Extending the Scope: Driving Change in Solid Tumour

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Thermo Fisher Scientific, Oncomine User Meeting, 15th November, 2019

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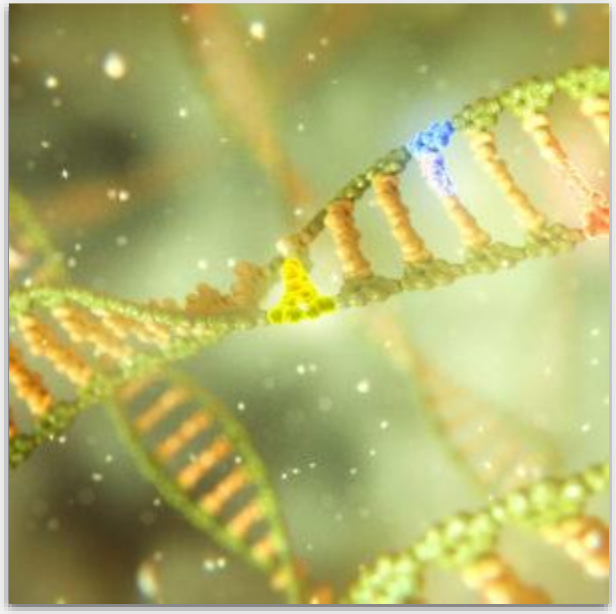
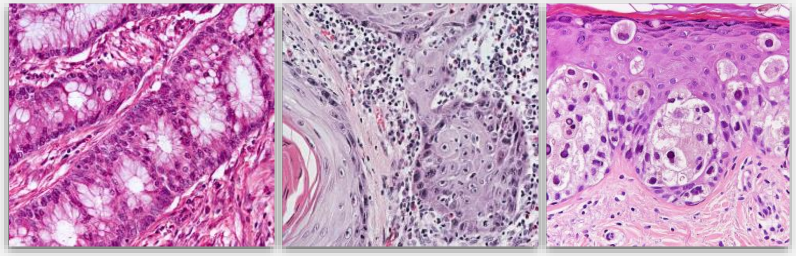
Speaker was provided travel and hotel support by Thermo Fisher Scientific for this presentation, but no remuneration

Introduction - SCMD

- Molecular Pathology laboratory in London, part of HCA Healthcare UK
- Offer a range of molecular assays:
  - Next Generation Sequencing (NGS) cancer research panels
  - Rapid Single Gene somatic variant analyses
  - Microsatellite Instability (MSI) status
  - *MLH1* promoter hypermethylation
  - B & T Cell Clonality
  - Endopredict (Breast Cancer)
- Majority of requests are NGS for Colorectal, NSCLC & Melanoma for treatment stratification (SOC)

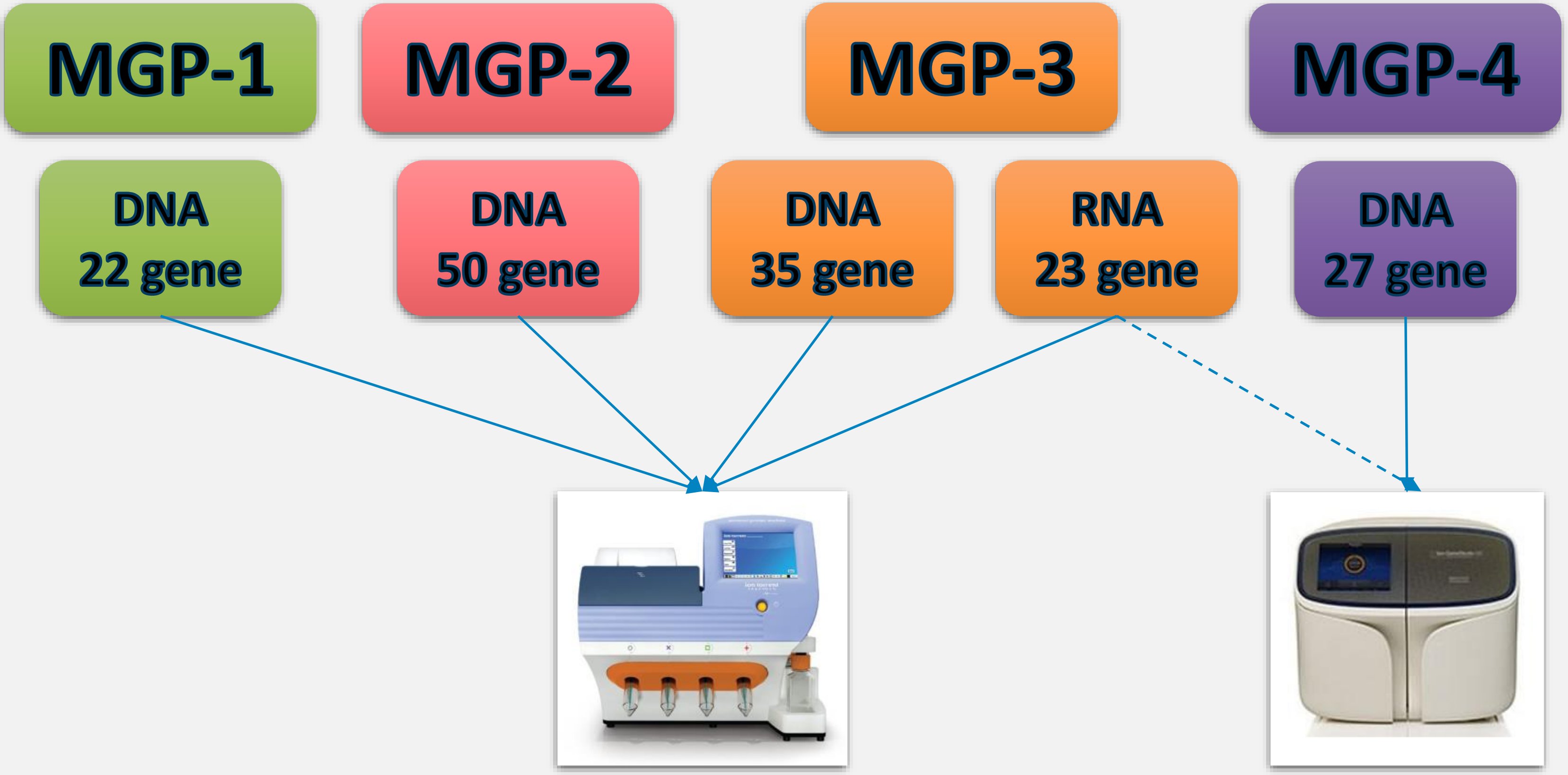




AmpliSeq / Ion Torrent Panels at SCMD - 2018

Live Under ISO 15189 Scope

In Development



MGP-1

DNA
22 gene

MGP-2

DNA
50 gene

MGP-3

DNA
35 gene

RNA
23 gene

MGP-4

DNA
27 gene

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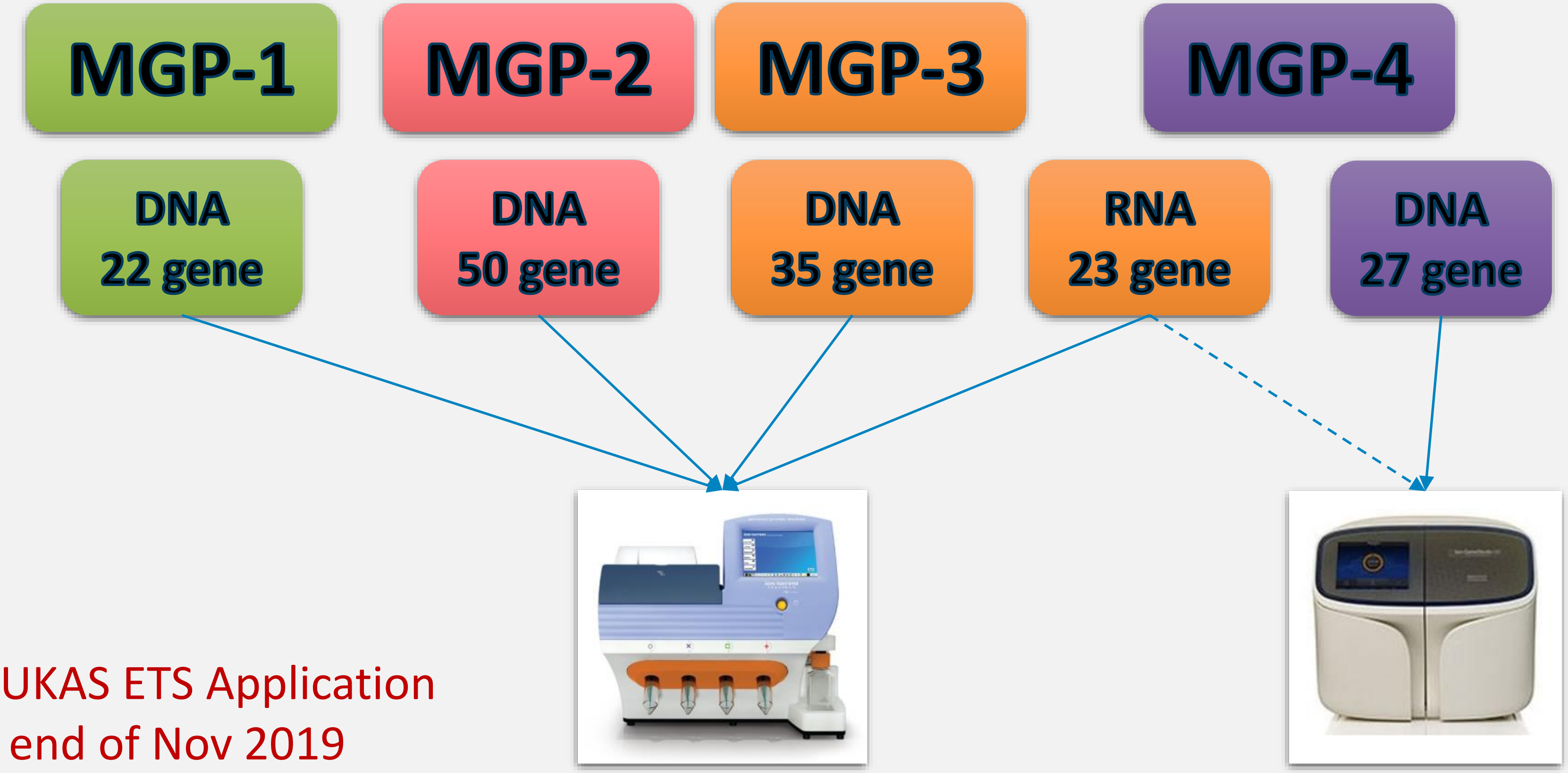
(Also In Development: DNA / RNA Thyroid Panel)

AmpliSeq / Ion Torrent Panels at SCMD – 2019

Live Under ISO 15189 Scope

In Implementation*



MGP-1

DNA
22 gene

MGP-2

DNA
50 gene

MGP-3

DNA
35 gene

RNA
23 gene

MGP-4

DNA
27 gene

*UKAS ETS Application
end of Nov 2019

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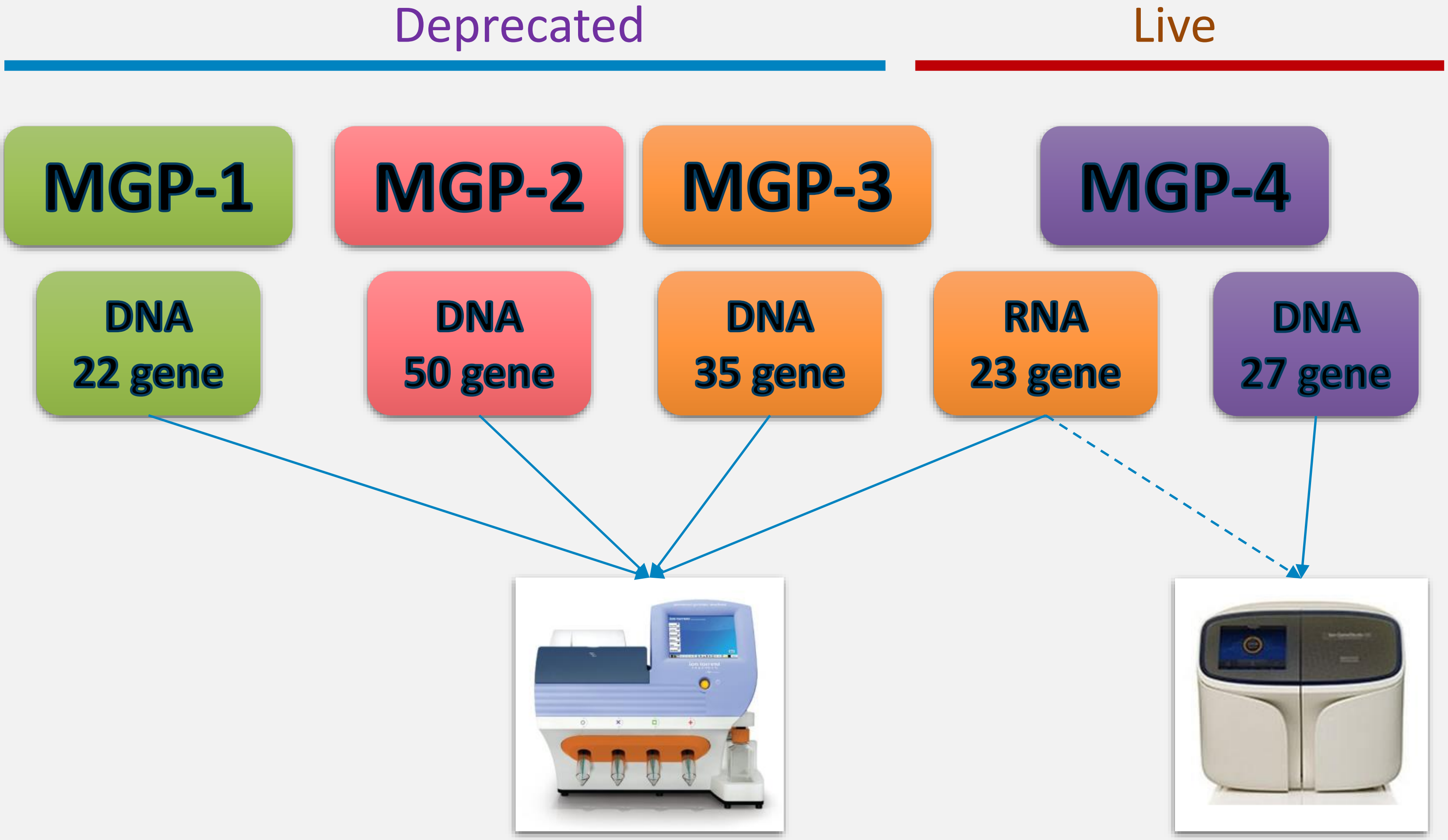
(Awaiting suitable samples: DNA / RNA Thyroid Panel)

AmpliSeq / Ion Torrent Panels at SCMD – Jan 2020



Deprecated

Live

MGP-1

MGP-2

MGP-3

MGP-4

DNA 22 gene

DNA 50 gene

DNA 35 gene

RNA 23 gene

DNA 27 gene

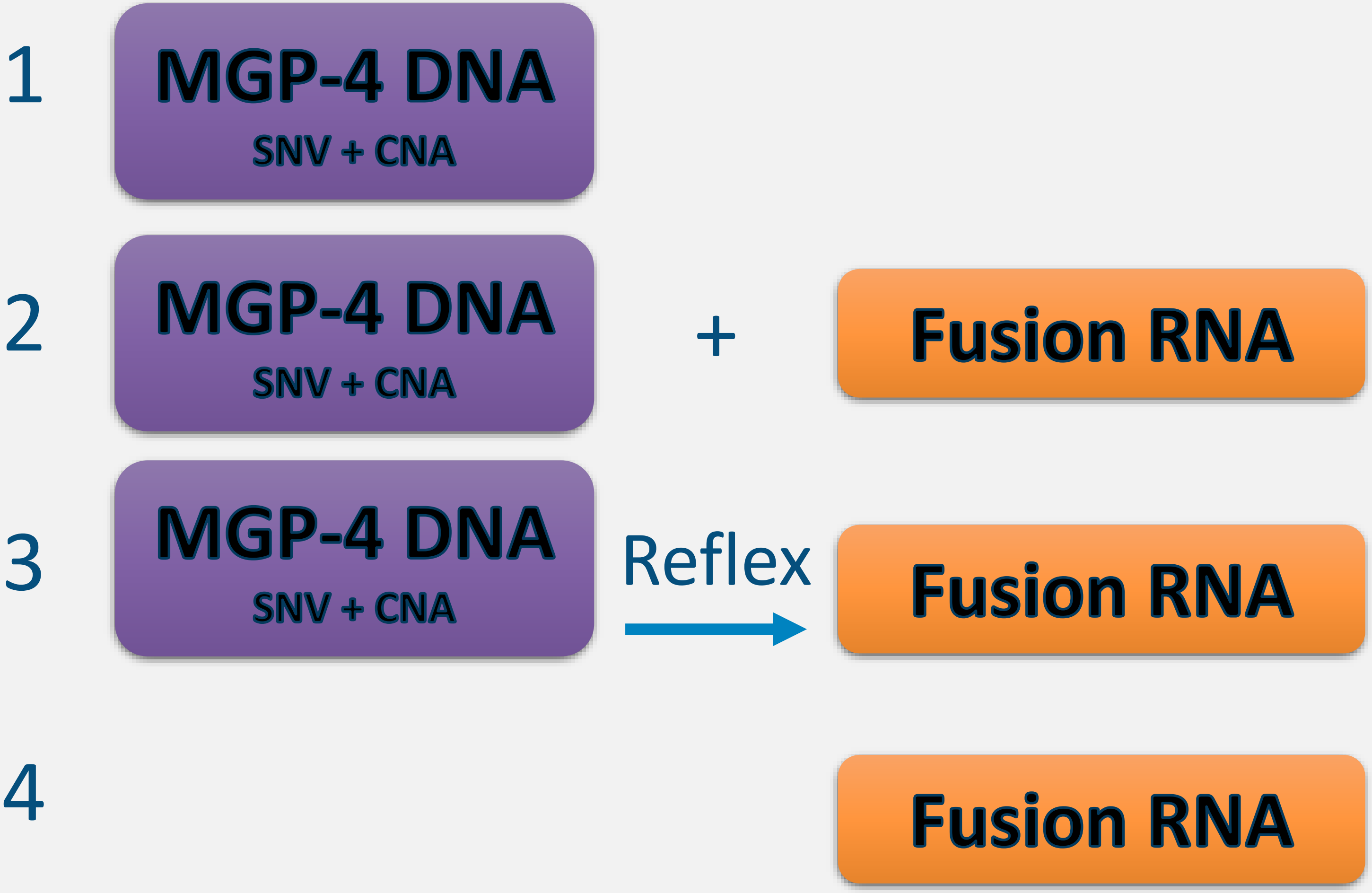
(Testing suitable samples?: DNA / RNA Thyroid Panel)

Custom AmpliSeq DNA Panel – MGP-4

27 Genes / 164 amplicons / 2776 hotspots (COSMIC v79)

	SCMD MGP-4 Panel						
	Gene	**Target Exons**	**Key Variant Loci**	**CNV**	**Associated Tumour**	**Responsive**	**Resistant**
1	*AKT1*	3	E17	-	Many	AKT / PI3K pathway inhibitors	BRAF Inhibitors
2	*ALK*	22,23,25	F1174, L1196, G1269	Amp	Lung / Glioma / other	ALK Inhibitors (depending on mutation)	ALK Inhibitors (depending on mutation)
3	*BRAF*	11,15	V600; G466, G469	-	Melanoma / CRC / other	BRAF & MEK inhibitors	EGFR mAb in CRC
4	*CDKN2A*	1b,2,3	R80; R58	Loss	Melanoma / other	CDK4/6 inhibitors	-
5	*CTNNB1*	3	T41,S45,S37	-	Endo/ CRC / other	MTOR inhibitors	Tankyrase inhibitors
6	*EGFR*	3,7,12,15,18-21	G719, T790, L858	Amp	Lung / other	EGFR TKIs	EGFR mAb in CRC
7	*ERBB2*	8,17,19,20,21	S310, L755	Amp	Lung / CRC / other	ERBB2 inhibitors	ERBB2 inhibitors (resitance loci)
8	*FBXW7*	4,7,8,9,10	R465, R505	Loss	CRC / other	MTOR inhibitors	Tubulin inhibitors
9	*GNA11*	4,5	Q209, R183	-	Melanoma	PI3K & MEK inhibitors	-
10	*GNAQ*	2,4,5	Q209, R183	-	Melanoma	PI3K & MEK inhibitors	-
11	*HRAS*	2,3,4	G12,G13,Q61	-	Cervix / other	MTOR, MEK & Farnesyltransferase inhibitors	-
12	*IDH1*	4	R132	-	Bilary / Glioma / other	PARP inhibitors; dasatinib	-
13	*IDH2*	4	R172	-	Bilary	Dasatinib	-
14	*KIT*	9,10,11,13,14,15,17	D816,	Amp	Melanoma / GIST / Other	TKIs	TKIs (resitance loci, D816)
15	*KRAS*	2,3,4,5,6	G12,G13,Q61,A146	Amp	Lung / CRC / other	combination MEK inhibitors	EGFR mAb in CRC; TKIs in Lung
16	*MAP2K1*	2,3,6	K57,P124,E203	-	Melanoma / CRC / other	MEK inhibitors	BRAF Inhibitors
17	*MET*	2,13,14,15,16,19, 21	Y1253, A1357	Amp	Lung / CRC / other	MET / ALK inhibitors	MET / ALK inhibitors (resistance loci); EGFR TKI
18	*MYD88*	3,4,5	L265, S219, S243	-	WM / Lymphoma	Ibrutinib	-
19	*NRAS*	2,3,4	G12, Q61	-	Melanoma / CRC / other	MEK inhibitors	BRAF Inhibitors; EGFR mAb
20	*PDGFRA*	12,14,15,18	D842V	-	GIST / Melanoma	TKIs	TKIs (resitance loci, D842)
21	*PIK3CA*	2,3,5,8,10,14,21	E454,H1047	Amp	Lung / CRC / Breast / other	PI3K & MTOR inhibitors	EGFR mAb in CRC
22	*PTEN*	1,2,3,4,5,6,7,8,9	R130; T319; R173; K267	Loss	Many	PARP inhibitors; PI3K pathway inhibitors	EGFR mAb in CRC
23	*RET*	3,10,11,15,16	M918,	Amp	Lung / Thyroid	RET inhibitors	Ponatinib
24	*SMAD4*	2,3,6,9,10,11,12	R361,R445,A118,R135	Loss	CRC / other	-	-
25	*SMARCA4*	5,17,19,26,35	T910, R1243,	Loss	Ovarian	EZH2 inhibitors	-
26	*STK11*	1,4,5,6,7,8	F354, P281, Q37	Loss	Lung / CRC / other	MEK inhibitors; MTOR inhibitors	PD-1 mAb
27	*TP53*	2-11	R175,R248,R273	Loss	Many	ATR & WEE1 inhibitors	CDK4/6 inhibitors

MGP-4 and Fusion Panel: 4 Options



1 **MGP-4 DNA** SNV + CNA

2 **MGP-4 DNA** SNV + CNA + **Fusion RNA**

3 **MGP-4 DNA** SNV + CNA Reflex → **Fusion RNA**

4 **Fusion RNA**

S5, Chef & MGP-4 Evaluation

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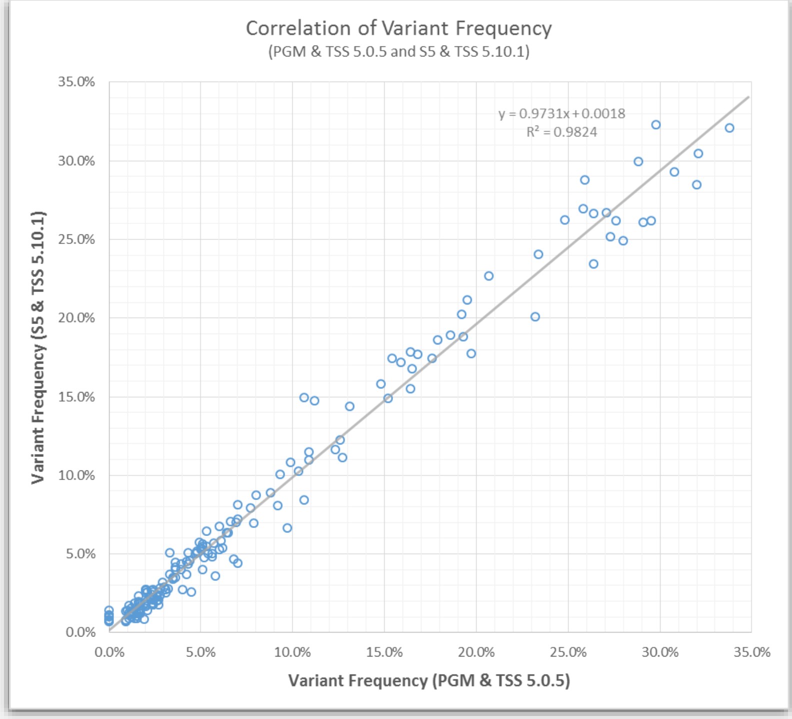
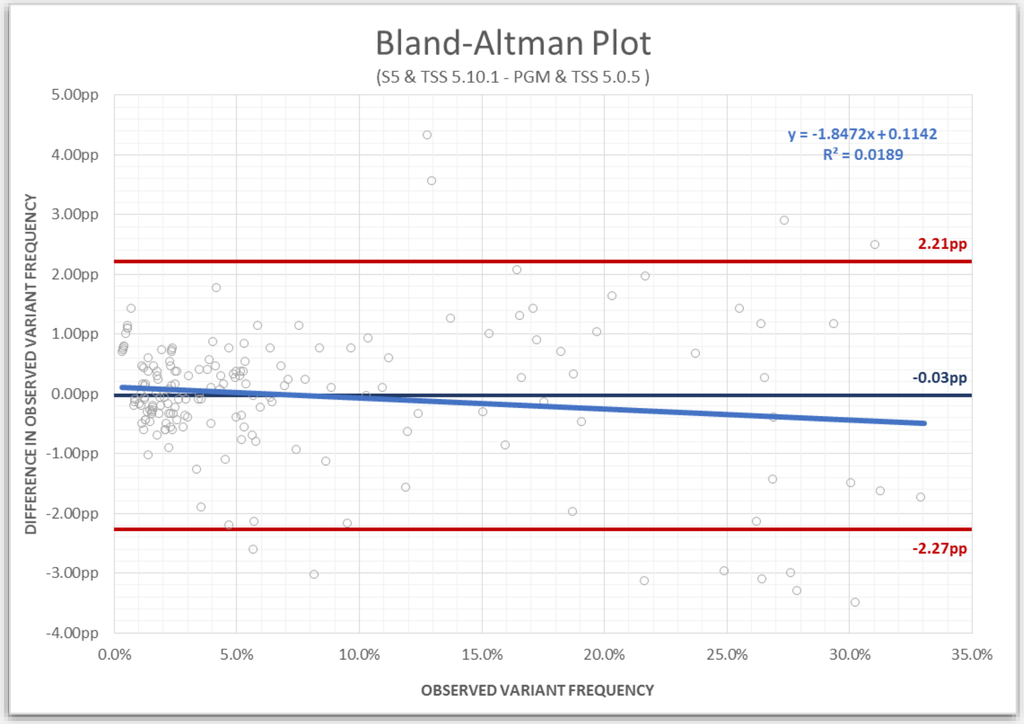
S5 & Chef Validation (MGP-3: S5 v PGM)

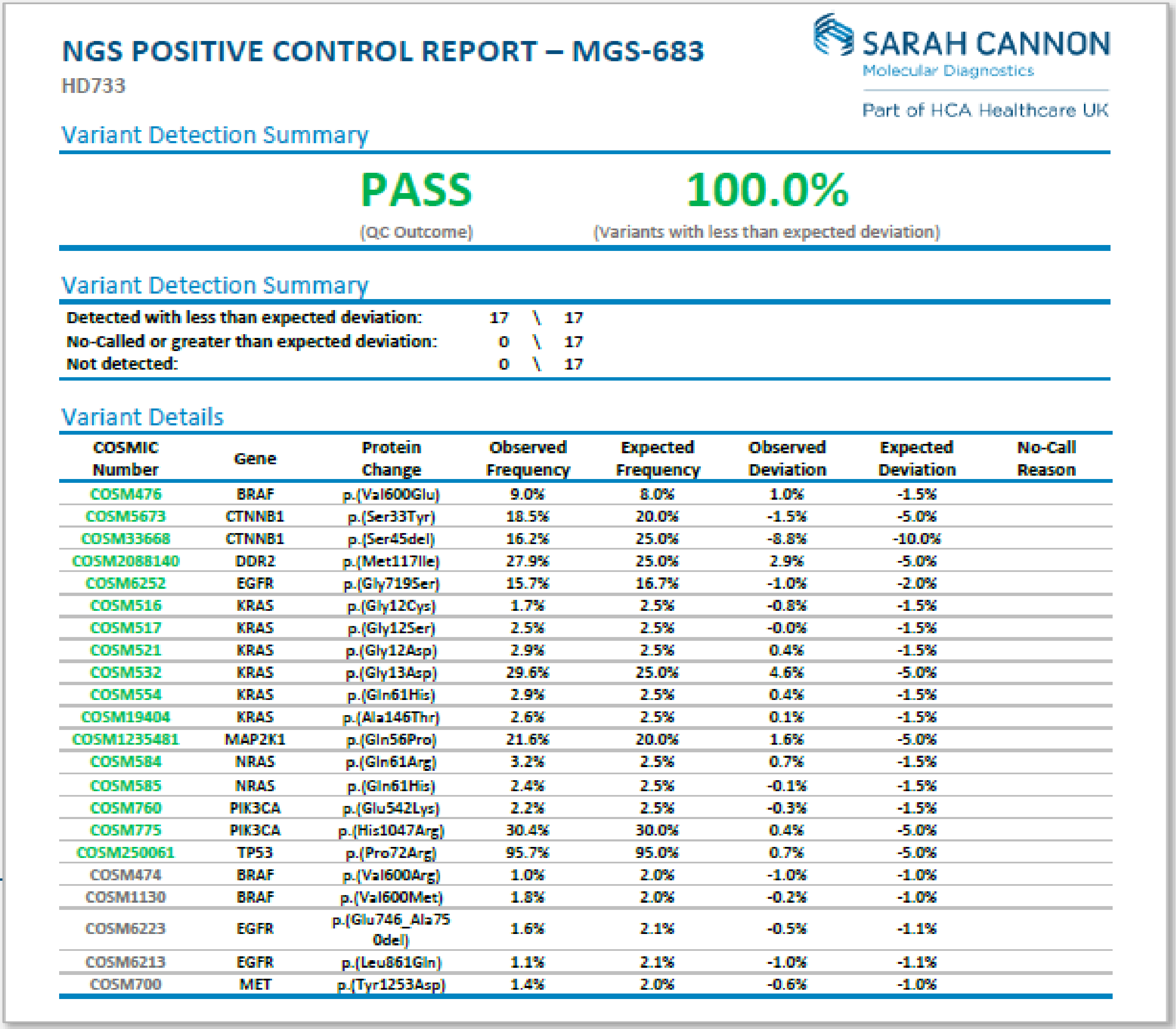
Evaluation set of Horizon Discovery Multiplex SNV Controls





HD701	Quantitative Multiplex Reference Standard
HD752	Wildtype Reference Standard
HD728-731	5% Tier Reference Standards
HD732-733	2.5% Tier Reference Standards
HD734	1.3% Tier Reference Standard
HD798	Formalin Compromised HD701 - Mild
HD799	Formalin Compromised HD701 - Moderate
HD803	Formalin Compromised HD701 - Severe

DNA SNV IQC



NGS POSITIVE CONTROL REPORT – MGS-683

HD733

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Variant Detection Summary

PASS
(QC Outcome)

100.0%
(Variants with less than expected deviation)

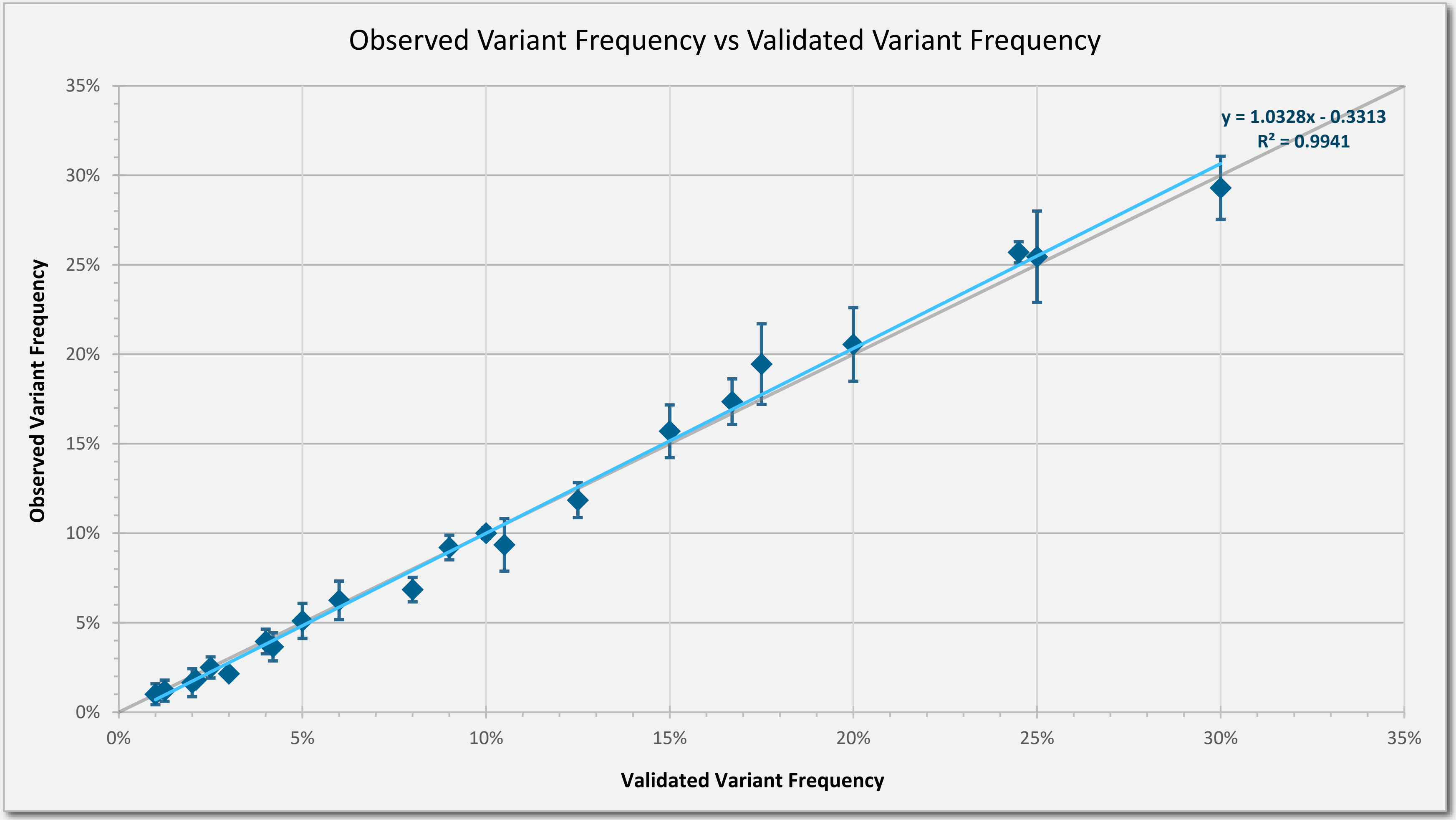
Variant Detection Summary

Detected with less than expected deviation:	17	\	17
No-Called or greater than expected deviation:	0	\	17
Not detected:	0	\	17

Variant Details

COSMIC Number	Gene	Protein Change	Observed Frequency	Expected Frequency	Observed Deviation	Expected Deviation	No-Call Reason
COSM476	BRAF	p.(Val600Glu)	9.0%	8.0%	1.0%	-1.5%	
COSM5673	CTNNB1	p.(Ser33Tyr)	18.5%	20.0%	-1.5%	-5.0%	
COSM33668	CTNNB1	p.(Ser45del)	16.2%	25.0%	-8.8%	-10.0%	
COSM2088140	DDR2	p.(Met117Ile)	27.9%	25.0%	2.9%	-5.0%	
COSM6252	EGFR	p.(Gly719Ser)	15.7%	16.7%	-1.0%	-2.0%	
COSM516	KRAS	p.(Gly12Cys)	1.7%	2.5%	-0.8%	-1.5%	
COSM517	KRAS	p.(Gly12Ser)	2.5%	2.5%	-0.0%	-1.5%	
COSM521	KRAS	p.(Gly12Asp)	2.9%	2.5%	0.4%	-1.5%	
COSM532	KRAS	p.(Gly13Asp)	29.6%	25.0%	4.6%	-5.0%	
COSM554	KRAS	p.(Gln61His)	2.9%	2.5%	0.4%	-1.5%	
COSM19404	KRAS	p.(Ala146Thr)	2.6%	2.5%	0.1%	-1.5%	
COSM1235481	MAP2K1	p.(Gln56Pro)	21.6%	20.0%	1.6%	-5.0%	
COSM584	NRAS	p.(Gln61Arg)	3.2%	2.5%	0.7%	-1.5%	
COSM585	NRAS	p.(Gln61His)	2.4%	2.5%	-0.1%	-1.5%	
COSM760	PIK3CA	p.(Glu542Lys)	2.2%	2.5%	-0.3%	-1.5%	
COSM775	PIK3CA	p.(His1047Arg)	30.4%	30.0%	0.4%	-5.0%	
COSM250061	TP53	p.(Pro72Arg)	95.7%	95.0%	0.7%	-5.0%	
COSM474	BRAF	p.(Val600Arg)	1.0%	2.0%	-1.0%	-1.0%	
COSM1130	BRAF	p.(Val600Met)	1.8%	2.0%	-0.2%	-1.0%	
COSM6223	EGFR	p.(Glu746_Ala750del)	1.6%	2.1%	-0.5%	-1.1%	
COSM6213	EGFR	p.(Leu861Gln)	1.1%	2.1%	-1.0%	-1.1%	
COSM700	MET	p.(Tyr1253Asp)	1.4%	2.0%	-0.6%	-1.0%	

MGP-4 on S5 Evaluation – Horizon Multiplex Control Set



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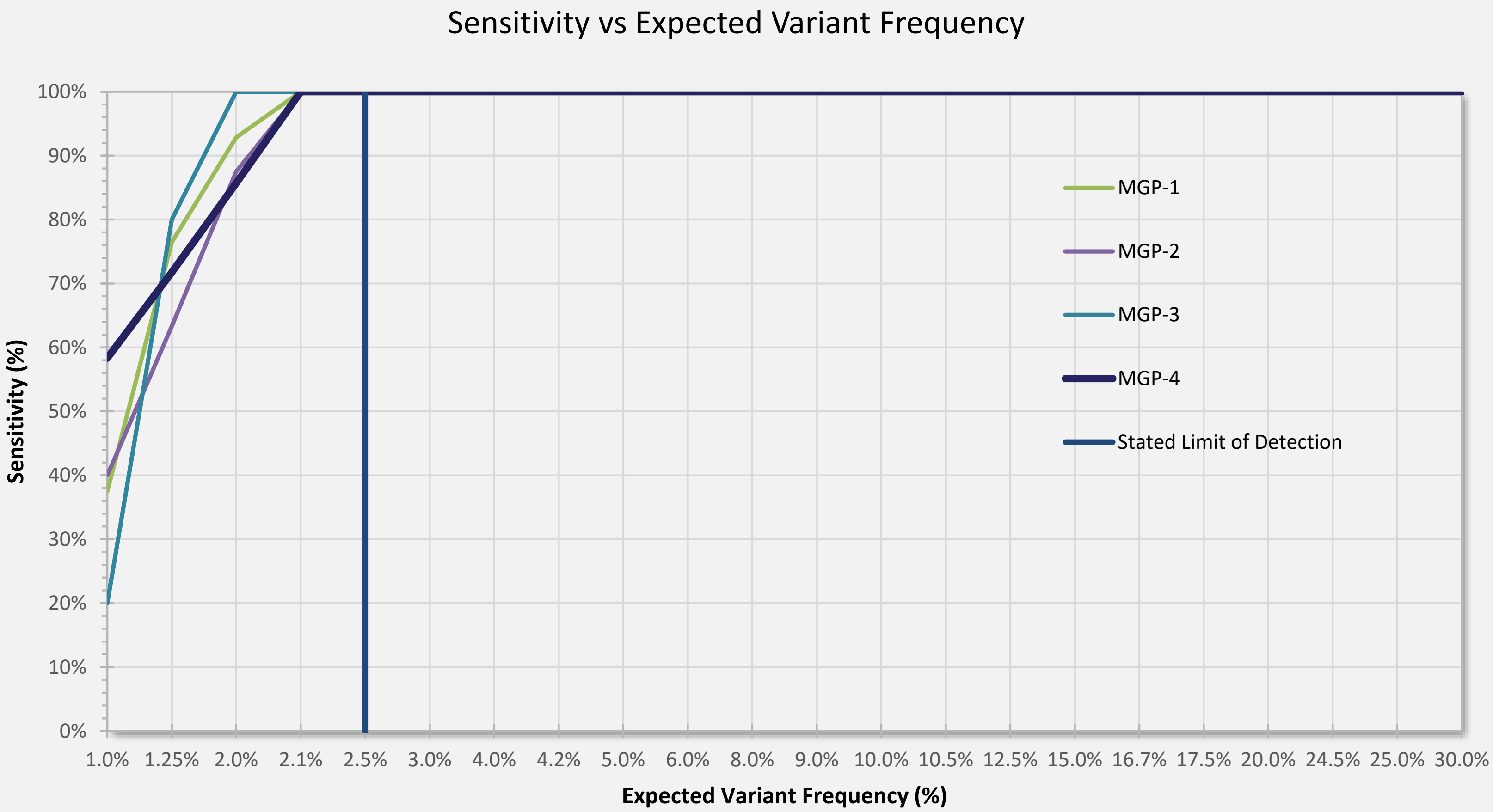
MGP-4 Evaluation - LOD



Copy Number Aberration Calling

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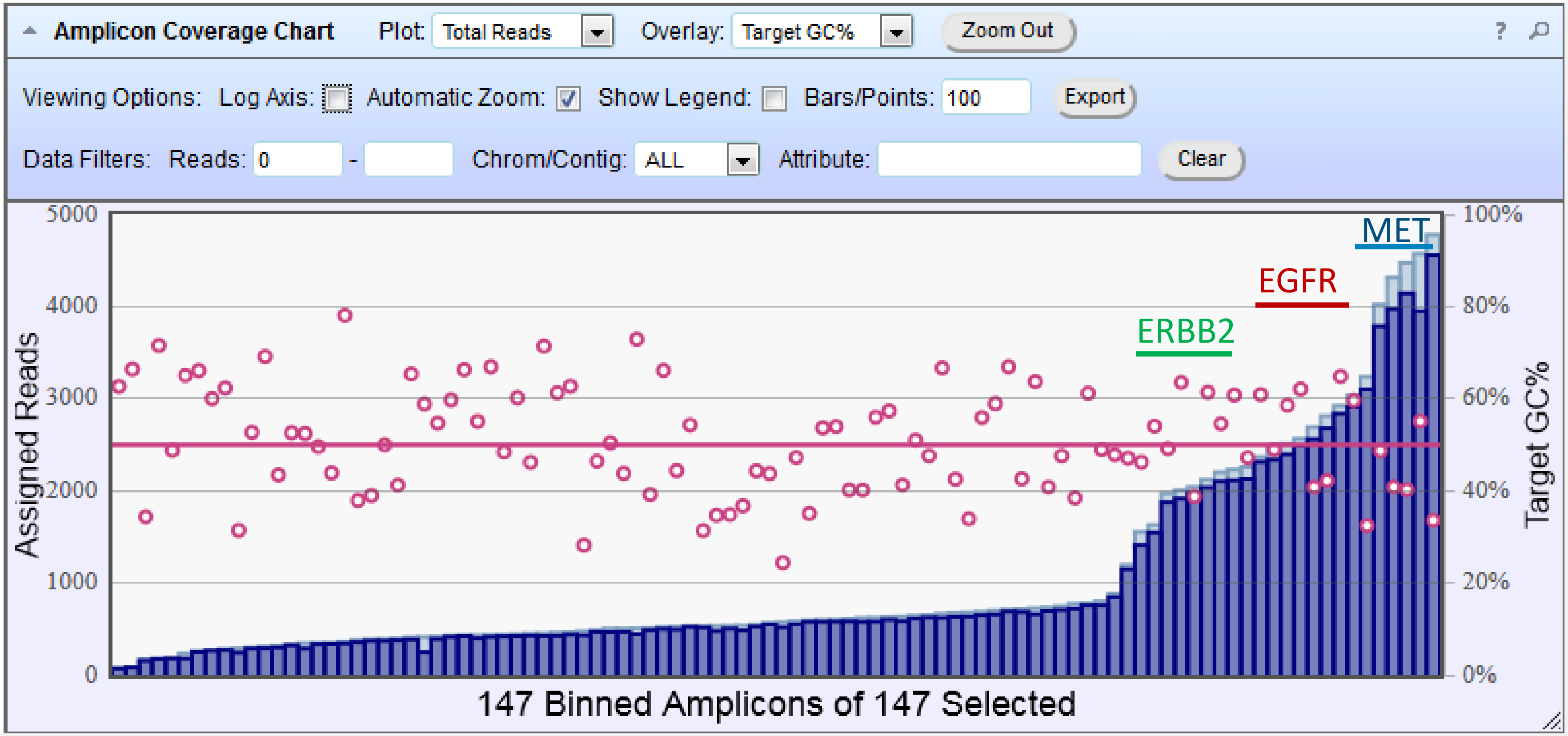
Copy Number Gain – DNA Gene Amplification

NSCLC Cancer Fusions	Data from MCG					
Gene	**Prevalance**	**Fusion Partners**	**Increased Sensitivity**	**Decreased Sensitivity**	**Restistance Markers**	**Other Cancers**
ALK	3-7%	EML4; KIF5B	crizotinib; ceritinib; pemetrexed	EGFR TKIs	L1196M; G1269A	thyroid
MET exon 14 skipping	~5%	MET	crizotinib; cabozantinib	?	?	CNS; UT
ROS	~2%	CD74; SLC34A2	crizotinib	EGFR TKIs	G2032R; D2033N	stomach
RET	~1%	KIF5B; CCDC6	cabozantinib	?	?	thyroid
FGFR3	0.5-2%	TACC3	FGFR/TORC inhibitors	?	?	
NTRK1	<1%	MPRIP; CD74	larotrectinib / Entrectinib /?crizotinib	?	?	thyroid
Amplifications						
MET Amplification	~2-4% (untreated)		crizotinib	EGFR TKIs	-	-
	~5-20% (treated + resistance)					
EGFR exon 18-25 dup	??	-	EGFR TKIs	-	-	-
ERBB2	~1%	-	-	-	-	-
FGFR1	1 - 6%		FGFR inhibitors	-	-	-

CNG – SeraCare CNV Controls

1. Breast CNV (ERBB2)
2. Lung & Brain CNV (MET + EGFR)



Amplicon Coverage Chart

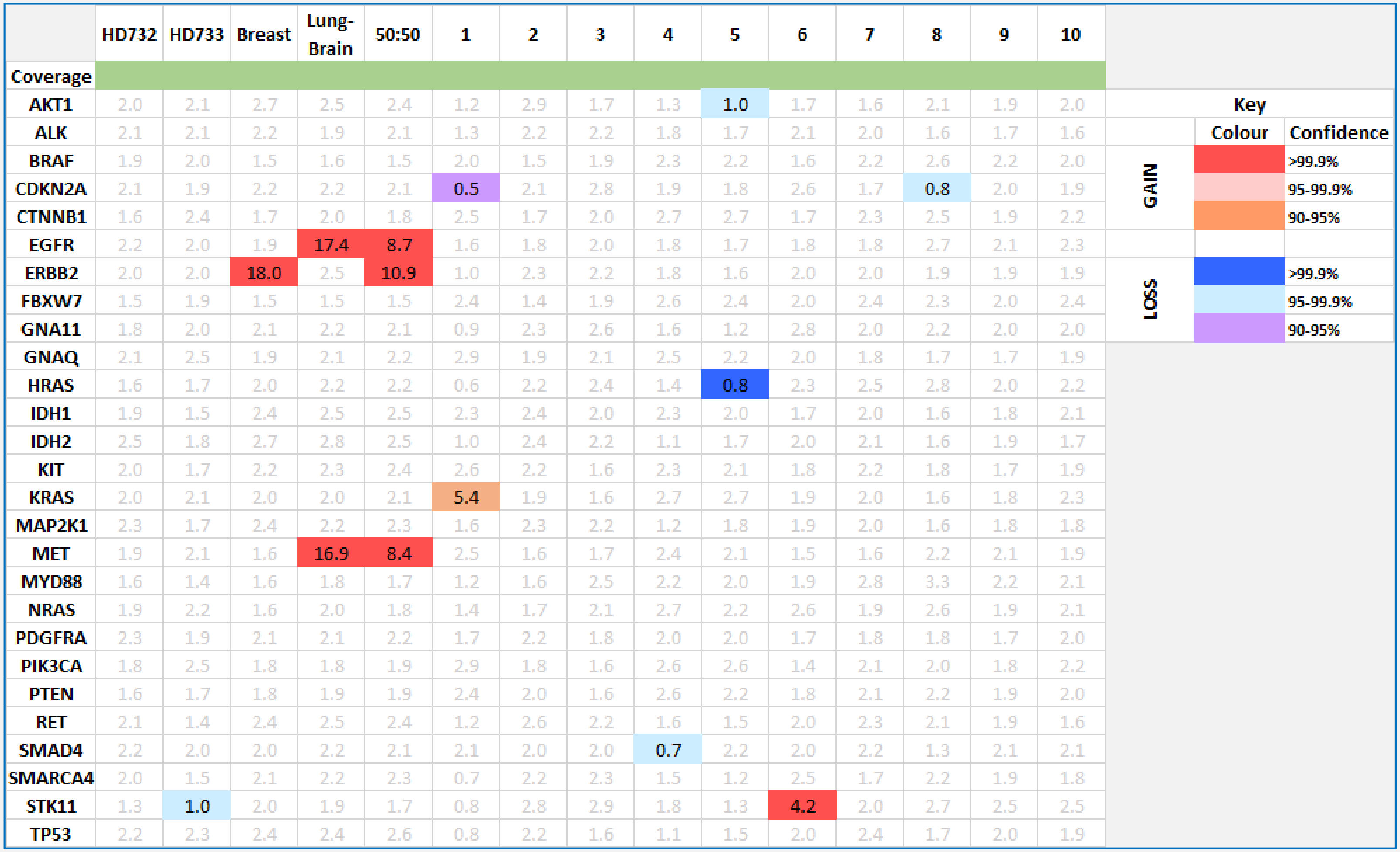
Plot: Total Reads

Overlay: Target GC%

Viewing Options: Log Axis: ☐ Automatic Zoom: ☑ Show Legend: ☐ Bars/Points: 100

Data Filters: Reads: 0 - Chrom/Contig: ALL Attribute:

CNA Calling Pipeline – Still Under Construction…!



	HD732	HD733	Breast	Lung-Brain	50:50	1	2	3	4	5	6	7	8	9	10
Coverage															
AKT1	2.0	2.1	2.7	2.5	2.4	1.2	2.9	1.7	1.3	1.0	1.7	1.6	2.1	1.9	2.0
ALK	2.1	2.1	2.2	1.9	2.1	1.3	2.2	2.2	1.8	1.7	2.1	2.0	1.6	1.7	1.6
BRAF	1.9	2.0	1.5	1.6	1.5	2.0	1.5	1.9	2.3	2.2	1.6	2.2	2.6	2.2	2.0
CDKN2A	2.1	1.9	2.2	2.2	2.1	0.5	2.1	2.8	1.9	1.8	2.6	1.7	0.8	2.0	1.9
CTNNB1	1.6	2.4	1.7	2.0	1.8	2.5	1.7	2.0	2.7	2.7	1.7	2.3	2.5	1.9	2.2
EGFR	2.2	2.0	1.9	17.4	8.7	1.6	1.8	2.0	1.8	1.7	1.8	1.8	2.7	2.1	2.3
ERBB2	2.0	2.0	18.0	2.5	10.9	1.0	2.3	2.2	1.8	1.6	2.0	2.0	1.9	1.9	1.9
FBXW7	1.5	1.9	1.5	1.5	1.5	2.4	1.4	1.9	2.6	2.4	2.0	2.4	2.3	2.0	2.4
GNA11	1.8	2.0	2.1	2.2	2.1	0.9	2.3	2.6	1.6	1.2	2.8	2.0	2.2	2.0	2.0
GNAQ	2.1	2.5	1.9	2.1	2.2	2.9	1.9	2.1	2.5	2.2	2.0	1.8	1.7	1.7	1.9
HRAS	1.6	1.7	2.0	2.2	2.2	0.6	2.2	2.4	1.4	0.8	2.3	2.5	2.8	2.0	2.2
IDH1	1.9	1.5	2.4	2.5	2.5	2.3	2.4	2.0	2.3	2.0	1.7	2.0	1.6	1.8	2.1
IDH2	2.5	1.8	2.7	2.8	2.5	1.0	2.4	2.2	1.1	1.7	2.0	2.1	1.6	1.9	1.7
KIT	2.0	1.7	2.2	2.3	2.4	2.6	2.2	1.6	2.3	2.1	1.8	2.2	1.8	1.7	1.9
KRAS	2.0	2.1	2.0	2.0	2.1	5.4	1.9	1.6	2.7	2.7	1.9	2.0	1.6	1.8	2.3
MAP2K1	2.3	1.7	2.4	2.2	2.3	1.6	2.3	2.2	1.2	1.8	1.9	2.0	1.6	1.8	1.8
MET	1.9	2.1	1.6	16.9	8.4	2.5	1.6	1.7	2.4	2.1	1.5	1.6	2.2	2.1	1.9
MYD88	1.6	1.4	1.6	1.8	1.7	1.2	1.6	2.5	2.2	2.0	1.9	2.8	3.3	2.2	2.1
NRAS	1.9	2.2	1.6	2.0	1.8	1.4	1.7	2.1	2.7	2.2	2.6	1.9	2.6	1.9	2.1
PDGFRA	2.3	1.9	2.1	2.1	2.2	1.7	2.2	1.8	2.0	2.0	1.7	1.8	1.8	1.7	2.0
PIK3CA	1.8	2.5	1.8	1.8	1.9	2.9	1.8	1.6	2.6	2.6	1.4	2.1	2.0	1.8	2.2
PTEN	1.6	1.7	1.8	1.9	1.9	2.4	2.0	1.6	2.6	2.2	1.8	2.1	2.2	1.9	2.0
RET	2.1	1.4	2.4	2.5	2.4	1.2	2.6	2.2	1.6	1.5	2.0	2.3	2.1	1.9	1.6
SMAD4	2.2	2.0	2.0	2.2	2.1	2.1	2.0	2.0	0.7	2.2	2.0	2.2	1.3	2.1	2.1
SMARCA4	2.0	1.5	2.1	2.2	2.3	0.7	2.2	2.3	1.5	1.2	2.5	1.7	2.2	1.9	1.8
STK11	1.3	1.0	2.0	1.9	1.7	0.8	2.8	2.9	1.8	1.3	4.2	2.0	2.7	2.5	2.5
TP53	2.2	2.3	2.4	2.4	2.6	0.8	2.2	1.6	1.1	1.5	2.0	2.4	1.7	2.0	1.9

Key

	Colour	Confidence
GAIN	🟥	>99.9%
	🟥	95-99.9%
	🟧	90-95%
LOSS	🟦	>99.9%
	🟦	95-99.9%
	🟪	90-95%

RNA Fusion Gene Validation & IQC

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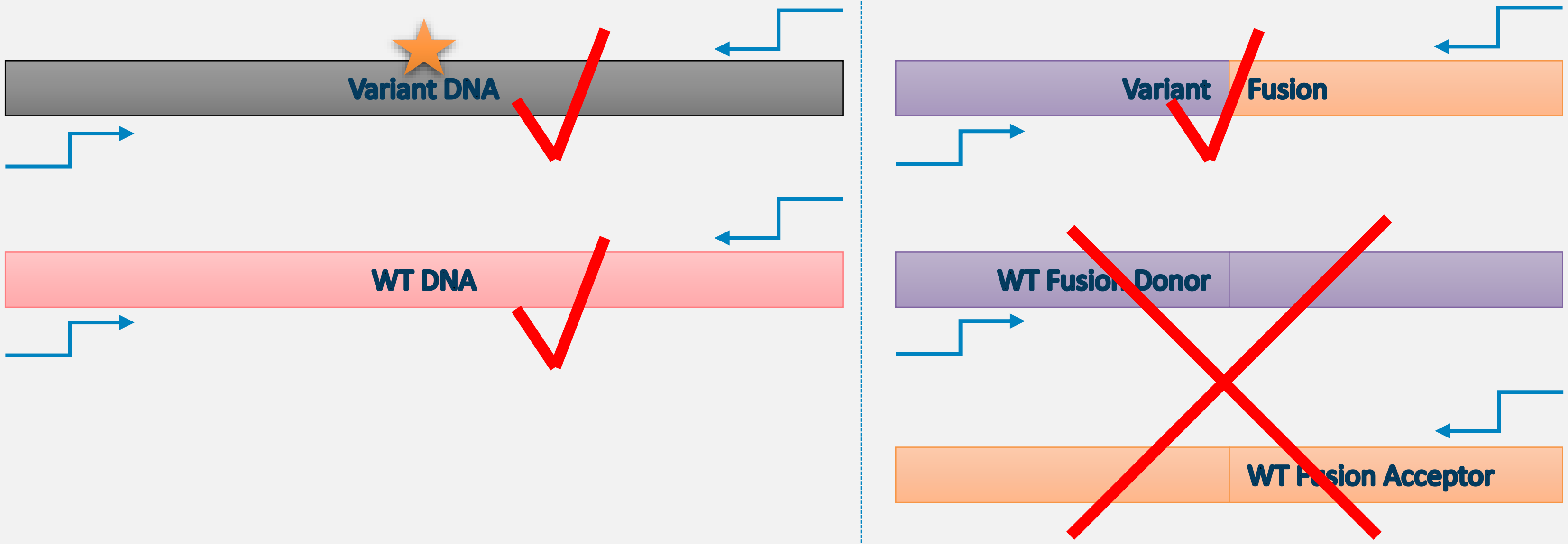
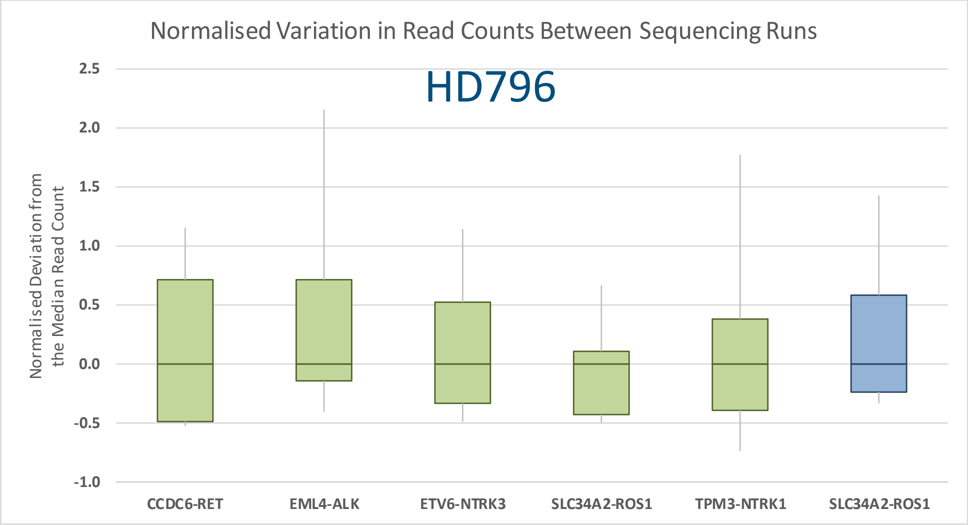
Issue of Fusion Gene IQC – absence of data!

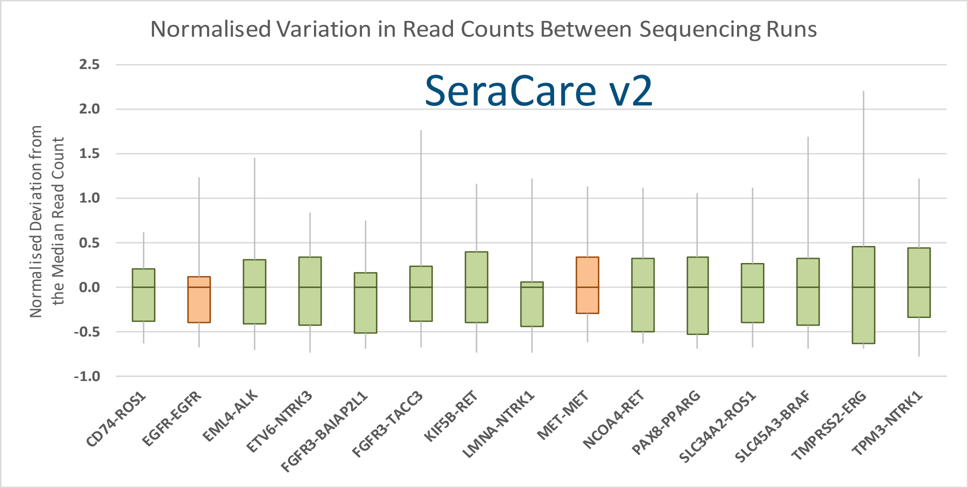
DNA / SNVs

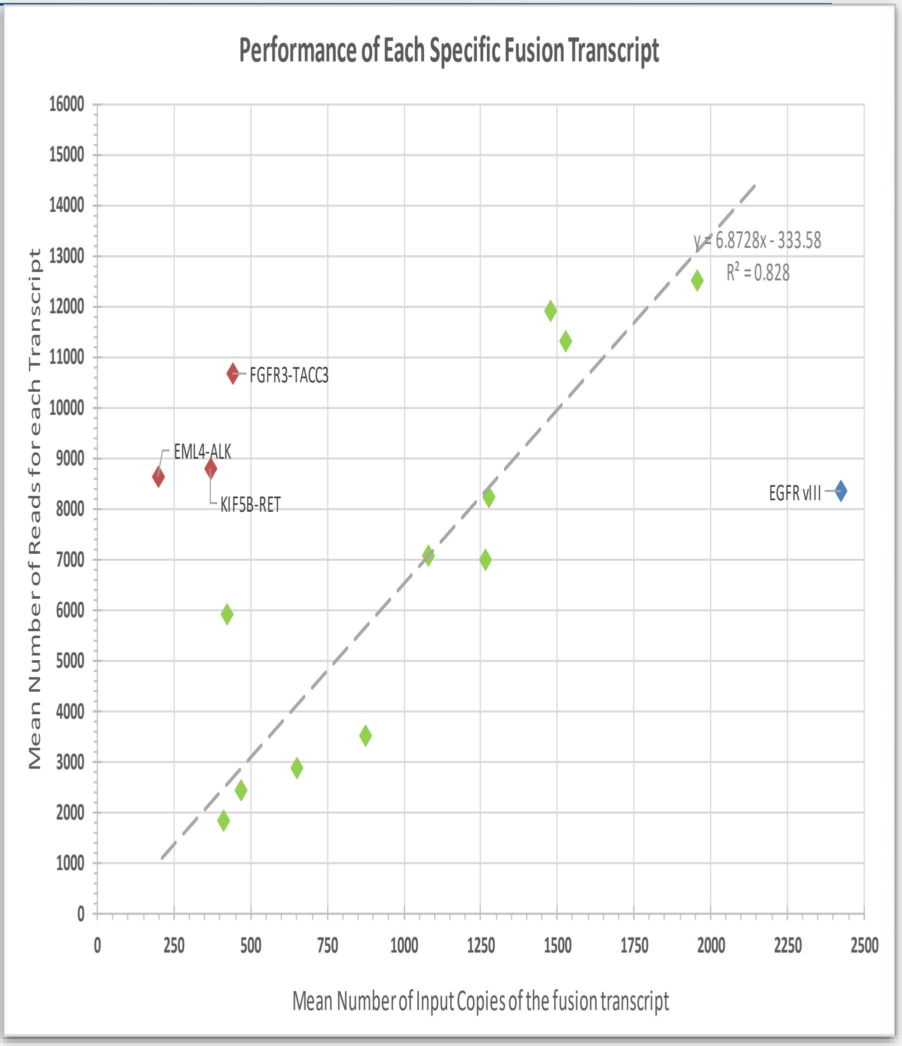
RNA / Fusions



Fusion Validation – HD796 & SeraCare Fusion v2


Normalised Variation in Read Counts Between Sequencing Runs


Normalised Variation in Read Counts Between Sequencing Runs


Performance of Each Specific Fusion Transcript

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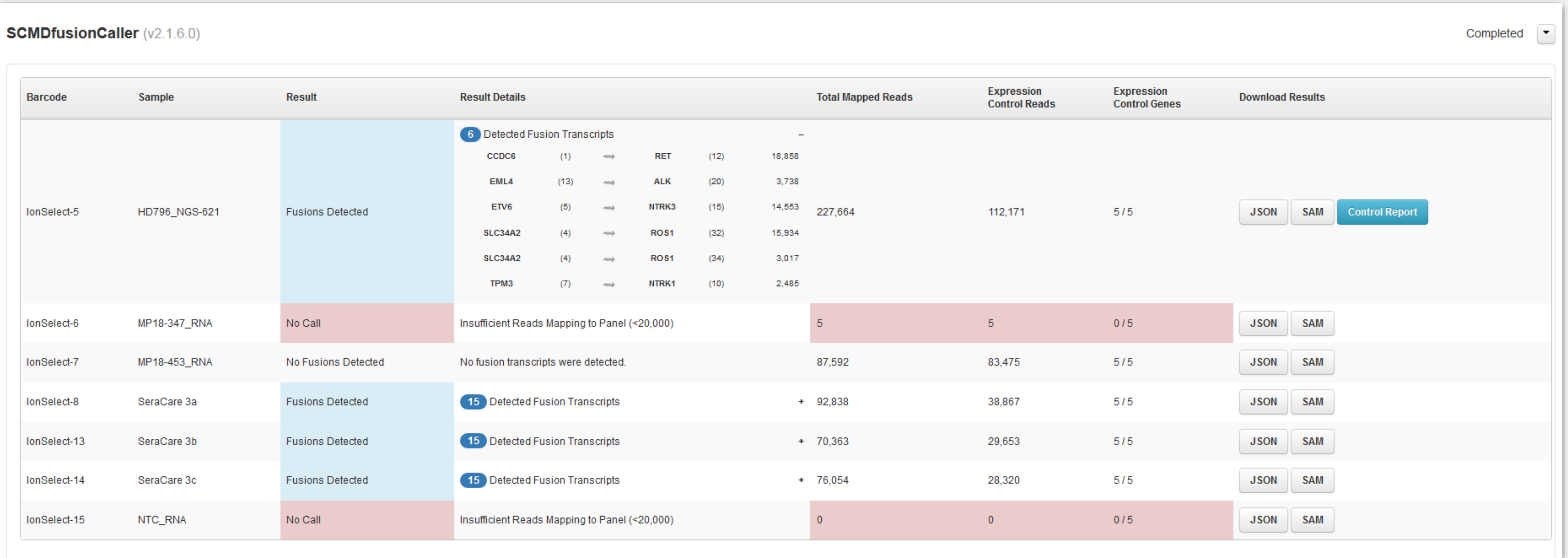
Horizon HD796 IQC – FFPE RNA Fusion Control Material

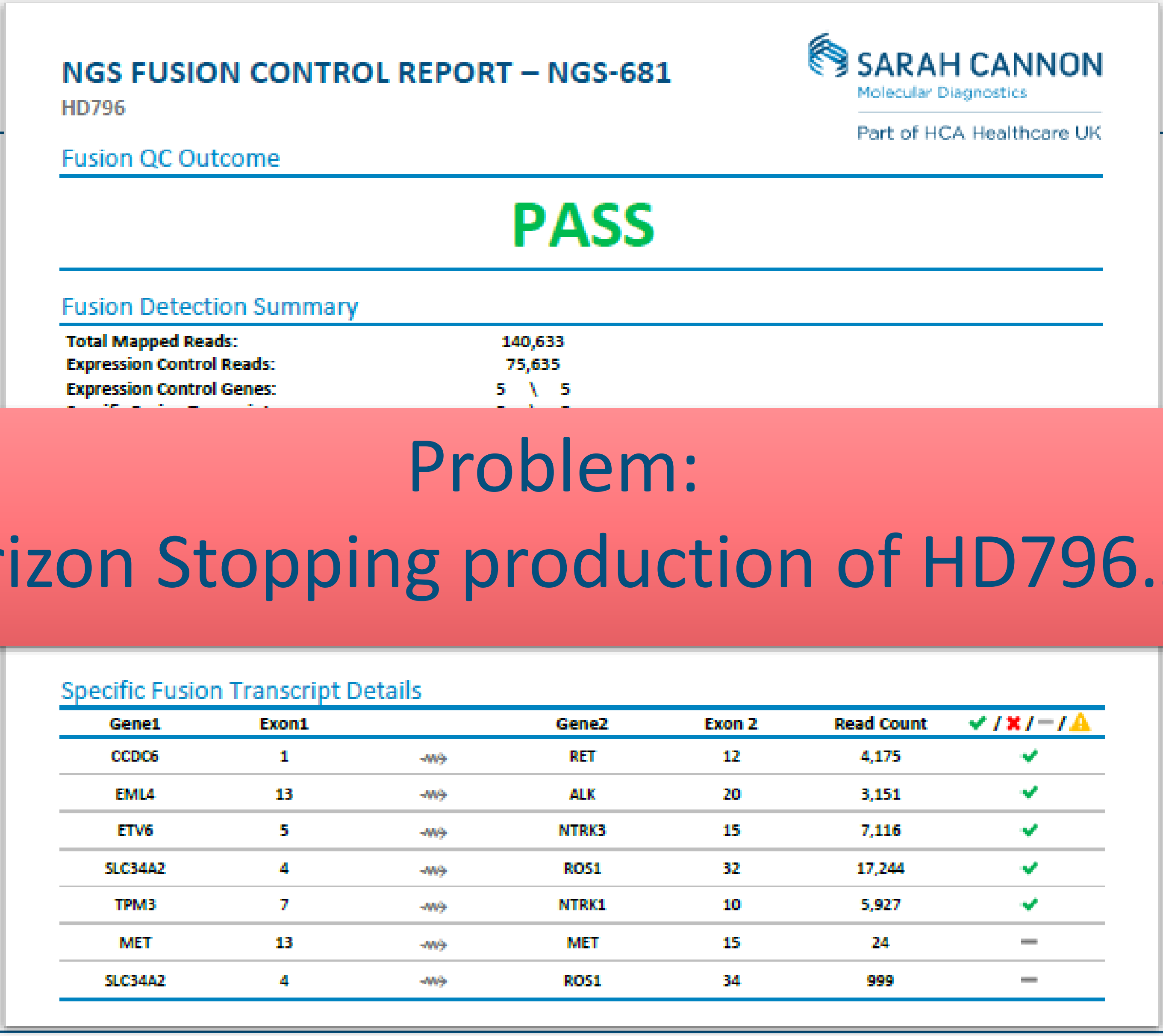
5' Partner	5' Partner Chromosome	3' Partner	3' Partner Chromosome	Translocation Name	Breakpoint Sequence cDNA
EML4 Exon 13	2	ALK Exon 20	2	EML4{ENST00000318522}:r.1_1751_ALK{ENST00000389048}:r.4080_6220 COSF463	EML4 ex13 ALK ex20 aggacctaaagtgtaccgcc
CCDC6 Exon 1	10	RET Exon 12	10	CCDC6{ENST00000263102}:r.1_535_RET{ENST00000355710}:r.2369_5659 COSF1272	CCDC6 ex1 RET ex12 agccagcgttaccatcgaggatccaaagtgg
SLC34A2 Exon 4	4	ROS1 Exon 32	6	SLC34A2{ENST00000382051}:r.1_429_ROS1 {NM_002944}:r.5448_7368 COSF1197	SLC34A2 ex4 ROS1 ex32 cttcagctggttggagctggagtcccaaataa
TPM3 Exon 7	1	NTRK1 Exon 10	1	TPM3{ENST00000368533}:r.1_717_NTRK1 {ENST00000392302}:r.1262_2609 COSF1330	TPM3 ex7 NTRK1 ex10 gacctggaagacactaacagcaca
ETV6 Exon 5	12	NTRK3 Exon 15	15	ETV6{ENST00000396373}:r.1_1283_NTRK3 {ENST00000394480}:r.1908_19984 COSF572	ETV6 ex5 NTRK3 ex15 agaatagcagatgtgcagcaca

In-house Fusion Caller Plugin – HD796 IQC



SCMDfusionCaller (v2.1.6.0)

Barcode	Sample	Result	Result Details	Total Mapped Reads	Expression Control Reads	Expression Control Genes	Download Results
IonSelect-5	HD796_NGS-621	Fusions Detected	6 Detected Fusion Transcripts – CCDC6 (1) ⇝ RET (12) 18,858 EML4 (13) ⇝ ALK (20) 3,738 ETV6 (5) ⇝ NTRK3 (15) 14,553 SLC34A2 (4) ⇝ ROS1 (32) 15,934 SLC34A2 (4) ⇝ ROS1 (34) 3,017 TPM3 (7) ⇝ NTRK1 (10) 2,485	227,664	112,171	5 / 5	JSON SAM Control Report
IonSelect-6	MP18-347_RNA	No Call	Insufficient Reads Mapping to Panel (<20,000)	5	5	0 / 5	JSON SAM
IonSelect-7	MP18-453_RNA	No Fusions Detected	No fusion transcripts were detected.	87,592	83,475	5 / 5	JSON SAM
IonSelect-8	SeraCare 3a	Fusions Detected	15 Detected Fusion Transcripts +	92,838	38,867	5 / 5	JSON SAM
IonSelect-13	SeraCare 3b	Fusions Detected	15 Detected Fusion Transcripts +	70,363	29,653	5 / 5	JSON SAM
IonSelect-14	SeraCare 3c	Fusions Detected	15 Detected Fusion Transcripts +	76,054	28,320	5 / 5	JSON SAM
IonSelect-15	NTC_RNA	No Call	Insufficient Reads Mapping to Panel (<20,000)	0	0	0 / 5	JSON SAM



NGS FUSION CONTROL REPORT – NGS-681

HD796

SARAH CANNON
Molecular Diagnostics
Part of HCA Healthcare UK

Fusion QC Outcome

PASS

Fusion Detection Summary

Total Mapped Reads:	140,633
Expression Control Reads:	75,635
Expression Control Genes:	5 \ 5

Problem:

Horizon Stopping production of HD796…!

Specific Fusion Transcript Details

Gene1	Exon1		Gene2	Exon 2	Read Count	✓ / ✗ / — / ⚠
CCDC6	1	⇝	RET	12	4,175	✓
EML4	13	⇝	ALK	20	3,151	✓
ETV6	5	⇝	NTRK3	15	7,116	✓
SLC34A2	4	⇝	ROS1	32	17,244	✓
TPM3	7	⇝	NTRK1	10	5,927	✓
MET	13	⇝	MET	15	24	—
SLC34A2	4	⇝	ROS1	34	999	—

SeraCare FFPE Fusion Reference Panel v4

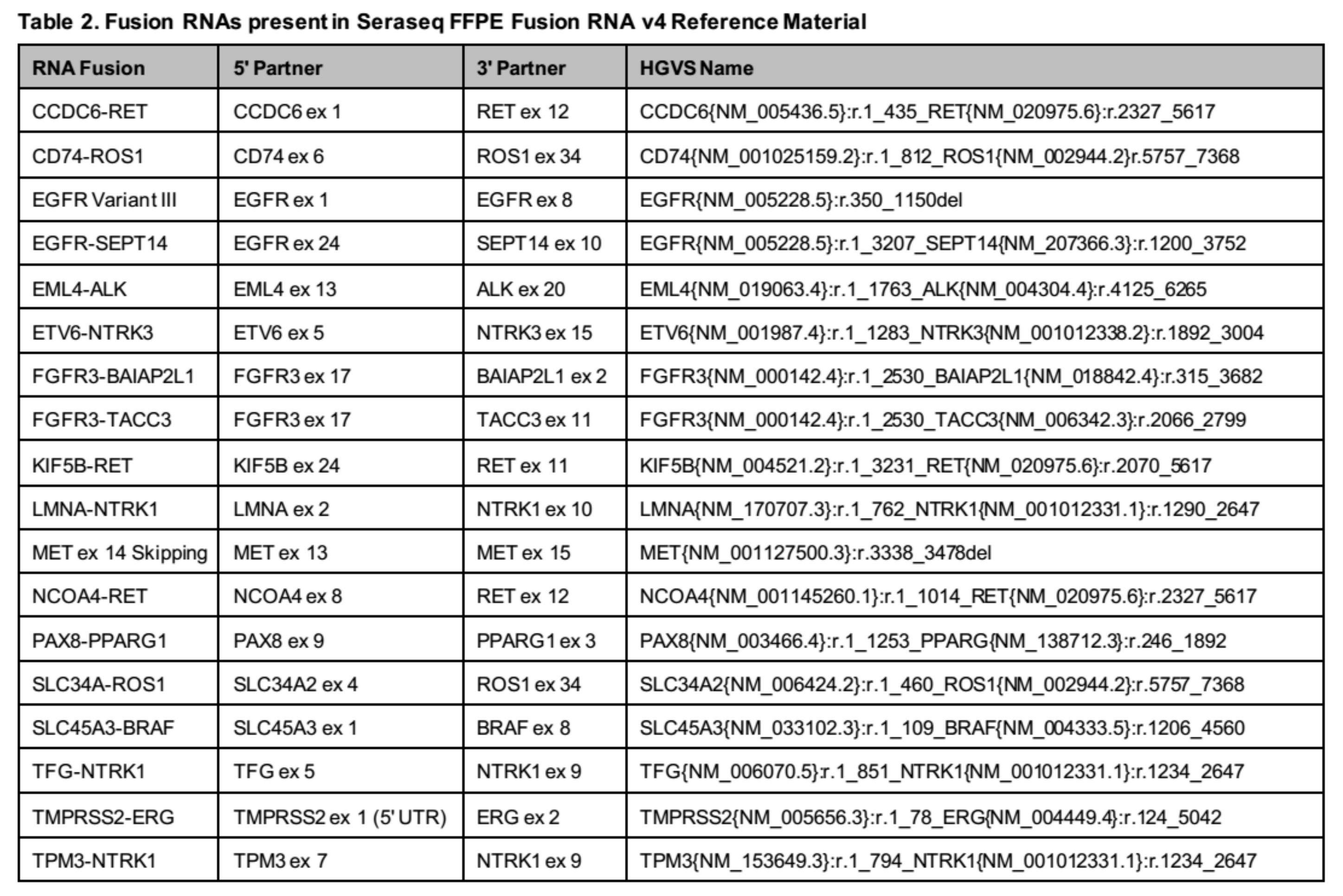
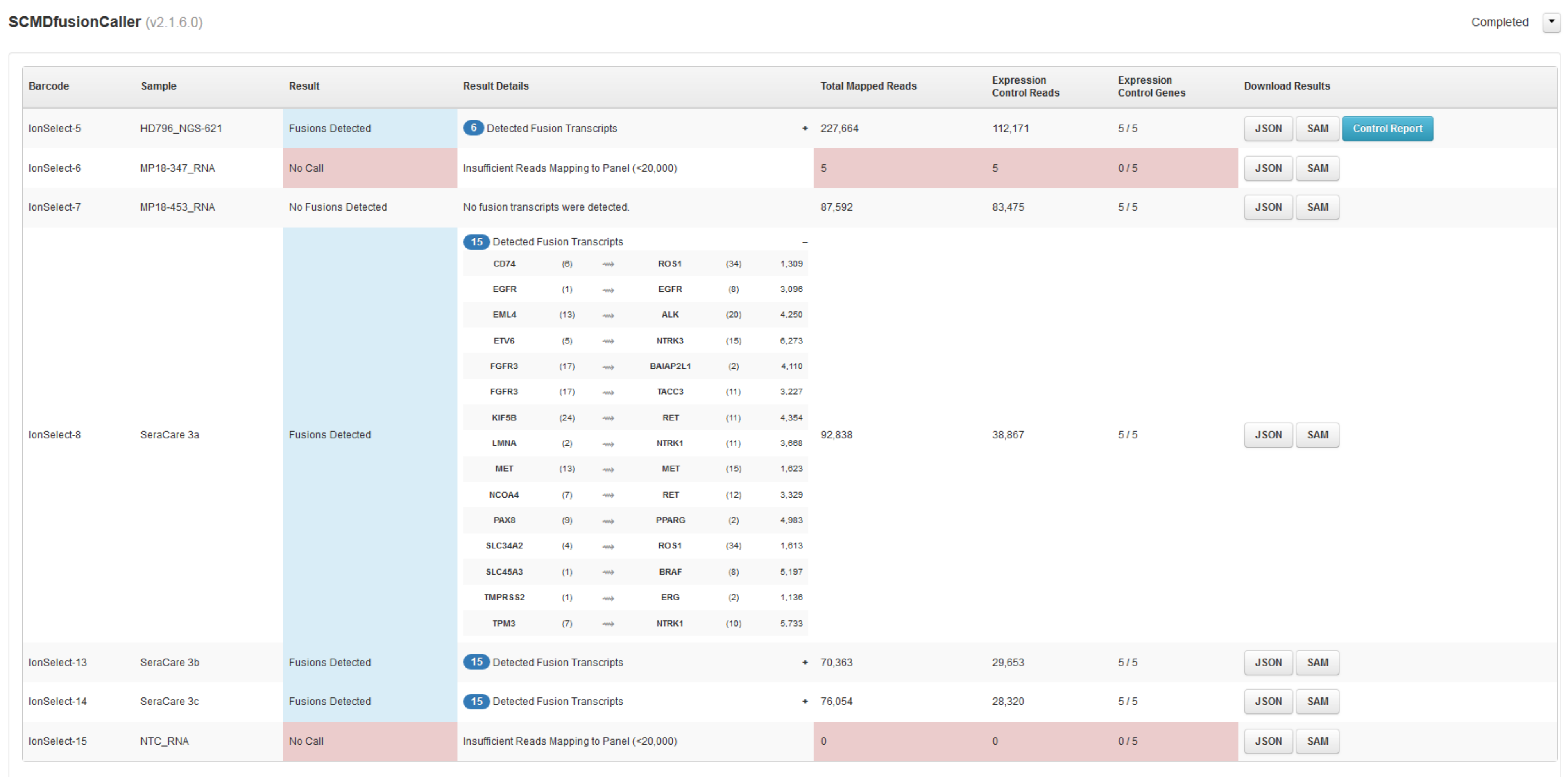


Table 2. Fusion RNAs present in Seraseq FFPE Fusion RNA v4 Reference Material

RNA Fusion	5' Partner	3' Partner	HGVS Name
CCDC6-RET	CCDC6 ex 1	RET ex 12	CCDC6{NM_005436.5}:r.1_435_RET{NM_020975.6}:r.2327_5617
CD74-ROS1	CD74 ex 6	ROS1 ex 34	CD74{NM_001025159.2}:r.1_812_ROS1{NM_002944.2}r.5757_7368
EGFR Variant III	EGFR ex 1	EGFR ex 8	EGFR{NM_005228.5}:r.350_1150del
EGFR-SEPT14	EGFR ex 24	SEPT14 ex 10	EGFR{NM_005228.5}:r.1_3207_SEPT14{NM_207366.3}:r.1200_3752
EML4-ALK	EML4 ex 13	ALK ex 20	EML4{NM_019063.4}:r.1_1763_ALK{NM_004304.4}:r.4125_6265
ETV6-NTRK3	ETV6 ex 5	NTRK3 ex 15	ETV6{NM_001987.4}:r.1_1283_NTRK3{NM_001012338.2}:r.1892_3004
FGFR3-BAIAP2L1	FGFR3 ex 17	BAIAP2L1 ex 2	FGFR3{NM_000142.4}:r.1_2530_BAIAP2L1{NM_018842.4}:r.315_3682
FGFR3-TACC3	FGFR3 ex 17	TACC3 ex 11	FGFR3{NM_000142.4}:r.1_2530_TACC3{NM_006342.3}:r.2066_2799
KIF5B-RET	KIF5B ex 24	RET ex 11	KIF5B{NM_004521.2}:r.1_3231_RET{NM_020975.6}:r.2070_5617
LMNA-NTRK1	LMNA ex 2	NTRK1 ex 10	LMNA{NM_170707.3}:r.1_762_NTRK1{NM_001012331.1}:r.1290_2647
MET ex 14 Skipping	MET ex 13	MET ex 15	MET{NM_001127500.3}:r.3338_3478del
NCOA4-RET	NCOA4 ex 8	RET ex 12	NCOA4{NM_001145260.1}:r.1_1014_RET{NM_020975.6}:r.2327_5617
PAX8-PPARG1	PAX8 ex 9	PPARG1 ex 3	PAX8{NM_003466.4}:r.1_1253_PPARG{NM_138712.3}:r.246_1892
SLC34A-ROS1	SLC34A2 ex 4	ROS1 ex 34	SLC34A2{NM_006424.2}:r.1_460_ROS1{NM_002944.2}:r.5757_7368
SLC45A3-BRAF	SLC45A3 ex 1	BRAF ex 8	SLC45A3{NM_033102.3}:r.1_109_BRAF{NM_004333.5}:r.1206_4560
TFG-NTRK1	TFG ex 5	NTRK1 ex 9	TFG{NM_006070.5}r.1_851_NTRK1{NM_001012331.1}:r.1234_2647
TMPRSS2-ERG	TMPRSS2 ex 1 (5' UTR)	ERG ex 2	TMPRSS2{NM_005656.3}:r.1_78_ERG{NM_004449.4}:r.124_5042
TPM3-NTRK1	TPM3 ex 7	NTRK1 ex 9	TPM3{NM_153649.3}:r.1_794_NTRK1{NM_001012331.1}:r.1234_2647

In-house Fusion Caller Plugin – switch to SeraCare IQC

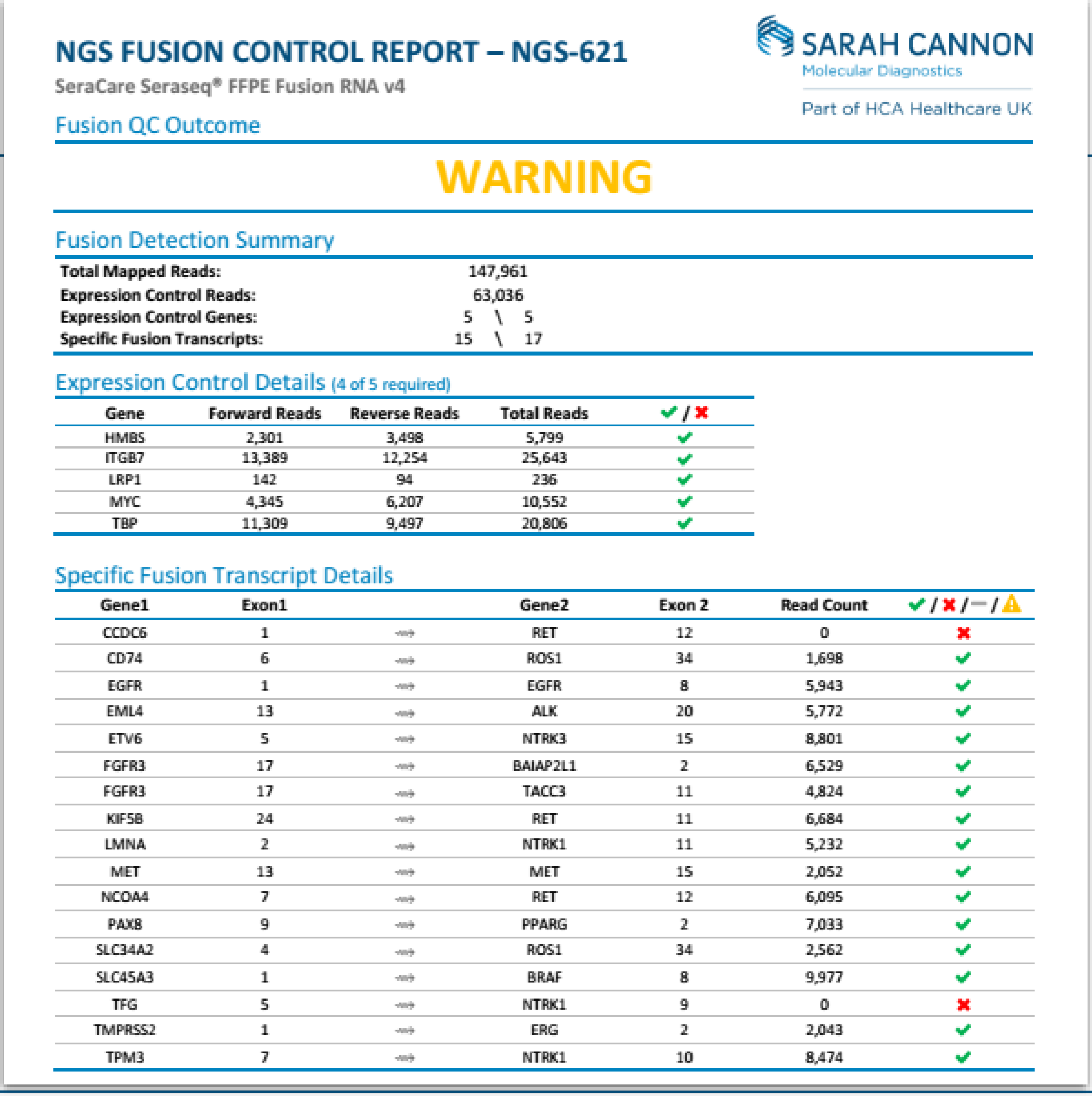


SCMDfusionCaller (v2.1.6.0)

Barcode	Sample	Result	Result Details		Total Mapped Reads	Expression Control Reads	Expression Control Genes	Download Results
IonSelect-5	HD796_NGS-621	Fusions Detected	6 Detected Fusion Transcripts	+	227,664	112,171	5 / 5	JSON SAM Control Report
IonSelect-6	MP18-347_RNA	No Call	Insufficient Reads Mapping to Panel (<20,000)		5	5	0 / 5	JSON SAM
IonSelect-7	MP18-453_RNA	No Fusions Detected	No fusion transcripts were detected.		87,592	83,475	5 / 5	JSON SAM
IonSelect-8	SeraCare 3a	Fusions Detected	15 Detected Fusion Transcripts	–	92,838	38,867	5 / 5	JSON SAM
IonSelect-13	SeraCare 3b	Fusions Detected	15 Detected Fusion Transcripts	+	70,363	29,653	5 / 5	JSON SAM
IonSelect-14	SeraCare 3c	Fusions Detected	15 Detected Fusion Transcripts	+	76,054	28,320	5 / 5	JSON SAM
IonSelect-15	NTC_RNA	No Call	Insufficient Reads Mapping to Panel (<20,000)		0	0	0 / 5	JSON SAM

IonSelect-8 – 15 Detected Fusion Transcripts:

CD74	(6)	⇝	ROS1	(34)	1,309
EGFR	(1)	⇝	EGFR	(8)	3,096
EML4	(13)	⇝	ALK	(20)	4,250
ETV6	(5)	⇝	NTRK3	(15)	6,273
FGFR3	(17)	⇝	BAIAP2L1	(2)	4,110
FGFR3	(17)	⇝	TACC3	(11)	3,227
KIF5B	(24)	⇝	RET	(11)	4,354
LMNA	(2)	⇝	NTRK1	(11)	3,668
MET	(13)	⇝	MET	(15)	1,623
NCOA4	(7)	⇝	RET	(12)	3,329
PAX8	(9)	⇝	PPARG	(2)	4,983
SLC34A2	(4)	⇝	ROS1	(34)	1,613
SLC45A3	(1)	⇝	BRAF	(8)	5,197
TMPRSS2	(1)	⇝	ERG	(2)	1,136
TPM3	(7)	⇝	NTRK1	(10)	5,733



NGS FUSION CONTROL REPORT – NGS-621

SeraCare Seraseq® FFPE Fusion RNA v4

SARAH CANNON
Molecular Diagnostics
Part of HCA Healthcare UK

Fusion QC Outcome

WARNING

Fusion Detection Summary

Total Mapped Reads:	147,961
Expression Control Reads:	63,036
Expression Control Genes:	5 \ 5
Specific Fusion Transcripts:	15 \ 17

Expression Control Details (4 of 5 required)

Gene	Forward Reads	Reverse Reads	Total Reads	✓ / ✗
HMBS	2,301	3,498	5,799	✓
ITGB7	13,389	12,254	25,643	✓
LRP1	142	94	236	✓
MYC	4,345	6,207	10,552	✓
TBP	11,309	9,497	20,806	✓

Specific Fusion Transcript Details

Gene1	Exon1		Gene2	Exon 2	Read Count	✓ / ✗ / — / ⚠
CCDC6	1	⇝	RET	12	0	✗
CD74	6	⇝	ROS1	34	1,698	✓
EGFR	1	⇝	EGFR	8	5,943	✓
EML4	13	⇝	ALK	20	5,772	✓
ETV6	5	⇝	NTRK3	15	8,801	✓
FGFR3	17	⇝	BAIAP2L1	2	6,529	✓
FGFR3	17	⇝	TACC3	11	4,824	✓
KIF5B	24	⇝	RET	11	6,684	✓
LMNA	2	⇝	NTRK1	11	5,232	✓
MET	13	⇝	MET	15	2,052	✓
NCOA4	7	⇝	RET	12	6,095	✓
PAX8	9	⇝	PPARG	2	7,033	✓
SLC34A2	4	⇝	ROS1	34	2,562	✓
SLC45A3	1	⇝	BRAF	8	9,977	✓
TFG	5	⇝	NTRK1	9	0	✗
TMPRSS2	1	⇝	ERG	2	2,043	✓
TPM3	7	⇝	NTRK1	10	8,474	✓

MET Exon 14 Skipping

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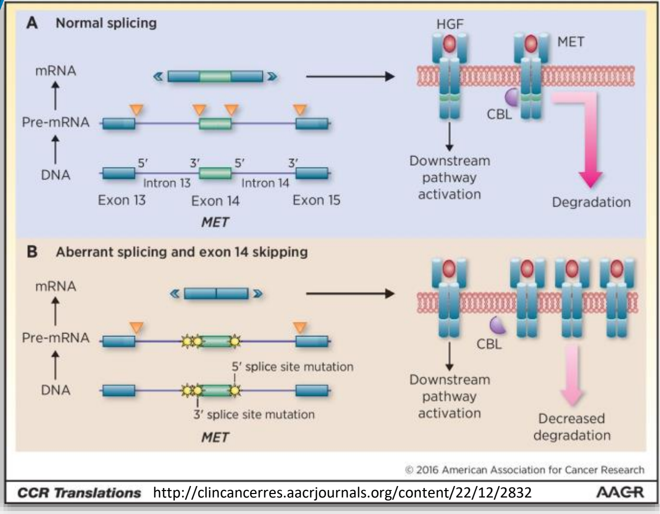
MET Exon 14 Skipping – Lung / CRC / Other

NSCLC Cancer Fusions	Data from MCG					
Gene	**Prevalance**	**Fusion Partners**	**Increased Sensitivity**	**Decreased Sensitivity**	**Restistance Markers**	**Other Cancers**
ALK	3-7%	EML4; KIF5B	crizotinib; ceritinib; pemetrexed	EGFR TKIs	L1196M; G1269A	thyroid
MET exon 14 skipping	~5%	MET	crizotinib; cabozantinib	?	?	CNS; UT
ROS	~2%	CD74; SLC34A2	crizotinib	EGFR TKIs	G2032R; D2033N	stomach
RET	~1%	KIF5B; CCDC6	cabozantinib	?	?	thyroid
FGFR3	0.5-2%	TACC3	FGFR/TORC inhibitors	?	?	
NTRK1	<1%	MPRIP; CD74	larotrectinib / Entrectinib /?crizotinib	?	?	thyroid
Amplifications						
MET Amplification	~2-4% (untreated)		crizotinib	EGFR TKIs	-	-
	~5-20% (treated + resistance)					
EGFR exon 18-25 dup	??	-	EGFR TKIs	-	-	-
ERBB2	~1%	-	-	-	-	-
FGFR1	1 - 6%		FGFR inhibitors	-	-	-

MET Exon 14 Skipping – detect at DNA & RNA level



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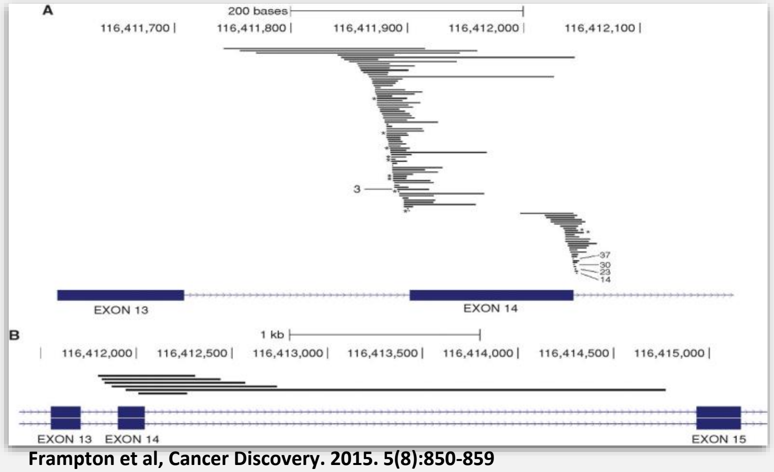
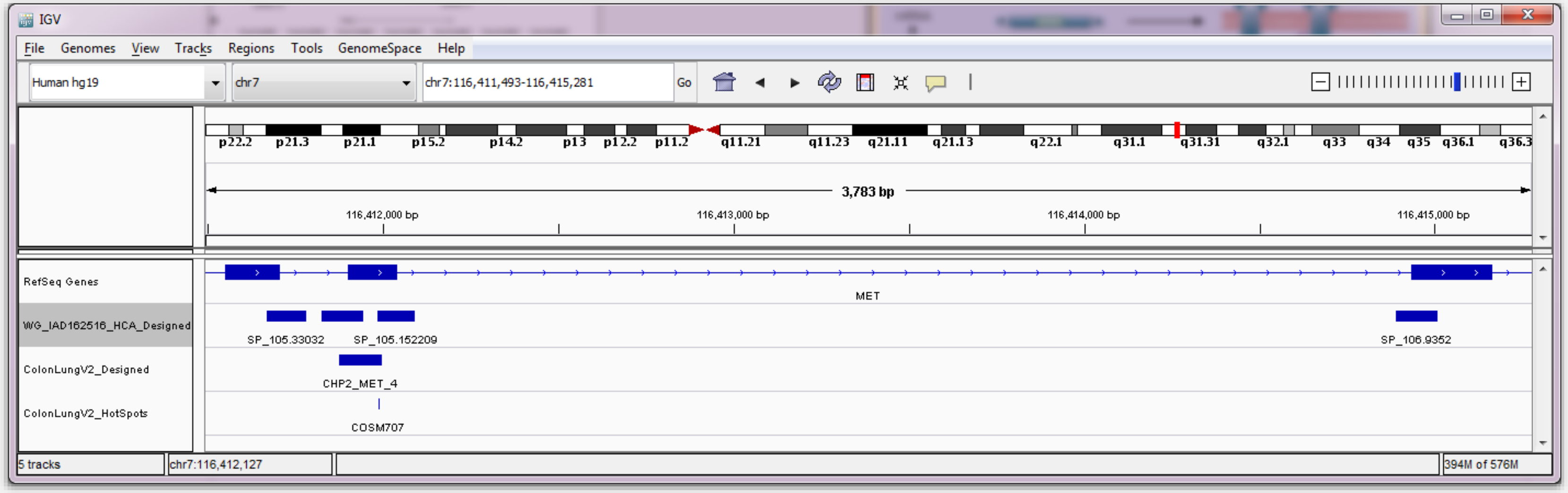
CCR Translations http://clincancerres.aacrjournals.org/content/22/12/2832 AACR



Frampton et al, Cancer Discovery. 2015. 5(8):850-859



Human hg19

chr7

chr7:116,411,493-116,415,281

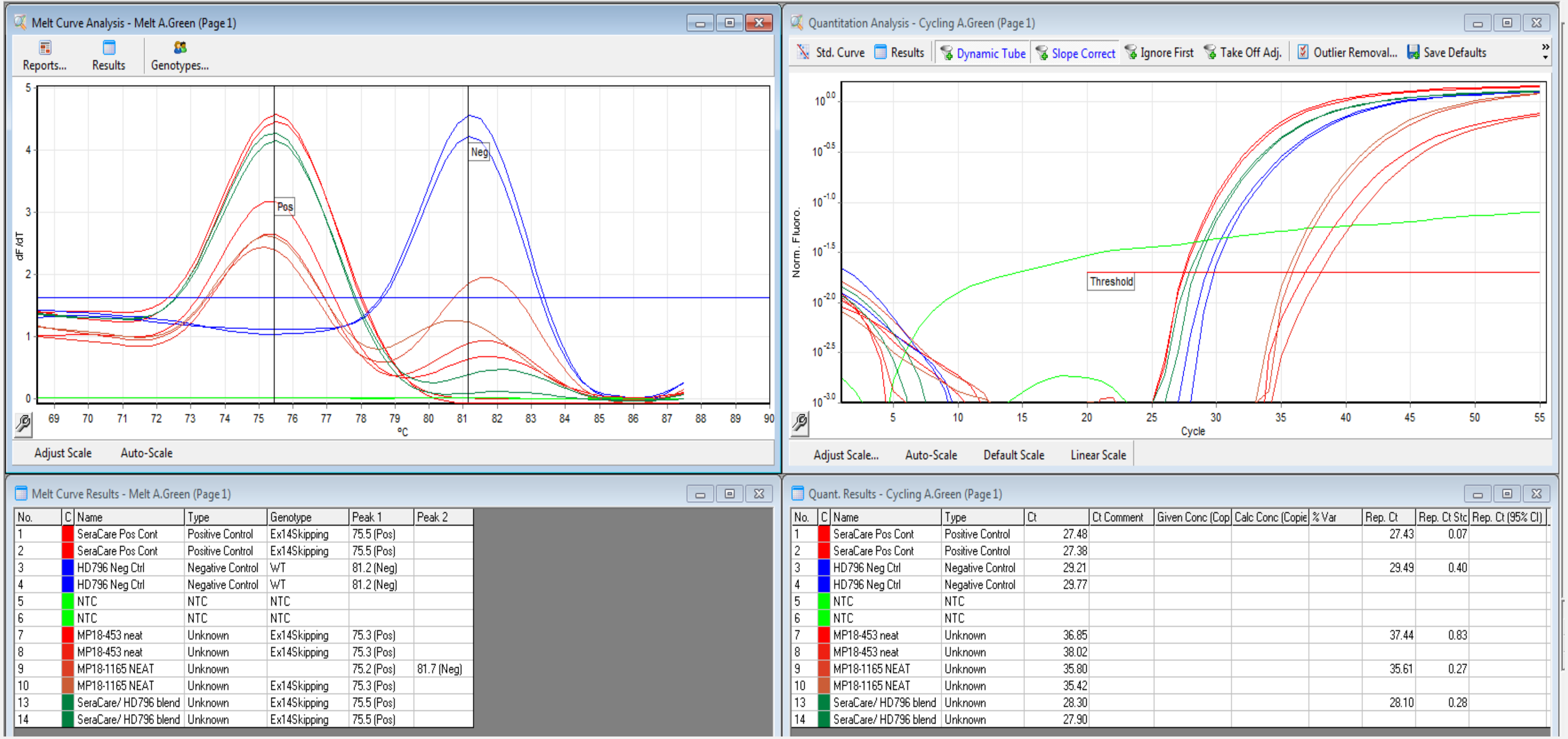
5 tracks

chr7:116,412,127

394M of 576M

MET exon 14 Skipping RT-qPCR Melt Curve Assay

For verification of novel DNA level events detected by MGP-4 (uses RNA)



No.	C	Name	Type	Genotype	Peak 1	Peak 2
1	🟥	SeraCare Pos Cont	Positive Control	Ex14Skipping	75.5 (Pos)	
2	🟥	SeraCare Pos Cont	Positive Control	Ex14Skipping	75.5 (Pos)	
3	🟦	HD796 Neg Ctrl	Negative Control	WT	81.2 (Neg)	
4	🟦	HD796 Neg Ctrl	Negative Control	WT	81.2 (Neg)	
5	🟩	NTC	NTC	NTC		
6	🟩	NTC	NTC	NTC		
7	🟥	MP18-453 neat	Unknown	Ex14Skipping	75.3 (Pos)	
8	🟥	MP18-453 neat	Unknown	Ex14Skipping	75.3 (Pos)	
9	🟧	MP18-1165 NEAT	Unknown		75.2 (Pos)	81.7 (Neg)
10	🟧	MP18-1165 NEAT	Unknown	Ex14Skipping	75.3 (Pos)	
13	🟩	SeraCare/ HD796 blend	Unknown	Ex14Skipping	75.5 (Pos)	
14	🟩	SeraCare/ HD796 blend	Unknown	Ex14Skipping	75.5 (Pos)	

No.	C	Name	Type	Ct	Ct Comment	Given Conc (Cop	Calc Conc (Copie	% Var	Rep. Ct	Rep. Ct Stc	Rep. Ct (95% CI)
1	🟥	SeraCare Pos Cont	Positive Control	27.48					27.43	0.07	
2	🟥	SeraCare Pos Cont	Positive Control	27.38							
3	🟦	HD796 Neg Ctrl	Negative Control	29.21					29.49	0.40	
4	🟦	HD796 Neg Ctrl	Negative Control	29.77							
5	🟩	NTC	NTC								
6	🟩	NTC	NTC								
7	🟥	MP18-453 neat	Unknown	36.85					37.44	0.83	
8	🟥	MP18-453 neat	Unknown	38.02							
9	🟧	MP18-1165 NEAT	Unknown	35.80					35.61	0.27	
10	🟧	MP18-1165 NEAT	Unknown	35.42							
13	🟩	SeraCare/ HD796 blend	Unknown	28.30					28.10	0.28	
14	🟩	SeraCare/ HD796 blend	Unknown	27.90							

On Scope Non-NGS Assays

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Biocartis Idylla – Complements NGS service

Rapid EGFR

Rapid CRC Screen
(KRAS / BRAF+NRAS)

Rapid BRAF

Rapid MSI
(7 novel microsatellite)

Lynch Syndrome Pre-Screen: MSI & *MLH1* Methylation

Verification of a Cartridge-based Microsatellite Instability (MSI) Platform and Cross-platform Assessment of Cases with Rare Germline Polymorphic Markers

Gareth Gerrard[1,2*], Diana Pelka[1], Nicole Gurunlian[1], Georgina Briggs[1], Jack Grant[1], Kevin Balbi[1], Phil Bennett[1]

[1]*Sarah Cannon Molecular Diagnostics, HCA Healthcare UK, London, UK;* [2]*Faculty of Medicine, Imperial College London, UK.*

Background

Microsatellite instability (MSI) testing is an important component of the Lynch Syndrome (LS) pre-screen for colorectal and endometrial cancers, and a pan-cancer biomarker for response to checkpoint-inhibitor immunotherapy.

We previously validated the Promega MSI v1.2 system, based around the capillary electrophoretic analysis of 5 quasi-monomorphic alleles in tumour-normal pairs.

However, Biocartis have recently launched the Idylla MSI CE-IVD solution, based on single-use cartridge qPCR analysis of 7 homopolymer regions in tumour-only samples (Figure 1).

We sought to verify this platform for clinical-diagnostic use, using multi-level synthetic controls; also to assess 3 samples that had previously shown germline polymorphisms in Promega alleles.

Figure 1. Biocartis Idylla single-use cartridge system

Methods

Seven MSI DNA controls (5 levels MSI-High (MSI-H); 2 MSI-Stable (MSS)) were acquired from Horizon Discovery (Table 1). These were assessed with both systems, using standard protocols and scored as MSI-H (2-or-more alleles unstable), or MSS (0 or 1 allele unstable). For the Promega system, 2ng sample DNA was analysed using an ABI 3500 genetic analyser in fragment analysis mode and used GeneMapper v5 software for the data analysis. For the Biocartis, one 10µM FFPE curl or 50ng DNA was used (1 cartridge per sample), with on-platform analysis. Additional data visualisation and generation of septopus plots was via the online Idylla Explore portal (Figure 2). The Analysis of the lowest signal-strength control was replicated 3 times on the Biocartis, with multiple operators to assess inter-run variability. Three tumour samples with previously identified germline polymorphic alleles (ovarian, BAT-25; endometrial, NR-21; colorectal, BAT-25; all MSS) were also assessed using the Biocartis system.

Table 1: Comparative results of 7 MSI control samples (Horizon Discovery) between Promega v1.2 system and Biocartis Idylla MSI 1.0

			Promega v1.2		ABI 3500 POP-7 / 50cm					BioCartis	MSI/1.0 CE-IVD							
	MSI Sample		BAT-26	NR-21	BAT-25	MONO-27	NR-24	Markers	Result	ACVR2A	BTBD7	DIDO1	MRE11	RYR3	SEC31A	SULF2	Markers	Result
1	MSIC1:	HD-D156	*	*	*	*	*	5/5	MSI-H	0.99	0.89	0.3	0.31	0.26	0.04	0.41	3/7	MSI-H
2	MSIC2:	HD-D157	**	**	**	**	**	5/5	MSI-H	1	1	0.98	0.61	0.98	1	0.98	7/7	MSI-H
3	MSIC3:	HD-D158	***	***	***	***	***	5/5	MSI-H	1	1	0.99	1	1	1	1	7/7	MSI-H
4	MSIC4:	HD-D159	****	****	****	****	****	5/5	MSI-H	1	1	1	1	1	1	1	7/7	MSI-H
5	MSIC5:	HD-D165	**	***	*	**	*	5/5	MSI-H	1	1	0.97	0.99	0.99	0.99	0.99	7/7	MSI-H
6	MSSC1:	HD-D155	S	S	S	S	S	0/5	MSS	0	0	0	0	0	0	0	0/7	MSS
7	MSSC2:	HD-D164	S	S	S	S	S	0/5	MSS	0	0	0	0.01	0	0	0	0/7	MSS

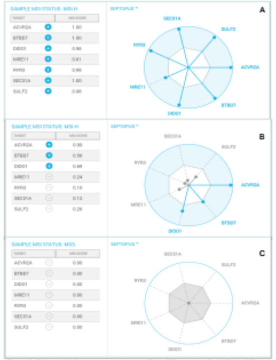


Figure 2. Biocartis Idylla septopus plots. A = MSI-H (7/7 markers); B = MSI-H (3/7); C = MSS (0/7)

Results

Control material results were 100% concordant between the Promega and Biocartis platforms (5 MSI-H; 2 MSS) (Table 1).

Replicated samples showed no significant differences between analytical runs (MSI-H; 3/7 markers positive (curl); 2/7 positive (DNA); ANOVA, P=0.53) (Table 2).

The three samples displaying single-marker germline polymorphisms were all reported as MSS (0/7 markers) by the Biocartis system (Table 3).

The additional septopus plots (Figure 2) show the signal strength from a high-signal sample (A: HD-D157; 7/7), low-signal sample (B: HD-D156; 3/7), and MSS sample (C: HD-D155).

Table 2: Results of repeated runs of single sample (HD-D156)

Run	Source	ACVR2A	BTBD7	DIDO1	MRE11	RYR3	SEC31A	SULF2	Markers	Result
1	Curl	0.99	0.89	0.3	0.31	0.26	0.04	0.41	3/7	MSI-H
2	DNA	0.9	0.58	0.27	0.4	0.22	0.03	0.23	2/7	MSI-H
3	DNA	0.99	0.56	0.69	0.24	0.1	0.12	0.26	2/7	MSI-H

Table 3: Results of 3 single-marker germline polymorphism samples analysed on Biocartis Idylla

	Sample	Primary	Promega	Markers	Polymorphic	Biocartis	Markers
1	MP18-1555	Ovarian	MSS	1/5	BAT-25	MSS	0/7
2	MP19-1122	Endometrial	MSS	1/5	NR-21	MSS	0/7
3	MP19-1140	CRC	MSS	1/5	BAT-25	MSS	0/7

Conclusions

Both platforms proved to be sensitive and cost-effective; however, the Biocartis benefited from a faster and more simplified workflow, and apparent reduced susceptibility to targets displaying potentially interfering polymorphism at the population level, thus obviating the requirement for co-analysis of paired-normal samples.

Acknowledgments: Thanks to Sian Constantine at Horizon Discovery for coordinating supply of control materials.

*email: gareth.gerrard@hcahealthcare.co.uk

MLH1 Promoter Hypermethylation: Development and Validation of a Methylation-Sensitive High-Resolution Melting Analysis (MS-HRM) Assay for use in a Lynch Syndrome Pre-Screen Pathway

Diana Pelka[1,2], Sasha Hansel[1], Ben Poskitt[1], David Moore[1,3], Phil Bennett[1], Gareth Gerrard[1,2*]

[1]*Sarah Cannon Molecular Diagnostics, HCA Healthcare UK, London, UK;* [2]*Faculty of Medicine, Imperial College London, London, UK;* [3]*Department of Histopathology, UCLH NHS Foundation Trust, London, UK*

Background

Lynch Syndrome (LS) is associated with germline mutations in genes encoding the mismatch repair proteins, leading to mismatch repair deficiency (dMMR). dMMR can also arise somatically by promoter methylation mediated silencing of the *MLH1* gene.

In colorectal cancer, there is a strong association between the $BRAF^{Val600Glu}$ mutation and *MLH1* promoter hypermethylation, thus analysis of these markers, along with high microsatellite instability (MSI-H), constitute components of the NICE-mandated LS pre-screen pathway in the UK.

In the absence of an out-of-the-box CE-IVD solution, we sought to develop and validate an MS-HRM based, *MLH1* promoter methylation assay for clinical use, using a kit consisting of: primers and controls from MethylDetect ApS, (Denmark); and bisulphite conversion and HRM-PCR mastermix kits from Qiagen.

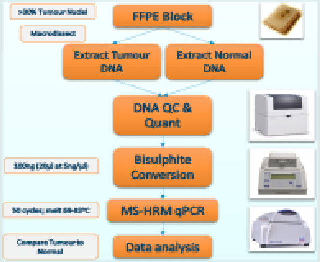


Figure 1. MS-HRM Workflow

Methods

Paired tumour & normal DNA was extracted from 26 FFPE samples (from 23 patients and 2 EQA samples) (Figure 1 & Table 1):

- 16 colorectal, 5 prostate, 2 endometrial, 1 bladder, 1 pancreas, and 1 unknown
- 13 samples were known MSI-H, 13 MSI-stable (MSS)
- 9 samples had known MMR by IHC status: 6 dMMR (5 MLH1+PMS2; 1 PMS2), 3 MMR-normal
- 17 samples had known BRAF status: 6 $BRAF^{Val600Glu}$, 11 $BRAF^{WT}$
- 1 sample from known LS; 3 from suspected LS. Samples 9 & 10 were synchronous tumours from the same patient.

20µL (5ng/µL) DNA was bisulphite converted (bsDNA) and 2-3µL used per MS-HRM reaction. These were run on a Qiagen Rotor-Gene Q with 2-4 technical replicates for 45-50 cycles, with a HRM melt start of 65-69ºC and finish of 83-95ºC. The paired-normal sample was used as the non-methylated baseline on a per-sample basis.

The data analysis was conducted using the on-board Rotor-Gene software and used both HRM melt curves, with a 1% Confidence Threshold and Melt-Peak genotype bins.

Orthogonal bisulphite Sanger sequencing was also conducted using *MLH1* specific primers, BigDye Direct Cycle Sequencing and XTerminator clean-up on an ABI 3500 Genetic Analyser.

Results

Repeated MS-HRM optimisation runs (initial test run, plus runs 1-3) showed that 3µL bsDNA input, with 4 technical replicates, x50 cycles, and 69-83ºC HRM melt range were the optimal parameters (Figure 2).

MLH1 methylation status was successfully assayed in all 26 samples, with no inter-run variation detected. 8 samples scored as methylated and 18 non-methylated (Table 1). The methylated samples were all MSI-H and included 3 dMMR (MLH1+PMS2), 5/6 $BRAF^{Val600Glu}$ samples, and none of the LS/possible LS samples. The non-methylated samples included: 4 MSH-High; 3 dMMR; and the 4 LS/possible LS samples. The one $BRAF^{Val600Glu}$ sample that was non-methylated (ID 6) was from a suspected LS patient, which showed a *PMS2* only dMMR profile, indicating that *MLH1* was not involved.

The orthogonal bisulphite-sequencing was concordant for 15/16 samples, failed for 9 samples, and 1 sample was not run because of low DNA level. The one discordant result (ID 2) was from a sample with a low methylation signal; likely to be below the sensitivity of bisulphite-sequencing (~20%).

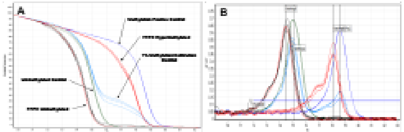
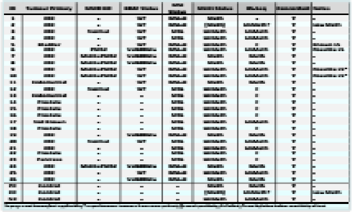


Figure 2: A, shows the MS-HRM melt curves for 2 paired tumour-normal samples: 1 hypermethylated and 1 unmethylated; B, shows the same data, but using melt-peak genotype binning.

Table 1. Sample details and results summary



Conclusions

The validation results for the *MLH1* promotor methylation by MS-HRM assay has shown the assay to be sensitive, specific, straightforward and robust, with no measureable inter-run variation. These data show the assay to be suitable for use downstream of MSI / MMR testing in a Lynch Syndrome Pre-Screen.

Acknowledgements: Many thanks to: Jack Grant, Nicole Gurunlian, Alba Santos, Georgina Briggs from SCMD for technical assistance. Thanks also to Tomasz Wojdacz at MethylDetect for advice and assistance.

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Acknowledgments

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