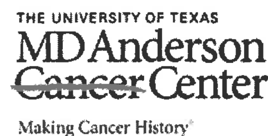
Date: January 9th, 2020

Department of Genomic Medicine

Cancer Immune Monitoring and Analysis Center

Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center



Director:

Andy Futreal, BS, PhD, Professor and Chair

SOP SureSelect^{XT} RNA Direct System For Preparation of Strand-Specific Sequencing Libraries from High-Quality or FFPE-Derived RNA Samples for the Illumina Platform

Table of Contents

A. **SCOPE** 1
B. **SAFETY PRECAUTIONS** 1
C. **SURESELECT^{XT} RNA DIRECT SYSTEM OVERVIEW** 1
D. **EQUIPMENT** 2
E. **MATERIALS** 3
F. **REAGENTS** 3
G. **PROCEDURE** 3

A. SCOPE

The scope of this SOP is to cover the automated sample processing using the Agilent NGS Workstation with the SureSelect^{XT} Low Input target enrichment workflow for samples to be sequenced using the Illumina paired-read sequencing platform. The procedure will cover both Whole Exome Sequencing and Targeted Sequencing library preparation.

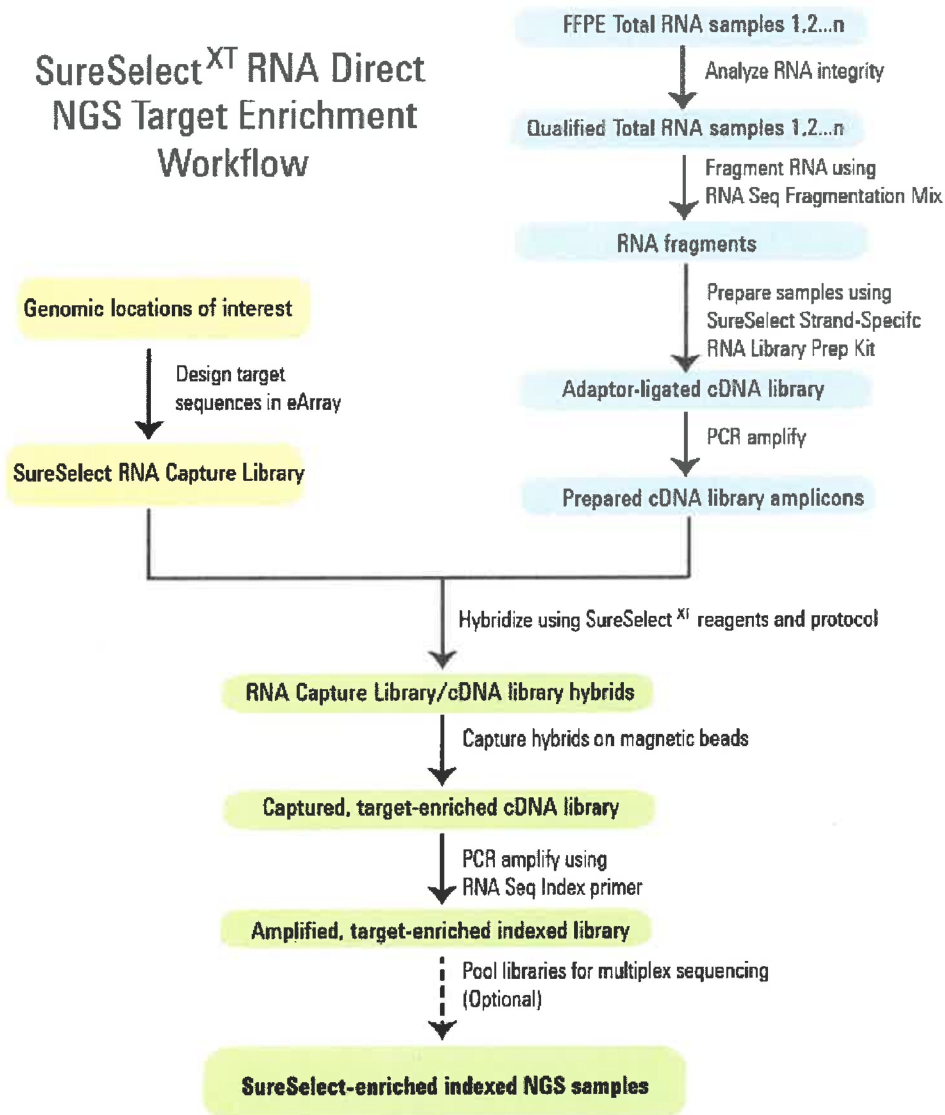
B. SAFETY PRECAUTIONS

Wear appropriate personal protective equipment (PPE) when working in the laboratory. Follow signs labeled on the instrument to prevent physical injury.

C. SURESELECT TARGET ENRICHMENT OVERVIEW

Prior to sequencing individual library preparations, hybridizations, and captures are performed. Depending on the target size of the SureSelect capture, multiple samples can be pooled and sequenced in a single lane using the 8-bp SureSelect^{XT} Low Input multiplex indexes. The SureSelect^{XT}

Low Input Library preparation is compatible with both high-quality gDNA prepared from fresh or fresh frozen samples and lower quality DNA prepared from FFPE samples, using a DNA input range to 10 to 200 ng DNA. The figure below represents the sample preparation workflow for SureSelect^{XT} Low Input NGS Target Enrichment.



D. EQUIPMENT

- Thermal cycler and accessories
- P2, P10, P20, P200 & P1000 single channel pipettes
- P10, P100, & P200 multichannel pipette
- Vortex mixer
- Qubit fluorimeter
- Covaris model E220
- Microcentrifuge
- Plate or strip centrifuge
- 96-well plate mixer
- Agilent 4200 TapeStation
- Applied Biosystems Quant Studio 6
- Agilent PlateLoc Thermal Microplate Sealer

- Vacuum concentrator
- -80°C freezer, -20°C freezer, and 4°C cooler
- Magnetic separator
- Agilent Bravo B liquid handling system (automated Library prepping)
- Ice Bucket

E. MATERIALS

- Robotic Pipetting Tips (Sterile, Filtered, 250μL)
- Applied Biosystems MicroAmp Optical 96-well Reaction Plate
- Applied Biosystems MicroAmp EnduraPlate Optical 96-Well Fast Clear Reaction Plate with Barcode
- Tube cap strips, domed
- Eppendorf twin.tec full-skirted 96-well PCR plates
- Thermo Scientific Reservoirs
- Nunc DeepWell Plates, sterile, 1.3-mL well volume
- Axygen 96 Deep Well Plate, 2 mL, Square Well (waste reservoirs; working volume 2.2 mL)
- Qubit Assay Tubes
- Eppendorf LoBind Tubes, 1.5-ml and 0.5-ml PCR clean
- P2, P10, P20, P200 & P1000 sterile, filtered tips
- Eppendorf P10 & P100 dual filtered, PCR clean and sterile tips
- Covaris 96 microTube Plate (P/N: 520078)
- 96-well plate foil seals
- 8-well tube strips and caps
- PPE: gloves, protective eyewear, and lab coat

F. REAGENTS

- Sure Select RNA Capture Library Select one library
- Sure SelectXT RNA Direct Reagent Kit Illumina platforms (ILM),
- Actinomycin D*
- Agencourt AMPure XP Kit
- Dynabeads M-270 Streptavidin Beads
- Buffer EB (10 mM Tris-Cl, pH 8.5) Qiagen p/n 19086
- 100% Ethanol, molecular biology grade
- Nuclease-free Water (not DEPC-treated)

* Actinomycin D should be obtained as a solid and prepared at 4 μg/μl concentration in DMSO then stored in single-use aliquots at –20°C, protected from light. The aliquots may be stored for up to one year before use.

G. PROCEDURE

Quality check for RNA samples: The CGL lab first conducted Sample Quality checks using the Tapestation. RNA was considered high quality if both ribosomal peaks were present on the Agilent Bioanalyzer trace, had a RIN of greater than 7.0 and a DV200 >70%. If the ribosomal peaks appeared degraded or absent, the RIN was below 7.0 and the DV200 was <70%, the sample was considered degraded or low quality. Samples with a DV200 below 20% are not recommended for this protocol.

Library preparation: The Agilent Sure Select^{XT} RNA Direct library preparation system was used to construct cDNA and Illumina sequencing libraries from total RNA following the manufacturer's protocol. The process is as follows:
Purified strand-specific double-stranded cDNA libraries are generated from either 100 ng of intact total RNA or 200 ng of degraded total RNA. Initially, total RNA were chemically fragmented using specific thermal cycling conditions based on the level of degradation and random primers annealed to the fragmented RNA in preparation for strand-specific cDNA generation. The first strand cDNA was synthesized and purified then the second strand was generated and the ends of the resulting cDNA fragments were repaired. Unique adapters were ligated to the ends of the cDNA fragments (For more information on this process, see below). The ligated products were amplified using 13 cycles of PCR. The resulting cDNA libraries were quantitated and library fragment sizes were assessed using the Agilent Tape Station DNAl000 tapes.

Hybridization: 200 ng of pre-captured cDNA library was then used to prepare for hybridization using the SureSelect^{XT} Target Enrichment protocol following the manufacturer's protocol. This protocol hybridizes the library with biotinylated RNA library baits. The biotinylated RNA library baits used for this process are the Agilent Human All-Exon v6 version. The captured libraries were then tagged with unique adapters and amplified using 12 cycles of PCR.
A qPCR quantitation was performed on all individual captured libraries to determine the concentration using the Applied Biosystems Quant Studio 6 and the KAPA Biosystems library quantification kit. The individual libraries were then pooled in equimolar amounts and the pool quantitated through qPCR. Final library pool were run on Agilent High Sensitivity screentapes to determine size adjusted concentration.

Cluster Generation by Bridge Amplification: Using the concentration from the Bio-Rad qPCR machine above, GpM of library was loaded onto a flowcell and amplified by bridge amplification using the Illumina cBot machine. A paired-end 100 cycle run was used to sequence the flowcell on a HiSeq 2500 Sequencing System and/or the NovaSeq 6000 (provides onboard clustering).

End Repair: converts the overhangs caused by fragmentation into blunt or polished ends. The 3' to 5' exonuclease activity of the End Repair mix will remove the 3' overhangs and polymerase activity fills in the 5' overhangs. The resulting blunt-end fragments will have a phosphate on the 5' ends and a hydroxyl group on the 3'ends.

Adaptor Ligation: Adaptor molecules are blunt-end ligated to the 5' and 3' end of each DNA fragment.

Genomics Laboratory

Curtis Gumbs, BS, Scientific Manager, Genomic Medicine

[Signatures] and [dates] 01/08/2020