced Analytical Technologies, part # DNF-473-0500 • Agilent Technologies, part # 4067-4626

Table 7 User-Supplied Equipment

Equipment	Supplier
DNA Engine Multi-Bay Thermal Cycler See <i>Thermal Cyclers</i> on page 81.	<ul style="list-style-type: none"> • Bio-Rad, part # PTC-0240G or • PTC-0220G, with Alpha Unit, ALS-1296CC
Fluorometric quantitation with dsDNA binding dye reagents	General lab supplier

Equipment	Supplier
One of the following: <ul style="list-style-type: none"> • Fragment Analyzer Automated CE System • 2100 Bioanalyzer Desktop System 	<ul style="list-style-type: none"> • Advanced Analytical Technologies, part # FSv2-CE2 or FSv2-CE10 • Agilent Technologies, part # G2940CA
High-Speed Microplate Shaker	VWR, catalog # <ul style="list-style-type: none"> • 13500-890 (110 V/120 V or • 14216-214 (230 V)
Magnetic stand-96	Life Technologies, part # AM10027
Microcentrifuge	General lab supplier
Microplate centrifuge	General lab supplier
MIDI plate insert for heating system Note: Two inserts are recommended to support successive heating procedures.	Illumina, catalog # BD-60-601

Equipment	Supplier
<p>One of the following: Note: Two systems are recommended to support successive heating procedures.</p> <ul style="list-style-type: none"> • SciGene TruTemp Heating System • Hybex Microsample Incubator 	<ul style="list-style-type: none"> • Illumina, catalog # <ul style="list-style-type: none"> • SC-60-503 (115 V) or • SC-60-504 (220 V) • SciGene, catalog # <ul style="list-style-type: none"> • 1057-30-0 (115 V) or • 1057-30-2 (230 V)
QuantiFluor dsDNA System or similar fluorometric-based DNA quantification system	Promega, catalog # E2670
SpectraMax M5 spectrofluorometer or similar fluorometric-based DNA quantification system	Molecular Devices, part # 0112-0159
Stroboscope	General lab supplier
Vortexer	General lab supplier
[Optional] Vacuum concentrator Note: Used to concentrate a pooled library. Another option is to use Amicon Ultra-0.5 centrifugal filter units.	General lab supplier

Thermal Cyclers

The following table lists the recommended settings for the Illumina recommended thermal cycler, as well as other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the TruSeq RNA Access Library Prep protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate