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Personalized Molecular Medicine®

Analysis and Characterization of Hematologic Cancers using a Comprehensive NGS Panel Comprised of DNA and RNA Baits Targeting 704 Genes

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Disclosures

Employee of Invivoscribe

Introduction

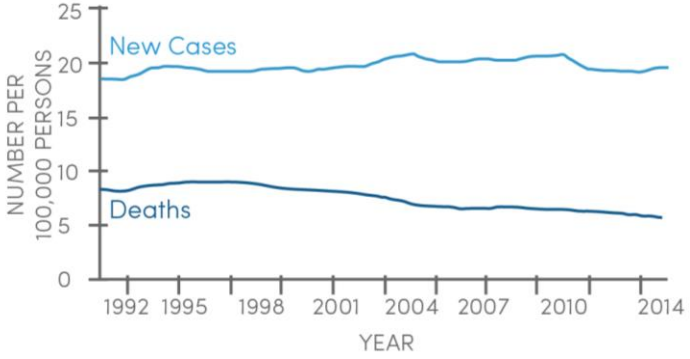
- Next-generation sequencing (NGS) of hematopoietic and lymphoid neoplasm genomes promises to revolutionize oncology.
- It is of critical importance that the **NGS platform** chosen, the **chemistry** utilized and the well vetted **bioinformatics** are then applied consistently for future adoption in clinical decisions.
- To illustrate the increased capacity and resolution of NGS for the comprehensive characterization of patients with hematologic cancers, we sequenced both clinical patient samples and contrived cell lines using a novel specific targeted strategy involving DNA and RNA.
- Understanding mutations in the context of clonal architecture may prove crucial for personalized therapies.

Non-Hodgkin Lymphoma

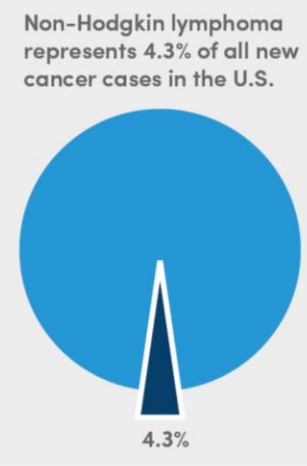
- NHL is the 7th most common cancer in the US (NCI)

Estimated New Cases in 2017	72,241
% of All New Cancer Cases	4.3%

Estimated Deaths in 2017	20,241
% of All Cancer Deaths	3.4%



Percent Surviving 5 Years
71.0%
2007–2013



MyHEME™ Sequencing Panel

Performance Characteristics of MyHEME

- Down to 1% allelic frequency coverage
- Analytical and Clinical validation ongoing with great than 95% sensitivity and specificity

An NGS panel that we use to interrogate:

✓ Genes
- The most comprehensive and current selection of genes relevant to all hematologic diseases.
- 571 DNA targets

✓ RNA
- Transcription RNA sequencing of the genes most relevant to all hematologic diseases.
- 361 gene transcripts

✓ Structural variants
- All known structural variants that are pathogenic in hematologic diseases.

✓ Clonality
- IGH, IGK, TRG Gene Rearrangements

DNA Targets (571 genes)

ABI1 ABL1 ABL2 ACSL6 ACVR1C ACVR2B ADGRG7 ADNP ADRBK1 AFF1 AFF3 AFF4 AKAP13 AKT1 ALK ANKHD1 ANKRD26 ARAF ARHGAP26 ARHGEF12 ARID1A ARID1B ARID2 ARID4B ARID5B ARNT ASXL1 ASXL2 ASXL3 ATG2B ATIC ATM ATP2B3 ATR ATRX AXL B2M BAP1 BAX BAZ2A BCL10 BCL11A BCL11B BCL2 BCL3 BCL6 BCL7A BCL9 BCOR BCORL1 BCR BIRC3 BLM BMI1 BRAF BRCA1 BRCA2 BRD4 BRIP1 BRPF1 BTG1 BTK BUB1 C15orf65 CACNA1E CALR CAMK1G CAMKK1 CAMTA1 CARD11 CARS CASC5 CBFA2T3 CBFB CBL CBLB CBLC CBX5 CBX7 CCDC6 CCND1 CCND2 CCND3 CD274 CD70 CD79A CD79B CDC42EP1 CDH23 CDK11B CDK16 CDK6 CDKN1B CDKN2A CDKN2B CDKN2C CDX2 CEBPA CEBPE CHD2 CHIC2 CIITA CLP1 CLTC CLTCL1 CNOT3 CPNE3 CREBBP CRLF2 CSF1R CSF3R CSMD1 CSTF2T CTNNA1 CTNNB1 CUL1 CUX1 CXCR4 CYP1A1 DCLK1 DDR2 DDX1 DDX10 DDX11 DDX23 DDX3X DDX41 DDX6 DEK DHX15 DHX29 DHX32 DICER1 DIS3 DNAJC11 DNM2 DNMT3A DNMT3B DOT1L DTX1 DUSP2 DYRK4 ECT2L EED EGFR EGR1 EGR2 EIF4A2 EIF4E2 ELF4 ELL ELN EP300 EPHA10 EPHA2 EPHA3 EPOR EPS15 ERBB2 ERBB3 ERG ESCO2 ETNK1 ETV3 ETV6 EWSR1 EZH2 EZR FAM175A FAM46C FANCA FANCC FANCD2 FANCE FANCF FANCG FAS FAT3 FBXO11 FBXW7 FCGR2B FCRL4 FEV FGFR1 FGFR1OP FGFR2 FGFR3 FGFR4 FH FHIT FIP1L1 FLI1 FLT1 FLT3 FNBP1 FOXO1 FOXO3 FOXO4 FOXP1 FSIP2 FSTL3 FUBP1 FUS GAS6 GAS7 GATA1 GATA2 GATA3 GLI1 GMPS GNA13 GNAS GNB1 GPHN GSKIP HDAC1 HDAC2 HDAC3 HFE HIP1 HIST1H1B HIST1H1E HIST1H3B HIST1H4I HLF HLX HNRNPK HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HOXD13 HRAS HSP90AA1 HSP90AB1 ICE1 IDH1 IDH2 IKBKB IKZF1 IKZF2 IKZF3 IKZF4 IL15 IL2 IL21R IL7R INPP5D INTS12 IRAK1 IRF1 IRF4 IRF8 ITK ITPKB JAK1 JAK2 JAK3 JMJD1C KAT6A KAT6B KDM2B KDM3B KDM5A KDM6A KDM8 KDR KDSR KIT KLHDC8B KLHL6 KMT2A KMT2B KMT2C KMT2D KRAS LASP1 LCK LCP1 LMO1 LMO2 LPP LRIG3 LRP1B LTB LUC7L2 LYL1 MAF MAFB MALT1 MAP2K1 MAP3K1 MAP3K13 MAPK1 MAU2 MDM2 MDM4 MDS2 MECOM MED1 MED12 MEF2B MET METTL3 MIR142 MIR155 MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MPL MSI2 MSN MST1R MTA2 MTCP1 MTOR MUC1 MUTYH MYB MYC MYD88 MYH11 MYH9 MYLK2 MYO3A NACA NBN NCKIPSD NCOA2 NF1 NF2 NFKB2 NFKBIE NIN NIPBL NKAP NLRP2 NOTCH1 NOTCH2 NPM1 NRAS NRG1 NRK NSD1 NT5C2 NUMA1 NUP214 NUP98 NXF1 OLIG2 P2RY8 PAFAH1B2 PAK1 PALB2 PAX5 PBRM1 PBX1 PCLO PCM1 PCSK7 PDCD1LG2 PDE4B PDE4DIP PDGFB PDGFRA PDGFRB PDS5A PDS5B PEG3 PER1 PHF6 PICALM PIGA PIK3CA PIK3CD PIM1 PIP4K2A PLCG1 PLRG1 PML POLR2A POLR3B POT1 POU2AF1 POU2F2 PRDM1 PRDM16 PRDM9 PRF1 PRKCG PRKD3 PRPF3 PRPF8 PRRX1 PSIP1 PTCH1 PTEN PTPN11 PTPN14 PTPN2 PTPN5 PTPN6 PTPRC PTPRF PTPRT PYGL RABEP1 RAD21 RALGDS RANBP17 RAP1GDS1 RARA RASA2 RBBP4 RBM15 RBMX REC8 REL RELN RET RHOA RHOH RMI2 RNF213 RNF217-AS1 ROBO1 RPL10 RPL22 RPL5 RPN1 RPS14 RPS15 RPS2 RPS6KA6 RPS6KB2 RUNX1 RUNX1T1 S1PR2 SAMHD1 SAP130 SBDS SCML2 SEPT5 SEPT6 SEPT9 SET SETBP1 SETD1A SETD2 SETDB1 SF3B1 SH2B3 SH2D1A SH3GL1 SKIV2L2 SMARCA2 SMARCA4 SMARCB1 SMC1A SMC3 SMC5 SMG1 SMO SNRNP200 SNX29 SNX7 SOCS1 SOS1 SP140 SPECC1 SPEN SPI1 SPOP SRP72 SRRM2 SRSF2 SRSF3 SRSF6 STAG1 STAG2 STAT3 STAT5B STAT6 STIL STK11 STK32A STK33 STK36 SUDS3 SUPT5H SUZ12 SYK SYNE1 TAF15 TAL1 TAL2 TBL1XR1 TCF3 TCL1A TCL6 TERC TERT TET1 TET2 TFG TFPT TFRC TGDS TLR2 TLX1 TLX3 TMEM255B TNF TNFAIP3 TNFRSF14 TNFRSF17 TOP1 TP53 TP63 TPM3 TPM4 TPMT TRA2B TRAF3 TRIM24 TRIO TRIP11 TSC1 TSC2 TTBK1 TTL TYK2 TYRO3 U2AF1 U2AF1L4 U2AF2 UBR5 VHL WAC WAPL WAS WEE1 WHSC1 WHSC1L1 WNK1 WNK3 WNK4 WT1 WWTR1 XPO1 XRCC1 ZBTB16 ZBTB33 ZBTB7B ZC3H18 ZMYM2 ZMYM3 ZNF292 ZNF384 ZNF471 ZNF521 ZRSR2

RNA Targets (361 genes)

ABI1 ABL1 ABL2 ACER1 ACSL6 ADD3 ADGRG7 AFF1 AFF3 AFF4 AHI1 ALK ANKRD28 AP2A2 ARHGAP20 ARHGAP26 ARHGEF12 ARHGEF17 ARNT ASXL1 ATF7IP ATIC AUTS2 BAALC BACH2 BAZ2A BCL10 BCL11A BCL11B BCL2 BCL2L1 BCL3 BCL5 BCL6 BCL7A BCL9 BCOR BCR BIRC3 BRD1 BRWD3 BTBD18 BTG1 C15orf65 CAPRIN1 CARS CASC5 CBFA2T3 CBFB CBL CCDC6 CCDC88C CCND1 CCND2 CCND3 CD274 CDK5RAP2 CDK6 CDX2 CEBPA CEBPB CEBPD CEBPE CEP170B CEP85L CHD6 CHIC2 CHST15 CIITA CLCA2 CLP1 CLTC CLTCL1 CNTRL CPSF6 CREBBP CRLF2 CUX1 DAB2IP DACH1 DACH2 DDX10 DDX6 DEK DMRT1 DTD1 DUSP22 EEFSEC EIF4A2 ELF4 ELL ELN EML1 ENAH EP300 EPOR EPS15 ERC1 ERG ERVK-6 ERVW-1 ETS1 ETV6 EWSR1 FAM46C FCGR2B FCRL4 FEN1 FGFR1 FGFR1OP FGFR1OP2 FGFR3 FIP1L1 FLT3 FNBP1 FOXO3 FOXO4 FOXP1 FRA7H FRYL FSTL3 FUS GAPDH GAS5 GAS6 GAS7 GATA1 GIT2 GLIS2 GMPS GOLGA4 GOLGA6A GOT1 GPHN GPR34 GRHPR HIP1 HIPK1 HIST1H4I HLF HMGA2 HOXA10 HOXA11 HOXA13 HOXA9 HOXC11 HOXC13 HOXD11 HOXD13 HRASLS5 HSP90AA1 HSP90AB1 ID4 IGF2BP1 IGH IGK IGL IKZF1 IL2 IL21R IL3 IQCG IRF4 IRS4 ITK JAK2 JAK3 KANK1 KAT6A KAT6B KDM5A KDSR KIAA1524 KIAA1549L KIF5B KMT2A KRAS KRT18P6 LASP1 LCK LCP1 LHX2 LHX4 LMBRD1 LMO1 LMO2 LNP1 LOC100289656 LPP LPXN LRMP LYL1 LYN MACROD1 MAF MAFB MALT1 MAML2 MAP3K9 MAPRE1 MBNL1 MBTD1 MDS2 MECOM MIR29A MKL1 MLF1 MLLT1 MLLT10 MLLT11 MLLT3 MLLT4 MLLT6 MN1 MNX1 MSI2 MSN MTCP1 MUC1 MYB MYC MYH11 MYH9 MYO18A NACA NAPA NBEAP1 NCKIPSD NCOA2 NCOA3 NCOR1 NDE1 NEBL NF1 NFKB2 NID2 NIN NIPBL NKX2-5 NOP2 NOTCH1 NPM1 NSD1 NTRK3 NUMA1 NUP214 NUP98 OLIG2 P2RY8 PAFAH1B2 PAK1 PAX5 PBX1 PCM1 PCSK7 PDCD1LG2 PDE4DIP PDGFB PDGFRA PDGFRB PER1 PHF21B PHF23 PICALM PIM1 PLAG1 PML POM121 POU2AF1 PPP1CB PRDM1 PRDM16 PRKAR1A PRKG2 PRRX1 PRRX2 PSIP1 PSMD2 PTPRR PVT1 RABEP1 RALGDS RANBP17 RANBP2 RAP1GDS1 RARA RBM15 RCSD1 RHOA RHOH RMI2 RNF213 RNF217-AS1 RPL22 RPN1 RUNX1 RUNX1T1 SARNP SART3 SEC31A SEPT2 SEPT5 SEPT6 SEPT9 SET SETBP1 SFPQ SH3D19 SH3GL1 SLCO1B3 SNHG5 SNX29 SORBS2 SPECC1 SPTBN1 SQSTM1 SRSF3 SSBP2 ST6GAL1 STAT5B STIL STRN SYK TAF15 TAL1 TAL2 TAOK1 TCF3 TCL1A TCL6 TCTA TET1 TFG TFPT TFRC THADA TLX1 TLX3 TMEM255B TNFRSF17 TOP1 TP53BP1 TP63 TPM3 TPM4 TRA TRAF1 TRB TRD TRIM24 TRIP11 TRPS1 TTL TYK2 USP16 USP42 WHSC1 WHSC1L1 XBP1 YPEL5 YTHDF2 ZBTB16 ZFP64 ZFPM2 ZFYVE19 ZMIZ1 ZMYM2 ZMYND11 ZNF384 ZNF521 ZNF687

Common Genes in Non-Hodgkin Lymphoma

The MyHEME sequencing panel includes genes with known mutations in a broad range of hematologic malignancies, including many causing non-Hodgkin lymphomas:

- DLBCL: *KMT2D* (*MLL2*), *BCL2*, *MYD88*, *HIST1H1E*, *PIM1*, *CREBBP*, *CARD11*, *TP53*, *TNFRSF14*
- BL: *MYC*, *DDX3X*, *TP53*, *GNA13*, *MLL3*, *RHOA*
- MCL: *SMARCA2*, *TP53*, *MLL2*, *UBR5*, *MLL3*, *CCND1*
- PTCL-NOS: *TET2*, *DNMT3A*, *GNA13*, *RHOA*

black=targeted by only DNA baits
red=targeted by DNA and RNA baits

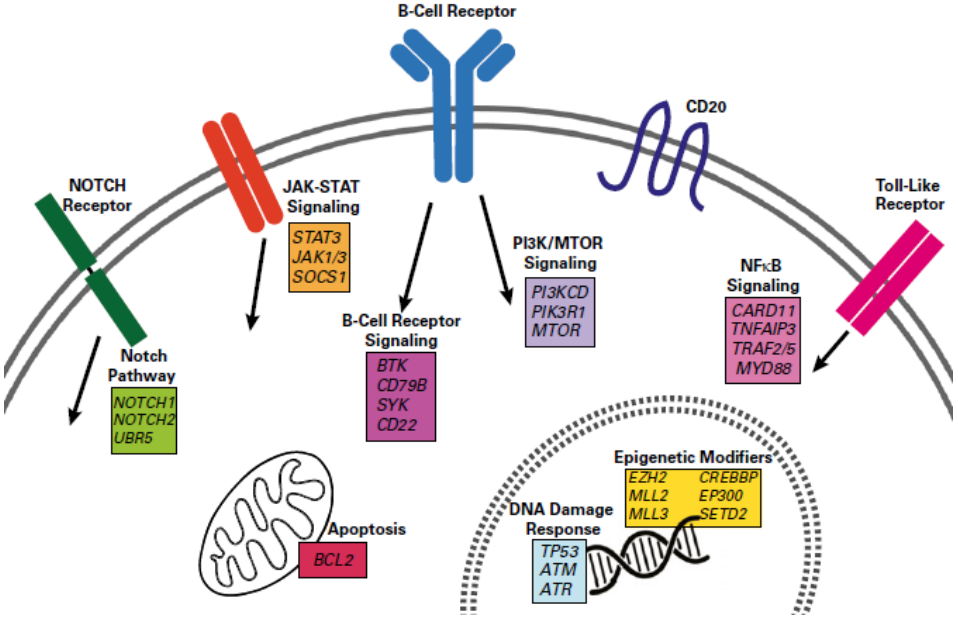
Frequently mutated genes across aggressive Non-Hodgkin Lymphoma subtypes from:
Moffitt AB, Dave SS: Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma. J Clin Oncol 35:955-962, 2017

MyHEME Methodology

Sample analysis methodology includes:

- Libraries constructed from 1µg of DNA or 0.1µg of RNA and sequenced on an Illumina® platform
- Variant analysis performed using MyInformatics™ software to detect SNVs, indels and SVs

DLBCL Genes Targeted by MyHEME



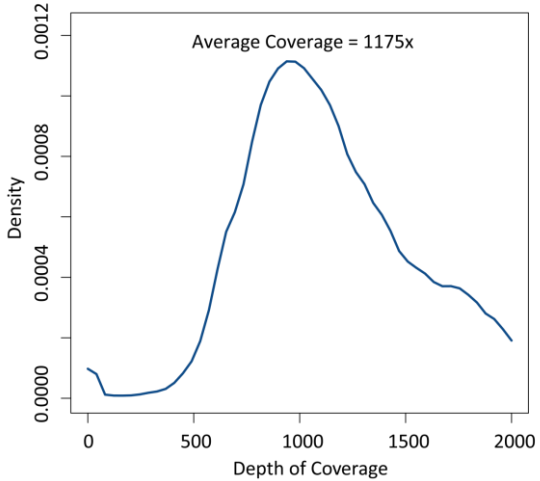
Signaling pathways in DLBCL from Moffitt AB, Dave SS: Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma. J Clin Oncol 35:955-962, 2017

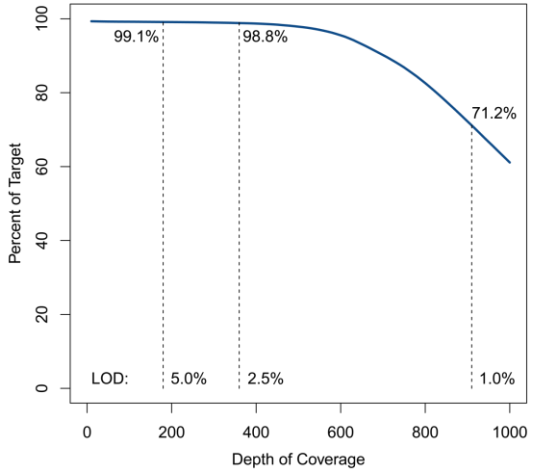
25 of 30 of genes in DLBCL pathways (highlighted by Moffitt and Dave) are targeted by MyHEME

DNA performance: Average read depth

DNA targets are sequenced to an average read depth of 1,175x (median = 1,088x)

- 99.1% of target bases meet minimum depth for an LoD of 5.0%
- 98.8% of target bases meet minimum depth for an LoD of 2.5%
- 71.2% of target bases meet minimum depth for an LoD of 1.0%

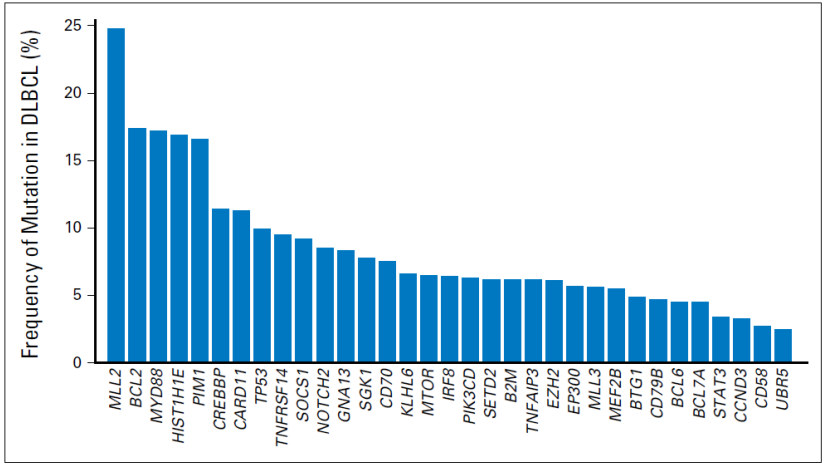




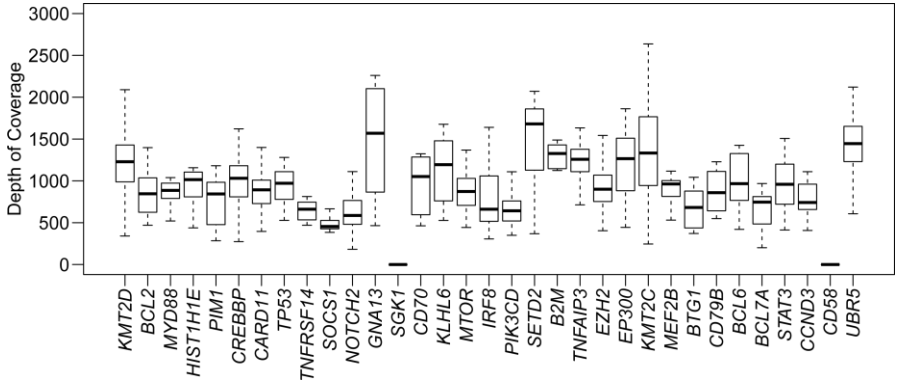
DNA performance: Average read depth

DLBCL targets are sequenced to an average read depth of 1,108x (median = 1,049x)

Most frequently mutated genes in DLBCL from Moffitt AB, Dave SS: Clinical Applications of the Genomic Landscape of Aggressive Non-Hodgkin Lymphoma. J Clin Oncol 35:955-962, 2017



MyHEME Coverage



- 31 of 33 most mutated genes in DLBCL are targeted by the MyHEME panel
- The 31 genes have average depth of coverage at or > 500x

DNA performance: Sensitivity and Specificity

We sequenced the NIST human reference sample NA12878

From GIAB consortium: extract high quality “truth” data overlapping MyHEME targets:

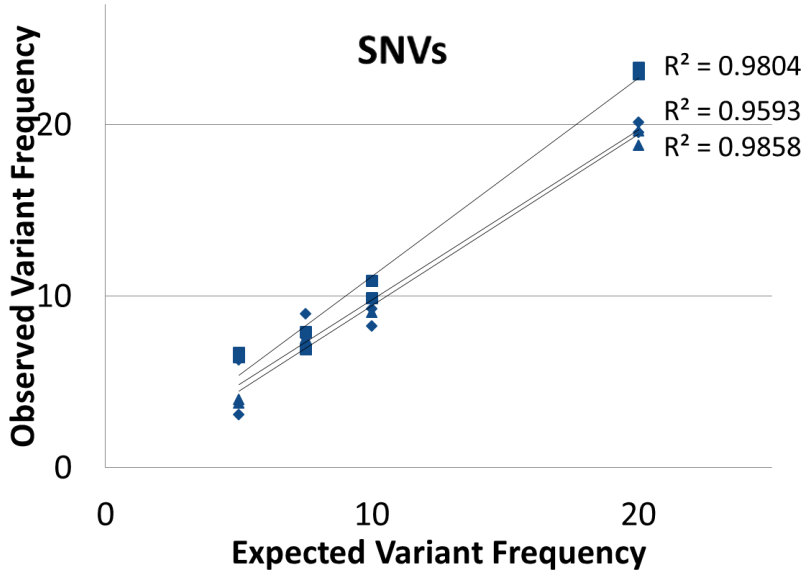
- 656 coding variants (640 SNVs and 16 indels)
- 2,171 non-coding variants (1,948 SNVs and 223 indels)
- 1,594,796 non-variant coding and 2,202,265 non-variant non-coding bases

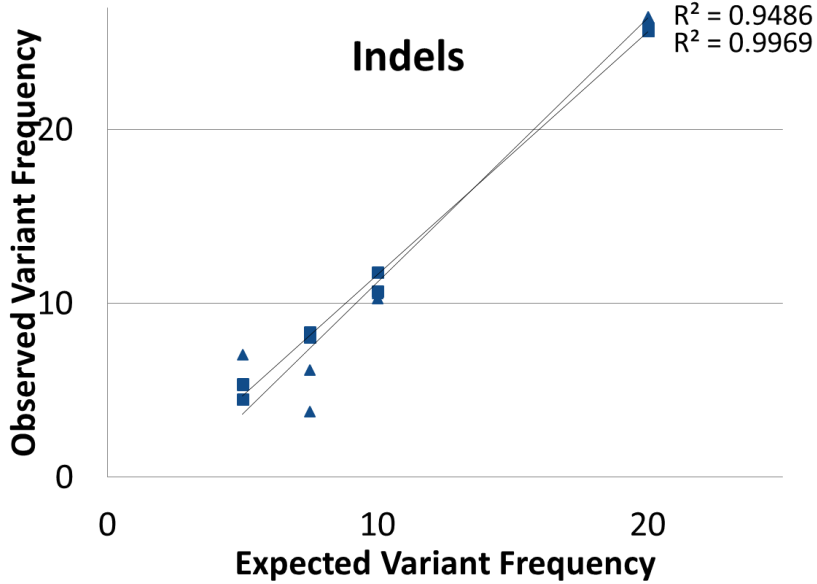
	SNVs with 2.5% LoD Threshold	Indels with 5.0% LoD Threshold
Sensitivity	99.8%	100%
Specificity	94.9%	97.7%

Using a SNV LoD threshold of 2.5% and an indel LoD threshold of 5.0% we achieve at least 99% sensitivity and 95% specificity for both SNVs and indels

DNA performance: Mutation detection in Cell Lines

- To determine the linearity of variant detection, we studied 3 SNV and 2 indel hematological pathogenic mutations in cell lines diluted at 5%, 7.5%, 10%, and 20%





RNA performance: Fusion detection in Cell Lines

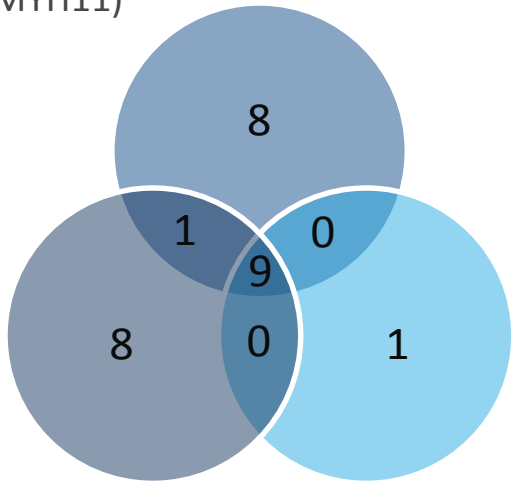
RNA targets are sequenced to an average read transcripts per million (TPM) of 2,256 (median = 743)

We sequenced 6 different cell lines containing a total of 10 known gene fusions:

- t(1;19)(TCF3;PBX)
- t(9;22)(BCR;ABL1) – b3a2 (e14/a2)
- t(15;17)(PML;RARA) – “L-form”
- t(9;22)(NUP214;XKR3)
- t(9;22)(BCR;ABL1)–b2a(e13/a2)
- t(8;21)(RUNX1;RUNX1T1)
- inv(16)(CBFB;MYH11)

red = reciprocal (count as 2 translocations)

- All fusions were detected in at least 2 of 3 chosen fusion finding programs
- Requiring detection in at least 2 of 3 programs increases specificity from 37% to 100% in these cell lines without reducing the sensitivity from 100%



Clinical Samples: 8 paired DNA/RNA samples

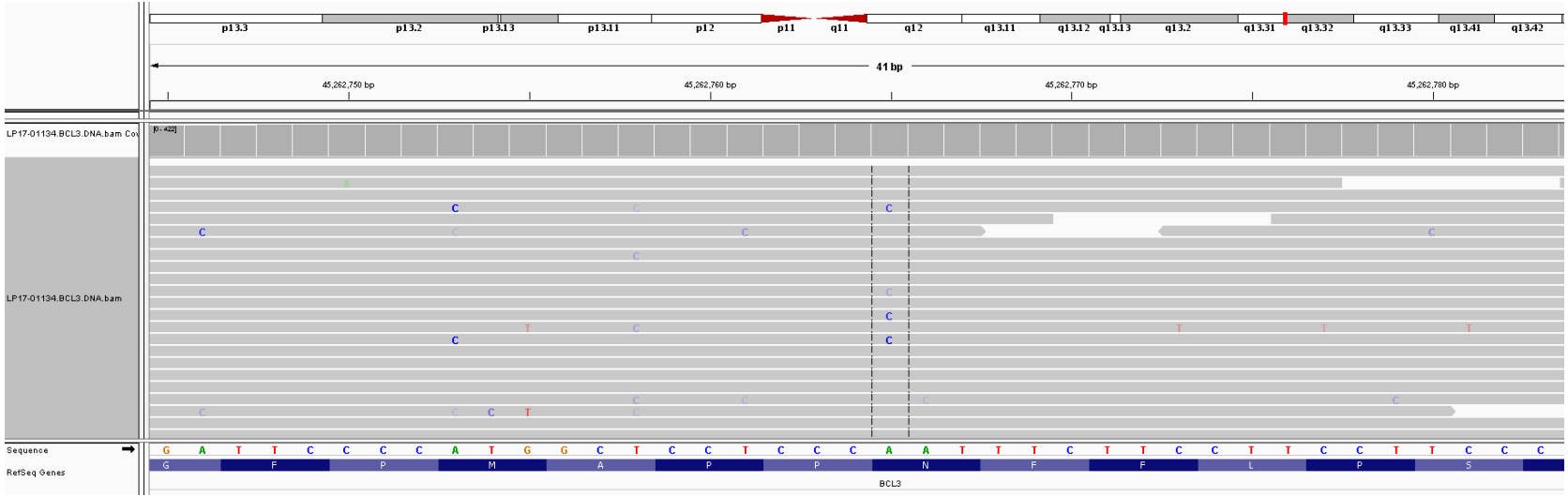
Due to overlap between DNA and RNA targets (228 genes), we can use paired results to cross-confirm novel mutations

- Increases specificity and removes sequencing artifacts
- Potentially identify allele specific transcription and/or sites of RNA-editing

Example: A novel *BLC3* mutation was detected near the detection threshold in the DNA of one patient that was predicted to be deleterious by SIFT.

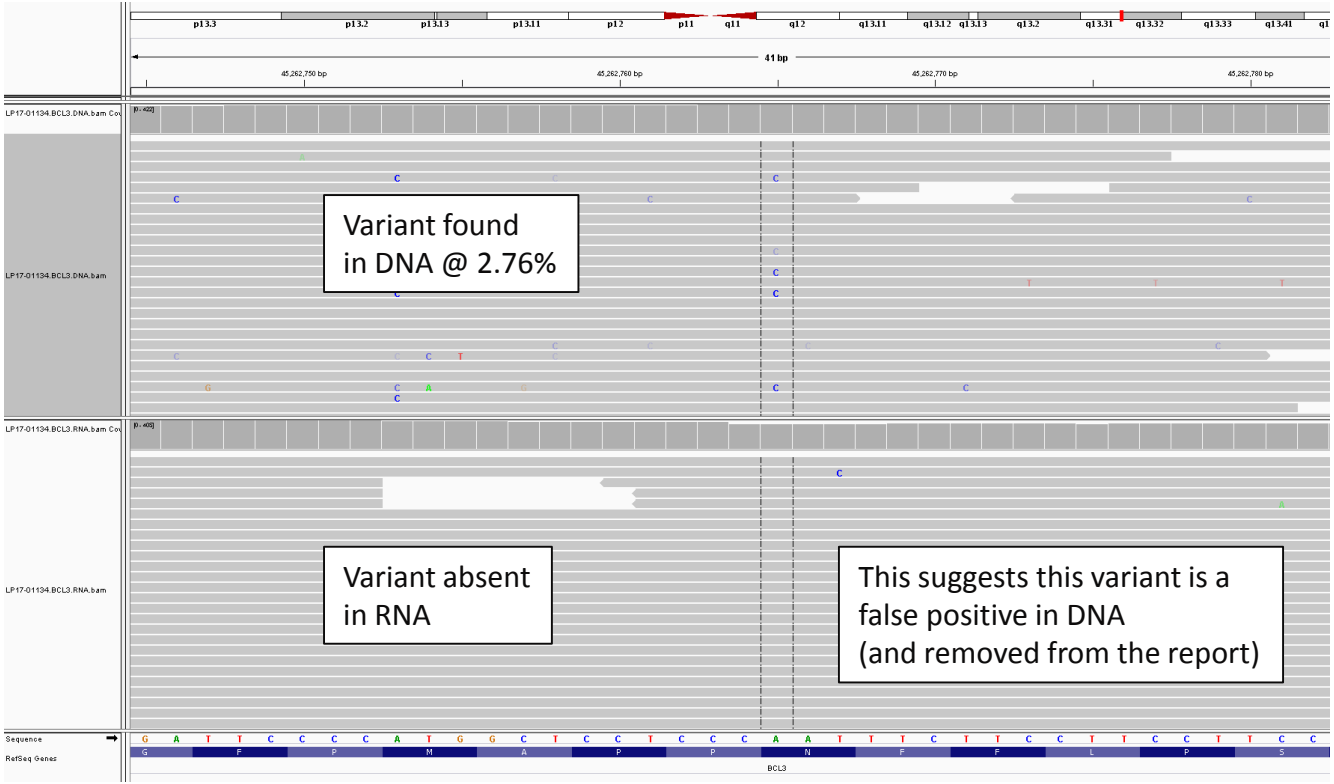
chr19:45,262,765 c.1258A>C; p.N420H (detected with a frequency of 2.76%)

A novel *BLC3* mutation was detected in DNA



Variant found
in DNA @ 2.76%

The *BLC3* mutation was not detected in RNA



Clinical Samples: 8 paired DNA/RNA samples

- We used RNA data to validate the DNA mutation calls in the 8 samples

Sample	SNV variants		Indel variants		SVs variants	
	In DNA	Match RNA	In DNA	Match RNA	In DNA	Match RNA
1	10	10/10	0	0	0	0
2	18	12/18	4	1/4	0	0
3	9	9/9	2	0/2	1	1/1
4	10	7/10	1	0/1	0	0
5	13	8/13	2	0/2	0	0
6	13	8/13	1	0/1	0	0
7	12	9/12	3	2/3	0	0
8	12	9/12	3	1/3	0	0

- Note, many indels are frameshifts and therefore may not be prevalent in RNA data.
- Used the RNA data to remove false positives and artifacts = improve specificity

Conclusions

Using an LoD threshold of 2.5% for SNVs and 5.0% for indels, we achieve:

- Variant Sensitivity > 99% for SNVs and indels
- Variant Specificity at 95% or greater for SNVs and indels

>98% of coding targets confidently meet an LoD of at least 2.5% VAF

Excellent linearity for detection of SNVs and indels

We are able to detect structural variants using both DNA and RNA:

- Combining 3 gene fusion programs improves sensitivity and specificity

Use DNA/RNA sequencing pairs to cross-confirm detected mutations and remove false positives and artifacts.

We demonstrated that MyHeme is a highly sensitive, accurate and reproducible assay that can comprehensively characterize mutations within samples from a variety of hematological malignancies, including Non-Hodgkin Lymphoma.