SOP Title:

Illumina TruSeq Stranded Total RNA Sample Prep

1.0 Purpose and Scope

This standard operating procedure (SOP) is for the preparation of Illumina TruSeq Stranded Total RNA Sample libraries from FFPE and fresh frozen samples at PM-OICR TGL for use on Illumina sequencers.

The procedure uses the Illumina TruSeq Stranded Total RNA Sample Preparation Kits and includes the following steps: the removal of ribosomal RNA (rRNA), first and second strand cDNA synthesis, adenylation of 3' ends, ligation of adapters, PCR amplification, and library validation. The procedure requires 200ng of FFPE or fresh frozen total RNA.

Related document:

- Manufacturer-supplied protocol: *TruSeq® Stranded Total RNA Sample Preparation Guide* (Revision E, October 2013) link to document

This SOP follows instructions provided by the manufacturer with minor modifications.

Related TGL documents:

- YYYY_MM_DD_SAMPLE SUBMISSION FORM_PI_Lastname_Firstname_TGL
- SOP_Qubit_Assay_for_Nucleic_Acid_Quantification.doc
- 17_06_16_TruSeq_Stranded_Total_RNA_Lot_Tracking_Sheet
- SOP_TapeStation_4200_V1.0
- SOP_KAPA_Library_Quantification_Illumina_Platforms_V1.0.2_production
- truseq-library-prep-pooling-guide-15042173-01
- truseq-stranded-total-rna-sample-prep-ls-euc-ltf-15031060-e (lot control sheet)

2.0 Materials

Workspace		
Library prep room for all the steps prior to enrichment PCR amplification		
Post-PCR area for enrichment PCR amplification and all the subsequent steps		
Equipment and associated consumables		
Agilent	Agilent TapeStation 4200	
	High Sensitivity RNA ScreenTape (7 tapes,112 samples)	5067-5579
	High Sensitivity RNA ScreenTape Sample Buffer	5067-5580
	High Sensitivity RNA ScreenTape Ladder	5067-5581
	High Sensitivity D1000 Screen Tape (7 tapes,112 samples)	5067-5584
	High Sensitivity D1000 Reagents	5067-5585
Eppendorf	Vacufuge Plus	022820001
Various	Thermal cycler (i.e. Applied Biosystems Veriti, BioRad T100, etc)	
Medstore	PCR STRIP(8), 0.2ml NEUTRAL, ATT FLAT CAP,120/pk	72.991.002
ThermoFisher	Dynamag (magnetic rack)	12321D
	Dynamag-96 (96 well magnetic rack)	123331D
Reagent kits		
Illumina	TruSeq Stranded RNA LT Kit- 48 Samples cDNA Synthesis PCR (Component 15032611)	RS-122-2301 (Set A adapters) or RS-122-2302 (Set B adapters)
	TruSeq Stranded RNA LT Kit- 48 Samples Ribo-Zero Gold (Component 15032619)	
	TruSeq Stranded RNA LT Kit- 48 Samples Index Set A (Component 15032612)	
	TruSeq Stranded RNA LT Kit- 48 Samples Index Set B (Component 15032613)	
Medstore	SuperScript II Reverse Transcriptase (10000units, 200units/ul)	18064014
Cedarlane	AMPure XP beads	A36881 (60 ml)
ThermoFisher	Qubit dsDNA HS Assay Kit	Q32854
	Qubit Assay Tubes	Q32856
Commonly used reagents		
Medstore	Nuclease-free water (Medstore)	W4502-1L
Medstore/Greenfield Specialty Alcohols	Ethanol anhydrous 100% (brown bottle), case of 12X500ml	P006EAAN
Medstore/Greenfield Specialty Alcohols	Ethanol anhydrous (4X4L white jug, cleaning only!)	P016EAAN

3.0 General Guidance:

- When opening a new kit, record all lot numbers in TGL master lot tracking sheet (R:\Lab_tracking_forms\truseq-stranded-total-rna-sample-prep-ls-euc-ltf-15031060-e, associate all samples to the master lot tracking sheet using a letter key ("A", "B") in sample tracking sheet
- Before beginning work every day, wipe down all pipettes and bench surfaces with peroxide wipes, then wipe with 70% ethanol (made from 4L white jug ethanol)
- Do not mix and match reagents from multiple kits! Aliquot reagents where appropriate to minimize freeze thaw cycles, and indicate each thaw with a dot on the top of the respective tube, especially first strand mix + SSII mix (section 2, first strand synthesis)
- Reagents/enzymes may be thawed and then placed on ice or in 4°C fridge until ready for use unless otherwise indicated
- rRNA Removal Beads and AMPure XP beads must be allowed to reach room temperature before use (30 mins at room temp)
- 80% ethanol wash solutions should be made fresh every day using molecular grade H_20 and anhydrous ethanol (brown bottle only!). Always use private ethanol and H_20 aliquots to minimize risk of contamination between technicians.
- Be sure beads are thoroughly bound to magnetic rack when washing, loss of beads will reduce diversity
- Wet beads that contain residual ethanol prior to elution in water or RSB will interfere with reactions (beads should almost appear to be cracking from dryness)
- Be sure not to carry over beads after elution. If beads do carryover, bind to magnetic rack and transfer again to fresh strip tube
- Use a 10ul pipette tip to remove residual liquid before adding 80% ethanol wash to Ampure XP beads. This will significantly reduce contaminating adapter in FFPE samples (ligation and PCR cleanup steps), and ensure optimal enzyme reactions occur.
- Record RINS, DV200 scores, quants, dilutions, and other QC information in individual sample sheets
- Enzyme solutions should be 'flick' mixed and briefly spun down prior to use, buffers should be vortexed and spun down,
- Reactions in strip tubes should be briefly spun to collect material at bottom of reaction well, especially before and after thermocycler incubations, and/or before Ampure XP additions (minifuge)

- Record all QC steps in original sample submission sheet, not running sample sheet, including MISO LIMS IDs, LIB and LDI #s, master lot tracking references, protocol version and tech performing prep/QC step in sample sheet
- Note use Ampure XP beads for all steps, use 80% ethanol for all wash steps
- Record a "hash mark" on kit box to indicate # of reactions used, and which index was used (limiting adapter in kit)
- Record all unusual observations, errors, or other issues in sample sheet

4.0 Procedure

Qubit material to verify concentration using Qubit HS RNA Assay kit and following SOP_Qubit_Assay_for_Nucleic_Acid_Quantification.docx. If sample concentration is excessively high, dilute stock solution and re-qubit to a reasonable concentration.

Run Agilent High Sensitivity RNA Tapestation and record DV200 and RIN. Use appropriate concentration. Do not go outside of recommended tape sample concentration (0.5-10 ng/ul), or RIN will not be accurate. Use these results to determine fragmentation times for RNA samples (see 9.0 Appendix).

PRE-PCR AREA

1. Ribo-Zero Deplete and Fragment RNA

Thaw the following reagents and keep on ice/ 4°C:

Elute, Prime, Fragment High Mix (EPH)
rRNA Removal Mix-Gold (RRM G)
Resuspension Buffer (RSB) (use an aliquot)

Bring the following reagents to room temperature before use:

Elution Buffer (ELB)
rRNA Binding Buffer (RBB)
rRNA Removal Beads (RRB)
AMPure XP beads (allow 30 minutes to reach room temperature)

Prepare fresh 80% EtOH.

1) Dilute 200 ng of total RNA to a final volume of 10 uL with nuclease-free water in PCR plate or strip. Dry dilute samples in Vacufuge if required.

2) Add 5 uL of rRNA Binding Buffer (RBB) to each well with sample.
3) Add 5 uL of rRNA Removal Mix- Gold. Pipette up and down 6 times to mix.
4) Place sealed PCR plate/strip in a thermal cycler and incubate with program "RNA-denature" as follows:
   68°C for 5 min
   Heated lid: 100°C.

5) Incubate plate/strip with the samples at room temperature for 1 minute.
6) Vortex rRNA Removal Beads to re-suspend the beads.
7) In a new PCR plate/strip, add 35 uL of rRNA Removal Beads for each sample.
8) Add entire volume of the sample (20 uL) to the wells containing rRNA Removal Beads. Pipette up and down quickly 20 times to mix the contents (set pipette to 45 uL), tap down any droplets on side of tube.
   NB: add sample to beads (not beads to sample) order is important!
9) Incubate the samples at room temperature for 1 minute.
10) Place the working PCR plate/strip on the magnetic stand and incubate for 1 minute.
11) Transfer supernatant from each well to a corresponding well of a new PCR plate/strip.
12) Place the new PCR plate/strip with the supernatants on the magnetic stand for 1 minute.
13) Repeat step 11 if there are any beads remaining in the wells of the PCR plate/strip.

If necessary, repeat magnetic stand incubation and transfer of supernatant to new plates/strips until there are no beads remaining (residual beads are a source of ribosomal contamination).

Clean up

Thaw the following reagents and keep on ice/ 4°C:
Elute, Prime, Fragment High Mix (EPF)
First Strand Synthesis Act D Mix (FSA)

14) Transfer samples to separate 1.5 mL tubes for cleanup.
15) Vortex AMPure XP beads until they are well dispersed and add to each sample:
    - 193 uL of beads (for FFPE samples DV200<65), **or**
    - 99 uL of beads (for non-degraded total RNA, DV200>65).

    Gently pipette up and down 10 times to mix.
16) Incubate samples at room temperature for 15 minutes.

17) Place samples on the magnetic stand at room temperature for 5 minutes, then remove and discard the supernatant. Use 10ul pipette tip to remove all residual supernatant before adding ethanol wash (critical step)!
18) With the samples on the magnetic stand, add 200 uL of 80% EtOH to the samples without disturbing the beads.
19) Incubate samples for 30 seconds and then remove and discard supernatant.
20) Dry the beads at room temperature for 15 minutes, or until ethanol has evaporated (beads almost cracking).
21) Add 11 uL of Elution Buffer (ELB) to each sample. Mix by pipetting the entire volume up and down 10 times. Visually confirm bead resuspension!
22) Incubate samples for 2 minutes at room temperature.
23) Place tubes on magnetic stand at room temperature for 5 minutes. Transfer 8.5 uL of supernatant from each tube to a new PCR plate/strip.
24) Add 8.5 uL of Elute, Prime, Fragment High Mix (EPF) to each sample. Mix by pipetting up and down the entire volume 10 times, briefly centrifuge.

Skip steps 25-26 if RNA is heavily degraded.

25) Place the PCR plate/strip in a thermal cycler and incubate with program "Frag-Prime" as follows:
    94°C for 0-8 min; 4°C HOLD**
    Heated lid: 100 °C.
    ** variable time: DV200<55, 0 fragmentation, 55<DV200<65 4 minute fragmentation, DV200>65, 8 minute fragmentation. Refer to 9.0 appendix of this document reference traces. Record fragmentation times in sample tracking sheets!

26) Remove the PCR plate/strip with the samples and centrifuge briefly.

2. First Strand cDNA Synthesis

Thaw First Strand Synthesis Act D Mix (FSA) and bring to room temperature.

1) When opening a new box, make a master mix of SuperScript II and First Strand Synthesis Act D (FSA)
   - 90 uL First Strand Synthesis Act D Mix (FSA) + 10 uL SuperScript II
   - This is sufficient for 12.5 reactions, never make smaller batches! Minimize freeze/thaws of mix, and track by indicating with a dot on the top of the master mix tube.

   Add 8 uL from the FSA + SSII master mix to each well of the PCR plate/strip containing the samples. Gently pipette the entire volume (25 uL) up and down 6 times, then centrifuge briefly.

First Strand Synthesis PCR Mix	**1x**
rRNA-depleted RNA sample	17
FSA + SSII mix	8
Total Reaction→	25

2) Place the PCR plate/strip in a thermal cycler and incubate with program "1st strand" as follows:

   25°C for 10 min; 42°C for 15 min; 70°C for 15 min; 4°C HOLD.
   Heated lid: 100°C; **proceed immediately to next step.**

3. Second Strand cDNA Synthesis

Thaw and keep on ice/4°C fridge:
Second Strand Marking Master Mix (SMM)
Resuspension Buffer (RSB)

Preheat thermal cycler to 16°C (pre-heat lid set to 30°C), program "2nd Strand"

1) Add 5 uL of Resuspension Buffer (RSB) and 20 uL of Second Strand Marking Mix (SMM) to each sample. Gently pipette the entire volume (50 uL) up and down 6 times, centrifuge briefly.
2) Place the PCR plate/strip on the preheated thermal cycler and incubate at **16°C for 1 hour**.
3) Remove the PCR plate/strip from the thermal cycler and bring to room temperature for 5 minutes.

Second Strand Synthesis PCR Mix	**1x**
Resuspension Buffer (RSB)	5
Second Strand Marking Master Mix (SMM)	20
First strand cDNA sample	25
Total Reaction→	50

Clean up

4) Vortex AMPure XP beads until re-suspended and then add 90 uL to each sample. Gently pipette the entire volume (140 uL) up and down 10 times.
5) Incubate at room temperature for 15 minutes.
6) Place the PCR plate/strip on a magnetic stand for 5 minutes then remove and discard 135 uL of the supernatant. Use 10ul pipette tip to remove all residual supernatant before adding ethanol wash (critical step)!
7) Keep the PCR plate/strip on the stand. Add 200 uL of fresh 80% EtOH without disturbing the beads.
8) Incubate for 30 seconds at room temperature. Remove and discard supernatant from each well.
9) Repeat steps 7 and 8 for a total of two 80% EtOH washes.
10) Keeping the PCR plate/strip on the magnetic stand, dry the beads at room temperature for 15 min, or until ethanol has evaporated (beads almost cracking).
11) Add 17.5 uL RSB to each sample well before removing from magnetic stand. Remove strip from stand and gently pipette the entire volume up and down 10 times (visually confirm beads fully re-suspended after drying!).
12) Incubate samples at room temperature for 2 mins.
13) Place PCR plate/strips on the magnetic stand for 5 mins. Transfer 15 uL of supernatant from each well to a corresponding well in a new PCR plate/strip.

SAFE STOP POINT. May store at -20 °C for up to 7 days, preferable to continue protocol.

4. Adenylate 3' Ends

Thaw the following and keep on ice/4°C fridge:

A Tailing Mix (ATL)
Resuspension Buffer (RSB)
RNA Adapter Indices (AR0XX), (follow recommended combinations, truseq-library-prep-pooling-guide)
Ligation Mix (LIG)
Stop Ligation Buffer (STL)

Remove AMPure XP Beads from storage and allow 30 minutes to come to room temperature.

1) Add 2.5 uL of RSB and 12.5 uL of A-Tailing Mix to each sample, briefly centrifuge.
2) Place the PCR plate/strip in a thermal cycler using program "ATAIL70"and incubate as follows:
   37 °C for 30 min; 70 °C for 5 min 4 °C HOLD
   Heated lid: 100 °C. **Proceed immediately to next step.**

3' Adenylation Reaction	**1x**
Resuspension Buffer (RSB)	2.5
A-tailing Mix	12.5
cDNA	15
Total Reaction-->	30

5. <u>Ligate Adapters</u>

Indicate consumption of each adapter on library kit box. Select indices according to Illumina recommended combinations (truseq-library-prep-pooling-guide). Timing and order of reagent additions is important.

Place the PCR plate/strip in a thermal cycler using program "LIG" and incubate as follows:
30 °C for 10 mins
Heated lid: 100 °C

1) Add 2.5 uL of RSB and 2.5 uL of Ligation Mix (LIG) to each sample.
2) Add 2.5 uL of the appropriate RNA Adapter Index to each well of the PCR plate/strip with samples. Gently pipette the entire volume (37.5 uL) up and down 10 times, briefly centrifuge.
3) Place the PCR plate/strips in the preheated thermal cycler. Incubate at **30 °C for 10 minutes**.
4) Add 5 uL of Stop Ligation Buffer (STL) to each sample. Gently pipette the entire volume (42.5 uL) up and down 10 times, briefly centrifuge.

Ligation Reaction	**1x**
Resuspension Buffer (RSB)	2.5
Ligation Mix (LIG)	2.5
RNA Adapter Index	2.5
3'-adenylated cDNA	30
Total Reaction-->	37.5

<u>Clean up</u>

5) Vortex AMPure XP beads until re-suspended and then add 42 uL of AMPure XP beads to each sample. Gently pipette the entire volume (84.5 uL) up and down 10 times, tap tubes to collect drops clinging to side of tubes.
6) Incubate at room temperature for 15 mins.
7) Place the PCR plate/strip on a magnetic stand at room temperature for 5 mins then discard 79.5 uL of supernatant per well. Use 10ul pipette tip to remove all residual supernatant before adding ethanol wash (critical step)!
8) Keeping the PCR plate/strip on the magnetic stand, add 200 uL of fresh 80% EtOH without disturbing the beads.
9) Incubate at room temperature for 30 seconds then discard the supernatant.

10) Repeat Steps 8 and 9 for a total of two 80% EtOH washes.
11) Dry the beads at room temperature for 15 mins, or until ethanol has evaporated (beads almost cracking).
12) Add 52.5 uL RSB to each well prior to removing from magnetic stand, then gently pipette the entire volume up and down 10 times. Visually confirm bead resuspension.
13) Incubate at room temperature for 2 mins.
14) Place the PCR plate/strips on a magnetic stand for 5 mins.
15) Transfer 50 uL supernatant to a new PCR plate/tube.
16) Add 50 uL of mixed AMPure XP beads to each sample. Gently pipette the entire volume (100 uL) up and down 10 times.
17) Incubate at room temperature for 15 minutes.
18) Place the PCR plate/strip on a magnetic stand at room temperature for 5 mins.
19) Remove and discard 95 uL supernatant from each well. Use 10ul pipette tip to remove all residual supernatant before adding ethanol wash (critical step)!
20) Keeping the PCR plate/strip on the magnetic stand, add 200 uL of fresh 80% EtOH without disturbing the beads.
21) Incubate at room temperature for 30 seconds then discard the supernatant.
22) Repeat Steps 20 and 21 for a total of two 80% EtOH washes.
23) Dry the beads at room temperature for 15 mins, or until ethanol has evaporated (beads almost cracking).
24) Add 22.5 uL RSB to each well before removing from magnetic stand. Remove strip/plate from magnetic rack and gently pipette the entire volume up and down 10 times. Visually confirm bead resuspension.
25) Incubate at room temperature for 2 minutes.
26) Place the PCR plate/strips on a magnetic stand for 5 minutes or until liquid is clear.
27) Transfer 20 uL of supernatant from each well to a new PCR plate/strip tube.

SAFE STOP POINT. Place in -20 ^{o}C.

6. Library Amplification

Move to Post PCR Work Area

Thaw the following and keep on ice/4°C fridge:

PCR Master Mix (PMM)
PCR Primer Cocktail (PPC)

Bring the following to room temperature:
Resuspension Buffer (RSB)
AMPure XP beads (allow 30 minutes to reach room temperature)

1) Add 5 uL of PCR Primer Cocktai (PPC) and 25 uL of PCR Master Mix (PMM) to each sample, pipette up and down to mix, and briefly centrifuge.
2) Place the PCR plate/strip in a thermal cycler using program "PCR" and incubate as follows:

Adapter-ligated cDNA PCR reaction	**1x**
PCR Primer Cocktail	5
PCR Master Mix	25
Adapter-ligated cDNA	20
Total Reaction-->	50

98 °C for 30 sec; 15 cycles {98 °C for 10 sec;60 °C for 30 sec;72 °C for 30 sec}72 °C for 5 mins; 4 °C HOLD.
Heated lid: 100 °C.

Clean up

3) Vortex AMPure XP beads until well dispersed. Add 50 uL of AMPure XP beads to each sample. Gently pipette the entire volume (100 uL) up and down 10 times.
4) Incubate at room temperature for 15 minutes.
5) Place the PCR plate/strip on a magnetic stand for 5 mins then discard 95 uL of supernatant. Use 10ul pipette tip to remove all residual supernatant before adding ethanol wash (critical step)!
6) Add 200 uL of freshly prepared 80% EtOH to each well, incubate for 30sec, then remove and discard supernatant.
7) Repeat step 6 for a total of two 80% EtOH washes.
8) Dry the beads at room temperature for 15 mins, or until ethanol has evaporated (beads almost cracking).
9) Add 32.5 uL of RSB to each sample prior to removing from magnetic rack. Remove samples from rack and gently pipette up and down 10 times to mix. Visually confirm resuspension.
10) Incubate at room temperature for 2 mins.

11) Place the PCR plate/strip on a magnetic stand for 5 mins.
12) Transfer 30 uL of supernatant to a labelled 1.5 mL tube.

7. Assess Quality and Quantity of Library

1) Use Qubit HS dsDNA assay using TGL SOP to quantify the cDNA library (SOP_Qubit_Assay_for_Nucleic_Acid_Quantification.docx.).
2) Use High Sensitivity D1000 screen tape and record average library size distribution (set region to 100bp- 1000bp). Average library size will be used to size correct the library after RT-qPCR quantification, and is used in LIMS sample IDs. Record Tapestation file ID in sample tracking sheet.

   Look for adapter contamination peaks at a size of approximately 100 bp (see Appendix for example). **Do not** proceed to RT-qPCR, normalization or library pooling if the samples are heavily contaminated by adapters.

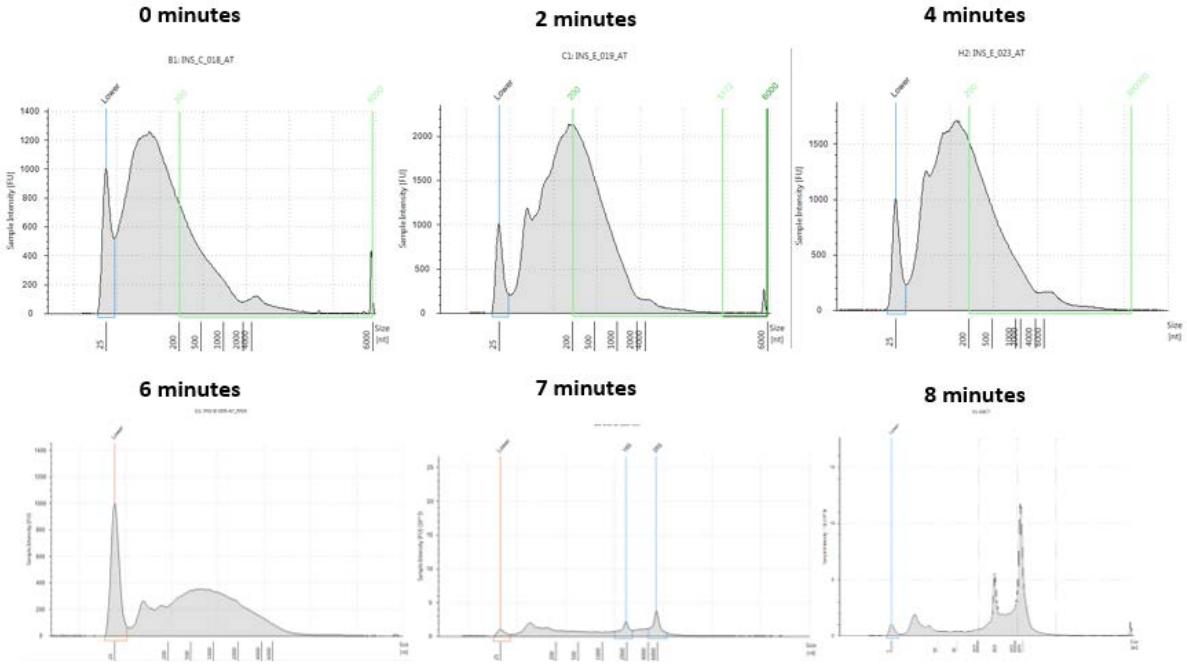
8. Sequencing

Libraries are sequenced at a depth of 80 million clusters, 75bpX6bpX75bp on the NextSeq550. Ideally five RNA samples are pooled per NextSeq550 High Output Kit.

9. Appendix

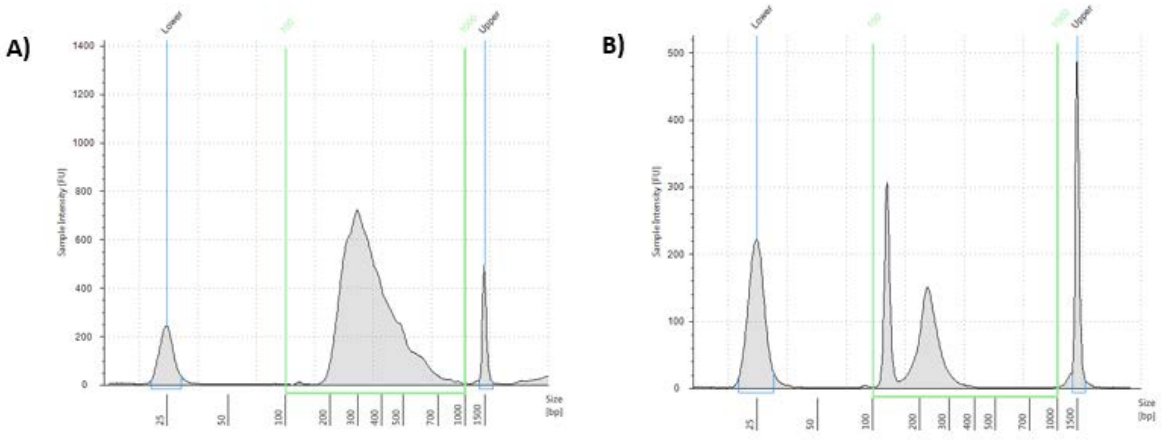
1) RNA Fragmentation Times.

Below are examples of total RNA (from FFPE samples) analyzed on the Tapestation and their associated fragmentation time. Samples with a DV200<55 are not fragmented. Samples with 55<DV200<65 should be fragmented for 4 minutes. Samples with DV200>65 are fragmented for 8 minutes.



2) Adaptor Contamination.

The following snapshots depict the difference seen between those samples with and without adaptor contamination, as identified by High Sensitivity D1000 Tapestation trace. Record adapter contamination in sample tracking sheet including % adapter contaminant (Tapestation analysis region selection).



A) High Sensitivity D1000Tapestation trace of a sample with no adapter contamination.
B) Trace of a sample with heavy adapter contamination at ~130 bp.

6.0 Revision History

Version Number	Date *(yyyy-mm-dd)*	History of Change
1.1	2017-06-13	First draft by Shihab Sarwar
1.2	2017-06-22	Update and Edits by Kayla Marsh
1.3	**2017-06-29**	**Edits by Dax Torti**

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

Consumables

Item	Lot Number
A-Tailing Control (CTA)	Lot #: _____
A-Tailing Mix (ATL)	Lot #: _____
Elute, Prime, Fragment High Mix (EPH)	Lot #: _____
Elution Buffer (ELB)	Lot #: _____
End Repair Control (CTE)	Lot #: _____
First Strand Synthesis Act D Mix (FSA)	Lot #: _____
Globin Removal Mix (GRM)	Lot #: _____
Ligation Control (CTL)	Lot #: _____
Ligation Mix (LIG)	Lot #: _____
PCR Master Mix (PMM)	Lot #: _____
PCR Primer Cocktail (PPC)	Lot #: _____
Resuspension Buffer (RSB)	Lot #: _____
rRNA Binding Buffer (RBB)	Lot #: _____
rRNA Removal Beads (RRB)	Lot #: _____
rRNA Removal Mix (RRM)	Lot #: _____
rRNA Removal Mix Gold (RRM G)	Lot #: _____
rRNA Removal Mix Plant (RRM P)	Lot #: _____
Second Strand Marking Master Mix (SMM)	Lot #: _____
Stop Ligation Buffer (STL)	Lot #: _____
80% Ethanol	Date Prepared: _____

Adapter Indices or RAP	Lot Number
RNA Adapter Index 1 (AR001)	Lot #: _____
RNA Adapter Index 2 (AR002)	Lot #: _____
RNA Adapter Index 3 (AR003)	Lot #: _____
RNA Adapter Index 4 (AR004)	Lot #: _____
RNA Adapter Index 5 (AR005)	Lot #: _____
RNA Adapter Index 6 (AR006)	Lot #: _____

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

Consumables

Adapter Indices or RAP	Lot Number
RNA Adapter Index 7 (AR007)	Lot #: _____
RNA Adapter Index 8 (AR008)	Lot #: _____
RNA Adapter Index 9 (AR009)	Lot #: _____
RNA Adapter Index 10 (AR010)	Lot #: _____
RNA Adapter Index 11 (AR011)	Lot #: _____
RNA Adapter Index 12 (AR012)	Lot #: _____
RNA Adapter Index 13 (AR013)	Lot #: _____
RNA Adapter Index 14 (AR014)	Lot #: _____
RNA Adapter Index 15 (AR015)	Lot #: _____
RNA Adapter Index 16 (AR016)	Lot #: _____
RNA Adapter Index 18 (AR018)	Lot #: _____
RNA Adapter Index 19 (AR019)	Lot #: _____
RNA Adapter Index 20 (AR020)	Lot #: _____
RNA Adapter Index 21 (AR021)	Lot #: _____
RNA Adapter Index 22 (AR022)	Lot #: _____
RNA Adapter Index 23 (AR023)	Lot #: _____
RNA Adapter Index 24 (AR024)	Lot #: _____
RNA Adapter Index 25 (AR025)	Lot #: _____
RNA Adapter Index 27 (AR027)	Lot #: _____
RNA Adapter Plate, 96plex (RAP)	Lot #: _____