Promega

Product Application

An NGS Workflow for FFPE Samples

Purify DNA from FFPE samples for targeted NGS sequencing.

Kits:

- Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)
- ProNex® DNA QC Assay ABI 7500/7500FAST (Cat.# NG1002)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)
- Accel-Amplicon 56G Oncology Panel for Illumina Platforms, v1 (Swift Biosciences, Cat.# AL-IL56G 48)
- SPRIselect Reagent (GE/Beckman Coulter, Cat.# B23317), plus reagents required by the manufacturer's protocol
- ProNex® NGS Library Quant Kit (Cat.# NG1201)
- MiSeq® Reagent Kit v3-600 cycle (Illumina, Cat.# MS-102-3003), plus reagent required by the manufacturer's protocol.

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM437, available at: **www.promega.com/protocols** or contact Technical Services at **techserv@promega.com.**

Sample Type(s): Human FFPE tissue

Input: 1 – 3 10µm sections of FFPE tissue

Equipment Required:

- Heat block
- Thermocycler
- Maxwell® RSC Instrument (Cat.# AS4500)
- Quantus™ Fluorometer (Cat.# E6150)
- Applied Biosystems 7500 Fast Real-time PCR System (Thermo Fisher)
- MiSeq® System (Illumina)

Protocol:

1. Pre-process FFPE sections and purify DNA using the Maxwell® RSC DNA FFPE Kit on the Maxwell® RSC Instrument as indicated in TM437.
2. Quantify DNA and perform quality control checks.
    a. qPCR-based analysis allows for specific quantification of amplifiable DNA, taking into account the fragmented and cross-linked nature of DNA from FFPE samples. For qPCR-based quantification, use the ProNex® DNA QC Assay. This assay includes three target sizes (75bp, 150bp, and 300bp), allowing quantification and analysis of sample degradation, as well as an internal positive control (IPC) to assess PCR inhibition.
    b. Dye-based quantification can be used to more rapidly estimate total DNA concentration. However, it may be skewed by non-amplifiable DNA (e.g. abasic, crosslinked, or fragmented), which may vary substantially between samples. For dye-based quantification, use the QuantiFluor® ONE dsDNA System.
3. Calculate DNA volumes needed for 10ng DNA template, as recommended for the Accel-Amplicon 56G Oncology Panel. This multiplex PCR has an average amplicon size of ~140bp and

recommends a qPCR-based method of quantification, so the 150bp target of the ProNex® DNA QC Assay may be used.

4. Assemble and cycle multiplex PCR reactions using the Accel-Amplicon 56G Oncology Panel according to the technical manual.
5. Clean up PCR reactions using SPRI Select Reagent, a DNA-binding magnetic bead, according to the technical manual. Method requires a magnetic stand and 80% Ethanol.
6. Perform the indexing reactions, ligating a unique combination of indices for each sample. Index sequences contain adaptors appropriate for Illumina platforms.
7. Clean up indexing reactions using SPRIselect Reagent according to the technical manual. Method requires a magnetic stand and PEG NaCl.
8. Quantify library yields using a qPCR method with primers specific to the Illumina adaptor sequence, such as ProNex® NGS Library Quant Kit.
9. Dilute and pool libraries to either 2nM or 4nM total concentration. The number of samples pooled and their relative concentrations will determine the expected read depth.
10. Denature and dilute the pooled library according to the technical manual, as appropriate for the NGS chemistry used.
11. Load denatured library on prepared reagent cartridge, prepare MiSeq® System (Illumina) and start sequencing.

Results: DNA was purified from 10µm sections of human lung tumor FFPE, quantified, and prepared for targeted NGS using the protocol above.

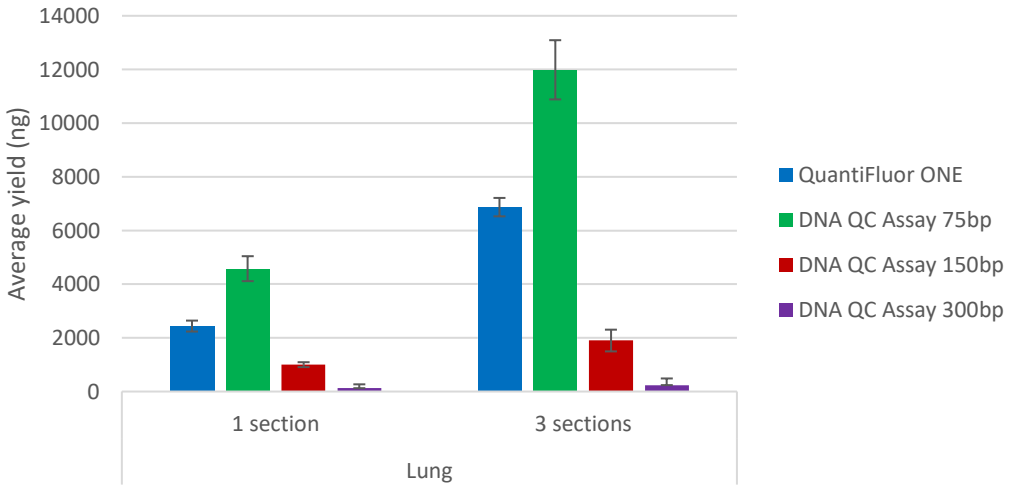

Figure 1. DNA was purified from FFPE samples using the Maxwell® RSC DNA FFPE Kit (Cat.# AS1450) on the Maxwell® RSC Instrument (Cat.# AS4500). Yields were quantified using the QuantiFluor® ONE dsDNA System (Cat.# E4871) and the ProNex® DNA QC Assay (Cat.# NG1002). Samples were pre-processed, pooled, and aliquotted into replicate purifications (n=4) to minimize variance due to sections.

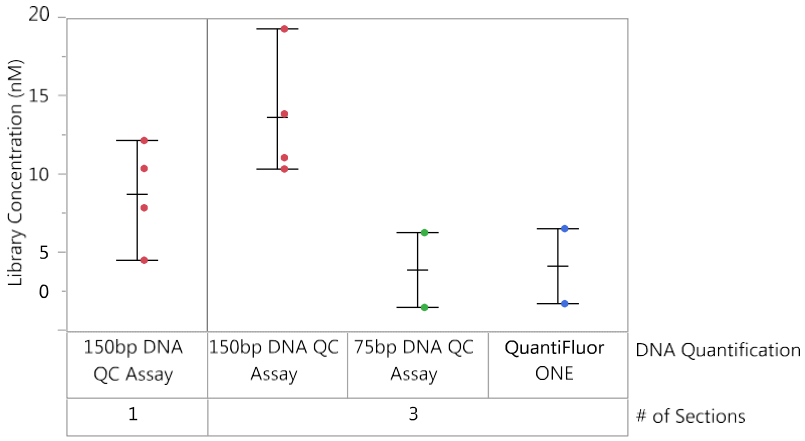

Figure 2. NGS libraries were prepared from purified lung FFPE DNA using the Accel-Amplicon 56G Oncology Panel (Swift Biosciences) with DNA input based on qPCR targets (150bp, 75bp) or dye-based methods, using the QuantiFluor® ONE dsDNA (Cat.# E4871). Library yields were measured using the ProNex® NGS Library Quant Kit (Cat.# NG1201). 10ng DNA inputs for the multiplex PCR panel were calculated using either the 150bp target of the ProNex® DNA QC Assay (n=4), the 75bp target of the ProNex® DNA QC Assay (n=2), or QuantiFluor® ONE dsDNA system (n=2) from the same DNA samples. NGS library concentration was measured in duplicate for each library and averaged.

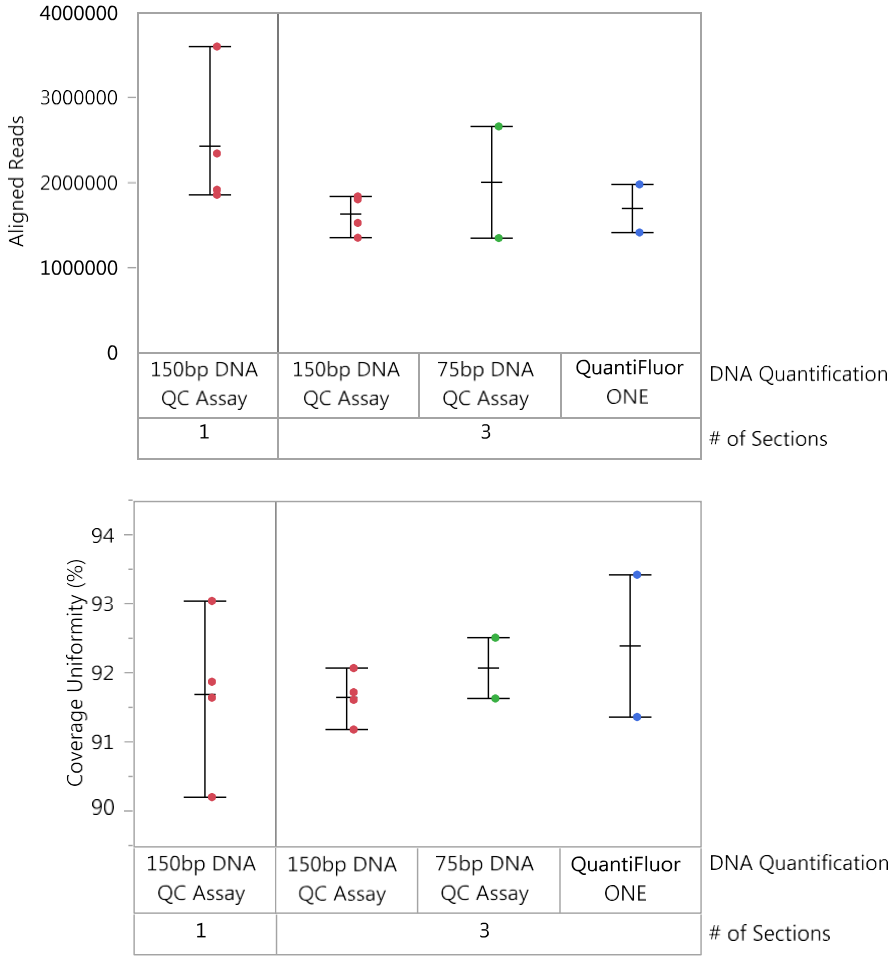

Figure 3. NGS libraries were diluted, pooled equally based on ProNex® NGS Library Quant Kit (Cat.# NG1201) quantification, and sequenced using a MiSeq® System (Illumina) with v3 chemistry (2 x 300 reads). Libraries were diluted and pooled at 125pM each (4nM total library pool), denatured and diluted to 15pM according to the MiSeq® technical manual, and loaded on a MiSeq® Reagent Kit v3. The resulting NGS data was analyzed by Swift Biosciences for number of reads aligned (top), percent of bases on target (data not shown), and coverage uniformity (bottom). Note: For this combination of FFPE sample, purification method, and NGS library preparation kit, performance was robust across quantification methods for the DNA eluates. However, samples with higher degradation, poorer purification, and/or less robust library preparation kits can show substantial differences in library concentration and coverage uniformity.