Rapid preparation of Illumina libraries using the NEBNext Ultra II FS DNA Library Prep Kit

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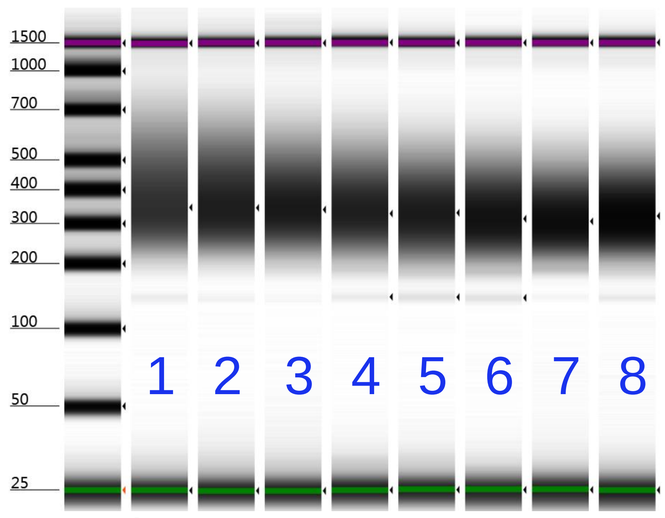
Background

- The IGM at NCH requires a cost-effective, rapid and robust Illumina NGS sample prep that is capable of automation and able to prepare unique libraries with low quality/quantity samples
- NEBNext Ultra II FS or NNUIIFS (New England Biolabs, Ipswhich MA) offers the rapid/sensitive NGS sample prep without time/ cost of mechanical shearing
- We report on the testing of the NNUIIFS 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.



TapeStation 2200 – D1000 HS

100 ng HG04217 HMW gDNA fragmented for:
Lanes 1 & 2: 12 min
Lanes 3 & 4: 15 min
Lanes 5 & 6: 18 min
Lanes 7 & 8: 21 min
- One PCR rxn per library
- 5 PCR cycles per library

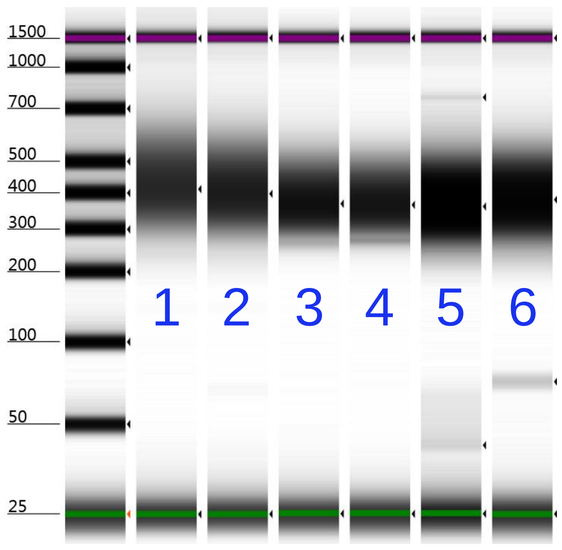
Final library yield
(1) & (2): 1122 ng & 1034 ng
(3) & (4): 1040 ng & 1040 ng
(5) & (6): 862.4 ng & 963.6 ng
(7) & (8): 924.0 ng & 983.4 ng

Varying Input DNA Quantity

To test the robustness of NNUIIFS 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.

NNUII FS Input Test:
HG04217 HMW gDNA
Lanes 1 & 2: 100 ng
Lanes 3 & 4: 10 ng
Lanes 5 & 6: 1 ng
- One PCR rxn per library
- 20 min fragmentation
- PCR cycles per library
  *100 ng: 4 cycles
  *10 ng: 7 cycles
  *1 ng: 10 cycles

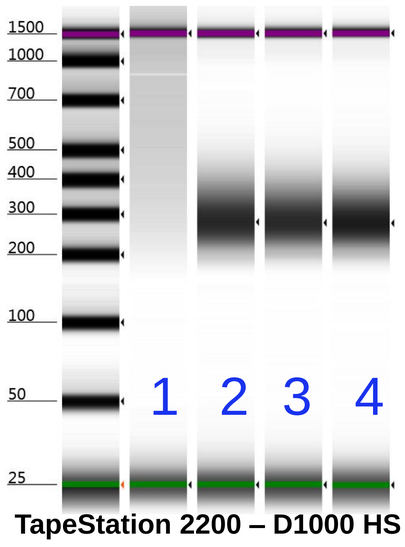
Final library yield
(1) & (2): 256.2 ng & 258.3 ng
(3) & (4): 291.9 ng & 281.4 ng
(5) & (6): 20.37 ng & 20.79 ng



TapeStation 2200 – D1000 HS

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 NNUIIFS protocol to prepare robust libraries from FFPE DNA.



TapeStation 2200 – D1000 HS

50ng HD803 Horizon DX Quantitative Multiplex Formalin Compromised (Severe) Reference Standard DNA:
Lane 1: Non-fragmented DNA
Lane 2: 9 min enzymatic fragmentation
Lane 3: 12 min enzymatic fragmentation
Lane 4: 15 min enzymatic fragmentation
- Two PCR rxns per library
- 5 PCR cycles per library

Final library yield
(1) 9 min frag: 94.16 ng
(2) 12 min frag: 112.2 ng
(3) 15 min frag: 88.88 ng

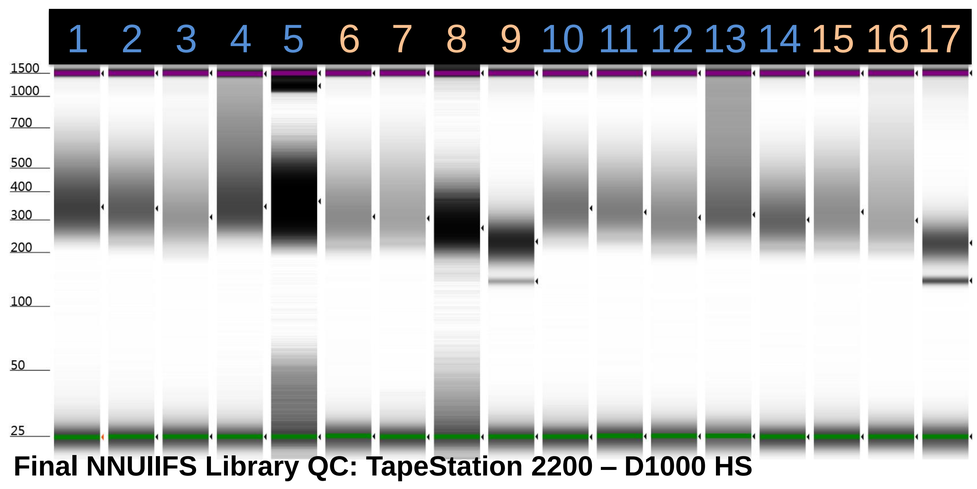
Fragmentation/Input Test Summary

- Input DNA: 10 ng and 100 ng DNA produced similar library yield and are suitable for WGS or Exome sequencing.
- 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.

NNUIIFS 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 – 19,396 genes)
- Sequencing target: 5x WGS and 500x Exome



Final NNUIIFS Library QC: TapeStation 2200 – D1000 HS

xGen Exome Research Panel v1.0 Sequencing Results

Lane	Sample ID	Tissue	Sample type	[DNA] ng/µl	Quality	Frag Time (min)	[Adapter] (µM)	# PCR rxns	# PCR Cycles	Coverage	Avg Insert	Stdev Insert	Total Reads (million)	Duplicate	Unmapped	Off-Target	On-Target
1	P1-A1A	Normal Brain	Frozen	50	>61kb	15	15	2	5	515.89	245	93	431.46	19.58%	1.00%	3.57%	63.50%
2	P1-B1A	Liver	Frozen	50	low MW	15	15	2	5	539.87	256	99	449.63	20.35%	0.31%	3.67%	63.82%
3	P1-C1A	Liver	Frozen	50	apoptotic DNA frag.	15	15	2	6	505.61	283	133	518.87	31.43%	0.49%	3.36%	51.93%
4	P1-D1	Adrenal	Frozen	empty	unknown	15	0.6	2	8	411.47	321	159	443.67	32.59%	1.20%	5.67%	50.94%
5	P1-E1C	Breast	FFPE	50	low MW	5	1.5	4	10	293.22	183	71	505.92	53.99%	0.26%	2.08%	33.56%
6	P2-F1A	Normal Liver	Frozen	50	>61kb	15	15	2	6	542.12	256	104	450.30	19.43%	0.30%	3.64%	63.83%
7	P2-G1A	Liver	Frozen	50	>61kb	15	15	2	6	531.57	267	116	489.48	25.84%	0.43%	4.15%	57.29%
8	P2-H1C	Breast	FFPE	50	low MW	5	1.5	4	14	6.33	120	27	302.64	94.55%	0.27%	1.03%	0.95%
9	P2-H1D	Breast	FFPE	50	low MW	5	1.5	4	14	6.06	119	28	251.49	94.15%	0.35%	1.18%	1.10%

Whole Genome Sequencing Results

Lane	Sample ID	Tissue	Sample type	[DNA] ng/µl	Quality	Frag Time (min)	[Adapter] (µM)	# PCR rxns	# PCR Cycles	Coverage	Avg Insert	Stdev Insert	Total Reads (million)	Duplicate	Unmapped
10	P1-A1B	Normal Brain	Frozen	50	>61kb	15	15	2	7	7.38	223	98	268.01	6.48%	0.90%
11	P1-B1B	Liver	Frozen	50	low MW	15	15	2	7	7.48	234	105	263.73	5.09%	0.49%
12	P1-C1B	Liver	Frozen	50	apoptotic DNA frag.	15	15	2	6	4.56	205	100	175.05	3.63%	0.46%
13	P1-A2	Adrenal	Frozen	empty	unknown	15	0.6	2	8	7.23	310?	182	236.15	3.67%	2.26%
14	P1-E1D	Breast	FFPE	50	low MW	15	1.5	4	10	4.87	174	82	210.77	5.25%	0.52%
15	P2-F1B	Normal Liver	Frozen	50	>61kb	15	15	2	6	7.26	218	103	270.30	4.93%	0.62%
16	P2-G1B	Liver	Frozen	50	>61kb	15	15	2	6	5.24	247	137	191.02	4.11%	1.29%
17	P2-H1B	Breast	FFPE	50	low MW	5	15	2	11	1.4	86	30	183.46	8.69%	21.17%

Summary

- 15 of 18 libraries successfully produce NNUIIFS 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 FFPE need to be determined to allow for better prediction of successful NGS library prep
- NNUIIFS sample prep generates robust for NGS libraries from standard and low quality/quantity DNA samples in manual experiments
- Next experiment: NNUIIFS NGS sample prep will be automated on the Agilent Bravo Liquid Handle Platform for high-throughput basic research and clinical research/diagnostic applications