Background

- The IGM at NCH requires a cost-effective, rapid and robust Illuma NGS sample prep that is capable of automation and able to prepare unique libraries with low quality/quantity samples.
- NEBNext Ultra II FS or NNUIFS (New England Biolabs, Ipswich MA) offers the rapid/sensitive NGS sample prep without time/ cost of mechanical shearing.
- We report on the testing of the NNUIFS kit (varying fragmentation time, input DNA quantity and quality) and the application of the protocol for a small cohort of challenging samples.

Varying Enzymatic Fragmentation Time

To test the effect of enzymatic fragmentation time on DNA size, duplicate 100 ng high molecular weight genomic DNA (HMW gDNA) from Coriell cell line HG04217 that was fragmented at 12, 15 18 and 21 minutes.

Varying Input DNA Quantity

To test the robustness of NNUIFS sample prep, duplicate libraries were prepared from 100 ng, 10 ng and 1 ng HMW gDNA from Coriell cell line HG04217 that was fragmented 20 minutes.

Low Quality FFPE DNA Test

Illumina NGS sample prep was demonstrated with 1, 10 and 100 ng HMW gDNA with fragmentation of 200-500 bp to 200-800 bp. We next tested the ability of the NNUIFS protocol to prepare robust libraries from FFPE DNA.

Fragmentation/Input Test Summary

- Input DNA: 10 ng and 100 ng DNA produced similar library yield and are suitable for WGS.
- Enzymatic Fragmentation: 15 minute fragmentation suitable for WGS and exome with HMW gDNA. Lower quality samples produce a similar fragmentation pattern in 5-15 min fragmentation.

NNUIFS Pilot Project: AURORA

AURORA is a project designed to explore primary breast tumors and metastatic lesions to better understand disease progression at the sequence level. Solid tumors can be challenging samples due to their size and the quality of nucleic acids after excision. Genomic DNA from frozen tissues and FFPE DNA, both of marginal quality, were prepared for whole genome and exome sequencing.

Experimental design:

- 50 ng DNA per library, two libraries per sample.
- Two samples were empty, tubes rinsed with Low EDTA TE.
- 5 min fragmentation for FFPE, 15 min fragmentation for other samples.
- Adapter concentration titrated for input DNA quality/quantity.
- One PCR reaction per library.
- Exome: xGen Exome Research Panel v1.0 (39 Mb – 19,936 genes).
- Sequencing target: 5x WGS and 500x Exome.

Summary

- 15 of 18 libraries successfully produce NNUIFS libraries.
- Two “empty” samples exceeded expectations with complex libraries.
- One sample (P2-H) failed to meet coverage metrics due to poor sample quality/lack of complexity.
- FFPE samples require further development to improve coverage metrics.
- QC standards for NNUIFS need to be determined to allow for better prediction of successful NGS library prep.
- NNUIFS sample prep generates robust for NGS libraries from standard and low quality/quantity DNA samples in manual experiments.
- Next experiment: NNUIFS GSN sample prep will be automated on the Agilent Bravo Liquid Handle Platform for high-throughput basic research and clinical research/diagnostic applications.