

CMGS Proof of Principle for Diagnostic Application of Next Generation Sequencing

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ACTTAG **CMGS** CGTGT CAGTACCGTACTTAGCGT
CGTACT **CMGS** ACGTGT CAGTACCGTACTCGTGT
TCAGTACCGTACTTAG **CLINICAL MOLECULAR** ACGTGT C
GTGT CAGTACCGTACT **GENETICS SOCIETY** ACGTGT CAG



Background

- ~70% of workload in molecular diagnostics essentially simple PCR based mutation scanning
- Ever increasing demand for greater capacity
- Clear potential for diagnostic application of NGS

- Rapidly developing field
- Lack of clarity about performance and suitability of NGS platforms and targeting methodologies

Setup

- Specific diagnostic question
 - NGS platform vendors
 - Associated technologies e.g. targeting
 - Service providers
- Considerations
 - Effective coverage of the ROI
 - Fluctuating referral rates (flexibility)
 - TAT
 - Cost
 - Effective capacity usage
 - Quality

Genes samples and variants

- 7 genes associated with hereditary colorectal cancer
- ROI including 50bp intronic buffers ~54Kbp
- 101 exons
- 141 samples from 9 different diagnostic laboratories
- 142 confirmed mutations
- Confirmation of primary legion in NGRL Wessex

Gene	RefSeq	Chr Location	Strand	Exons	Variants
MUTYH	NM_001128425	1p34.3-p32.1	-	16	4
MSH6	NM_000179	2p16	+	10	21
MSH2	NM_000251	2p22-p21	+	16	50
EPCAM	NM_002354	2p21	+	9	2
MLH1	NM_000249	3p21.3	+	19	34
APC	NM_000038	5q21-q22	+	16	28
PMS2	NM_000535	7p22	-	15	3
Totals				101	142

Proposals

- Collect data on:
 - Sample requirements
 - Instrumentation
 - Workflow
 - Processing time
 - Capacity of the system (ROI, sample numbers)
 - Flexibility
 - Cost

Workflows and status

Company	n	Targeting	Sequencing	Samples dispatched	Targeting	Sequencing	Analysis
Agilent	141	SureSelect	AB SOLiD4	Complete	Complete	Ongoing	
Raindance	8	Emulsion PCR	Roche 454	Complete	Complete	Ongoing	
			Illumina	Complete	Complete	Complete	Ongoing
			SOLiD4	Complete	Complete	Complete	Ongoing
			Ion Torrent?	Complete	Complete	Seeking collaborator	
MWG	10	PCR	Roche 454	Complete	Status uncertain		
Illumina	96	Array	Illumina (GAll, HiSeq)	Complete	Ongoing		
		Amplicon	Illumina (GAll, HiSeq)	Complete	Ongoing		
Fluidigm	48	Access array	Roche 454	Complete	Complete	Seeking collaborator	
			Illumina	Complete	Complete	Seeking collaborator	

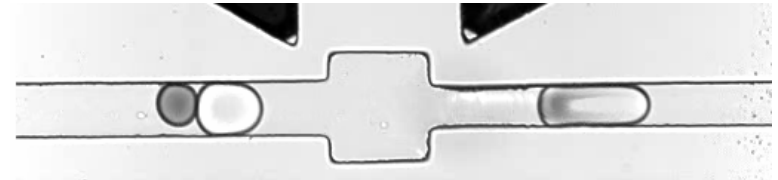
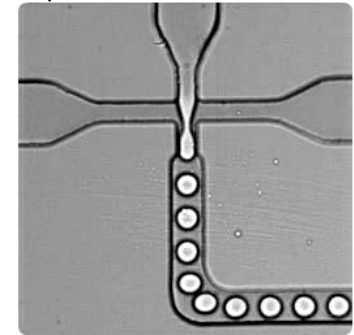
Data analysis

- By supplier where appropriate
- Raw data and mapped data collected
- Range of analysis packages incl. NextGene, CLC work bench
- Key measurement parameters:
 - Sensitivity
 - Specificity
 - Selectivity
 - Depth of sequencing required
 - Capacity required to achieve depth

Raindance

➤ Technology

- Engineered emulsion PCR
- Massive parallelisation
- Single tube



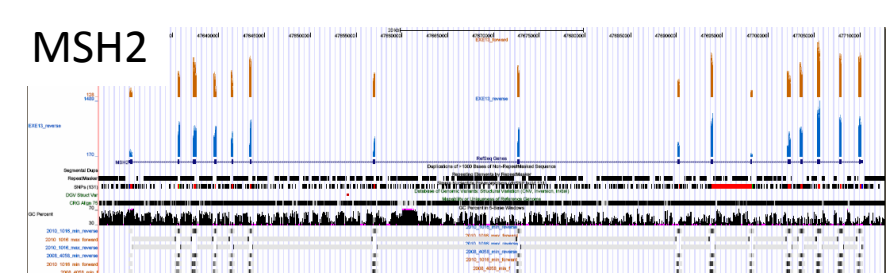
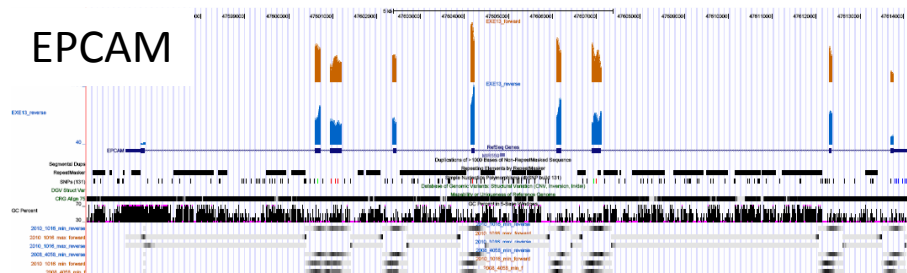
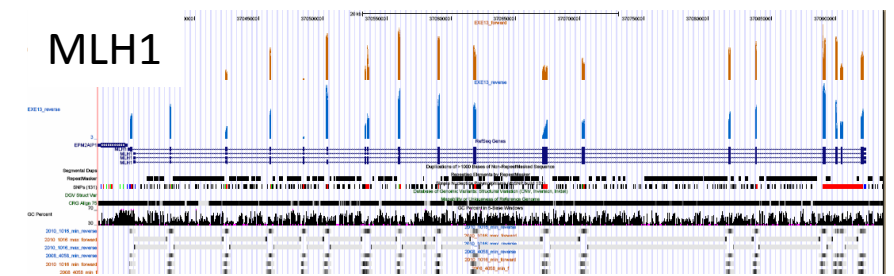
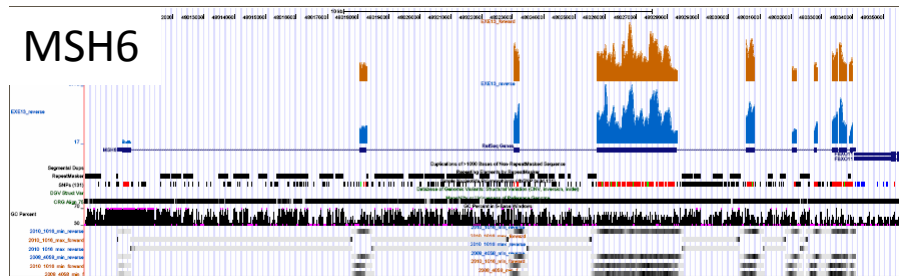
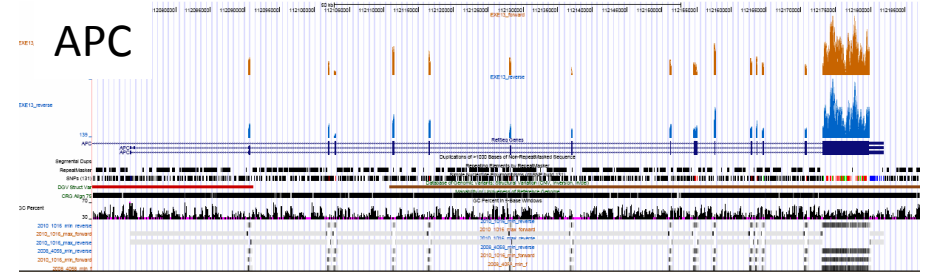
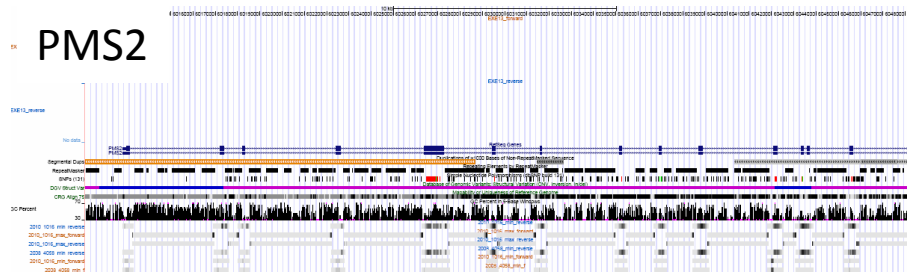
Droplet Merging

➤ Study workflows

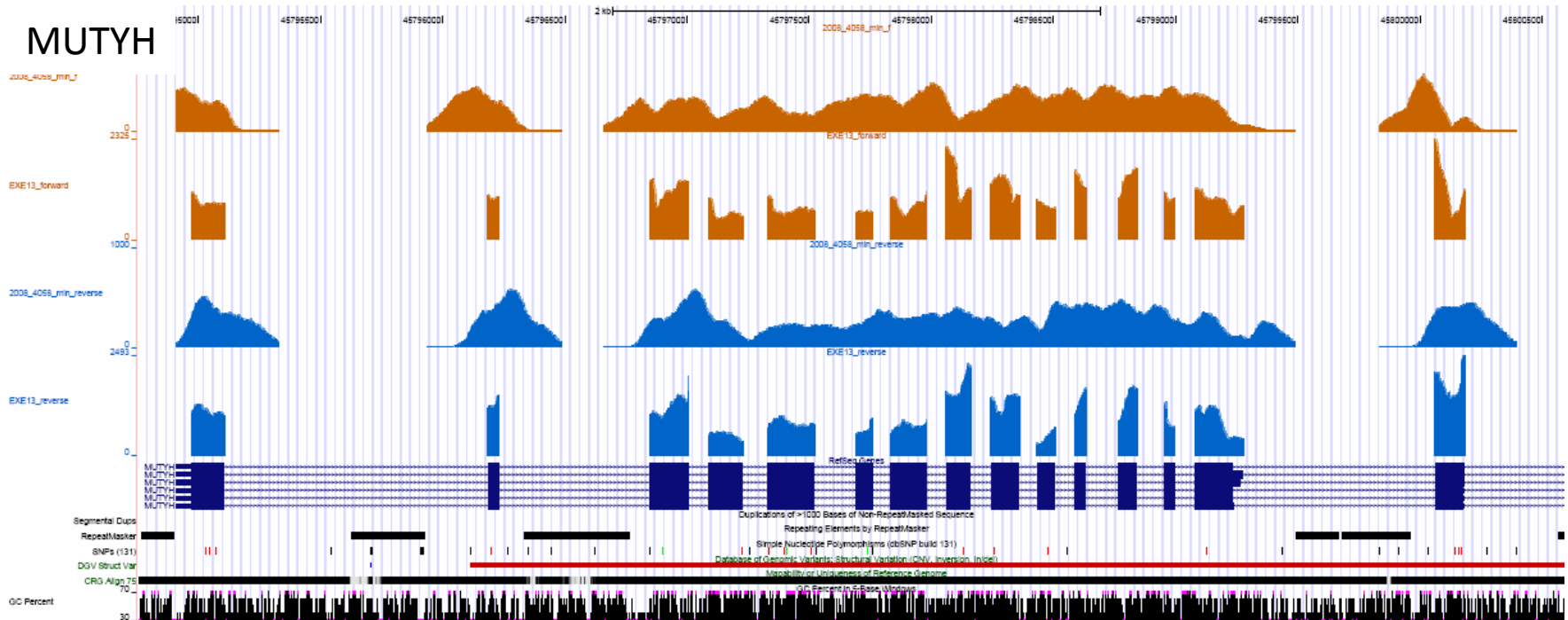
- Emulsion PCR (merge)
- Split amplicon library
- ½ for direct sequencing on Roche 454
- ½ concatenated and resheared (~200 bp) > sequencing on Illumina and SOLiD
- Material for Ion Torrent sequencing?



Raindance on Illumina GAI

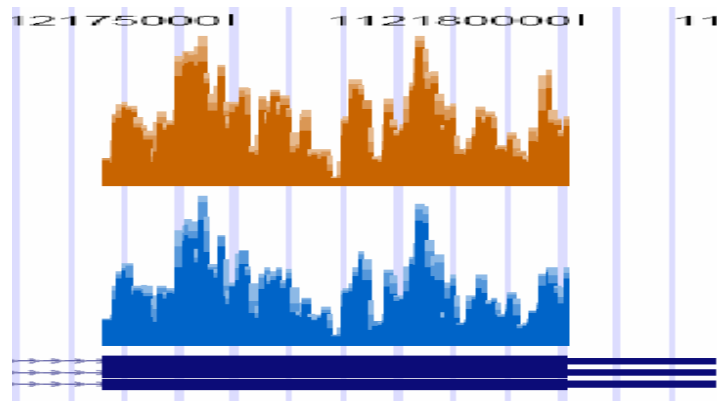


Raindance vs array target



Very Preliminary observations

- Raindance > SOLiD
 - ROI is well covered (Where appropriately targeted)
 - Quite significant variation in coverage in regions of overlapping amplicon
- All expected primary legions detected but much more work required
- Analysis with appropriate RefSeq!



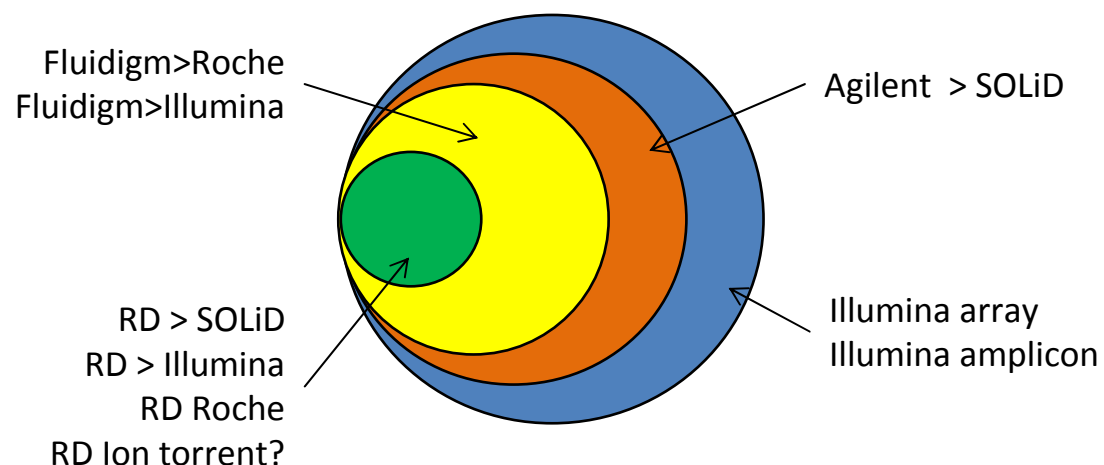
MUTYH:

NM_001128425.1:c.1187G>A
NM_012222.2:c.1178G>A
NM_001048173.1:c.1103G>A
NM_001048172.1:c.1106G>A
NM_001048171.1:c.1145G>A
NM_001048174.1:c.1103G>A

Summary

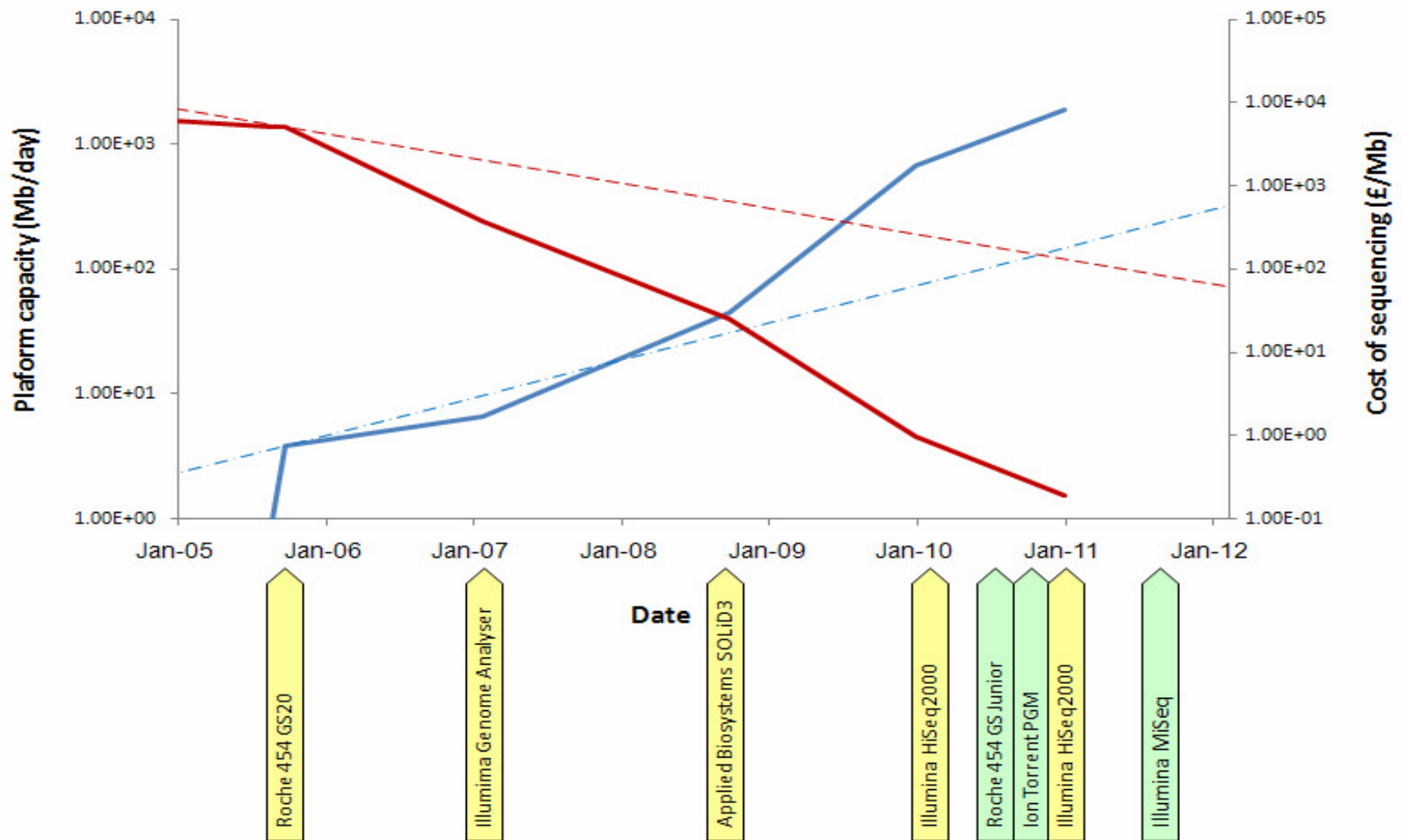
➤ **Study to assess and compare a wide range of parameters across the key technologies:**

- Targeting
- Sequencing
- Analysis



➤ **Currently at data collection and analysis stage**

Rise (capacity) and fall (cost) of NGS



Acknowledgements

➤ **Laboratories**

- Birmingham
- Cambridge
- Cardiff
- Edinburgh
- Exeter
- Liverpool
- Newcastle
- Nottingham
- Salisbury

➤ **NGRL(W)**

- Gemma Watkins

➤ **Targeting**

- Raindance
- Agilent
- Fluidigm
- Illumina

➤ **Sequencing**

- NewGene
- Graham Taylor (Leeds)
- Jo morgan (Leeds)
- Roche
- Illumina
- Applied Biosystems