Next-Generation Sequencing for DLBCL Classification

Abstract 11559

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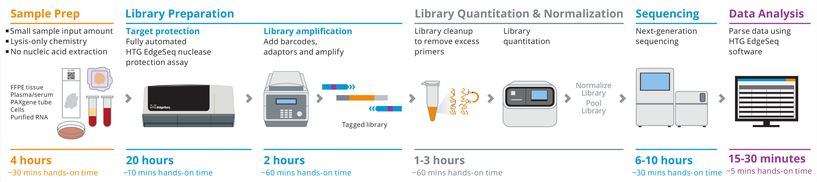
Background

Diffuse large B-cell lymphoma (DLBCL) can be molecularly classified as germinal center (GCB) and activated (ABC) B-cell-like, which traditionally define low- and high-risk patient groups when treated with R-CHOP combination therapy [1, 2, 3]. The HTG EdgeSeq system combines HTG's proprietary quantitative nuclease protection assay chemistry with Next-Generation Sequencing (NGS) to enable semi-quantitative analysis of hundreds of targeted probes in a single assay. We developed a novel HTG EdgeSeq DLBCL Cell of Origin (DLBCL COO) Assay that simultaneously measures the transcript levels of 96 genes using small clinical samples with an objective for accurate subtyping of GCB and ABC DLBCL using NGS.

HTG EdgeSeq System Workflow



HTG EdgeSeq DLBCL COO Assay RUO (96 probes)

Drug targets	Markers				HK	Control
AKT1	BAG5	CD8A	MAL	PTPRC	ACTB	ANT
BTK	BAK1	CDK16	MAP2K7	REL	DDX5	Pos1
CD19	BCL2	CDKN1B	MAP3K13	RRM2	EEF1G	
CD22	BCL6	ENTPD1	MKI67	SERPINA9	EIF4A1	
CD23	CASP7	FCGR3A	MME	SMS	GAPDH	
CD274	CCND1	FOXP1	MYBL1	SPI1	PPIA	
CD276	CCND2	FUT8	MYC	SPN	PRKG1	
CD37	CCT7	GRB2	NCAM1	SREBF1	RPL19	
CD70	CD15 (FUT4)	HLA-DRA	NCOA1	STAT6	RPL4	
CTLA4	CD3D	IL13	NF2	SUV39H2	RPL6	
EZH2	CD4	IL16	PAICS	TCF3	RPS29	
LAG3	CD47	IL4I1	PAX5	TCL1A	TBP	
MS4A1	CD5	IRF4	PDCD1LG2	TRAF1		
PDCD1	CD68	ITPKB	PIM1	TYMS		
STAT3	CD79A	LMO2	POU2AF1	VDAC1		
TNFRSF8 (CD30)	CD86	LRMP	POU2F2	ZHX2		

Classifier Development

Objective: Develop a classifier for subtyping DLBCL samples into two classes, ABC and GCB. Additionally, a third subtype, denoted as unclassified, is reported for samples which could not be characterized as either ABC or GCB.

Design: Retrospective study on a total of 256 DLBCL samples that were previously characterized by the Affymetrix Gene Chip-based gene expression.

Methods: Dilutions of each sample lysate were made so that no more than the equivalent of 5 mm^2 of FFPE tissue was present in each sample well. This sample amount was selected to meet customer requirements regarding the maximum acceptable FFPE tissue input amount.

Statistical Approach: Model development (training) included model/variable selection, parameter tuning and an internal evaluation of testing of the final model that defined the subtypes. The assessment (testing) includes estimation of prediction error. Each of these steps used a randomly selected and mutually exclusive set of samples from the study population.

Samples: A total of 172 samples were randomly selected for model development, 86 ABC and 86 GCB. The remaining 85 samples (36 ABC, and 49 GCB) were used for classifier/model assessment.

Classifier Results

A cross-validation was performed via bootstrap resampling of the training set and showed a low propensity for over-fitting (< 1%). All samples passed process control metrics.

Classifier Performance on the Model Training Set

HTG EdgeSeq DLBCL COO Assay

GEP	ABC	GCB	UNC
ABC	82	2	2
GCB	3	81	2

Agreement between the HTG EdgeSeq assay and GEP classified samples was 97% when unclassified samples were excluded, and 94.8% when unclassified results were included.

Classifier Performance on the Assessment Set

HTG EdgeSeq DLBCL COO Assay

GEP	ABC	GCB	UNC
ABC	32	2	2
GCB	6	42	2

The subsequent model assessment used to demonstrate expected performance resulted in 90% agreement to GEP. All samples passed process control metrics.

IHC Comparison Study

Objective: Assess the agreement of the HTG EdgeSeq DLBCL COO Assay compared to IHC methodologies.

Design: Retrospective study on two cohorts previously characterized via IHC and the HTG EdgeSeq DLBCL COO Assay. The first cohort consisted of 132 samples previously characterized as ABC or GCB via the Visco-Young method [4]. A second cohort of 24 samples were characterized using the Choi method [3].

Methods: Agreement rates, excluding unclassified samples, were calculated for each cohort.

Visco-Young IHC	HTG EdgeSeq DLBCL COO Classifier ABC	GCB	UNC
ABC	60	12	6
GCB	9	40	5

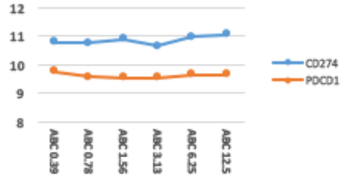
Overall Agreement to IHC	83%
Unclassified rate	6%

Choi	HTG EdgeSeq DLBCL COO Classifier ABC	GCB	UNC
ABC	11	1	1
GCB	1	10	0

Overall Agreement to IHC	91%
Unclassified rate	4%

Consistency In Sample Titration

Single DLBCL sample was titrated from 12.5 mm^2 to 0.39 mm^2. The expression of two low-expression genes, PD-1 and PDL-1, was consistent down to 0.39 mm^2.



Internal System Validation

Objective: Assess the deployed assay, software, and classifier.

Design: Retrospective study in a subset of samples used in classifier development to evaluate the consistency of the assay. Unclassified samples are excluded from this assessment.

Methods: An equivalent of approximately 5 mm^2 of FFPE tissue was present in each sample well. This set was run and sequenced on three separate days in independent testing cycles. Each of these runs was classified using the final integrated classification software.

Day 1 Result	ABC	GCB
ABC	39	0
GCB	0	39
DNP	2	

Day 2 Result	ABC	GCB
ABC	38	0
GCB	0	40
DNP	2	

Day 3 Result	ABC	GCB
ABC	40	0
GCB	0	40
DNP	0	

Across the 80 total tested tumors on three separate days, all 236 results, which passed QC, matched their expected classifications. This testing indicates a stable day-to-day performance of the DLBCL subtype classifications.

Diagnostic Reproducibility

Objective: Determine the sample level classification reproducibility using different processors and input volumes to simulate multi-site processor deployment.

Design: Fourteen DLBCL FFPE samples at two sample input amounts (1.5mm^2/well & 5mm^2/well) were run in triplicate across three HTG EdgeSeq processors. The samples were independently processed and sequenced in three discrete processing runs.

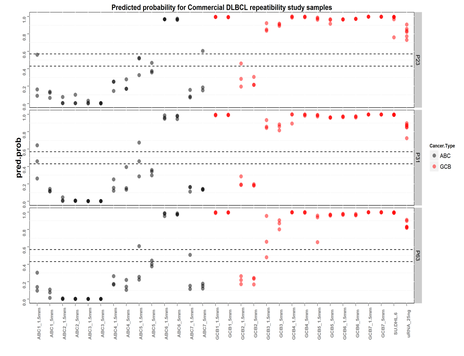
Methods: Overall agreement (OA) was calculated for each processor and sample input amount.

		Percent Pass QC	Agreement	N
Processor	Instrument 1	98.8%	94.0%	83
	Instrument 2	96.4%	97.5%	81
	Instrument 3	95.2%	97.5%	80
Input amount	1.5 mm	93.7%	94.9%	118
	5 mm	100.0%	97.6%	126

A total of 97% of samples (244/252) passed the QC metrics for subsequent classification.

99% agreement was obtained when unclassified samples were excluded (1 total misclassification).

97% agreement was obtained when unclassified results were included (8 total unclassified results, 6 of these obtained from Sample 5).



Conclusions

We developed a classifier for DLBCL ABC/GCB subtyping based on targeted NGS and demonstrated a high degree of agreement with GEP and a low sample failure rate, despite low tissue input requirements.

References:
1. Lenz G, et al., *N Engl J Med* 359(22):2313-2323 (2008).
2. Hans CP, et al., *Blood*;103(1):275-282 (2004).
3. Choi WW, et al., *Clin Cancer Res*;15(17):5494-5502 (2009).
4. Visco C, et al., *Leukemia* 26(9):2103–2113 (2012).

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