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Abstract 1575745

A Novel Lab Developed Test Simultaneously Assays Heme and Solid Tumors and Shows Good Performance on Degraded Samples at Low Minimum Input

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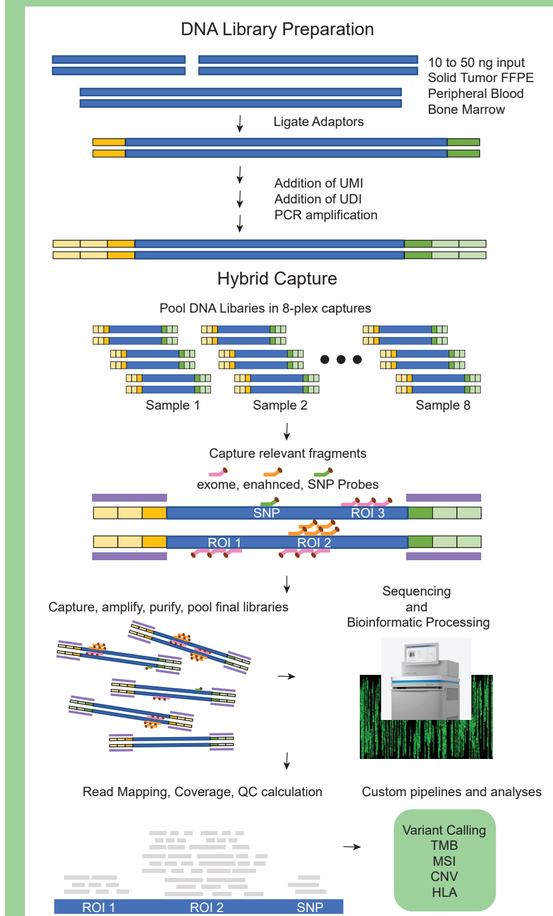
Background Lab developed tests 'LDT' simultaneously evaluate many different genomic alterations. We employ recent advances in nucleic acid extraction, library preparation, and next generation sequencing to address limited sample quantity and variable quality which currently severely limit patient access to LDTs.

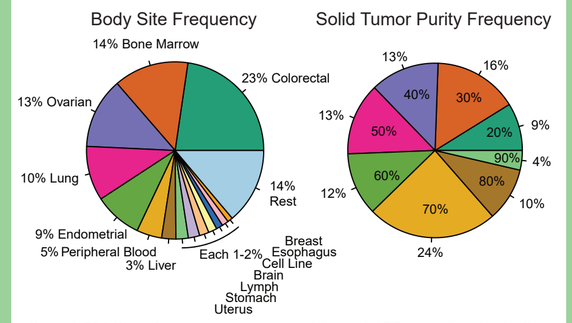
Methods DNA and RNA are dual extracted from a single sample. A novel DNA library generation method with unique dual indexes and molecular identifier sequences were used to create the novel assay. The DNA libraries undergo hybrid capture and sequencing. Additionally, the RNA is used to perform RNAseq. Libraries are sequenced on NovaSeq 6000 and bioinformatically processed with custom pipelines for variant calling for SNVs, InDels, TMB, MSI, CNVs, and HLA profiling. We compare FFPE and Heme performance to validated amplicon assays.

Results: We made over 800 libraries from more than 700 unique clinical samples, including 100 heme samples from peripheral blood or bone marrow and more than 600 FFPE samples from breast, colon, lung, lymph, ovarian, and prostate (ranging from 20 to 90% tumor). Our workflow allows unified processing of heme and FFPE samples down to 10ng input. Poor-quality samples that did not yield sufficient nucleic acid or failed QC in the amplicon assays were rescued. Samples met a minimum of 500x average coverage at over 1,000 cancer genes and 80x average coverage across the exome. Variant calling to 5% AF reached 99% accuracy (98% sensitivity and 99% specificity). TMB concordance met 93% accuracy (95% sensitivity and 91% specificity). MSI detection reached 97% accuracy (95% sensitivity and 100% specificity). CNVs were detected at 100% accuracy with high correlations for MET and ERBB2 amplifications in solid tumors (Pearson, r = 0.999 and r = 0.997, p value <= 1.9x10-13). HLA genotyping of 11 loci was performed to four-digit resolution in heme and FFPE samples and reached 98% concordance.

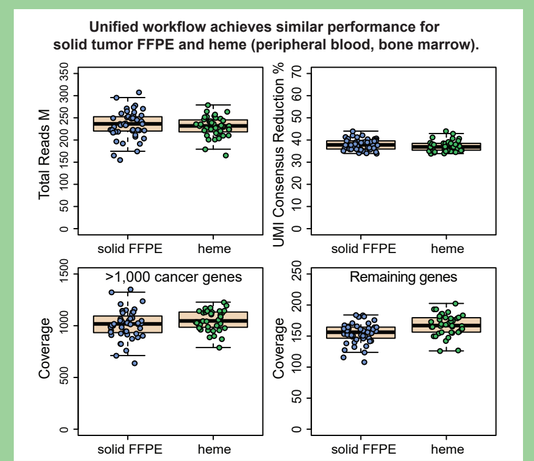
Conclusion We have developed a novel LDT that characterizes solid tumors and heme samples with as little as 10 ng of DNA. We demonstrate high accuracy for variant calling, TMB, MSI and CNV.

We present a novel Lab Developed Test with unified workflow and performance for heme and solid tumor FFPE samples. Over 1,500 libraries were constructed from more than 1,000 samples. We demonstrate good performance for variant calling, TMB, MSI, CNV and HLA. Poor quality samples achieve good performance at low input.

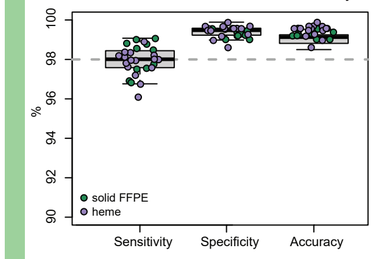




Over 1,500 libraries were constructed from 1,155 samples, including 920 solid tumor FFPE samples, 211 heme samples, and 24 cell lines or controls. One quarter (25%) of solid tumor samples had tumor purities from 20 and 30%, and half (50%) of the solid tumors had purities ranging from 20-50%.



27 samples were evaluated for SNV/Indel detection and all reached 99% accuracy.

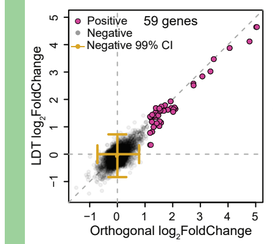


PCR	MSS	MSI-H
MSS	24	0
MSI-H	2	39

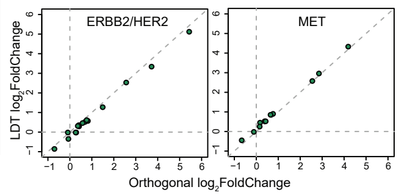
65 solid tumor FFPE samples were evaluated for MSI and reached 97% accuracy (95% sensitivity and 100% specificity).

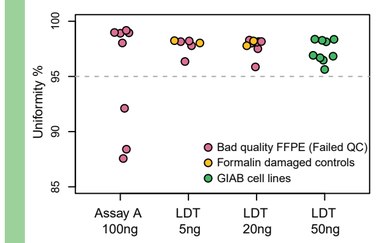
Orthogonal	High	Low
High	40	2
Low	4	43

89 solid tumor FFPE samples were evaluated for TMB and reached 93% accuracy (95% sensitivity and 91% specificity).



117 solid tumor FFPE samples were evaluated for CNVs and reached 99.8% accuracy.





Poor quality samples with bad uniformity (100ng) on another NGS assay and formalin damaged controls had good uniformity on this assay (5 and 20ng) and matched cell line controls at 50ng input. 5 additional samples failed the other assay (low coverage) but passed QC for this assay.

HLA genotypes were evaluated in 16 heme and 24 FFPE samples, and results were 98% concordant.

HLA Gene	Concordant	Discordant	% Concordant
DPA1	80	0	100
DPB1	79	1	99
DQA1	80	0	100
DQB1	78	2	98
DRB1	79	1	99
DRB3	50	2	96
DRB4	42	0	100
DRB5	27	2	93
HLA-A	76	4	95
HLA-B	78	2	98
HLA-C	76	4	95
	745	18	98

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