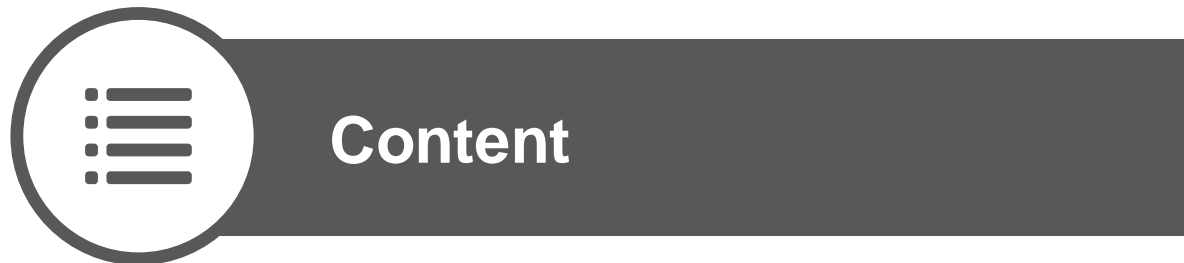




# Oncomine Childhood Cancer Research Assay

Customer Facing PPt

April 16 2018





# Childhood Cancer Is Different

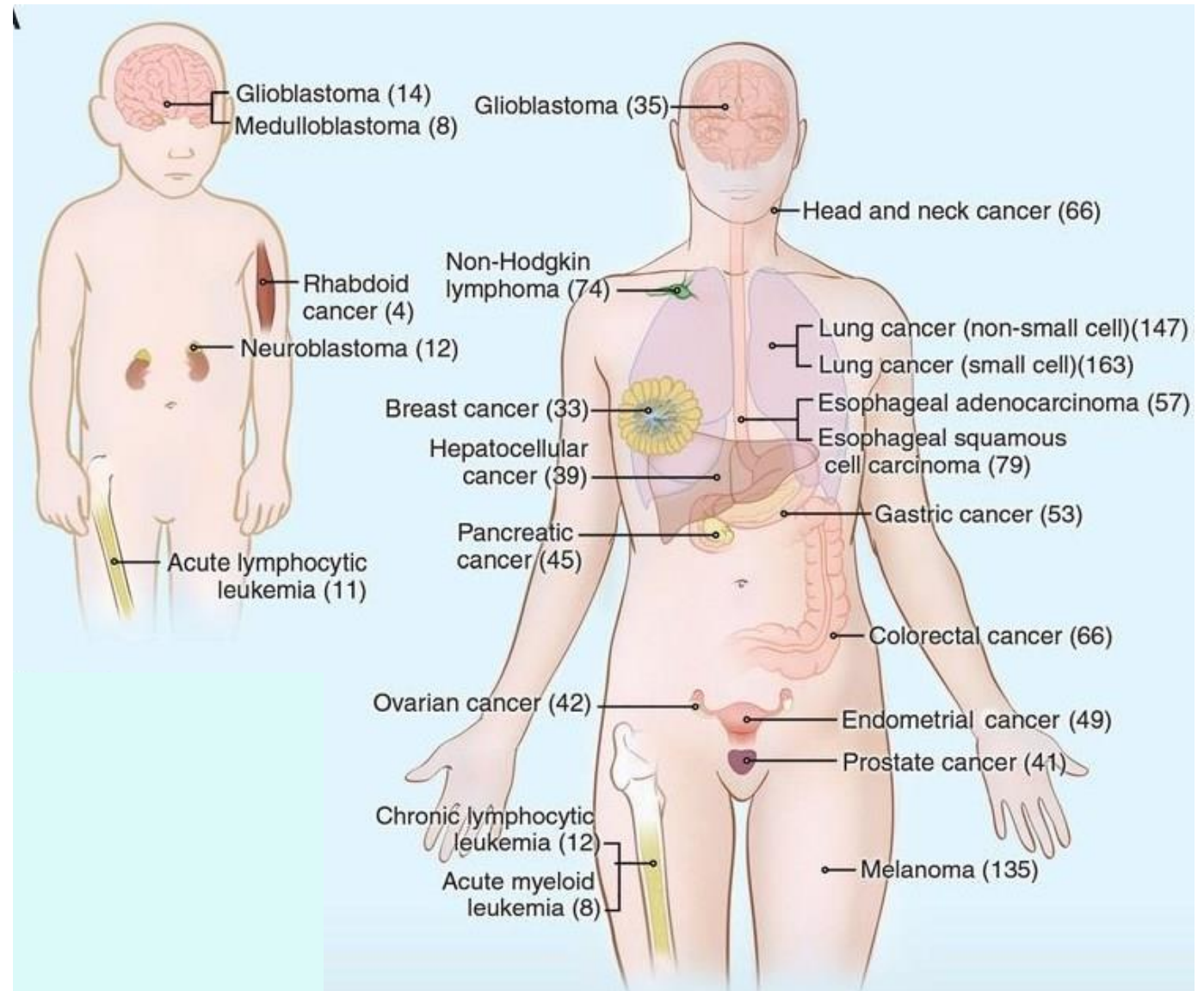


- Every year, 300,000 cases of cancer are diagnosed in children and teens under the age of 19 globally (over 15,000 cases are diagnosed annually in the United States, where cancer remains the most common cause of death by disease for children).
- Cancers impacting children and young adults (up to age 20) are fundamentally different from cancers impacting the adult population.
- Existing tools for comprehensive genomic profiling, developed for adult cancers, are not suitable for childhood and adolescent cancer research.



# Childhood Cancer Is Different

- Adult cancers are largely carcinoma, derived from epithelia with the most common cause being the accumulation of mutations over time, often caused from external sources
- Childhood and young adult cancers are rarely carcinoma most usually being mesenchymal or neuro-ectodermal in origin, developmental in character with few mutations.
- Childhood and young adult cancers also have fewer genomic aberrations than cancer in adults.



• Bert Vogelstein et al. *Science* 2013;339:1546-1558 . The genomes of a diverse group of adult (right) and pediatric (left) cancers. Numbers in parentheses indicate the median number of nonsynonymous mutations per tumor



# The Oncomine Childhood Cancer Research Assay (OCCRA) Is Unique



Designed to enable comprehensive genetic profiling of the most common childhood and adolescent cancers



Includes tumor-specific gene fusions, over-expressed genes, amplified genes, known gene mutations, insertions, and deletions—based on collaboration with leading scientists and pediatric oncologists



Robust performance is verified for multiple sample types, including FFPE, blood, and bone marrow



Free access to the International Childhood Oncology Network (ICON) and to a variant database, enabling researchers to collect, compare, and interrogate results

# New Addition to Broad Oncomine Solutions Portfolio

All Ion Torrent™ Oncomine™ solutions have a comprehensive end-to-end workflow that includes bioinformatics



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All OncoPrint Childhood Cancer Research Assay customers will be invited to become a member of ICON, established by Thermo Fisher Scientific and CHLA.

## Membership in this network will be free, and offers:

- 1 Connection with all other members to facilitate the optional sharing of protocols, best practices, and samples to enable rapid validation and ongoing quality assessment
- 2 Access to the Variant Database Platform
  - Allows interrogation of variants and comparison to other site results
  - Accumulate all OncoPrint Childhood Cancer Research Assay data to amass an invaluable database for future use
  - Link out to public databases
  - Future iterations of Ion Reporter Software intended to have a push button associated with OCCRA run files, allowing seamless upload

“The Ion Torrent Oncomine Childhood Cancer Research Assay is the first cancer panel designed for all forms of childhood cancer, including leukemia, brain tumors, and a diverse group of solid tumors. Of particular importance, it assays both RNA and DNA, allowing for the detection of both gene fusions and DNA mutations that are important in childhood cancer.”

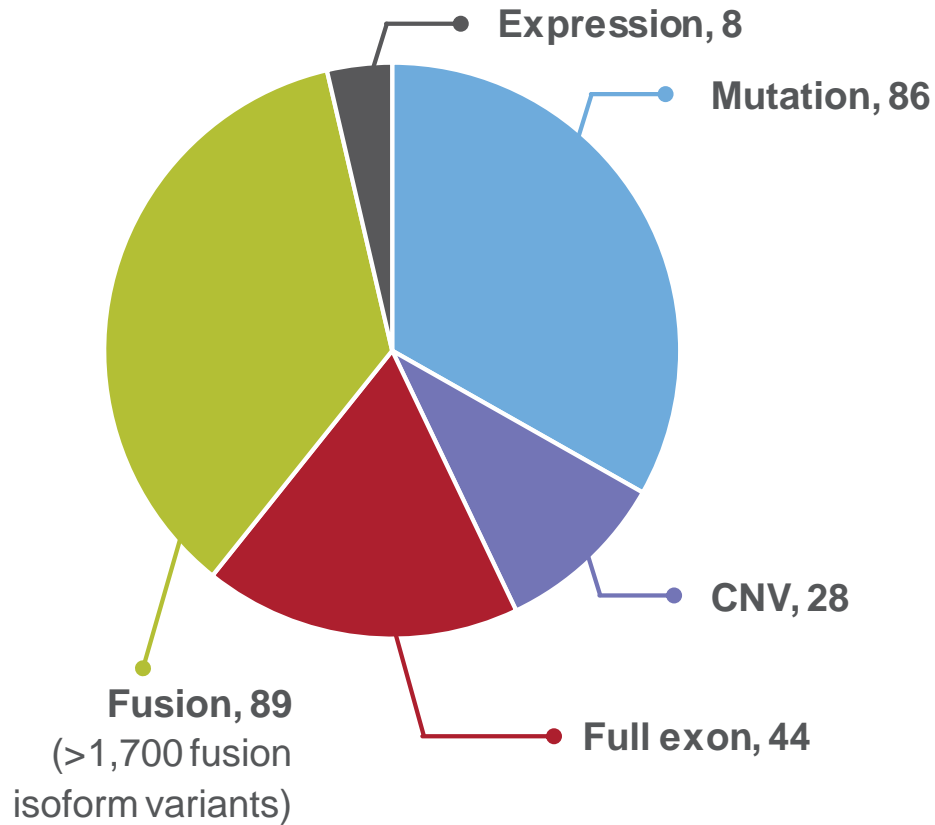


**Timothy Triche, MD, PhD**  
Founding Director,  
Children’s Hospital Los  
Angeles Center for  
Personalized Medicine



# Key Facts About the Cancer Driver Aberrations Covered

203 unique genes, 3,069 DNA primer pairs (2 pools), and 1,701 (2) RNA isoforms (2 pools)



Comprehensive coverage of all relevant mutations in each of the 86 genes

Full-gene coverage specifically for tumor suppressor genes, as deleterious mutations can occur anywhere in the coding sequence

Fusion cancer drivers are relatively more common in many childhood cancers such as sarcomas and hematological malignancies, so they are covered by a large panel

# Oncomine Childhood Cancer Research Assay Content

## Comprehensive mutation coverage

(86 genes)

ABL1	ERBB2	KDM4C	RAF1
ABL2	ERBB3	KDR	RET
ALK	ERBB4	KIT	RHOA
ACVR1	ESR1	KRAS	SETBP1
AKT1	EZH2	MAP2K1	SETD2
ASXL1	FASLG	MAP2K2	SH2B3
ASXL2	FBXW7	MET	SH2D1A
BRAF	FGFR1	MPL	SMO
CALR	FGFR2	MSH6	STAT3
CBL	FGFR3	MTOR	STAT5B
CCND1	FLT3	MYC	TERT
CCND3	GATA2	MYCN	TPMT
CCR5	GNA11	NCOR2	USP7
CDK4	GNAQ	NOTCH1	ZMYM3
CIC	H3F3A	NPM1	
CREBBP	HDAC9	NRAS	
CRLF2	HIST1H3B	NT5C2	
CSF1R	HRAS	PAX5	
CSF3R	IDH1	PDGFRA	
CTNNB1	IDH2	PDGFRB	
DAXX	IL7R	PIK3CA	
DNMT3A	JAK1	PIK3R1	
EGFR	JAK2	PPM1D	
EP300	JAK3	PTPN11	

## CNV

(28 genes)

ABL2	MYC
ALK	MYCN
BRAF	PDGFRA
CCND1	PIK3CA
CDK4	
CDK6	
EGFR	
ERBB2	
ERBB3	
FGFR1	
FGFR2	
FGFR3	
FGFR4	
GLI1	
GLI2	
IGF1R	
JAK1	
JAK2	
JAK3	
KIT	
KRAS	
MDM2	
MDM4	
MET	

## Full exon coverage

(44 genes)

APC	NF2
ARID1A	PHF6
ARID1B	PRPS1
ATRX	PSMB5
CDKN2A	PTCH1
CDKN2B	PTEN
CEBPA	RB1
CHD7	RUNX1
CRLF1	SMARCA4
DDX3X	SMARCB1
DICER1	SOCS2
EBF1	SUFU
EED	SUZ12
FAS	TCF3
GATA1	TET2
GATA3	TP53
GNA13	TSC1
ID3	TSC2
IKZF1	WHSC1
KDM6A	WT1
KMT2D	XIAP
MYOD1	
NF1	

## Fusion and expression

(97 genes)

ABL1	FLT3	MYB	PDGFB	TSPAN4
ABL2	FOSB	MYBL1	PDGFRA	UBTF
AFF3	FUS	MYH11	PDGFRB	USP6
ALK	GLI1	MYH9	PLAG1	WHSC1
BCL11B	GLIS2	NCOA2	RAF1	YAP1
BCOR	HMGA2	NCOR1	RANBP17	ZMYND11
BCR	JAK2	NOTCH1	RARA	ZNF384
BRAF	KAT6A	NOTCH2	RECK	
CAMTA1	KMT2A	NOTCH4	RELA	<b>Gene</b>
CCND1	KMT2B	NPM1	RET	<b>expression</b>
CIC	KMT2C	NR4A3	ROS1	
CREBBP	KMT2D	NTRK1	RUNX1	BCL2
CRLF2	LMO2	NTRK2	SS18	BCL6
CSF1R	MAML2	NTRK3	SSBP2	FGFR1
DUSP22	MAN2B1	NUP214	STAG2	FGFR4
EGFR	MECOM	NUP98	STAT6	IGF1R
ETV6	MEF2D	NUTM1	TAL1	MET
EWSR1	MET	NUTM2B	TCF3	MYCN
FGFR1	MKL1	PAX3	TFE3	MYC
FGFR2	MLLT10	PAX5	TP63	TOP2A
FGFR3	MN1	PAX7	TSLP	

>1,700 fusion isoform variants

# All Major Leukemia Translocations Represented

Acute lymphoblastic leukemia

ETV6-RUNX1, BCR-ABL1, MLL-AFF4, CDKN2A

Ph+ -like B-precursor ALL

ABL1, ABL2, CSF1R, PDGFRB, CRLF2, FLT3, KRAS

Acute myelogenous leukemia

FLT3, NPM1, KIT, IDH1, IDH2, DNMT3A, RUNX1, TET2, CEBPA

Acute promyelocytic leukemia

PML-RAR $\alpha$

# Childhood/Adolescent Brain Tumors and Types

<b>AT/RT, cribriform neuroepithelial tumor, schwannoma</b>	<b>Medulloblastoma, WNT, RT (rhabdoid tumor)</b>	<b>Medulloblastoma</b>	<b>Ependymoma</b>	<b>Ependymoma, meningioma</b>
SMARCB1	SMARCA4	GLI2, MSH6, MYCN, PTCH1, SUFU	RELA	NF2
<b>Astrocytoma</b>	<b>Glioblastoma</b>	<b>Glioma, astrocytoma grade I–IV, ependymoma grade 3–4</b>	<b>Glioma, astrocytoma grade I–IV, oligoastrocytoma</b>	<b>Pilocytic astrocytoma</b>
FGFR1, HIST1H3B, MDM2, PTPN11, TERT, TP53	MDM4	PTEN	H3F3A	BRAF, NTRK2



# Childhood/Adolescent Sarcoma Fusions

<b>Rhabdomyosarcoma (embryonal and alveolar)</b>	<b>Ewing sarcoma</b>	<b>Synovial cell sarcoma</b>	<b>Infantile (congenital) fibrosarcoma</b>	<b>Desmoplastic small round cell tumor</b>	<b>Alveolar soft part sarcoma</b>
PAX3/7-FOXO1	EWS-FLI1/ERG	SS18-SSX1/2/4	ETV6-NTRK	EWS-WT1	TFE3-ASPSCR1 (ASPL)
<b>Clear cell sarcoma (melanoma of soft parts)</b>	<b>Inflammatory myofibroblastic tumor</b>	<b>Fibromyxoid sarcoma</b>	<b>Dermatofibrosarcoma protuberans</b>	<b>Epithelioid sarcoma</b>	<b>Angiomatoid fibrous histiocytoma</b>
EWS-ATF1, EWS-CREB1	ALK-TPM3/4, CLTC	FUS-CREB3L2/1	COL1A-PDGFB	SMARCB1	EWS-CREB1
<b>Epithelioid hemangioendothelioma</b>	<b>Mesenchymal chondrosarcoma</b>	<b>Malignant peripheral nerve sheath tumor</b>	<b>Undifferentiated sarcoma</b>	<b>Midline carcinoma</b>	
WWTR1-CAMTA1	HEY1-NCOA2	NF1/NF2 mut	BCOR-CCNB3, CIC-DUX4	NUTM1-BRD4	

# End-To-End Workflow Optimized for Childhood Cancer Research

- Variety of sample types verified: blood and bone marrow, fresh/frozen tissue, and FFPE tissue
- Up to 8 samples can be multiplexed on a single chip
- 2–3 days from sample to report—fast turnaround time is important in pediatric cancer research

## Powerful

Efficient with challenging samples

## Simple

Automated library and templating options

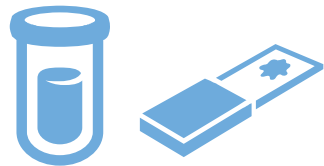
## Flexible

1 to 8 samples can be run on the same chip with DNA and RNA

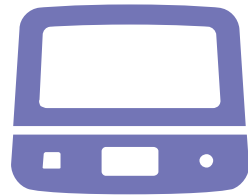
## Streamlined

Built-in informatics

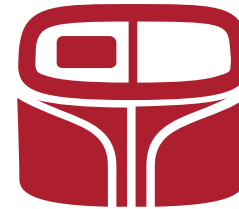
Ion AmpliSeq™ technology



Ion Chef™ System



Ion GeneStudio™ S5 System



Ion Reporter™ Software



The optimized bioinformatics pipeline will result in filtered lists of cancer driver mutations in .vcf file format that are uploadable to a dedicated childhood cancer variant platform

For Research Use Only. Not for use in diagnostic procedures.

# Average Run-Level Performance Data

	Average Total Run Reads	Average Uniformity	Average Mapped Reads per Barcode (DNA)	Average Polyconality	Average Low Quality	Average Median Run Read Length
S5 Manual Library Prep	80 million	96.5%	8.5 million	26%	8%	117
S5 Chef Library Prep	70 million	96.5%	7.5 million	43%	14%	117

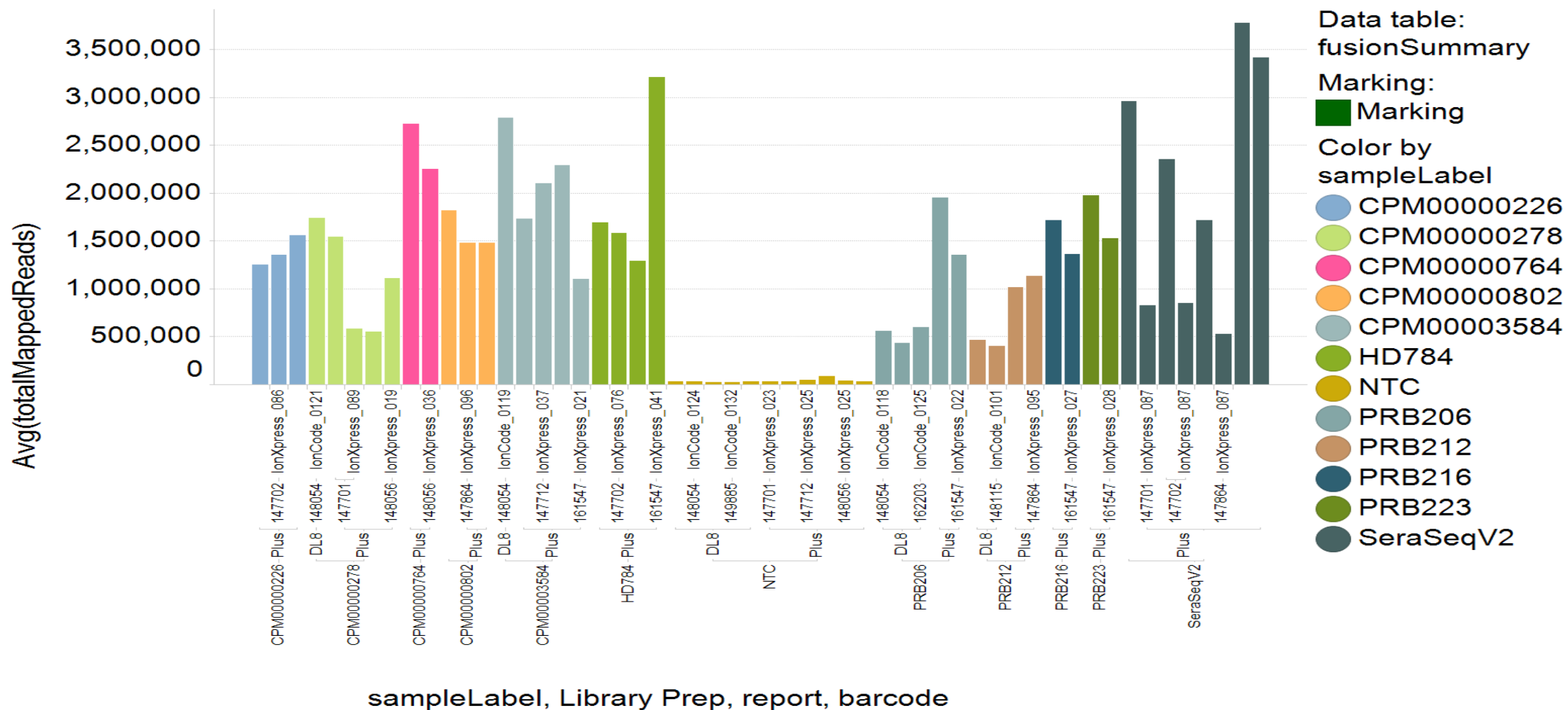
- Consistent performance across platforms
  - Ion S5 Manual Library Prep plexy = 7 samples + 1 NTC per Ion 540 Chip
  - Ion S5 Chef Library Prep plexy = 7 samples + 1 NTC per Ion 540 Chip

## Results of verification in Children Hospital Los Angeles on over 500 samples \*

- >5000X average coverage for DNA variants
- Average uniformity >95%
- Average mapped reads >2,000,000 for RNA fusions

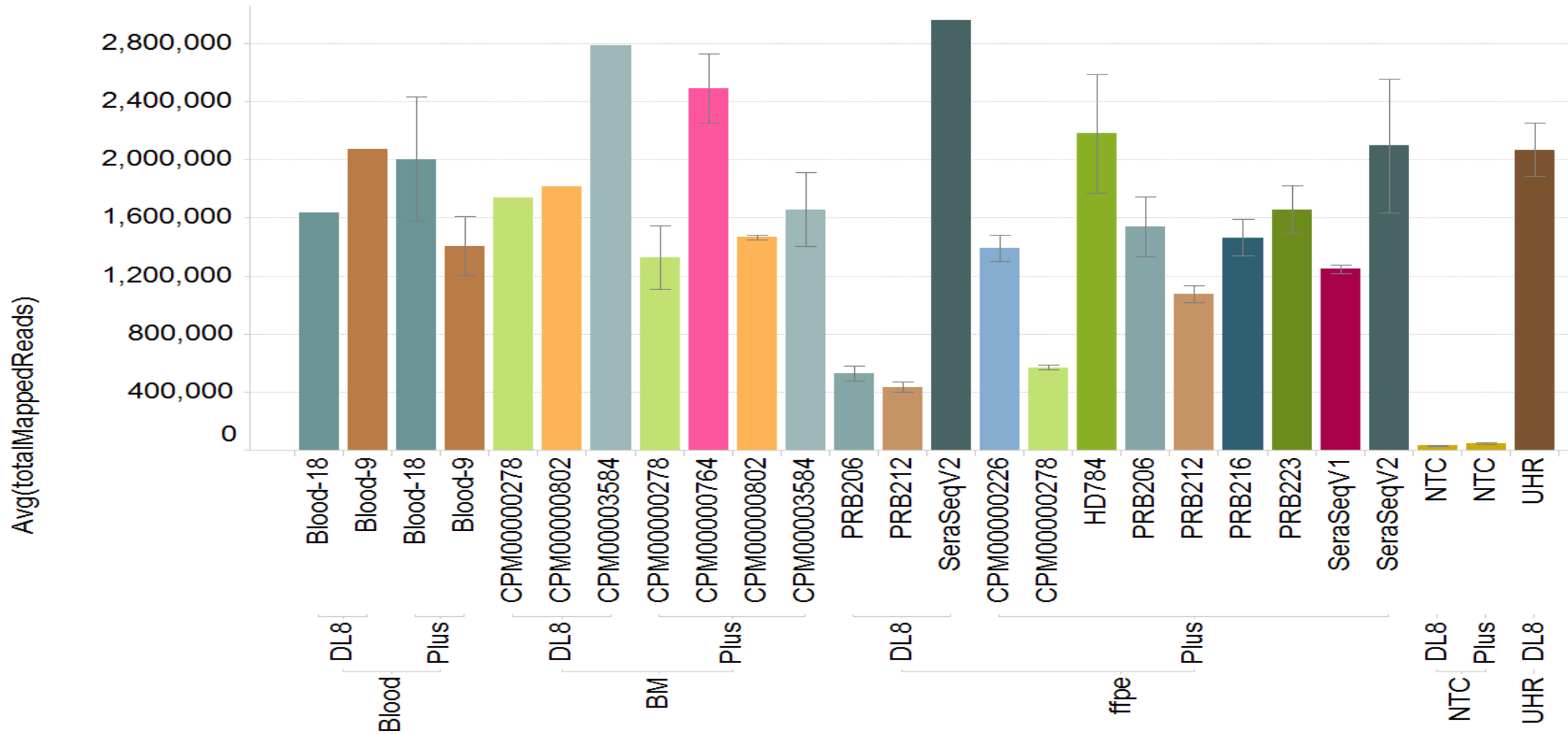
\*Data presented at AMP Europe Conference in April 2017

# DNA: Mapped Reads





# RNA: Total Mapped Reads



## OncoPrint Childhood Cancer Research Assay & ICON will enable progress and standardization of the childhood and young adult cancer research across the world

This assay is a **unique tool** designed specifically for use in childhood and young adult cancer research

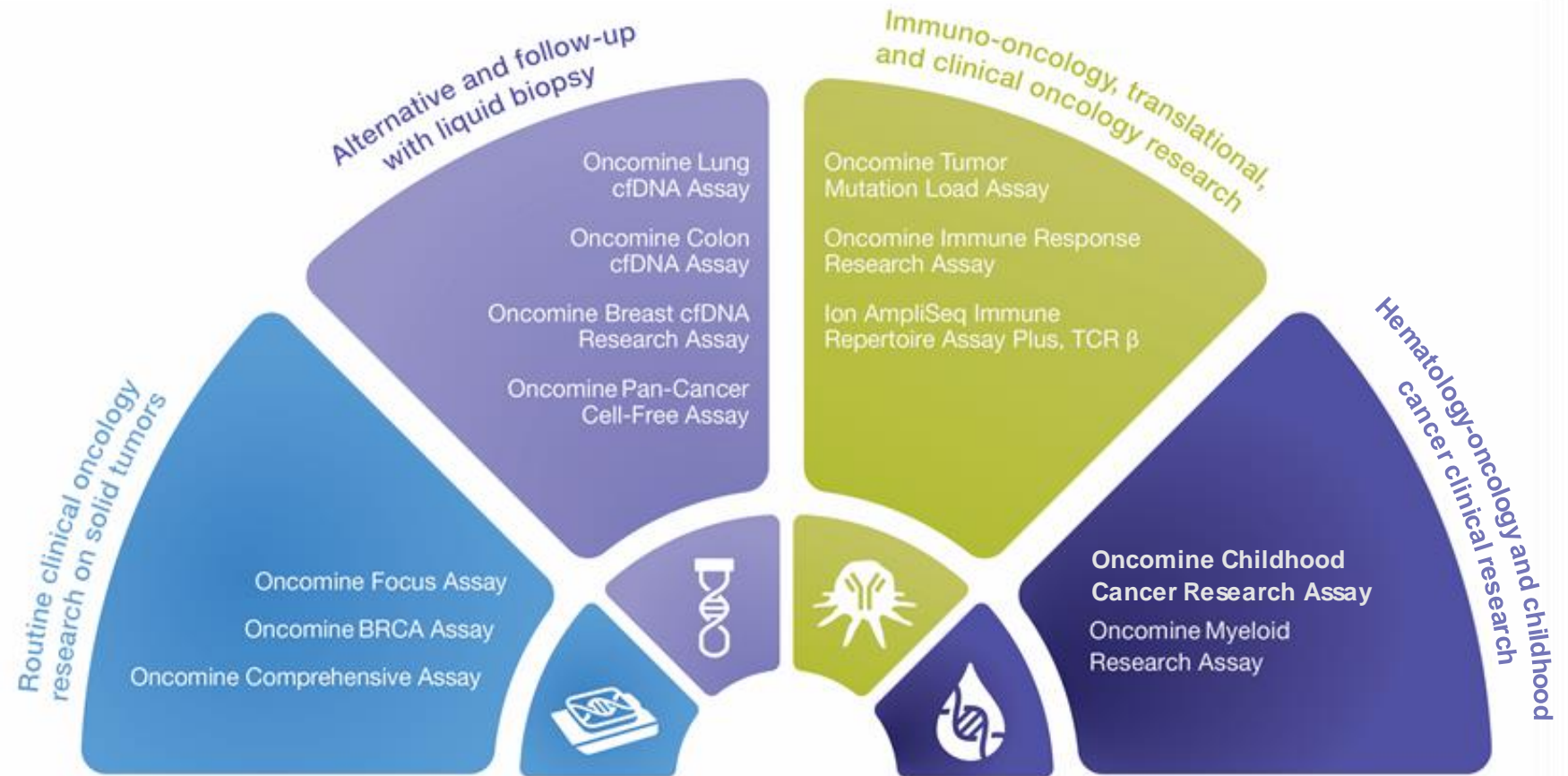
It has a **complete** end-to-end workflow, including informatics

**The content**, defined in collaboration with leading pediatric oncologists, covers key genetic aberrations across the whole spectrum of childhood cancers

Membership in **ICON** enables access to the variant database and collaboration with other researchers while collecting valuable findings, sharing protocols, best practices, and samples to enable rapid validation and ongoing quality assessment

# Complete Set of OncoPrint Assays for Clinical Research

OncoPrint assays provide comprehensive tools not only for solid tumor clinical research but also for hematology-oncology clinical research and translational research in immuno-oncology.





Thank you

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# Kit Configuration and Ordering Information



Oncomine™ Childhood Cancer Research Assay  
(Chef Ready Library x2, Equalizer and bar codes are included)

Content	Oligos & Library reagents
Number of pools	2 DNA, 2 RNA
Sample ID	Included
Blood/bone marrow compatible	Yes
FFPE compatible	Yes
Validation on clinical samples	Yes
Manufacturing QC	Enhanced



Oncomine™ Childhood Cancer Research Assay  
(Manual Library).  
Library Plus x2  
Equalizer and bar codes NOT included

Part Number	SAP Description	Samples
A36114	ONCOMINE CH CANCER RESASSY DNA	x24
A36483	ONCOMINE CH CANCER RESASSY RNA	x24

# Small Variant Detection

## SNV Detection

Hotspot

True Pos	False Pos	False Neg	%Sens	%PPV	%Repr*
387	1	8	98.0	99.7	96.5

**Samples:** AOHC synthetic control (MegaMix 5%) with 102 SNV Hotspot variants and Horizon 200, 728, and AF10 cell lines with 20 SNV Hotspot variants among them.

De novo

True Pos	False Pos	False Neg	%Sens	%PPV	%Repr*
342	1	0	100	99.7	100

**Samples:** AOHC synthetic control (MegaMix 5%) with 112 SNV De novo variants and Horizon 200, and AF10 cell lines with 2 SNV De novo variants among them.

## Indel Detection

Hotspot

True Pos	False Pos	False Neg	%Sens	%PPV	%Repr*
52	0	0	100	100	100

**Samples:** AOHC synthetic control (MegaMix 10%) with 5 Indel Hotspot variants and AF10 cell line with 3 Indel Hotspot variants.

De novo

True Pos	False Pos	False Neg	%Sens	%PPV	%Repr*
137	0	19	87.8	100	91.0

**Samples:** CPM00000028 bone marrow samples with 1 Indel De novo variant, AOHC synthetic control (MegaMix 20%) with 11 Indel De novo variants and Horizon 200 cell line with 1 Indel De novo variant.

# Copy Number Variants Detection in FFPE samples

True Pos	False Pos	False Neg	True Neg	%Sens	%Spec	%Repr*
40	0	0	32	100	100	100

Clinical Research Sample	Cellularity (LL)	Amplified Gene	FISH CNV	OCCRA	Neutral Gene	FISH CNV	OCCRA	Novel AMPs	
								Gene	OCCRA
365	90%	CCND1	13	13.14, 13.61, 13.03, 12.28	CDK4	1.9	1.93, 1.84, 1.93, 1.90		
1181615B	99%	EGFR	52.9	77.78, 78.42, 79.46, 81.80	MET	ND	2.77, 2.86, 2.87, 2.82		
		CDK4	14	14.09, 13.24, 13.31, 13.82	FGFR1	1.9	1.98, 1.73, 1.98, 1.89		
1193124B	100%	CDK6	16.8	7.86, 7.90, 7.82, 8.13	CDK4	3.3	2.24, 2.26, 2.23, 2.23	PDGFRA	8.74, 8.61, 8.66, 9.00
								KIT	6.34, 6.29, 6.59, 6.34
1196647B	80%	PIK3CA	10.5	8.80, 9.50, 9.88, 10.56	MET	3.2	2.15, 2.24, 2.30, 2.38		
1197046B	65%	CCND1	6.1	7.12, 7.69, 7.11, 7.66	EGFR	2.1	2.49, 2.43, 2.48, 2.58		
1198367B	70%	MYC	12.1	10.96, 10.41, 10.71, 11.27	CDK6	2.4	2.03, 2.03, 2.03, 2.11	CCND1	25.66, 26.76, 25.04, 25.73
1194253B	75%	PIK3CA	13.2	10.61, 11.41, 12.11, 11.17	MYC	2.5	1.56, 1.61, 1.63, 1.67		
1197339B	70%	CCND1	12.0	8.04, 8.13, 8.33, 8.84	PIK3CA	ND	2.44, 2.84, 2.54, 2.57	FGFR2	15.79, 15.86, 15.80, 16.09
1199087B	75%	CCND1	6.2	8.00, 7.77, 6.84, 8.12	CDK6	3.2	2.93, 2.76, 2.93, 2.93	EGFR	5.32, 5.51, 5.71, 5.37
								IL7R	6.41, 7.04, 7.48, 7.52
								PIK3CA	5.29, 5.41, 5.32, 5.19
								TERT	7.07, 12.59, 9.11, 7.25



**ThermoFisher**  
S C I E N T I F I C

# Oncomine Childhood Cancer Research Assay

Performance Data

April 11 2018

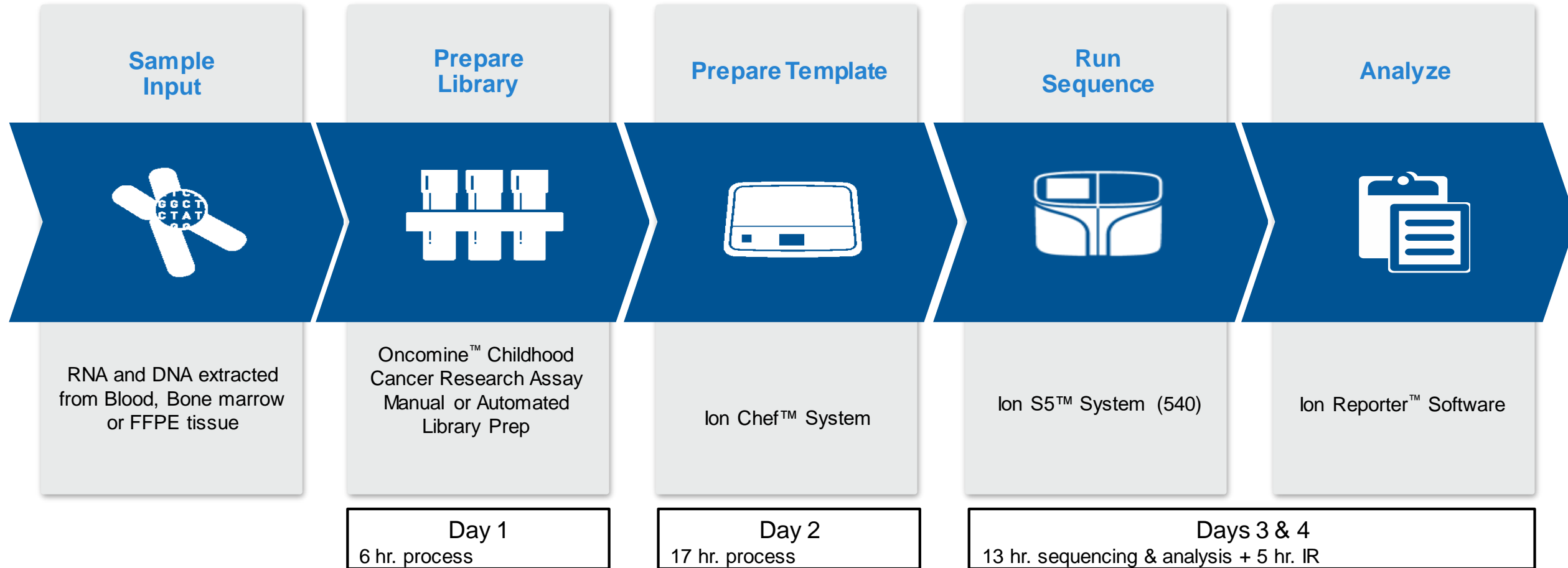
The world leader in serving science



Oncomine™ Childhood Cancer Research Assay													
<b>Number of pools</b>	4												
<b>Total Amplicons</b>	DNA: 3,060 (excluding Sample ID) RNA: 1,701												
<b>Amplicons/pool</b>	<table border="1"> <tr> <td>DNA pool 1: 1537</td> <td>Hotspot</td> <td>537</td> </tr> <tr> <td>DNA pool 2: 1532</td> <td>CDS</td> <td>2205</td> </tr> <tr> <td></td> <td>CNV</td> <td>142</td> </tr> <tr> <td></td> <td>CNV/Hotspot</td> <td>176</td> </tr> </table> RNA pool 1: 1006 (950 primers) RNA pool 2: 695 (581 primers)	DNA pool 1: 1537	Hotspot	537	DNA pool 2: 1532	CDS	2205		CNV	142		CNV/Hotspot	176
DNA pool 1: 1537	Hotspot	537											
DNA pool 2: 1532	CDS	2205											
	CNV	142											
	CNV/Hotspot	176											
<b>Avg. Amplicon insert length (range)</b>	DNA: 114 bp (52 - 216) RNA: 97 bp (34 - 222)												
<b>Sample ID</b>	Included, +9 amplicons												
<b>Libraries per sample</b>	2 (1 DNA, 1 RNA)												
<b>Sample Input</b>	10ng per pool (20ng DNA, 20ng RNA)												

# OCCRA Workflow

- RNA and DNA from same sample
  - Seven DNA samples plus one negative control –PLUS-
  - Seven RNA samples plus one negative control per chip



**PASS**

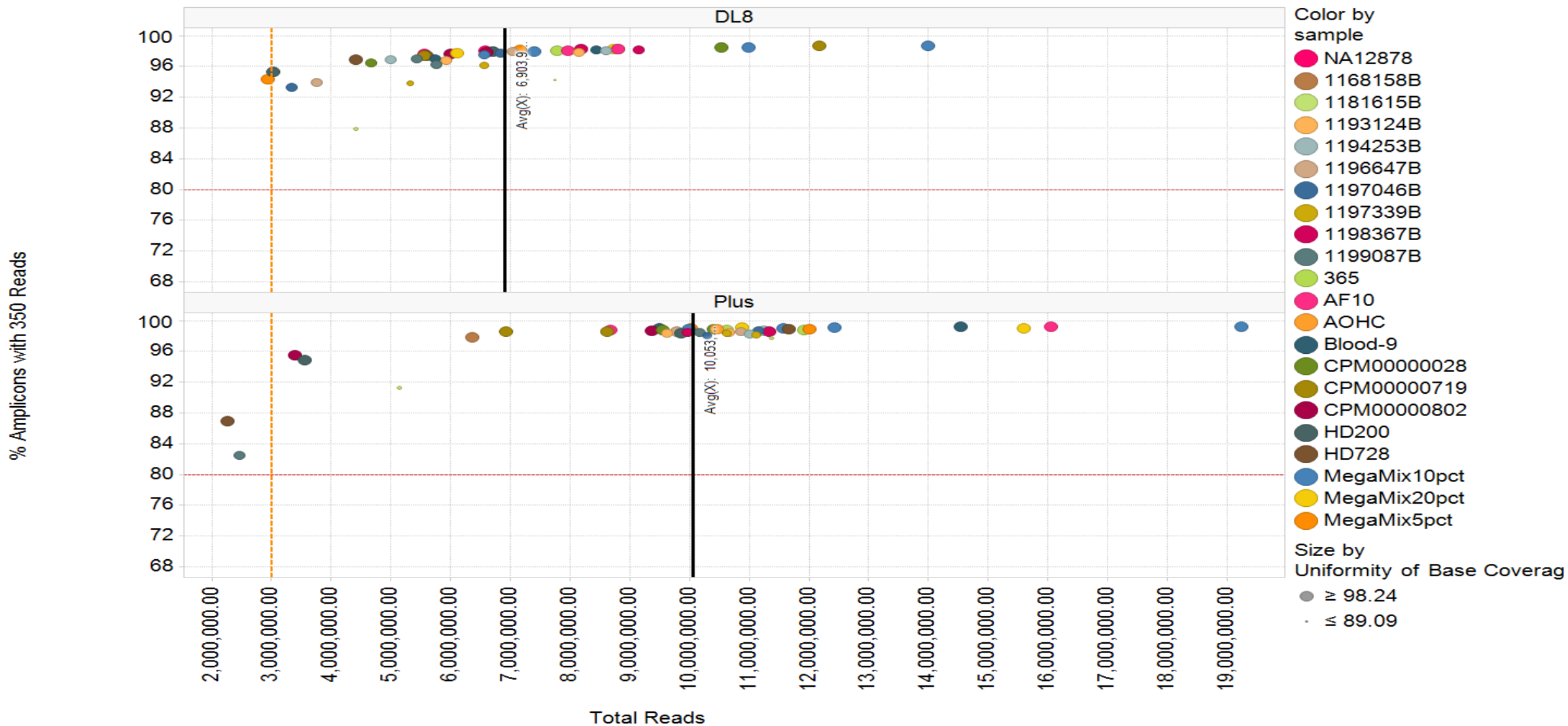
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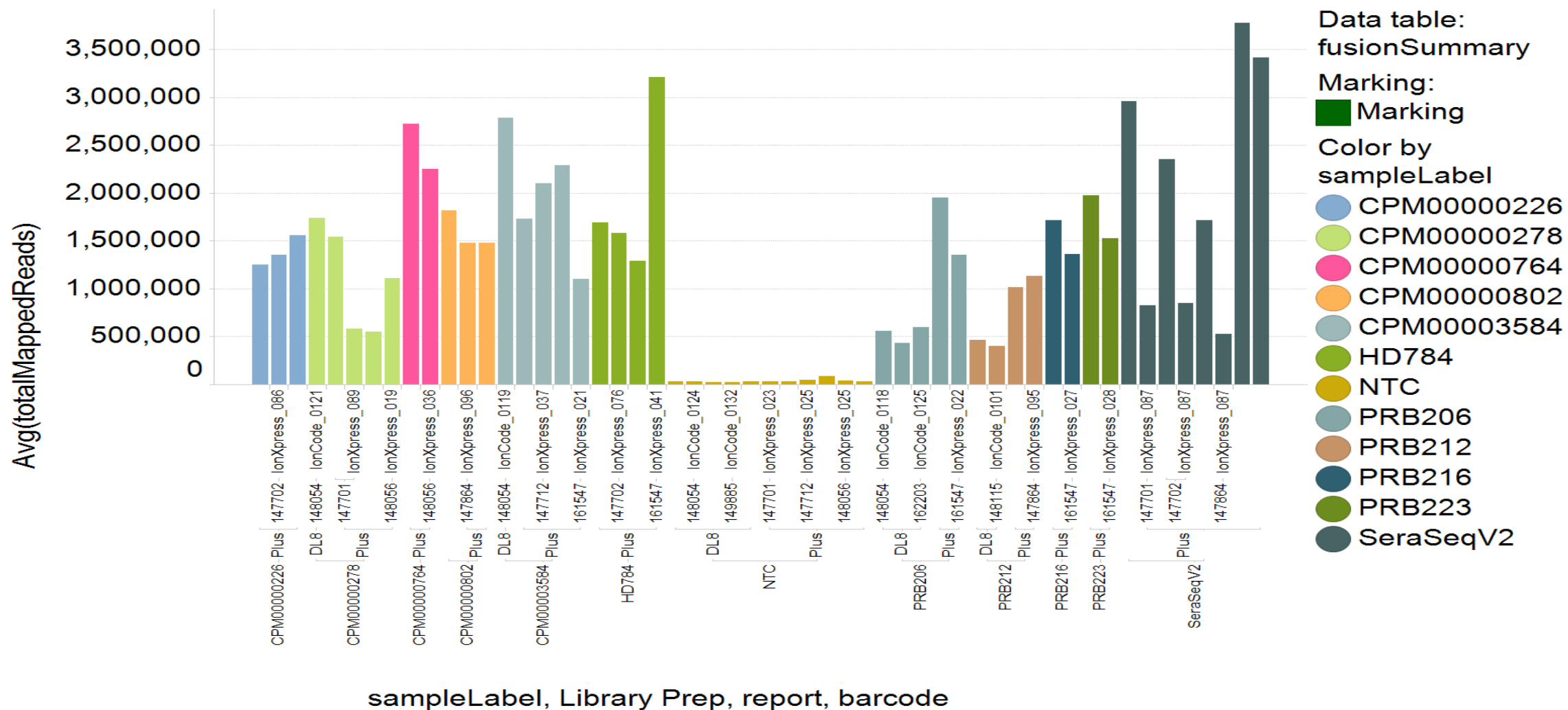
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# DNA: Amplicon Coverage vs Total Reads

% Amplicons with 350 Reads vs. Total Reads



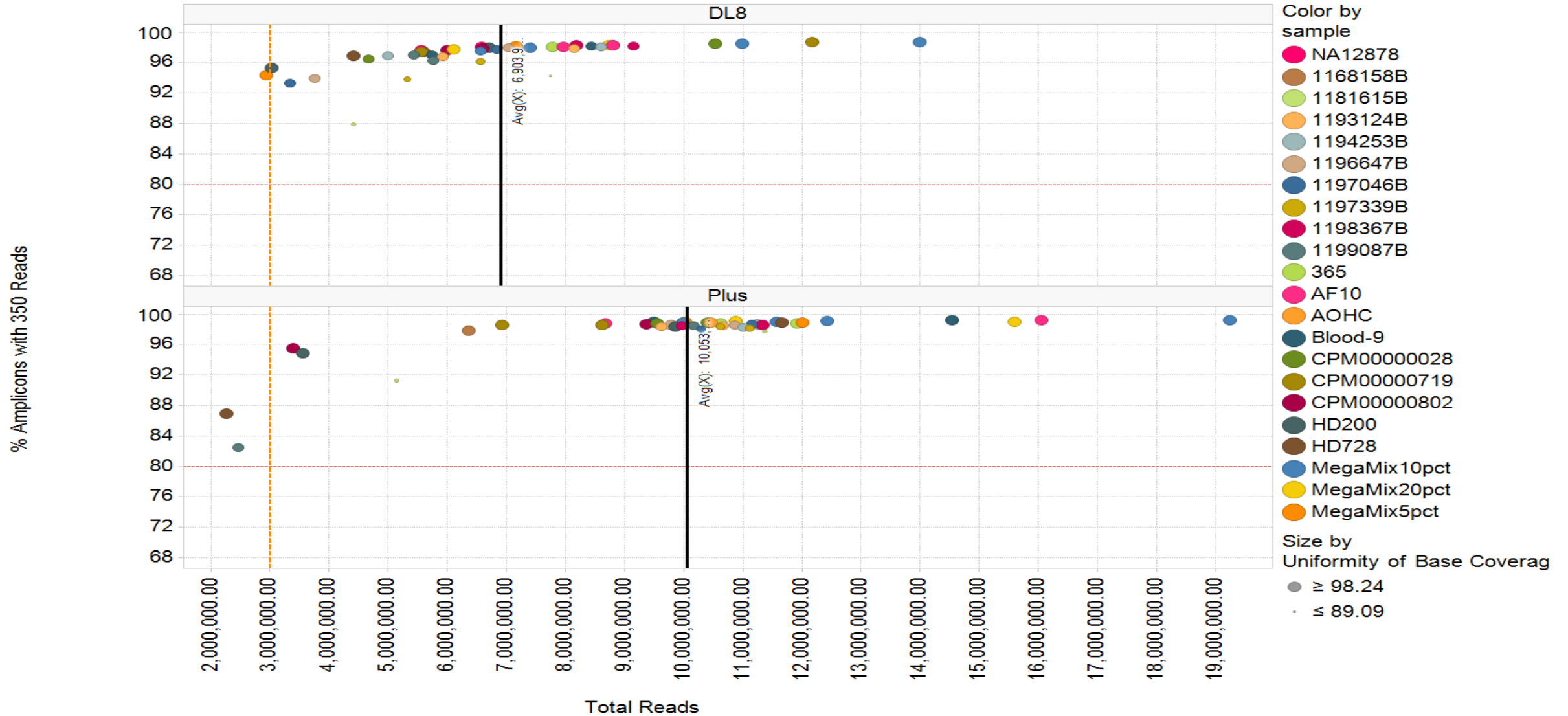
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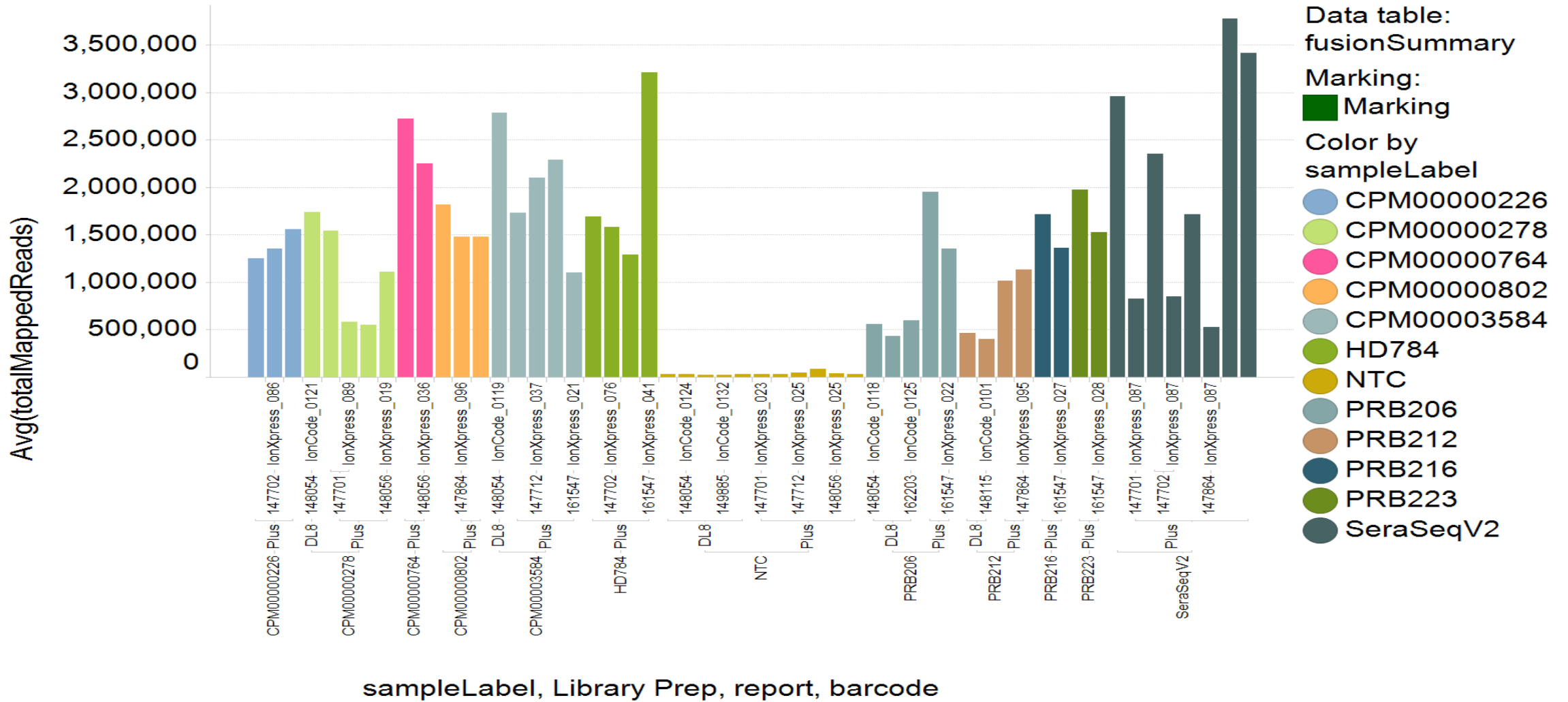


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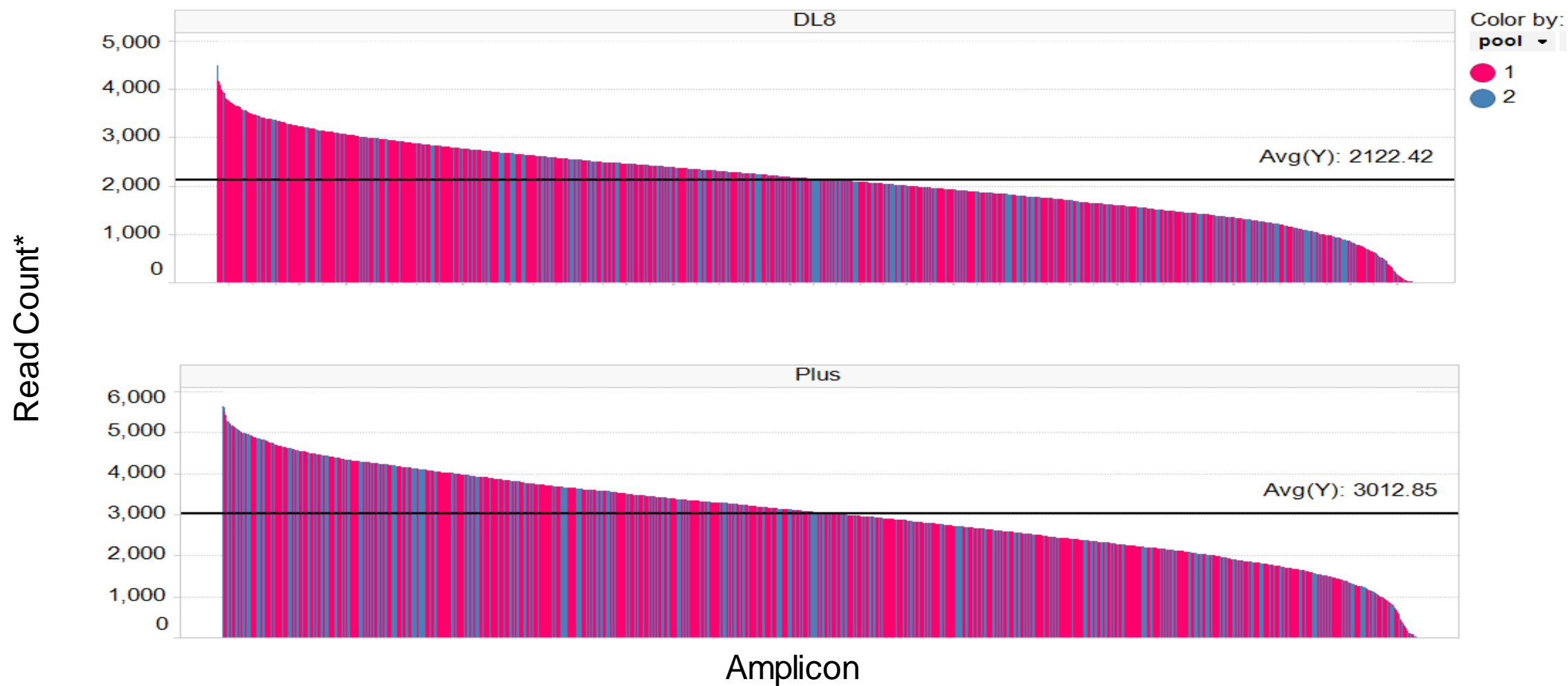
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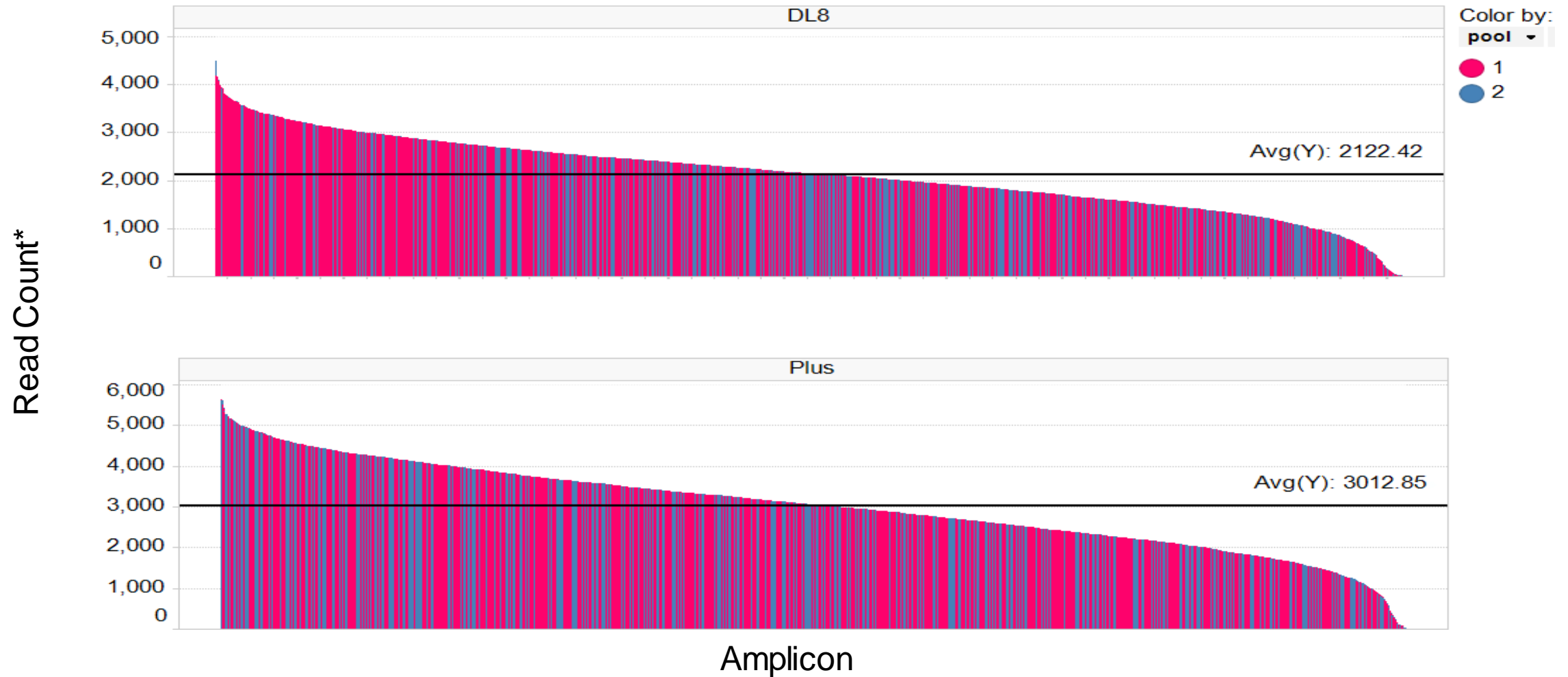


# DNA: Amplicon Coverage Depth



\*High outlier values stemming from high-level amplicification removed

# DNA: Amplicon Coverage Depth



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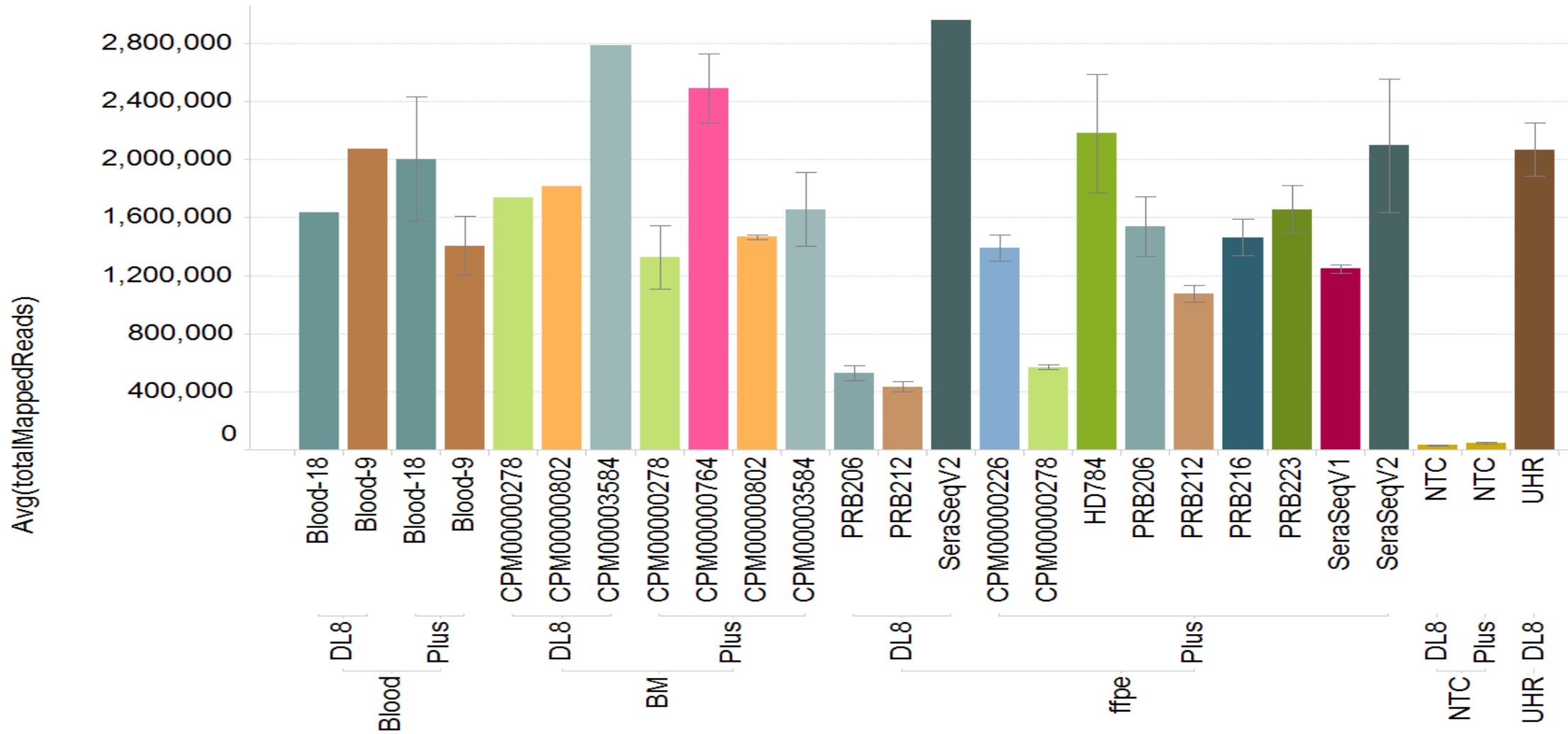
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		CDK4	14	14.09, 13.24, 13.31, 13.82	FGFR1	1.9	1.98, 1.73, 1.98, 1.89		
1193124B	100%	CDK6	16.8	7.86, 7.90, 7.82, 8.13	CDK4	3.3	2.24, 2.26, 2.23, 2.23	PDGFRA	8.74, 8.61, 8.66, 9.00
								KIT	6.34, 6.29, 6.59, 6.34
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1198367B	70%	MYC	12.1	10.96, 10.41, 10.71, 11.27	CDK6	2.4	2.03, 2.03, 2.03, 2.11	CCND1	25.66, 26.76, 25.04, 25.73
1194253B	75%	PIK3CA	13.2	10.61, 11.41, 12.11, 11.17	MYC	2.5	1.56, 1.61, 1.63, 1.67		
1197339B	70%	CCND1	12.0	8.04, 8.13, 8.33, 8.84	PIK3CA	ND	2.44, 2.84, 2.54, 2.57	FGFR2	15.79, 15.86, 15.80, 16.09
1199087B	75%	CCND1	6.2	8.00, 7.77, 6.84, 8.12	CDK6	3.2	2.93, 2.76, 2.93, 2.93	EGFR	5.32, 5.51, 5.71, 5.37
								IL7R	6.41, 7.04, 7.48, 7.52
								PIK3CA	5.29, 5.41, 5.32, 5.19
								TERT	7.07, 12.59, 9.11, 7.25



# RNA: Total Mapped Reads



# Fusions Detected

## Clinical Research Samples

Sample	Expected Fusion	Sample Type
CPM00000226	KIAA1549-BRAF.K14B9.COSF483	BM
CPM00000278	ETV6-RUNX1.E5R3	BM
CPM00000764	ETV6-RUNX1.E5R4	BM
CPM00000802	PML-RARA.P3R3	BM
CPM00003584	ETV6-RUNX1.E5R4	BM
PRB206	PML-RARA.P6R3	FFPE
PRB212	EWSR1-FLI1.E7F6	FFPE
PRB216	ETV6-NTRK3.E5N15.COSF571.1	FFPE
PRB223	BCR-ABL1.B14A2	FFPE

## Controls

Sample	Expected Fusion
HD784	CCDC6-RET.C1R12.COSF1271
	EML4-ALK.E13A20.COSF408.1
	SLC34A2-ROS1.S4R32.COSF1196
SeraSeq FFPE v2	CD74-ROS1.C6R34.COSF1200
	EGFR-SEPT14.E24S10
	EML4-ALK.E13A20.COSF408.1
	ETV6-NTRK3.E5N15.COSF571.1
	FGFR3-BAIA.P2L1.F17B2.COSF1346
	FGFR3-TACC3.F17T11.COSF1348
	KIF5B-RET.K24R11.COSF1262
	LMNA-NTRK1.L2N11
	NCOA4-RET.N7R12.COSF1491
	SLC34A2-ROS1.S4R34.COSF1198
	SLC45A3-BRAF.S1B8.COSF871
	TPM3-NTRK1.T7N10.COSF1329
	EGFR-EGFR.E1E8.DeIPositive.2
	MET-MET.M13M15

True Pos	False Pos*	False Neg	%Sens	%PPV	%Repr
140	0	0	100	100	100

# Seraseq RNA FFPE Control

## Seraseq FFPE Control Truth

### Fusion RNA

EML4-ALK  
KIF5B-RET  
NCOA4-RET  
CD74-ROS1  
SLC34A2-ROS1  
TPM3-NTRK1  
FGFR3-BAIAP2L1

Not assayed

PAX8-PPARG1  
FGFR3-TACC3  
ETV6-NTRK3  
LMNA-NTRK1  
SLC45A3-BRAF

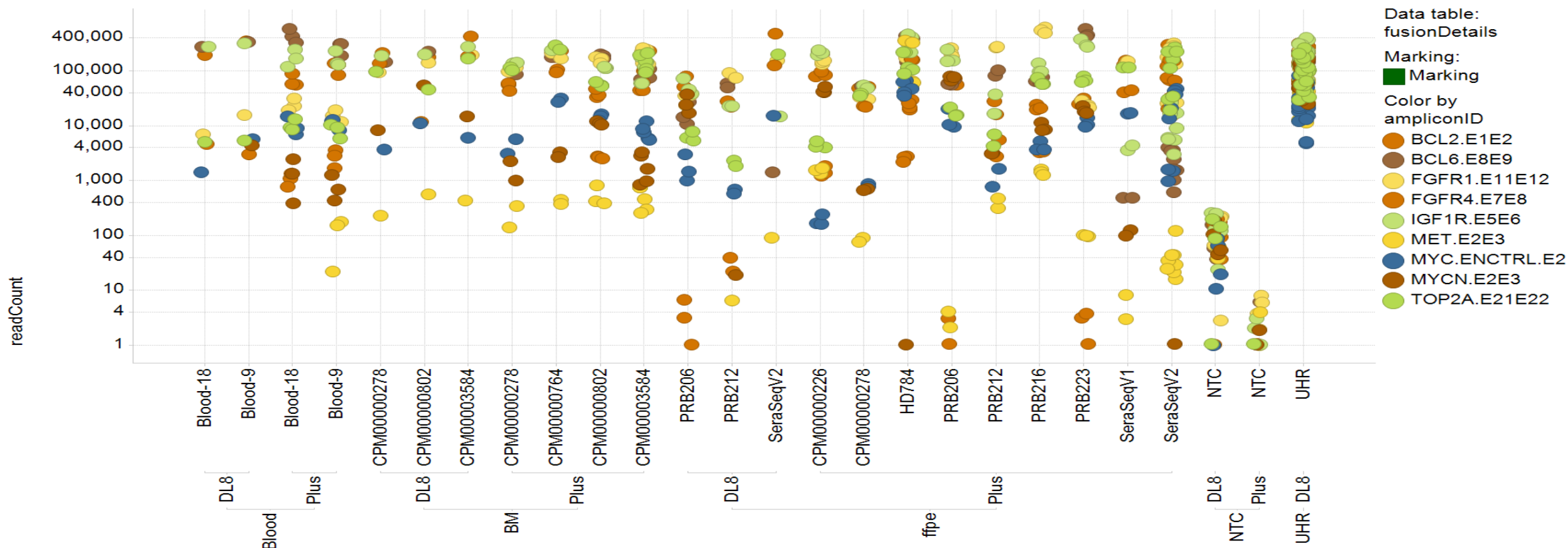
Not assayed

TPMRSS2-ERG  
EGFR-SEPT14  
MET exon 14 skipping  
EGFR Variant III  
(del exons 2-7)

## Replicates

	Plus		Plus		Plus		Plus		Plus		Plus		DL8			
	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)	Avg([r...)	Avg(re...)		
TPM3-NTRK1.T7N10.COSF1329	6.60 %	54,685	5.66 %	85,413	6.46 %	34,235	4.85 %	183,235	4.13 %	141,029	4.07 %	134,420	6.11 %	104,941	7.63 %	225,789
FGFR3-TACC3.F17T11.COSF1348	4.78 %	39,602	4.65 %	72,359	4.91 %	26,034	4.01 %	151,669	3.93 %	134,167	3.95 %	130,291	2.04 %	35,101	5.67 %	167,821
KIF5B-RET.K24R11.COSF1262	3.29 %	27,294	3.51 %	57,325	3.33 %	17,662	4.03 %	152,458	3.87 %	132,217	3.91 %	129,140	3.87 %	66,469	4.07 %	120,439
ETV6-NTRK3.E5N15.COSF571.1	4.33 %	35,923	4.72 %	78,129	4.37 %	23,162	2.69 %	101,767	3.18 %	108,786	3.20 %	105,521	3.65 %	62,755	4.20 %	124,191
EGFR-SEPT14.E24S10	2.52 %	20,858	2.75 %	46,517	2.53 %	13,424	2.73 %	103,257	3.21 %	109,647	3.15 %	103,974	2.33 %	40,060	3.17 %	93,909
LMNA-NTRK1.L2N11	1.88 %	15,607	1.58 %	23,203	1.98 %	10,513	1.77 %	66,731	2.03 %	69,283	2.01 %	66,320	1.24 %	21,368	2.20 %	65,188
FGFR3-BAIAP2L1.F17B2.COSF1346	1.41 %	11,669	1.54 %	25,358	1.36 %	7,223	1.83 %	69,085	2.00 %	68,200	1.95 %	64,452	1.57 %	27,037	1.45 %	42,800
SLC45A3-BRAF.S1B8.COSF871	1.26 %	10,419	1.27 %	20,354	1.39 %	7,363	1.31 %	49,669	1.23 %	42,112	1.25 %	41,436	1.13 %	19,386	1.76 %	52,147
EML4-ALK.E13A20.COSF408.1	0.57 %	4,765	0.87 %	16,192	0.57 %	3,022	1.41 %	53,339	1.57 %	53,507	1.56 %	51,509	1.03 %	17,719	0.82 %	24,293
NCOA4-RET.N7R12.COSF1491	1.10 %	9,115	1.17 %	18,950	1.05 %	5,547	1.23 %	46,662	1.40 %	47,835	1.36 %	44,797	0.78 %	13,366	0.39 %	11,419
SLC34A2-ROS1.S4R34.COSF1198	0.49 %	4,067	0.52 %	8,718	0.47 %	2,470	0.62 %	23,357	0.66 %	22,615	0.66 %	21,816	0.54 %	9,269	0.26 %	7,811
CD74-ROS1.C6R34.COSF1200	0.24 %	2,010	0.29 %	5,079	0.24 %	1,269	0.59 %	22,220	0.65 %	22,230	0.65 %	21,336	0.28 %	4,888	0.18 %	5,240
FGFR3-TACC3.F17T10	0.01 %	76	0.07 %	1,660	0.01 %	46	0.04 %	1,360	0.04 %	1,510	0.05 %	1,584	0.01 %	194	0.02 %	549
EML4-ALK.E20A20.COSF409.1	0.00 %	0	0.01 %	332	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.05 %	857	0.03 %	748
EML4-ALK.E6aA20.AB374361	0.00 %	0	0.00 %	48	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	45	0.01 %	282
FGFR3-TACC3.F18T11del5	0.01 %	64	0.00 %	45	0.01 %	34	0.00 %	72	0.00 %	49	0.00 %	43	0.00 %	0	0.00 %	69
FGFR3-TACC3.F17ins1T10	0.00 %	0	0.00 %	7	0.00 %	0	0.00 %	10	0.00 %	11	0.00 %	11	0.00 %	1	0.00 %	2
ETV6-RUNX1.E5R4	0.00 %	0	0.00 %	1	0.00 %	0	0.00 %	1	0.00 %	0	0.00 %	1	0.00 %	0	0.00 %	10
EML4-ALK.E13A20.COSF1062.2	0.00 %	2	0.00 %	1	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	1
ETV6-RUNX1.E5R3	0.00 %	1	0.00 %	0	0.00 %	0	0.00 %	3	0.00 %	0	0.00 %	0	0.00 %	0	0.00 %	0
EGFR-EGFR.E1E8.DelPositive.2	1.09 %	9,035	1.05 %	16,886	1.09 %	5,759	1.56 %	58,933	1.92 %	65,653	1.99 %	65,711	0.97 %	16,622	1.47 %	43,452
MET-MET.M13M15	0.05 %	443	0.17 %	3,670	0.05 %	269	0.62 %	23,249	0.77 %	26,231	0.75 %	24,793	0.27 %	4,668	0.13 %	3,957

# RNA: Gene Expression (Raw Reads)





**ThermoFisher**  
S C I E N T I F I C

# Oncomine Childhood Cancer Research Assay

Content Details

April 11 2018

The world leader in serving science

# Expression Controls, Gene Expression, Transcript Variants

Assay Type	Genes
GeneExpression	BCL2, BCL6, FGFR1, FGFR4, IGF1R, MET, MYC, MYCN, TOP2A
ExpressionControl	ABCF1, ARF1, EIF2B1, FBXW2, HMBS, ITGB7, LRP1, PSMB2, PUM1, RAB7A, SYMPK, TBP, TRIM27, VPS29
RNAExonVariant	BRAF, EGFR, MET, NOTCH1, RELA

## Expression Gene Pool Assignment:

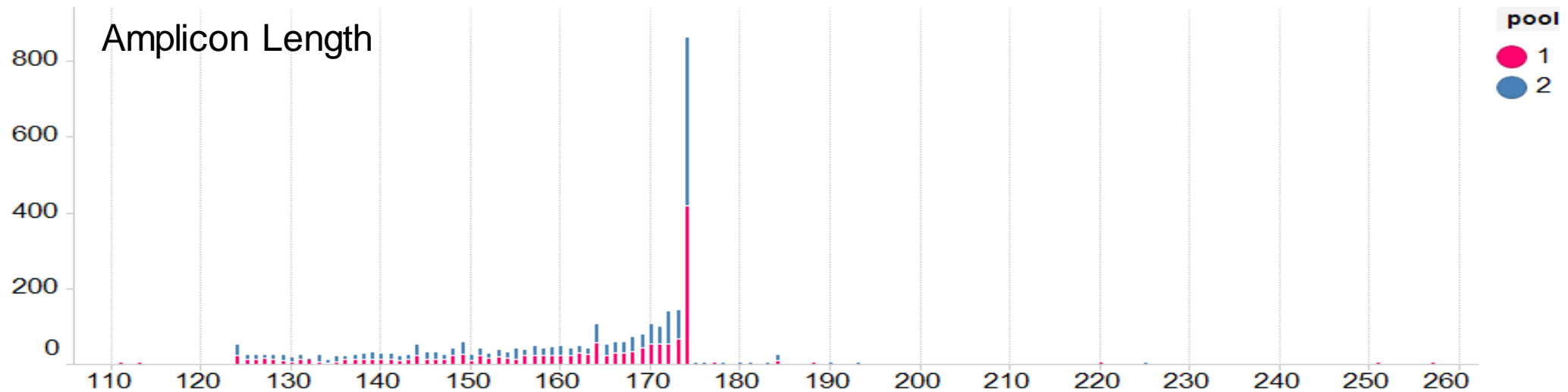
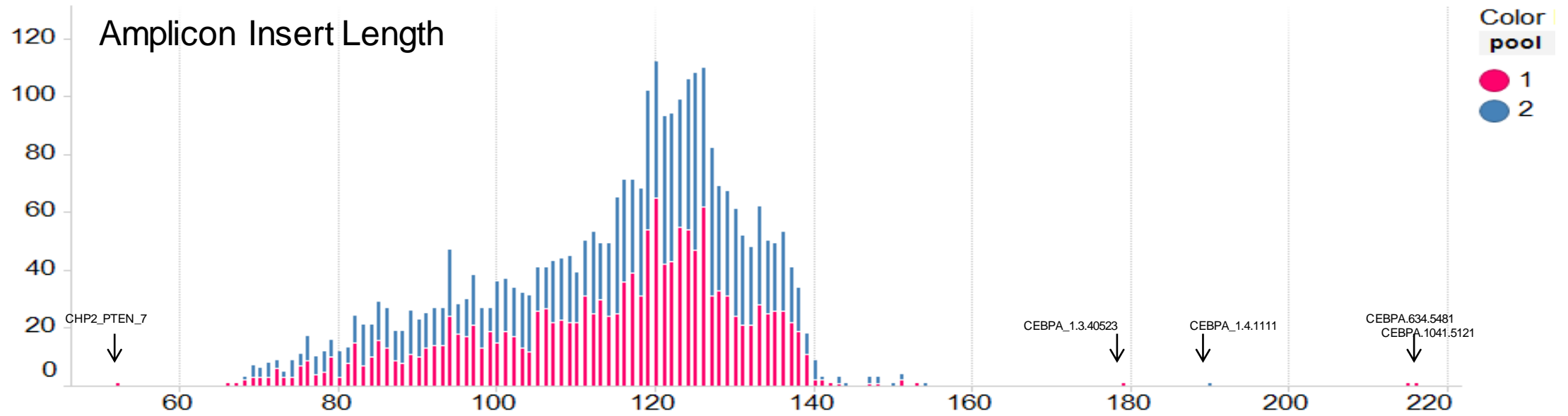
Type	Pool 1	Pool 2
GeneExpression	FGFR1 FGFR4 MYCN MET IGF1R TOP2A BCL2 BCL6 MYC	None
ExpressionControl	TRIM27 RAB7A SYMPK VPS29 ARF1	EIF2B1 PUM1 PSMB2 FBXW2 ABCF1 LRP1 HMBS TBP ITGB7
	Total: 14	Total: 9

## RNAExonVariant Isoforms:

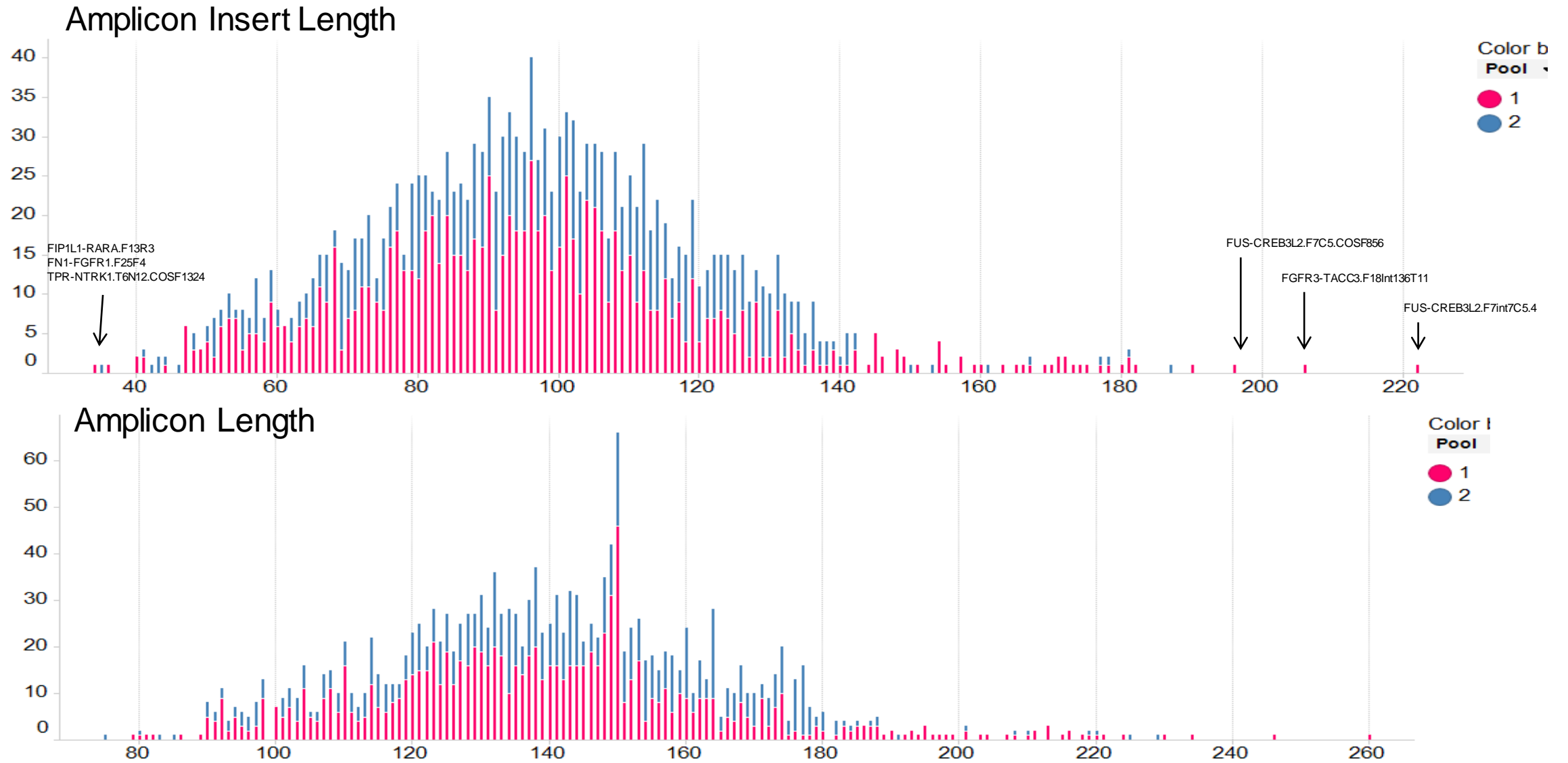
Gene	Transcript Variants	Comment
BRAF	exon18-exon10	kinase domain duplication
BRAF	exon1-exon11	
BRAF	exon1-exon9	
EGFR	exon13-exon15	
EGFR	exon1-exon8	
EGFR	exon24-exon28	
EGFR	exon25-exon18	kinase domain duplication
EGFR	exon3-exon5	
MET	exon13-exon15	
MET	exon 17-exon20	
NOTCH1	exon2-exon28	
RELA	exon1-exon3	



# DNA Size Distribution



# RNA Size Distribution



# Oncomine Childhood Cancer Fusion Assays (3' Acceptor, ABL1 to NCOA2)

3' Acceptor	5' Donor	# partners	# isoforms
ABL1	BCR, CABIN1, EML1, ETV6, FOXP1, INPP5D, NUP214, PRDM12, RALGPS1, RANBP2, RCSD1, RNF213, SFPQ, SNX2, SPECC1L, ZMIZ1	16	64
ABL2	ETV6, PAG1, RCSD1, ZC3HAV1	4	5
ALK	A2M, ACTG2, ATIC, ATRNL1, BEND5, BIRC6, C2orf44, CAD, CARS, CCDC88A, CENPF, CEPBZ, CLIP1, CLTC, COL25A1, DCTN1, EIF2AK3, EML4, FN1, GFPT1, GTF2IRD1, GTF3C2, HIP1, ITSN2, KANK2, KCNQ5, KIF5B, KLC1, KTN1, LMNA, MALAT1, MAPRE3, MCFD2, MEMO1, MROH2B, MSN, MYH9, NCOA1, NPM1, PICALM, PPFIBP1, PPM1B, PPM1G, PPP1CB, PPP1R21, PPP4R3B, PRKAR1, PRKAR1A, PRKAR1B, RANBP2, RNF213, RRBP1, SATB1, SEC31A, SPTBN1, SQSTM1, STK32B, STRN, TERT, TFG, TPM1, TPM3, TPM4, TPR, TRAF1, VCL	66	119
BCL11B	ZEB2	1	2
BCOR	ZC3H7B	1	1
BRAF	AGAP3, AGK, AGTRAP, AKAP9, AP3B1, ARMC10, ATG7, BAIAP2L1, BBS9, BCL2L11, BTF3L4, C7orf73, CCDC6, CCDC91, CCNY, CDC27, CEP89, CLCN6, CLIP2, CUL1, CUX1, DGKI, DYNC1I2, EML4, EPS15, ERC1, FAM114A2, FAM131B, FCHSD1, FXR1, GATM, GHR, GNAI1, GTF2I, HERPUD1, KCTD7, KDM7A, KIAA1549, KLHL7, LSM12, LSM14A, MACF1, MAD1L1, MKRN1, MKRN10, MYRIP, MZT1, NFIC, NRF1, NUB1, NUDCD3, NUP214, PAPSS1, PARP12, PICALM, PLIN3, PLXNA2, PPFIB2, RAD18, RBMS3, RNF11, RNF130, RP2, SLC12A7, SLC45A3, SND1, SOX6, STRN3, TANK, TAX1BP1, TMEM178B, TMPRSS2, TRIM24, TRIM4, UBN2, ZC3HAV1, ZKSCAN5, ZNF767P, ZSCAN30	79	118
CAMTA1	WWTR1	1	10
CREBBP	KAT6A, KAT6B, KMT2A	3	13
CRLF2	P2RY8	1	4
CSF1R	MEF2D, RBM6, SSBP2	3	4
EGFR	CAND1, SEC61G	2	2
FGFR1	BAG4, BCR, CNTRL, CPSF6, CUX1, ERLIN2, ERVK3_1, FGFR1OP, FGFR1OP2, FN1, LRRFIP1, MYO18A, RANBP2, SQSTM1, TPR, TRIM24, WHSC1L1, ZMYM2	18	28
FGFR2	APIP, CD44, COL25A1, CTNBN1, PARK2, PDHX, SLC45A3, SNX19	8	12
FGFR3	ETV6	1	1
FLT3	ETV6, SPTBN1	2	6
FOSB	SERPINE1, ZFP36	2	2
GLI1	ACTB	1	2
GLIS2	CBFA2T3	1	2
HMGA2	FHIT, LPP, RAD51B	3	3
JAK2	ATF7IP, BCR, BICD2, EBF1, ETV6, OFD1, PAX5, PCM1, PPFIBP1, RIC1, SEC31A, SPAG9, SSBP2, STRN3, TERF2, TPM3, TPR	17	38
KMT2A	NUP98	1	1
LMO2	RAG2	1	1
MAML2	C11orf95, CRTCL1, CRTCL3, KMT2A	4	5
MECOM	ETV6, PSMD2, RPN1, RUNX1	4	5
MET	BAIAP2L1, C8orf34, CAPZA2, CLIP2, DCTN1, EPS15, KIF5B, LRRFIP1, OXR1, PPFIBP1, PTPRZ1, SCAF11, SLC34A2, TFG, TPR, TRIM4, ZKSCAN1	17	21
MKL1	RBM15	1	2
MLLT10	DDX3X, HNRNPH1, KMT2A, NAP1L1, NUP98, PICALM	6	31
MN1	ETV6	1	2
MYBL1	C8orf34	1	1
MYH11	CBFB, KMT2A	2	20
NCOA2	AHRR, ETV6, HEY1, KAT6A, PAX3, SRF, TEAD1, VGLL2	8	11

# Oncomine Childhood Cancer Fusion Assays (3' Acceptor, NCOR1 to ZNF384)

3' Acceptor	5' Donor	# partners	# isoforms
NCOR1	PAX5	1	1
NOTCH1	MIR143HG, SEC16A	2	3
NOTCH2	SEC22B	1	1
NOTCH4	NSD1	1	1
NR4A3	EWSR1, TAF15, TCF12, TFG	4	9
NTRK1	ARHGEF2, BCAN, CD74, CEL, CHTOP, EPHB2, EPS15, GSN, IRF2BP2, LMNA, MPRIP, MRPL24, NFASC, PPL, RNF213, SQSTM1, SSBP2, TFG, TP53, TPM3, TPR	21	50
NTRK2	AFAP1, AGBL4, DAB2IP, ETV6, NACC2, NAV1, QKI, SLMAP, SQSTM1, TP63, TRIM24, VCL	12	13
NTRK3	AKAP13, BTBD1, COX5A, EML4, ETV6, FAT1, LYN, MYO5A, RBPMS, TFG	10	15
NUP214	NOTCH1, SET	2	2
NUTM1	BRD3, BRD4, CIC, WHSC1L1	4	6
NUTM2B	YWHAE	1	1
PDGFB	COL1A1	1	42
PDGFRA	BCR, CDK5RAP2, DIP2C, ETV6, FIP1L1, FOXP1, KDR, KIF5B, SCAF11, STRN, TNKS2	11	30
PDGFRB	ATF7IP, BIN2, CAPRIN1, CCDC6, CCDC88C, CEP85L, CPSF6, DTD1, EBF1, ERC1, ETV6, GIT2, GOLGA4, GOLGB1, HIP1, KANK1, MPRIP, MYO18A, NDE1, NIN, PDE4DIP, PRKG2, RABEP1, SART3, SPECC1, TNIP1, TP53BP1, TPM3, TRIP11, WDR48, ZEB2	31	41
PLAG1	CHCHD7, COL1A2, COL3A1, CTNNB1, HAS2, LIFR, TCEA1	7	10
RAF1	AGGF1, ATG7, B4GALT1, CLCN6, CNTLN, ESRP1, FYCO1, GOLGA4, HACL1, LMNA, MPRIP, NFIA, PAPP7, PDZRN3, QKI, SRGAP3, TRAK1, TRIM33	18	19
RARA	ADAMTS17, BCOR, FIP1L1, IRF2BP2, MED1, NABP1, NPM1, NUMA1, PML, PRKAR1A, STAT5B, TBL1XR1, ZBTB16	13	45
RECK	PCSK5	1	1
RELA	C11orf95, ZMYND8	2	4
RET	ACBD5, AFAP1, AKAP13, BCR, CCDC6, CUX1, ERC1, FGFR1OP, FKBP15, FRDM4A, GOLGA5, HOOK3, KIAA1217, KIAA1468, KIF5B, KTN1, MYH10, MYH13, NCOA4, PCM1, PRKAR1A, RUFY2, SPECC1L, TBL1XR1, TRIM24, TRIM27, TRIM33	27	54
ROS1	CCDC30, CCDC6, CD74, CEP85L, CLIP1, CLTC, ERC1, EYS, EZR, GOPC, HLA_A, KIAA1598, LIMA1, LRIG3, MSN, MYO5A, NCOR2, NFKB2, PPF1BP1, PWWP2A, SDC4, SLC22A16, SLC34A2, TFG, TMEM106B, TPD52L1, TPM3, TSPAN3, YWHAE, ZCCHC8	30	47
RUNX1	ETV6	1	4
SS18	MEF2D	1	1
STAT6	NAB2	1	29
TAL1	STIL	1	3
TFE3	ASPSR1, CLTC, DVL2, LUC7L3, MED15, NONO, PARP14, PRCC, SFPQ, YAP1	10	22
TP63	TBL1XR1	1	3
TSLP	IQGAP2	1	1
USP6	CDH11, CNBP, COL1A1, MYH9, OMD, THRAP3	6	11
WHSC1	ST6GAL1	1	1
YAP1	C11orf95	1	1
ZNF384	ARID1B, BMP2K, CREBBP, EP300, EWSR1, TAF15, TCF3	7	18

# Oncomine Childhood Cancer Fusion Assays (5' Donor, AFF3 to NOTCH1)

5' Donor	3' Acceptor	# partners	# isoforms
AFF3	BCL2	1	1
ALK	PTPN3	1	1
BCOR	CCNB3, L3MBTL2, MAML3	3	3
BCR	LZTR1	1	1
CCND1	FSTL3	1	1
CIC	DUX4, FOXO4	2	6
CRLF2	CSF2RA	1	1
DUSP22	PTK7	1	1
EGFR	GNS, MCL, PSPH, RAD51, SEPT14, VOPP1	6	6
ETV6	ACSL6, ANLN, ARNT, ATP2B1, CDX2, CHIC2, DUSP16, FRK, GOT1, INO80D, ITPR2, LRP6, LYN, PER1, PRDM16, PTPRR, SYK	17	19
EWSR1	ATF1, CREB1, CREB3L1, CREB3L2, CREM, DDIT3, ERG, ETV1, ETV4, FEV, FLI1, KLF15, NFATC1, NFATC2, PATZ1, PBX1, PBX3, POU5F1, SMARCA5, SP3, WT1, YY1, ZNF444	23	68
FGFR1	ADAM32, HOOK3, TACC1, WHSC1L1	4	5
FGFR2	ACSL5, AFF3, AHCYL1, BICC1, CASP7, CCAR2, CCDC6, CIT, CLIP2, COL14A1, CREB5, FAM76A, KCTD1, KIAA1217, MGEA5, NOL4, OFD1, OGDH, OPTN, PCM1, PPHLN1, SHTN1, TACC3, WARS, ZMYM4	25	28
FGFR3	AES, BAIAP2L1, ELAVL3, FBXO28, JAKMIP1, TACC3	6	33
FUS	ATF1, CREB3L1, CREB3L2, DDIT3, ERG, FEV, KLF17, POU5F1	8	71
HMGA2	ALDH2, CCNB1IP1, CHMP1A, COX6C, EBF1, FHIT, LHFP, LPP, NFIB, PPAP2B, RAD51B, WIF1	12	19
KAT6A	EP300, NCOA3	2	3
KMT2A	ABI1, ABI2, ACACA, ACER1, ACTN4, ADARB2, AFF1, AFF3, AFF4, APBB1IP, ARHGAP26, ARHGEF12, ARHGEF17, ATG16L2, BTBD18, C11orf88, CASC5, CASP8AP2, CBL, CDK6, CEP170B, CT45A2, DAB2IP, DCP1A, DCPS, ELL, ENAH, EP300, EPS15, FLNA, FNBP1, FOXO3, FOXO4, FRYL, GAS7, GMPS, GPHN, KIAA1524, LASP1, LPP, MAPRE1, MEF2C, MLLT1, MLLT11, MLLT3, MLLT4, MLLT6, MYO18A, MYO1F, NCKIPSD, NEBL, NKAIN2, NRIP3, PDS5A, PICALM, RABGAP1L, RNF115, RPS3, SARNP, SEPT11, SEPT2, SEPT5, SEPT6, SEPT9, SH3GL1, SMAP1, SORBS2, TECR, TET1, TIRAP, TOP3A, USP2, UVRAG, ZFYVE19	74	173
KMT2B	GPS2	1	1
KMT2C	PRKAG2	1	3
KMT2D	ADCY9	1	1
MAN2B1	RNASEH2A	1	1
MEF2D	BCL9, DAZAP1, HNRNPUL1	3	6
MN1	BEND2	1	1
MYB	NFIB, PCDHGA1, QKI, RAD51B, TYK2	5	18
MYBL1	NFIB	1	5
MYH9	IL2RB	1	1
NCOR1	LYN	1	1
NOTCH1	GABBR2, SDCCAG3, SNHG7	3	3

# Oncomine Childhood Cancer Fusion Assays (5' Donor, NPM1 to ZMYND11)

5' Donor	3' Acceptor	# partners	# isoforms
NPM1	MLF1	1	1
NTRK1	DYNC2H1	1	1
NTRK3	ETV6, HOMER1	2	2
NUP214	DEK, XKR3	2	2
NUP98	ADD3, CCDC28A, CCT8, DDX10, GSX2, HHEX, HMGB3, HOXA11, HOXA13, HOXA9, HOXC11, HOXC13, HOXD11, HOXD13, IQCG, KDM5A, LNP1, NSD1, PHF23, POU1F1, PRRX1, PRRX2, PSIP1, RAP1GDS1, RARG, SETBP1, TOP1, TOP2B, WHSC1L1	29	44
PAX3	FOXO1, FOXO4, MAML3, NCOA1	4	5
PAX5	ASXL1, AUTS2, BRD1, DACH1, DACH2, ELN, ESRRB, FOXP1, GOLGA6A, HIPK1, KIAA1549L, KIF3B, MLLT3, NOL4L, PML, POM121, SLC01B3, SOX5, TAOK1, ZNF521	20	29
PAX7	FOXO1	1	1
RAF1	C9orf153	1	1
RANBP17	TLX3	1	1
RUNX1	AFF3, CBFA2T2, CBFA2T3, CEP76, CLCA2, EVX1, KIAA1549L, LPXN, MACROD1, MRPS6, NOL4L, PRDM16, PRDX4, RPL22, RUNX1T1, SH3D19, TRPS1, USP16, USP42, YTHDF2, ZFPM2, ZNF687	22	37
SS18	REPS2, SSX1, SSX2, SSX4	4	14
SSBP2	FER	1	1
STAG2	MAP7D3(5' Donor, AFF3 to NOTCH1)	1	2
TCF3	HLF, PBX1, TFPT	3	9
TFE3	GRIPAP1, SFPQ	2	2
TSPAN4	CD151	1	1
UBTF	MAML3	1	2
YAP1	FAM118B, MAMLD1	2	3
ZMYND11	MBTD1	1	1





## Informatics Details

# OncoPrint Myeloid IR 5.6 and IR 5.10 TVC parameters

	<b>SNP</b>	<b>Indel</b>	<b>Hotspot</b>
Min AF	2%	2%	3%
Min Coverage	20	20	15
Strand Bias	0.9	0.85	0.96

ampliseq\_childhood\_cancer\_research\_panel\_dna\_540\_w2.1\_somatic\_low stringency\_parameters.json

# OncoPrint Myeloid IR 5.6 and IR 5.10 Fusion parameters

Parameter	Value
Min Read Count	40
Min Read Count Non-Targeted	1000
Total Mapped Reads	250,000
Total Mapped Reads per Pool	50,000
Min Read Length	60

AmpliSeq-childhood\_cancer\_research\_panel\_fusion\_540\_w 2.1-RNA CountsActor.ini

# OncoPrint Childhood Cancer Research Assay IR 5.6 Files

DNA	DNA Target BED file	OCCRA.20170711.designed.bed
	Hotspot/Blacklist BED file	OCCRA.20180306.hotspots.blist.bed
	Parameters	ampliseq_childhood_cancer_research_panel_dna_540_w2.1_somatic_lowstringency_parameters.json
RNA	Fusions FASTA file	OCCRA_030718_Reference_Reference.fasta
	Fusions target BED file	OCCRA_030718_Reference_Reference.bed
	Fusions properties file	OCCRA_030718_ReferenceReference.properties.txt

- IR 5.6 has a bug that will cause the blacklist file, if provided separately, to be deleted if workflow is copy-edited. We are thus providing a combined hotspot/blacklist BED.

# OncoPrint Childhood Cancer Research Assay IR 5.10 Files

DNA	DNA Target BED file	OCCRA.20170711.designed.bed
	Hotspot BED file	OCCRA.20180306.hotspots.bed
	Blacklist BED file	OCCRA.20170621.mask.bed
	Parameters	ampliseq_childhood_cancer_research_panel_dna_540_w2.1_somatic_lowstringency_parameters.json
RNA	Fusions FASTA file	OCCRA_030718_Reference_Reference.fasta
	Fusions target BED file	OCCRA_030718_Reference_Reference.bed
	Fusions properties file	OCCRA_030718_ReferenceReference.properties.txt