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A single stranded DNA library preparation workflow used for hybridization capture based WES assay showed superior uniformity

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Background: In many instances in Precision Oncology Medicine fragmented DNA from formalin-fixed paraffin-embedded (FFPE) samples is the only option. Such degraded samples may need to be sequenced using next generation sequencing (NGS) [1]. Standardized clinical NGS assays strive for a minimum of 500× coverage depth [2]. However, coverage isn't the only metric required for a successful NGS analysis. Highly uniform DNA libraries allow clinical labs to generate more hits from their screens, saving both time and money across the course of diagnosis thereby ultimately benefiting patient care. The commercially available single stranded DNA library prep kit tested in this study renders input molecules single-stranded before ligation therefore it recovers template molecules containing DNA nicks and labile lesions thus maximizing library complexity.

Methods: 10 solid tumor FFPE derived DNA samples went through three different NGS workflows: 100ng of DNA input in an amplicon-based assay 325 gene panel (Assay A), 60ng input for a 500 gene mechanical sheared-based double strand DNA library preparation with hybridization capture assay (~500 gene panel, Assay B), 50ng and 10ng input for an enzymatic shear-based single stranded DNA library preparation with hybridization capture assay (WES with 1000+ enhanced gene content, Assay C). Data from assays A and C was processed with in-house bioinformatic pipeline, while Assay B's data was processed with a commercially available bioinformatic pipeline that was bundled with the assay. NGS QC metrics were compared across the three datasets.

Results: The 10 FFPE samples got an average uniformity 84% from Assay A and 61% from Assay B, both of which failed to meet Neo's internal uniformity metric. This novel single stranded DNA library preparation with hybridization capture workflow (Assay C) showed superior QC metrics for uniformity (average 99%) and percent of target with at least 100x coverage (average 100%) compared to the other two workflows even with as low as 10ng input.

Conclusions: Employing a novel commercially available DNA library preparation workflow in an NGS assay, NeoGenomics was able to rescue poor quality samples that had previously failed to meet our internal uniformity metric on other NGS assays and generate robust and reportable results with as low as 10ng DNA input.

[1] Nagahashi et al., J Surg Res. 2017 Dec; 220: 125–132.
[2] Cappello et al., J Pers Med. 2022 May; 12(5): 750.

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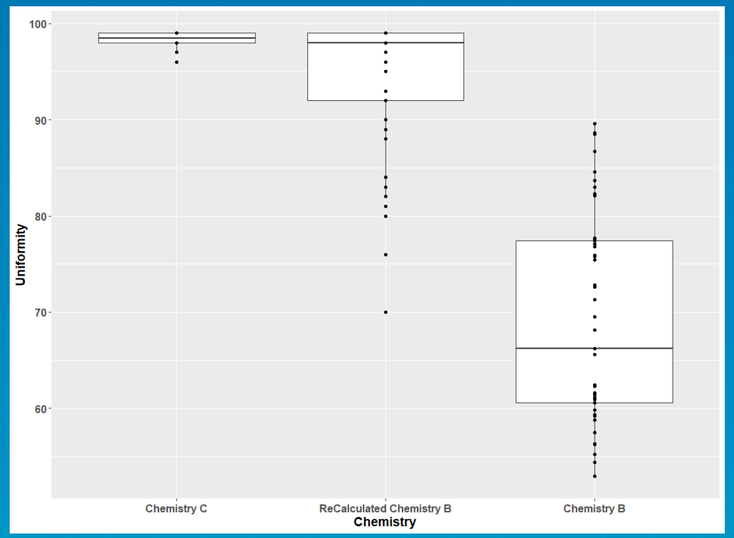
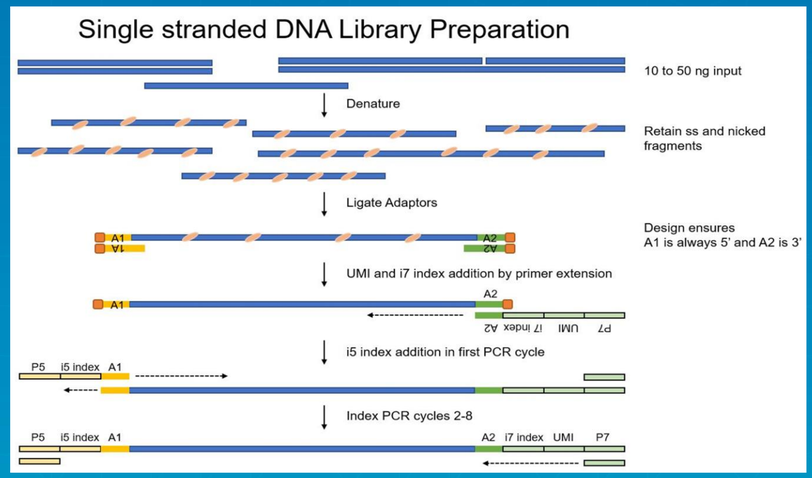
Higher uniformity and percent targets above 100X from Chemistry C

Sample ID	Chemistry A		Chemistry B		Chemistry C			
	100ng Uniformity	100ng % Targets >=100x	60ng Uniformity	60ng % Targets >=100x	50ng uniformity	50ng % Targets >=100x	10ng uniformity	10ng % Targets >=100x
Endo-01	67	99	59	95	99	100	99	100
Endo-02	69	99	69	97	99	100	99	100
Endo-03	66	99	66	95	99	100	99	100
Endo-04	55	96	63	93	99	100	99	100
Endo-05	94	100	85	99	99	100	99	100
Endo-06	99	99	99	99	99	99	99	100
Endo-07	98	100	99	99	99	100	99	100
Endo-08	97	100	97	99	99	100	99	100
Endo-09	96	100	92	99	99	100	99	100
Endo-10	99	100	99	99	99	100	99	100

More samples' uniformity comparison



- Chemistry B original output - PCT_TARGET_0.4X_MEAN with clapsed BAM
- ReCalculated Chemistry B - PCT_TARGET_0.2X_MEAN with unclasped BAM
- Chemistry C - PCT_TARGET_0.2X_MEAN with unclasped BAM



A single stranded DNA library preparation workflow

With this novel sing stranded DNA library preparation workflow in an NGS assay, NeoGenomics was able to rescue poor quality samples that had previously failed to meet our internal uniformity metric on other NGS assays and generate robust and reportable results with as low as 10ng DNA input