Accessing Genomic Alternations in Chronic Lymphocytic Leukemia using an NGS-based Comprehensive Genomic Profiling Assay(#1303)

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Background

- For detecting copy number abnormalities (CNAs), fluorescence in situ hybridization (FISH) and conventional cytogenetics (CC) are the gold standard
- NGS is emerging as a comprehensive assay that can detect CNAs as well as SNVs, INDELs and loss of heterozygosity (CN-LOH) at much higher resolution
- Thus, identifying CNA events in addition to mutations and RNA fusions may help characterize the highly complex genetic landscape of hematologic malignancies

A **comprehensive genomic profiling** (CGP) approach to interrogate **hematologic malignancies** using a novel **multimodal next generation sequencing** assay

- **297 genes (DNA)**
- 213 genes (RNA)
- **Genomic backbone in 14 chromosomes**

→ **SNV, INDEL, CNV**

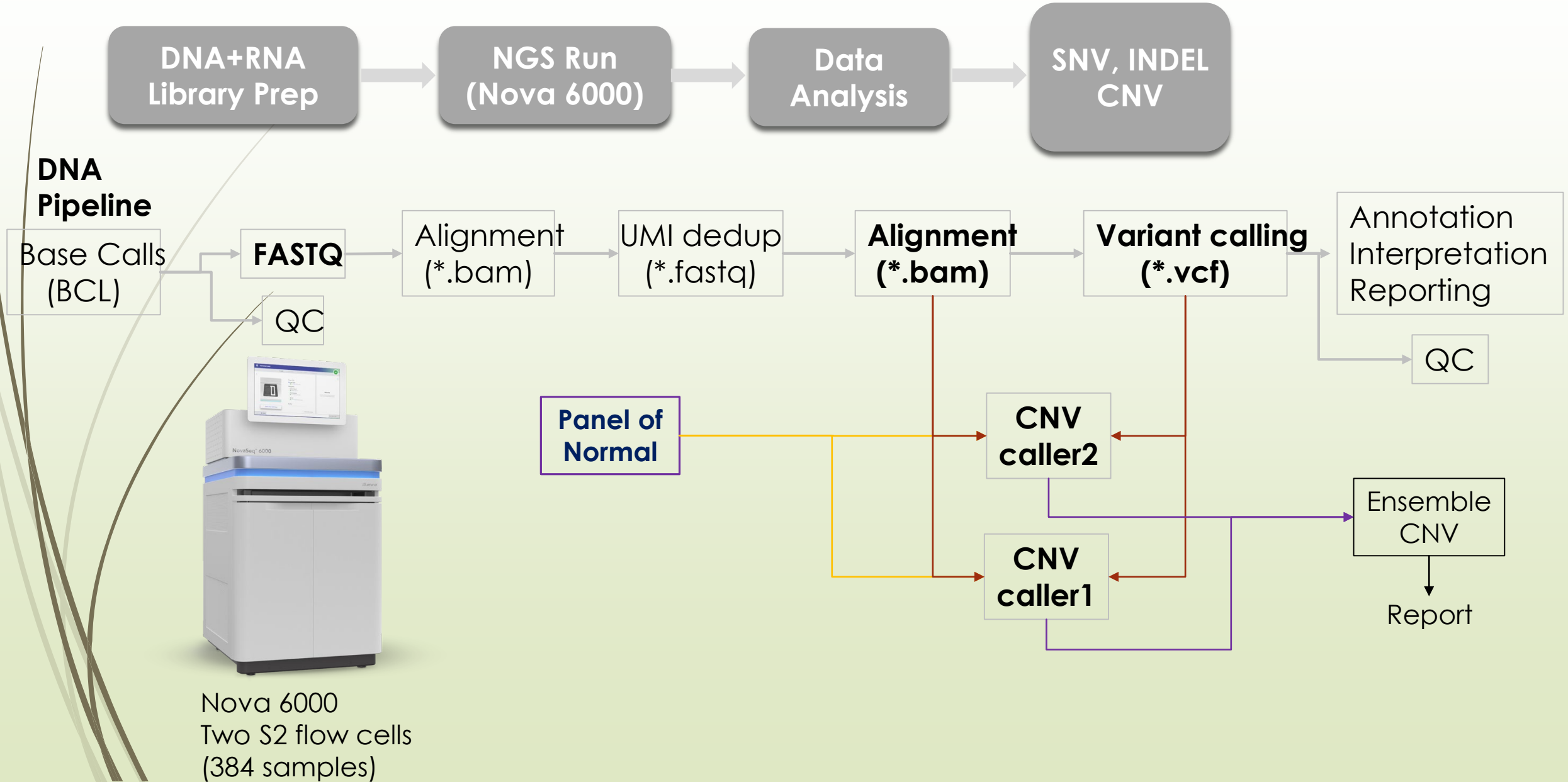
ABI1	ABL1	ABL2	ACTN4	ADAMTS17	AFDN	AFF1	AFF3	AGGF1	ALK
ARHGAP26	ARHGEF12	ATF7IP	ATIC	ATP2A1	ATP5L	BCL11B	BCL2	BCL3	BCL6
BCR	BIN2	BIRC3	CALR	CAPRIN1	CASC5	CBFB	CBL	CCDC6	CCDC88C
CCND1	CCND2	CCND3	CD274	CDK6	CDKN2A	CEBPA	CEBPD	CEBPE	CEBPG
CEP85L	CHD1	CHIC2	CHMP2A	CIITA	CNTRL	COL1A1	CPSF6	CREBBP	CRLF2
CSF1R	CTLA4	CXCR4	DEK	DTD1	DUSP22	EBF1	EIF4A1	ELL	EML1
EP300	EPOR	EPS15	ERC1	ERG	ERVK3-1	ETV6	FGFR1	FGFR1OP	FGFR1OP2
FGFR3	FIP1L1	FLT3	FNBP1	FOXO4	FOXP1	FRYL	FUS	GAS7	GIT2
GLIS2	GOLGA4	GPHN	GPI	GUSB	HIP1	HLF	HNRNPA2B1	ID4	IKZF1
IKZF2	IKZF3	IL3	IRF4	IRF8	JAK2	KANK1	KAT6A	KLF2	KMT2A
LAIR1	LDHA	LMNA	LRRFIP1	MAF	MAFB	MALT1	MAML2	MAP4	MECOM
MEF2D	MKL1	MLF1	MLLT1	MLLT10	MLLT11	MLLT3	MLLT6	MUC1	MYB
MYC	MYH11	MYO18A	MYO1F	NDE1	NF1	NFKB2	NIN	NOTCH1	NOTCH2
NPM1	NRIP1	NTRK1	NTRK2	NTRK3	NUP214	NUP98	P2RY8	PAG1	PAX5
PBX1	PCM1	PDCD1	PDCD1LG2	PDE4DIP	PDGFRA	PDGFRB	PGD	PICALM	PLAG1
PML	PRDM16	PRDM9	PRKG2	PTK2B	PVT1	RAB7A	RABEP1	RARA	RBM15
RBM6	RCSD1	ROS1	RPL19	RPL5	RPN1	RUNX1	RUNX1T1	SART3	SEMA6A
SEPT2	SEPT3	SEPT5	SEPT6	SEPT9	SET	SETD2	SNX2	SPECC1	SPTBN1
SQSTM1	SSBP2	STIL	SYNRG	TACC1	TAL1	TBL1XR1	TCF3	TCL1A	TCL1B
TERF2	TET1	TFG	TLX1	TLX3	TP53BP1	TP63	TPM3	TPR	TRAC
TRIM24	TRIP11	TYK2	UBE2R2	VCP	WDR48	ZBTB16	ZCCHC7	ZEB2	ZMIZ1
ZMYM2	ZNF384	ZNF703							

Hematologic malignancies

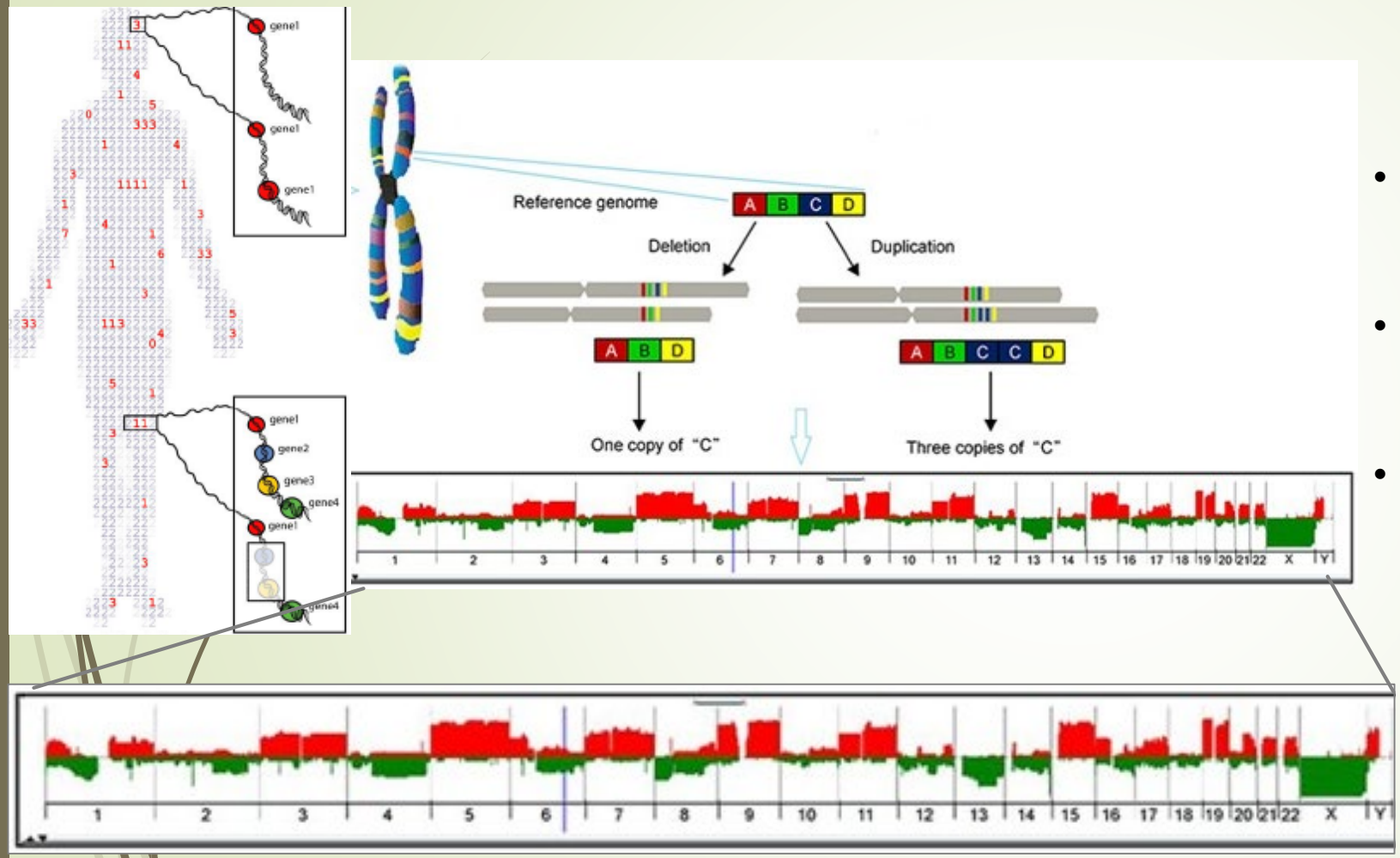
- JMML
- AML
- **CLL**
- MDS/CMML
- MPN
- Myeloid Disorders

ABL1	ABL2	AKT1	AKT2	AKT3	ALK	ANKRD26	APC	ARAF	ARHGEF1
ARID1A	ARID1B	ARID2	ASXL1	ASXL2	ATG2B	ATM	ATP2B	ATRX	AXL
B2M	BAP1	BCL1	BCL2	BCL2L11	BCL6	BCOR	BCORL	BCR	BIRC3
BLM	BRAF	BRCA1	BRCA2	BRIP1	BTK	C17orf97	CALR	CARD11	CBFB
CBL	CBLB	CBLC	CCND2	CCND3	CD273	CD274	CD33	CD79A	CD79B
CDC25C	CDK2	CDK4	CDK6	CDKN1B	CDKN2A	CDKN2B	CEBPA	CHEK2	CIC
CIITA	CND2	CREBBP	CRLF2	CSF1R	CSF3R	CTC1	CTCF	CTNNB1	CUX1
CXCR4	CYLD	DAXX	DCK	DDX3X	DDX41	DIS3	DKC1	DNMT1	DNMT3A
E2A	EBF1	EED	EGFR	EGLN1	EGR1	ELANE	EP300	EPCAM	EPHA2
EPHA7	EPOR	ERBB2	ERBB3	ERCC4	ETNK1	ETV6	EZH2	FAM46C	FAM5C
FANCA	FANCB	FANCC	FANCD2	FANCE	FANCF	FANCG	FANCI	FANCL	FANCM
FAS	FAT1	FBXW7	FGFR2	FGFR3	FLT2	FLT3	FOXO1	FUBP1	G6PC3
GAB2	GATA1	GATA2	GATA3	GFI1	GNA12	GNA13	GNAQ	GNAS	GNB1
GSKIP	HAX1	HIF1A	HIST1H1E	HNRNPK	HRAS	ID3	IDH1	IDH2	IGF1R
IKBKB	IKZF1	IKZF3	IL7R	IRAK4	IRF4	ITPKB	JAK1	JAK2	JAK3
KDM6A	KDR	KEAP1	KIT	KLF2	KLHL6	KMT2A	KMT2C	KMT2D	KRAS
LUC7L2	MALT1	MAP2K1	MAP3K1	MAP3K14	MAPK1	MCL1	MDM2	MDM4	MED12
MEF2B	MET	MLH1	MPL	MSH2	MSH6	MTOR	MYC	MYCN	MYD88
NBN	NF1	NFKBIE	NHP2	NOP10	NOTCH1	NOTCH2	NOTCH3	NPM1	NRAS
NSD1	NT5C2	NTRK1	NTRK2	NTRK3	NUP98	P2RY8	PALB2	PAX5	PDGFRA
PDGFRB	PHF6	PIGA	PIK3CA	PIK3CD	PIK3R1	PIM1	PLCG1	PLCG2	PML
PMS2	POT1	PPM1D	PRDM1	PRPF40B	PRPF8	PRPS1	PTCH1	PTEN	PTPN11
PTPRC	RAC1	RAD21	RB1	RBBP6	REL	RHEB	RHOA	RICTOR	RIPK1
RIT1	RPL11	RPL35A	RPL5	RPN1	RPS10	RPS15	RPS17	RPS26	RPS7
RTEL1	RUNX1	S1PR2	SAMD9	SAMD9L	SAMHD1	SBDS	SETBP1	SETD2	SF1
SF3A1	SF3B1	SGK1	SH2B3	SLX4	SMAD4	SMARCB1	SMC1A	SMC3	SMO
SOCS1	SPEN	SRP72	SRSF2	STAG2	STAT3	STAT5B	STAT6	STK11	SUZ12
TBL1XR1	TCAB1	TERC	TERT	TET2	TET3	THPO	TINF2	TLR2	TNFAIP3
TNFRSF14	TP53	TP63	TRAF2	TRAF3	TSC1	TSC2	U2AF1	U2AF2	UBR5
VHL	WAS	WT1	XPO1	ZFHX4	ZMYM3	ZRSR2			

Multimodal NGS workflow of LDT assay



CNA Detection



- CNV measured by the ratio of tumor to normal DNA abundance
- Certain CNVs not only prognostic, but also predictive biomarker
- Loss of heterozygosity (LOH) is due to allelic imbalance (i.e. heterozygous germline to homozygous somatic mutation

Aouiche et al. *Quantitative Biology.* 6: 99–112(2018)

CNV detection in Chronic Lymphocytic Leukemia (CLL)

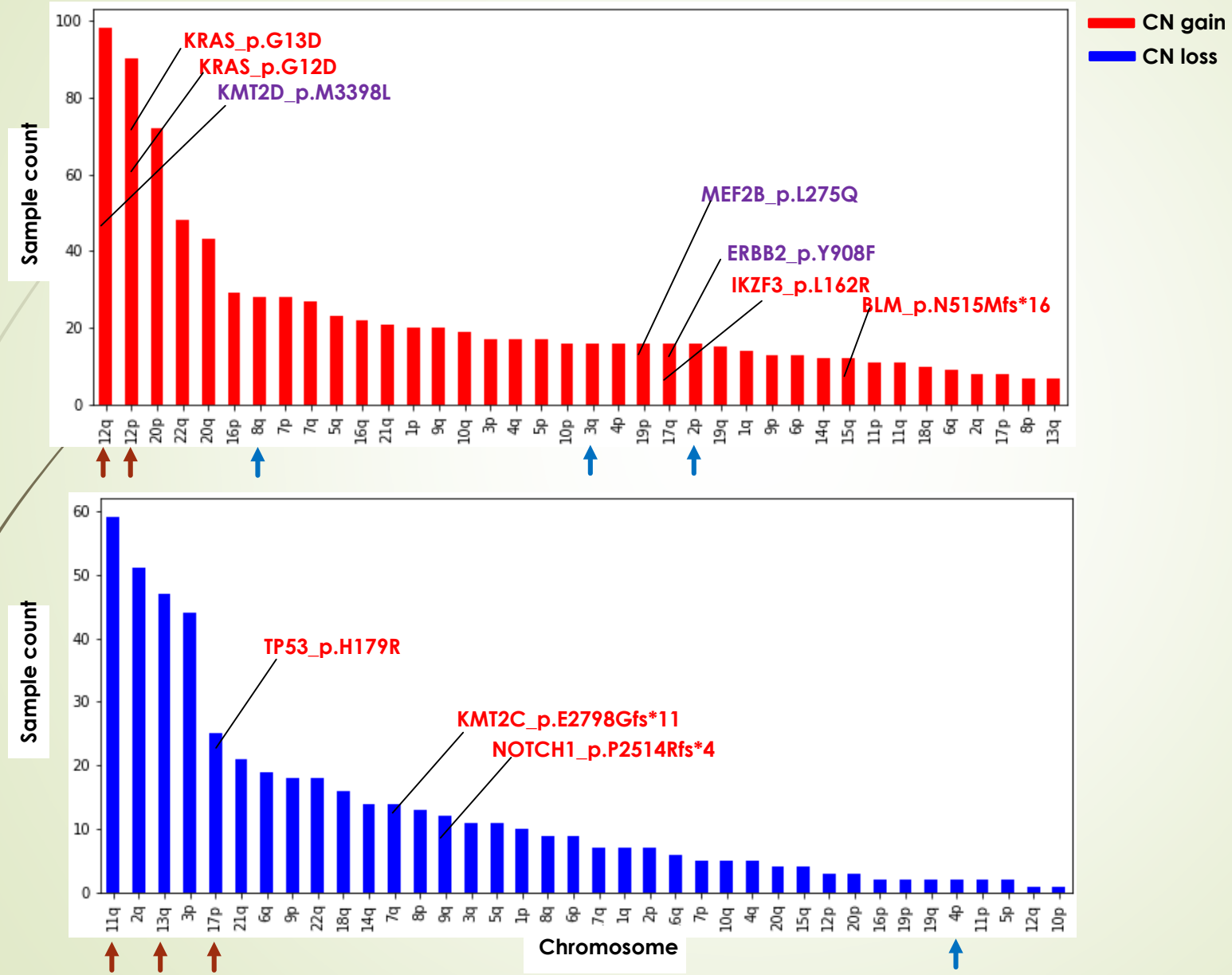
Established for prognosis

Chromosome	Abnormality	Prevalence(%)	Genes	Prognostic significance
11q22.3	loss	10-20	ATM, BIRC3, MRE11, H2AFX	Unfavorable
12	gain	10-20	Unknown	Intermediate
13q14	loss	50-60	DLEU1, DLEU2	Favorable
17p13.1	loss	5-15	TP53	Unfavorable

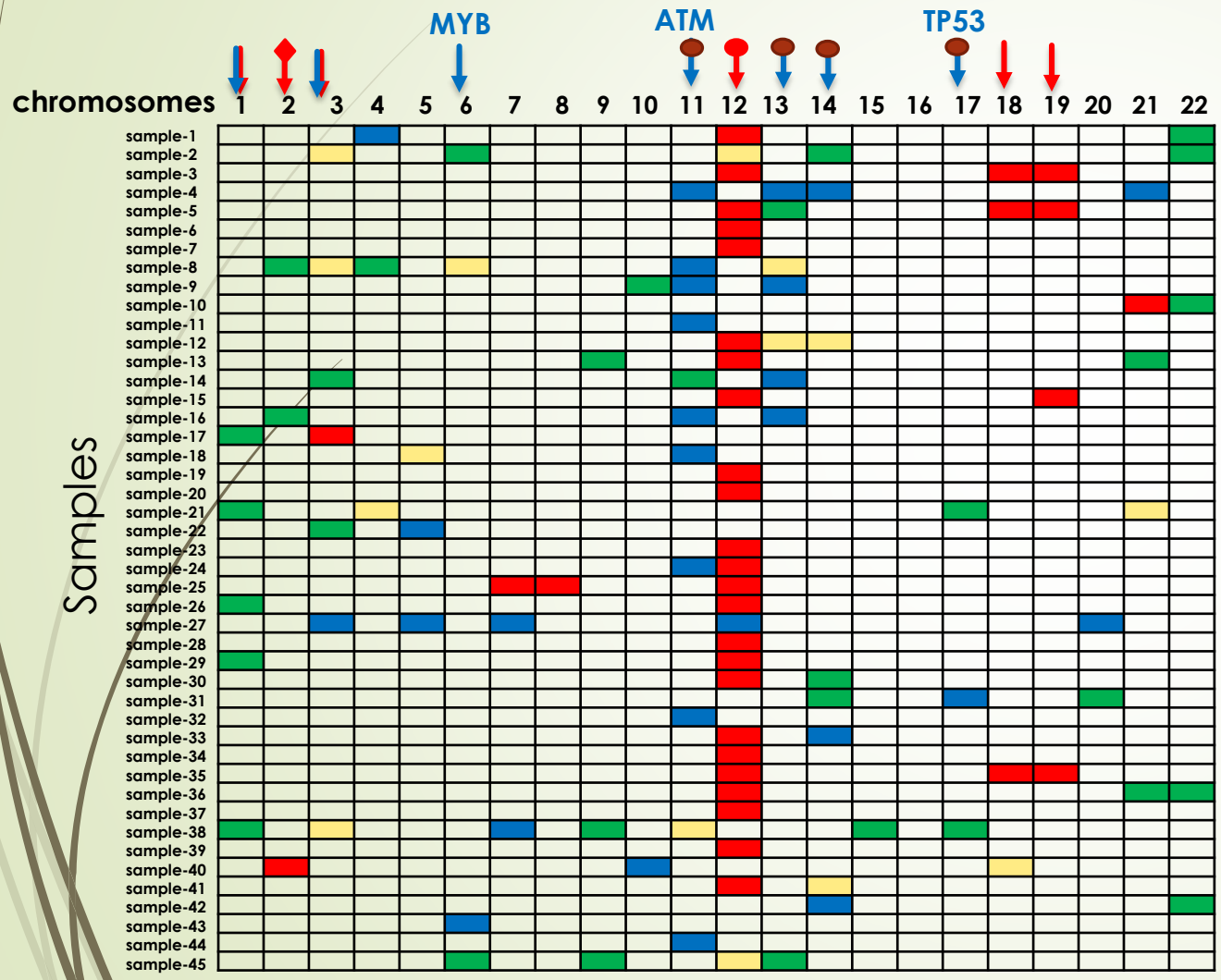
Suspected for prognosis

Chromosome	Abnormality	Prevalence(%)	Genes	Prognostic significance
2p12p25.3	gain	5-30	ACP1, **MYCN**, ALK, REL, BCL11A	Unfavorable
3q	gain	2-19	Unknown	Unfavorable
4p15.2p16.3	loss	14	Unknown	Unfavorable
8q24.1	gain	5	MYC	Unfavorable

Frequency of copy number gains and losses in CLL (n=236)



CNA detection in Chronic Lymphocytic Leukemia (CLL)



gain
loss
Cytogenetic only
NGS only

Suspected for prognosis

Established for prognosis

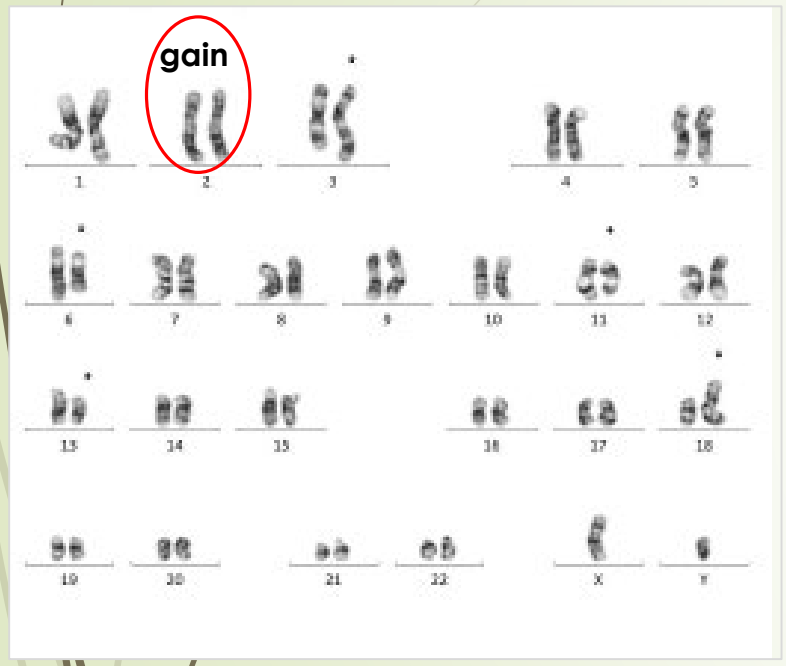
Partially established for prognosis

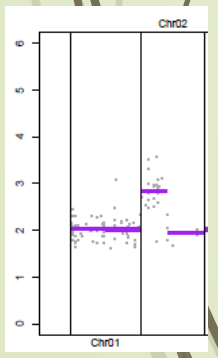
- Comparing NGS CNV with 45 karyotyping (abnormalities)

- 36/45 (80%) in full agreement
- 6/45 (13%) in partial agreement
- 3/45 (6%) in no agreement

2p gain in CLL

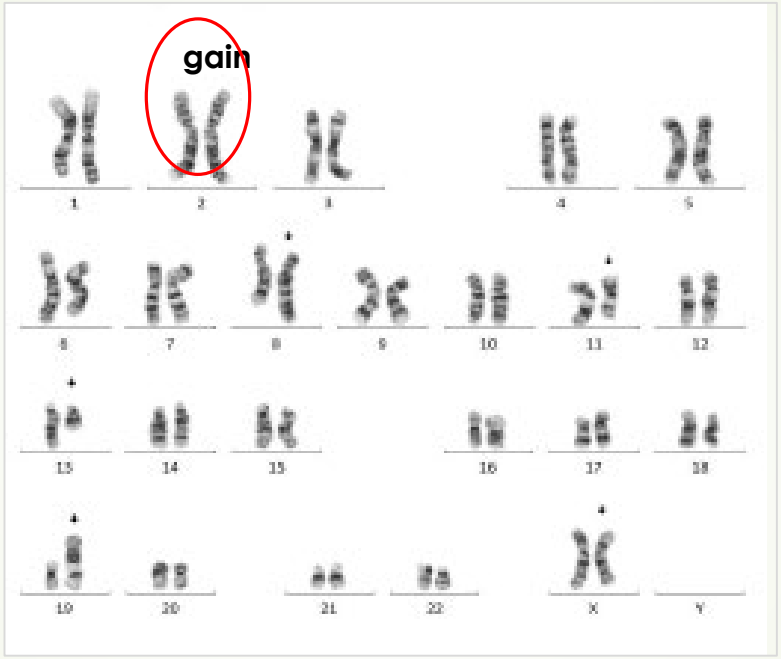
case1

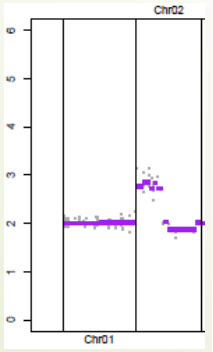




Chr	Pos	ID	REF	ALT	Gene
2	25994416	rs372407443	A	G	ASXL2
2	48030639	rs1361078163	A	AC	MSH6

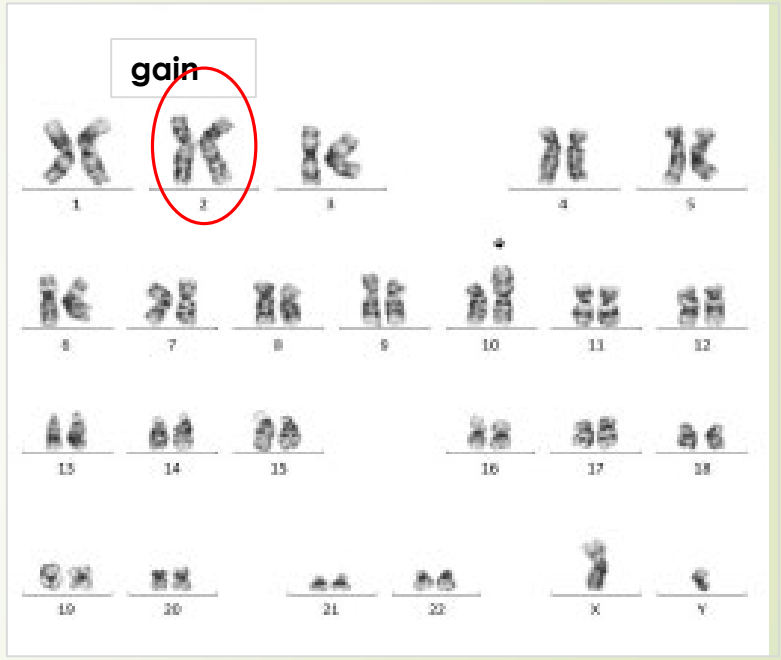
case2

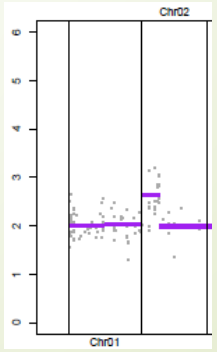




Chr	Pos	ID	REF	ALT	Gene
2	25469913	rs2276599	C	T	DNMT3A
2	25994412	rs71399322	A	AG	ASXL2
2	29446202	rs4622670	G	A	ALK
2	29449819	rs2293563;COSM148824	C	T	ALK
2	47601106	rs1126497	T	C	EPCAM
2	47630550	rs2303426	C	G	MSH2
2	47739551	rs2303424	A	G	MSH2

case3





Chr	Pos	ID	REF	ALT	Gene
2	25994416	rs372407443	A	G	ASXL2
2	47601106	rs1126497	T	C	EPCAM
2	47630550	rs2303426	C	G	MSH2
2	47703500	rs2303428;COSM133154	T	C	MSH2
2	48010558	rs1042820	C	A	MSH6
2	48018081	rs1800932	A	G	MSH6
2	48023115	rs1800935	T	C	MSH6

Conclusions

- NGS and CC results agree with 91% accuracy
- NGS and FISH results agree with 77% accuracy
- CN-LOH was mostly detected on chromosomes 13q, 17p and 22q
- Several pathogenic and VUS mutations were detected that may correlate with the CNAs

DISCLOSURE

I have relevant financial relationships with the materials and results in this presentation.

- Financial
  - Principal Scientist of Research and Development at NeoGenomics Laboratories
  - Shareholder of Neogenomics Laboratories