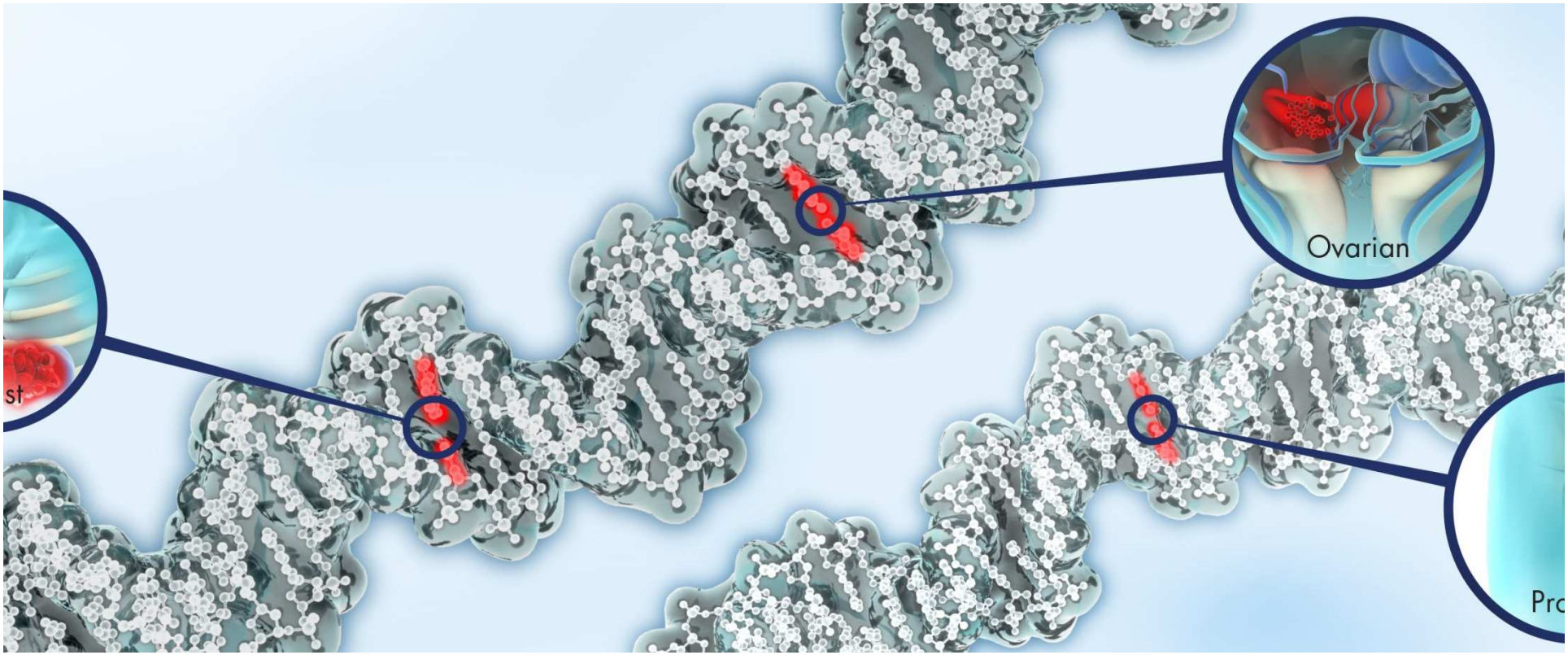

Because every base counts: sample-to-insight NGS workflow for BRCA1 and BRCA2 testing

Raed Samara, PhD

Global Product Manager

Legal disclaimers

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- For up-to-date licensing information and product-specific disclaimers, see the respective **QIAGEN kit handbook or user manual**. QIAGEN kit handbooks and user manuals are available at www.QIAGEN.com or can be requested from QIAGEN Technical Services or your local distributor.

Outline

BRCA1 and BRCA2 Genes

QIAGEN “Sample-to-Insight” Solution for Next Generation Sequencing challenges

Performance and Application Data

- Dr. Nicola Normanno,
  Chief of the Laboratory Of Pharmacogenomics, Centro Ricerche Oncologiche, Italy
- Dr. Reinhard Büttner
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Summary

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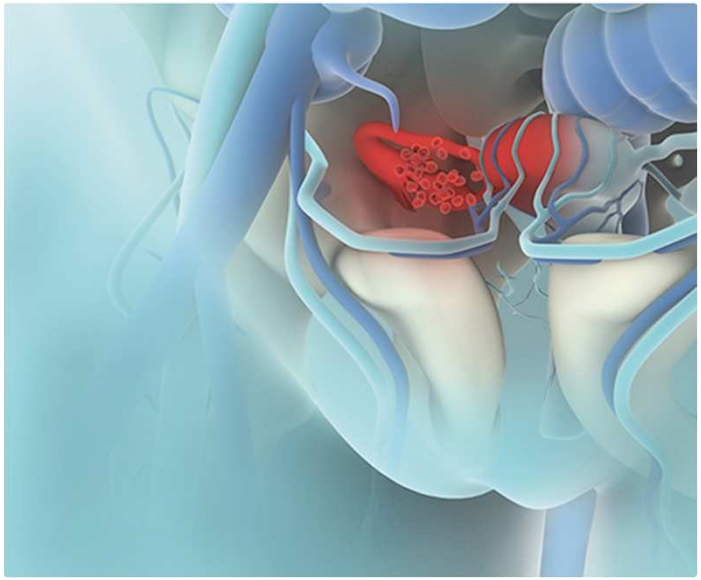
Summary

BRCA1 and BRCA2 are key tumor suppressors



BRCA1 and BRCA2 genes have DNA repair and transcriptional regulation functions.

- ~0.25% of US women (375,000) carry a mutation in BRCA genes
  - At very high risk of hereditary breast and ovarian cancer
    - 40% - 80% lifetime breast cancer risk
    - 11% - 40% lifetime ovarian cancer

- A diagnosis is made following molecular genetic testing in an individual or family with a germline *BRCA1* or *BRCA2* mutation
- **Knowledge of risk allows prevention**
- Prognosis for BRCA1/2-related cancer depends on the stage at which the cancer is diagnosed

BRCA1 and BRCA2: New development

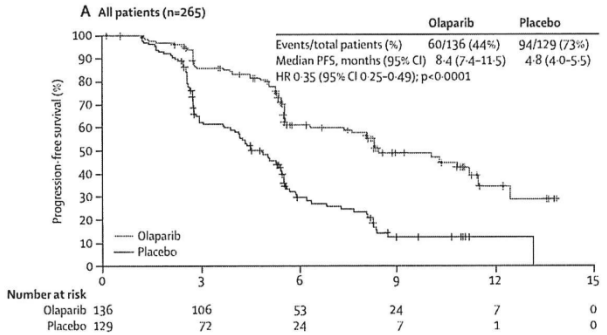
Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by *BRCA* status in a randomised phase 2 trial

Jonathan Ledermann, Philipp Harter, Charlie Gourley, Michael Friedlander, Ignace Vergote, Gordon Rustin, Clare L Scott, Werner Meier, Ronnie Shapira-Frommer, Tamar Safra, Daniela Matei, Anitra Fielding, Stuart Spencer, Brian Dougherty, Maria Orr, Darren Hodgson, J Carl Barrett, Ursula Matulonis

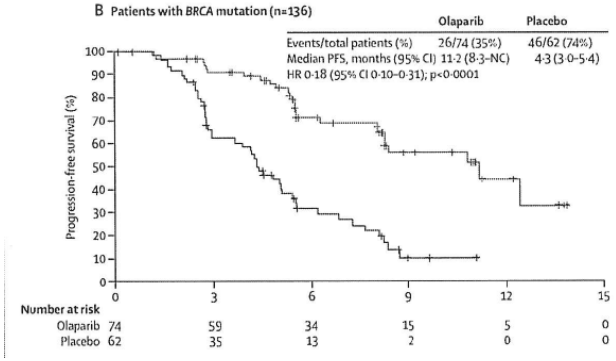
- Maintenance monotherapy with the PARP inhibitor olaparib significantly prolonged progression-free survival (PFS) versus placebo in patients with platinum-sensitive recurrent serous ovarian cancer
- Of patients with a BRCA mutation, median PFS was significantly longer in the olaparib group than in the placebo group

PFS in Monaten	
Olaparib	Placebo
8.4	4.8
11.2	**4.3**
7.4	5.5

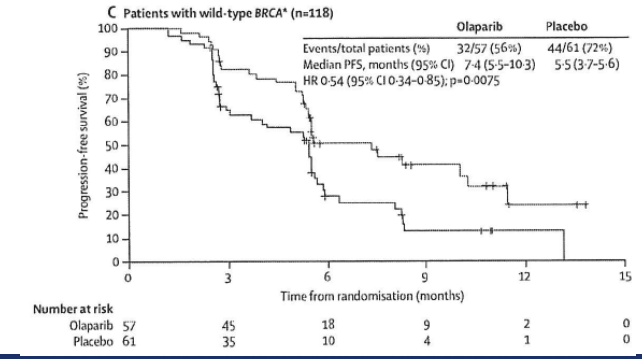
All patients



BRCA mut+



BRCA mut-

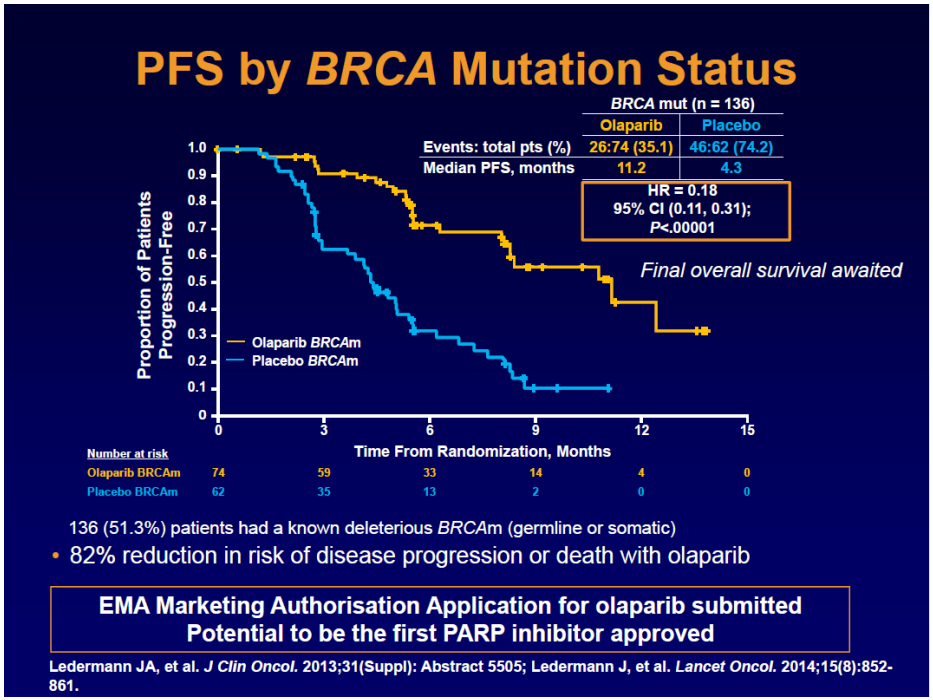


Lynparza – positive CHMP opinion

Lynparza™ (olaparib) receives positive CHMP opinion in the EU for the maintenance treatment of BRCA-mutated platinum sensitive relapsed ovarian cancer

Friday, 24 October 2014

AstraZeneca today announced that the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) has adopted a positive opinion recommending the marketing authorisation of Lynparza™ (olaparib) as monotherapy for the maintenance treatment of adult patients with platinum sensitive relapsed BRCA-mutated (germline and/or somatic) high grade serous epithelial ovarian, fallopian tube or primary peritoneal cancer who are in response (complete or partial) to platinum-based chemotherapy. Olaparib is a poly ADP-ribose polymerase (PARP) inhibitor that exploits tumour DNA repair pathway deficiencies to preferentially kill cancer cells.



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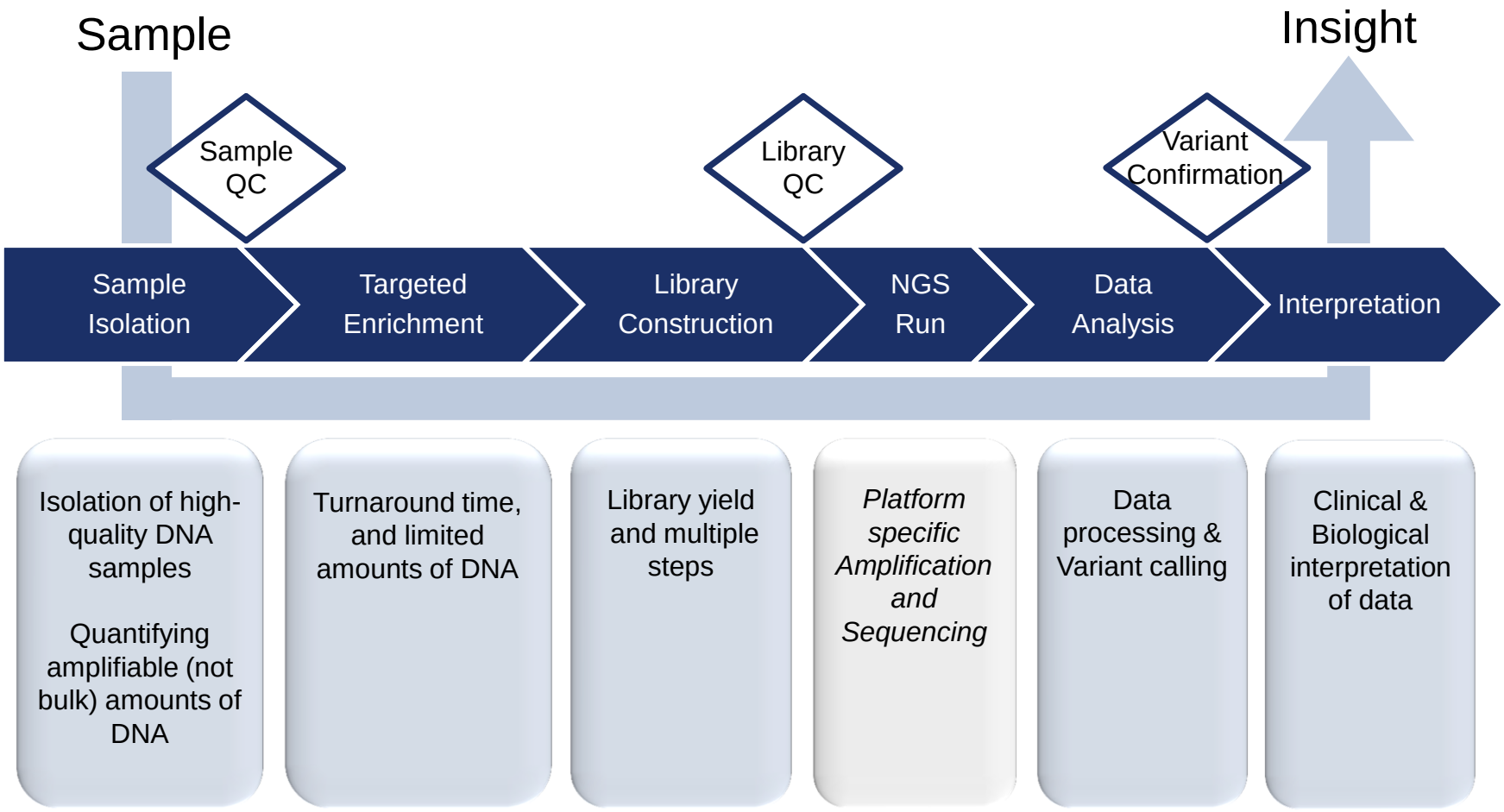
Director, Institute of Pathology, Cologne University Hospital, Germany

Summary

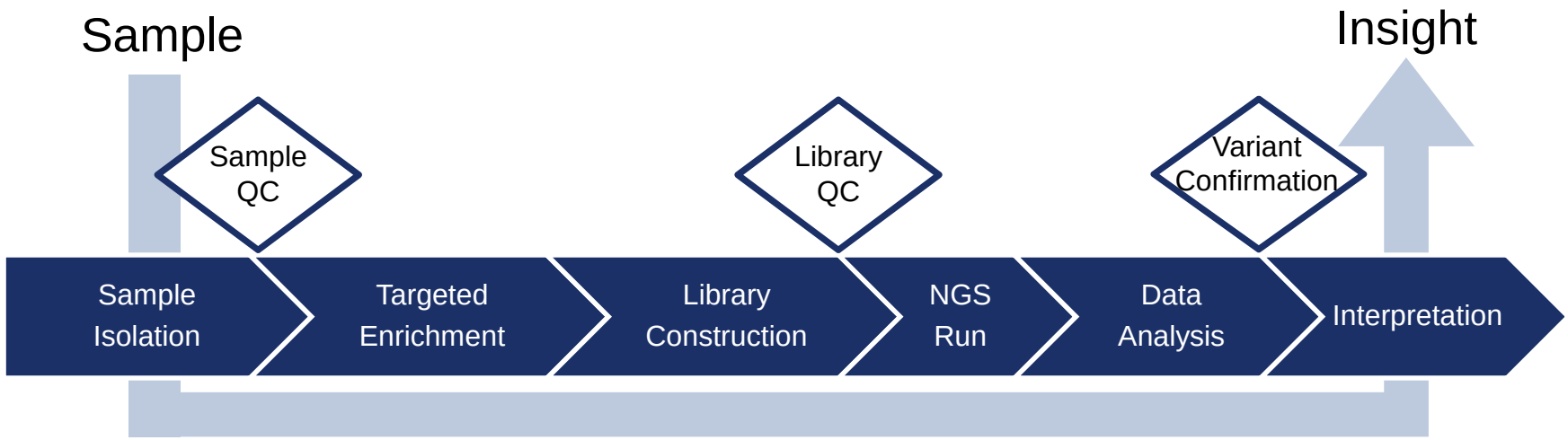
Universal NGS workflow: from sample to insight

To overcome NGS challenges



Universal NGS workflow: from sample to insight

Integrated solutions to address NGS challenges



GeneRead DNA FFPE kit GeneRead DNA QuantiMIZE kit	GeneRead DNAseq Targeted Panels V2 **[BRCA1/2 Panel]** GeneRead DNAseq PCR Kit V2	GeneRead DNA Library Core & Amp Kits GeneRead Adapter 12-plex GeneRead Size selection Kit GeneRead DNASeq Library Quant Array	*Platform specific Amplification and Sequencing*	CLC Cancer Research Workbench GeneRead DNAseq V2 Data analysis	Ingenuity Variant Analysis

GeneRead DNA FFPE Kit

- **The use of DNA isolated from FFPE tissues remains challenging**
  - Low DNA quality
  - Presence of base damage leading to artifactual single nucleotide changes
    - >50% of artifacts are C>T changes resulting from deamination
  - Increase false positive rate

- **GeneRead DNA FFPE Kit**
  - Enzymatically remove artifacts during DNA isolation step and provide more confidence in SNP calling
  - Automate on QIAcube

Kerick *et al. BMC Medical Genomics* 2011, 4:68
http://www.biomedcentral.com/1755-8794/4/68

BMC Medical Genomics

RESEARCH ARTICLE Open Access

Targeted high throughput sequencing in clinical cancer Settings: formaldehyde fixed-paraffin embedded (FFPE) tumor tissue, input amount and tumor heterogeneity

Martin Kerick[1†], Melanie Isau[1,2†], Bernd Timmermann[1], Holger Sültmann[3], Ralf Herwig[1], Sylvia Krobitsch[1], Georg Schaefer[4,5], Irmgard Verdorfer[5,6], Georg Bartsch[4], Helmut Klocker[4], Hans Lehrach[1] and Michal R Schweiger[1*]

See also:
Williams et al., 1999. A high frequency of sequence alterations is due to formalin fixation of archival specimens. Am J Pathol 155(5): 1467-1571
Yost et al., 2012. Identification of high-confidence somatic mutations in whole genome sequence of formalin-fixed breast cancer specimens. NAR 40(14): e108

GeneRead DNA FFPE Kit eliminates false positives

Example: Liver carcinoma patient FFPE sample

Chrom	Position	COSMIC ID	dbSNP ID	Gene name	Ref	Var	QIAamp FFPE	GeneRead FFPE
chr1	226595647	COSN392383	rs907187	PARP1	C	G	0.95	1.00
chr2	29416481	COSM1130802	rs1881420	ALK	T	C	1.00	1.00
chr2	**48032105**	**COSM13342**	**-**	**MSH6**	**C**	**T**	0.13	0.00
chr3	30713126	COSM149346	rs11466512	TGFBR2	T	A	0.51	0.45
chr3	47125385	COSM149376	rs4082155	SETD2	G	A	0.59	0.63
chr4	**1807130**	**COSM327089**	**-**	**FGFR3**	**C**	**T**	0.16	0.00
chr4	55152040	COSM22413	rs2228230	PDGFRA	C	T	0.63	0.67
chr4	55595519	COSM12708	rs121913516	KIT	C	T	0.31	0.56
chr5	35861068	COSM149813	rs1494558	IL7R	T	C	0.53	0.50
chr5	35871190	COSM149814	rs1494555	IL7R	G	A	0.49	0.47
chr5	35875593	COSN167436	rs987106	IL7R	A	T	0.66	0.49
chr5	180036871	COSN167671	rs2242219	FLT4	C	G	0.60	0.59
chr7	55214348	COSM42978	rs2072454	EGFR	C	T	1.00	1.00
chr9	21968199	COSM14251	rs11515	CDKN2A	C	G	0.99	0.99
chr9	139397707	COSM33747	rs10521	NOTCH1	G	A	1.00	1.00
chr11	64572018	COSM255213	rs2959656	MEN1	T	C	0.99	1.00
chr12	**121426785**	**COSM46438**	**-**	**HNF1A**	**G**	**A**	0.20	0.00
chr16	**3828705**	**COSM970602**	**-**	**CREBBP**	**C**	**T**	0.10	0.00
chr17	41244000	COSM148277	rs16942	BRCA1	T	C	0.40	0.52

15-year-old liver carcinoma FFPE. DNA extracted with QIAamp FFPE kit or GeneRead DNA FFPE Kit. DNA amplified using Human Comprehensive Cancer GeneRead DNAseq Gene Panel

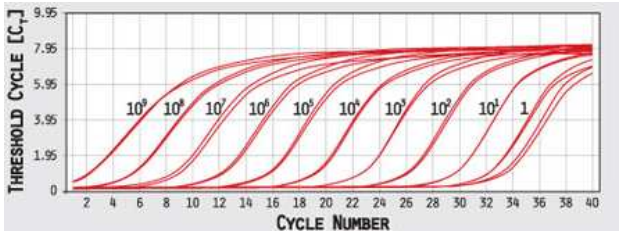
Pay attention to what you are measuring

Challenge:
Inaccurate quantification of input DNA can lead to poor target enrichment and NGS library yield, eventually wastage of your precious sample and time.

gDNA

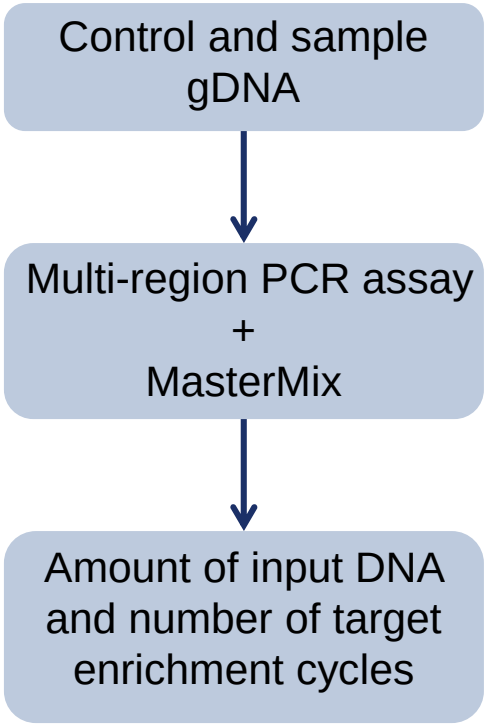
Measure any form of DNA

Measure double stranded DNA
(even 20 bp)



Measure PCR amplifiable DNA

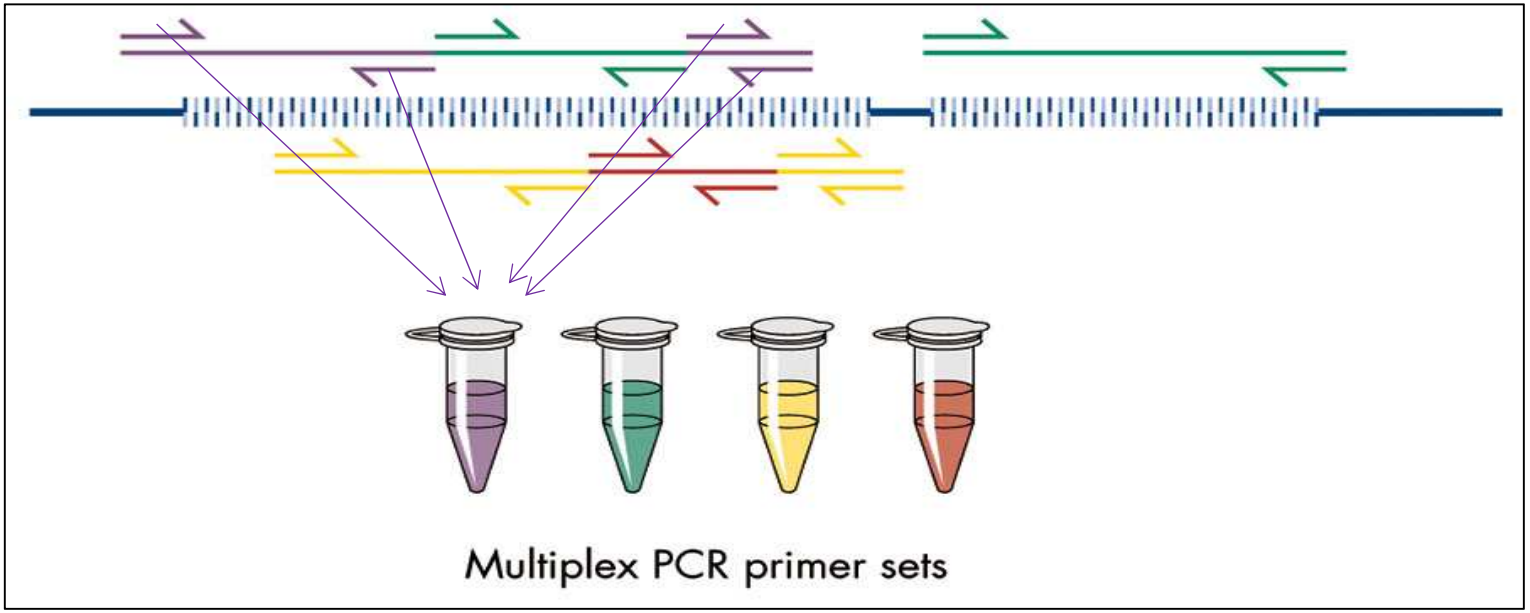
A qPCR based kit to measure amplifiable, functional DNA



- Uses:
  - Two qPCR assays to query more than 40 genomic loci that are randomly distributed in the genome
- Provides:
  - Quantification of only PCR amplifiable DNA
  - Guidance to rescue low quality DNA sample
    - Appropriate amount of input DNA
    - Number of target enrichment cycles
- Ensure reproducible high post-amplification yield

Challenges: sequence every base with little amounts of DNA

- Multiplex PCR-enabled enrichment of gene(s) of interest
- Provides primer sets for any region, gene, or set of genes in the human genome
- Overlapping primers are divided into 4 tubes



Clinically Relevant Panels

Largest collection of wet-bench verified gene panels

Reference databases:

The Cancer Genome Atlas
National Comprehensive Cancer Network
COSMIC
Cancer Genome Census
OMIM®
ClinVar (NCBI)

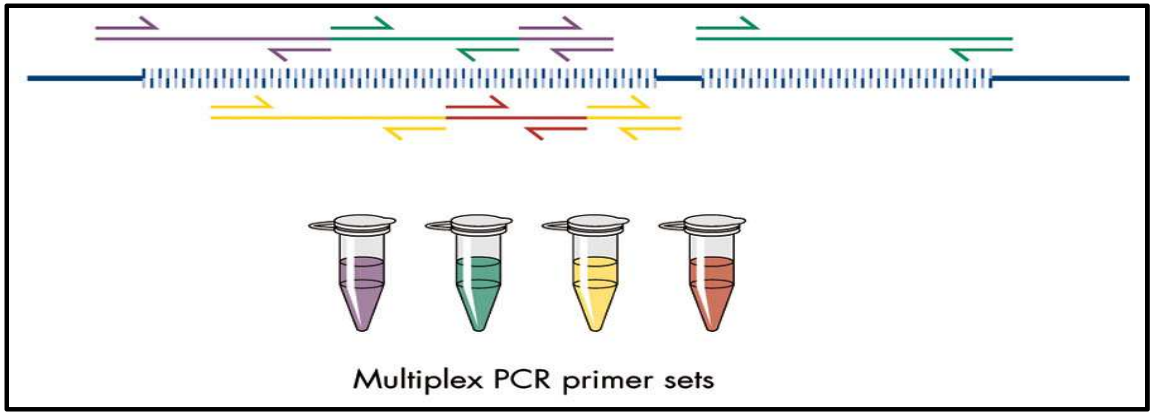
INGENUITY SYSTEMS

Type	Panel name
Solid tumor	Clinically Relevant Tumor
	Tumor Actionable Mutations
Hematologic malignancies	Myeloid Neoplasms
Tissue-specific	Breast Cancer
	Colorectal Cancer
	Liver Cancer
	Lung Cancer
	Ovarian Cancer
	Prostate Cancer
	Gastric Cancer
	Cardiomyopathy
Comprehensive	Cancer Predisposition
	Comprehensive Cancer
	Carrier Testing
Custom	Online custom builder
Gene focused	**BRCA1 and BRCA2**

GeneRead DNAseq BRCA1 and BRCA2 panel

Design and specifications

Overlapping amplicon design



100% coverage

Experimentally-verified 100% coverage	
Regions targeted	Coding regions + 20 bp intron-exon junctions
# of bases targeted	21,472
Average amplicon length	150 bp
Primer primer pools	4
Total input DNA	40 ng
Number of amplicons	250
Specificity	99%
Uniformity (0.2x mean)	97%
% of bases callable	100%

GeneRead DNAseq BRCA1 and BRCA2 panel

Overlapping amplicon design

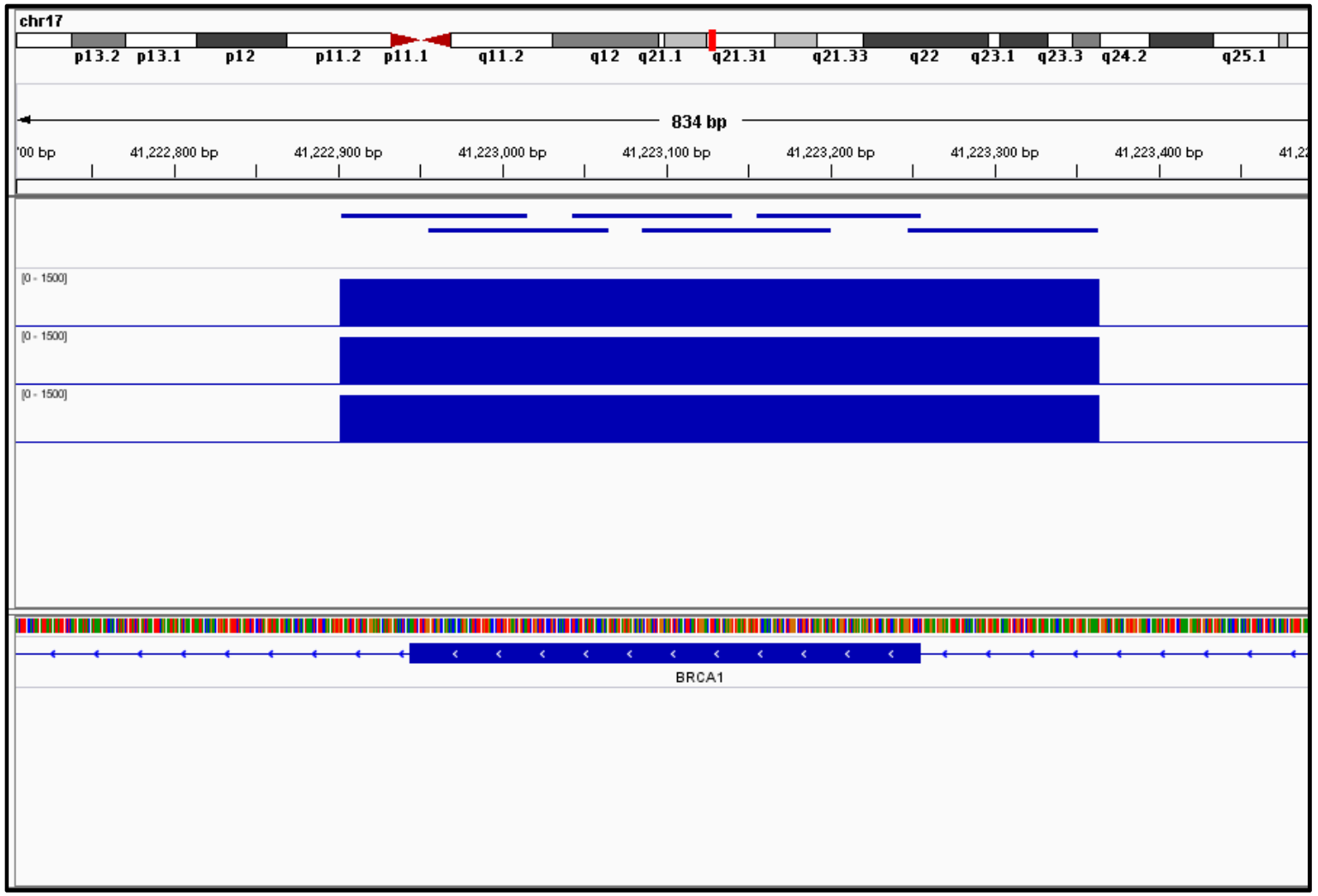
Amplicons

PGM, NA12878 DNA

PGM, FFPE sample

MiSeq, NA12878 DNA

RefSeq Genes



GeneRead DNAseq BRCA1 and BRCA2 panel

BRCA1: 100% coverage

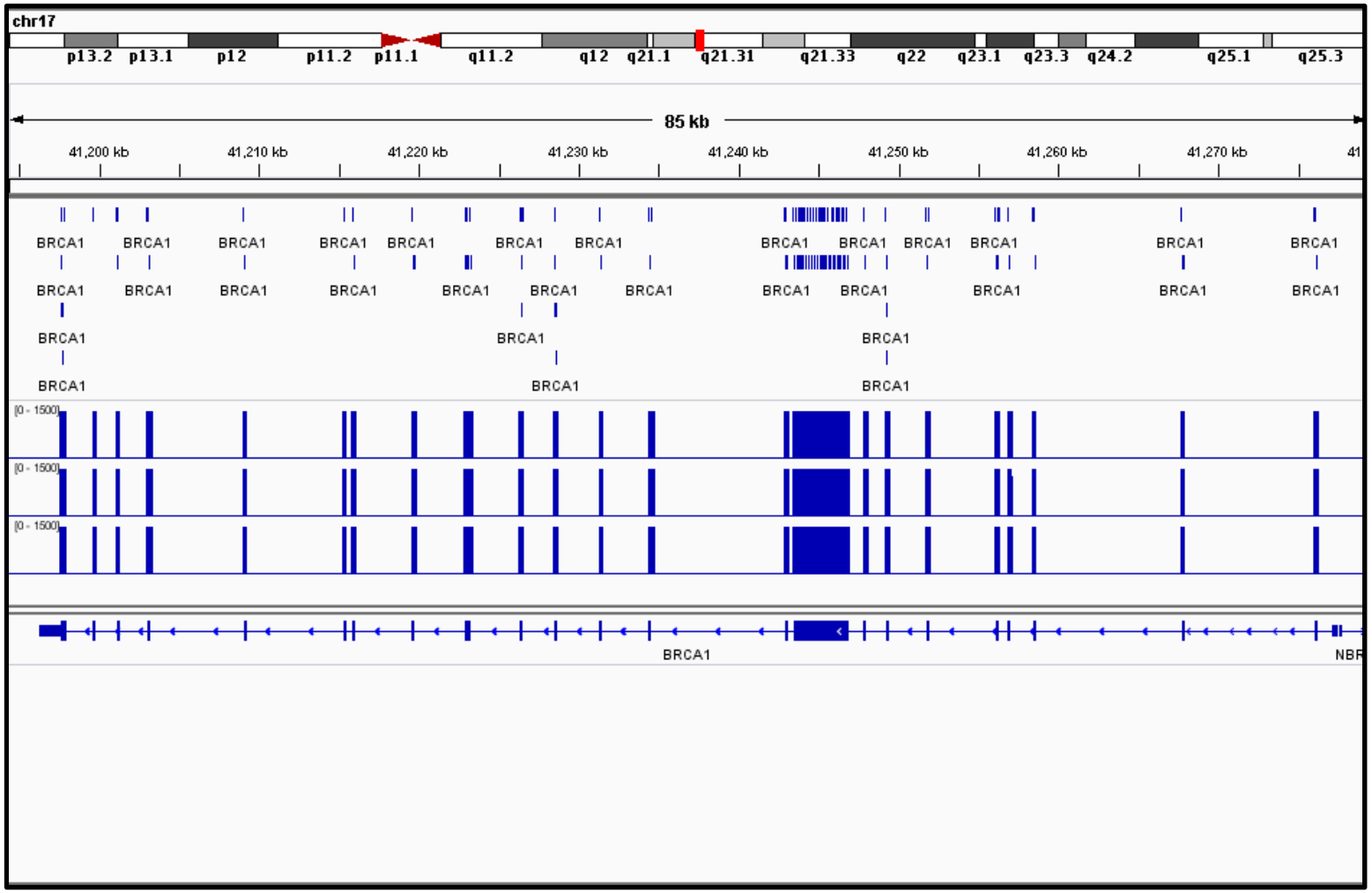
Amplicons

PGM, NA12878 DNA

PGM, FFPE sample

MiSeq, NA12878 DNA

RefSeq Genes



GeneRead DNAseq BRCA1 and BRCA2 panel

BRCA2: 100% coverage

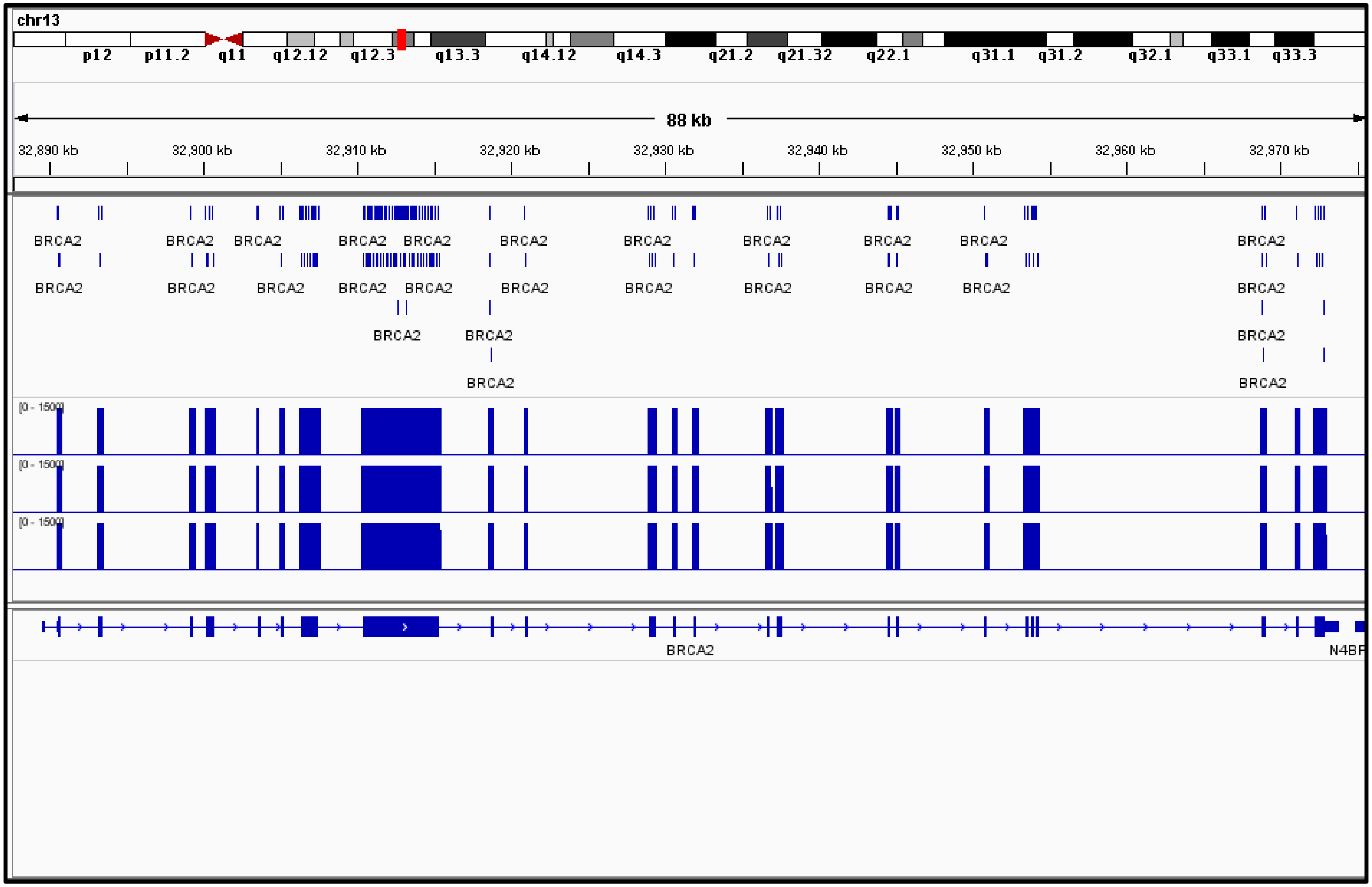
Amplicons

PGM, NA12878 DNA

PGM, FFPE sample

MiSeq, NA12878 DNA

RefSeq Genes



Every base counts

Avoid blind spots at the ends of amplicons

- In order to accurately call variants near the ends of reads, primer sequences have to be present for read mapping and alignment
- Physically removing primers results in blind spots causing false positives.

Vijaya Satya and DiCarlo *BMC Genomics* 2014, **15**:1073
http://www.biomedcentral.com/1471-2164/15/1073

BMC Genomics

METHODOLOGY ARTICLE Open Access

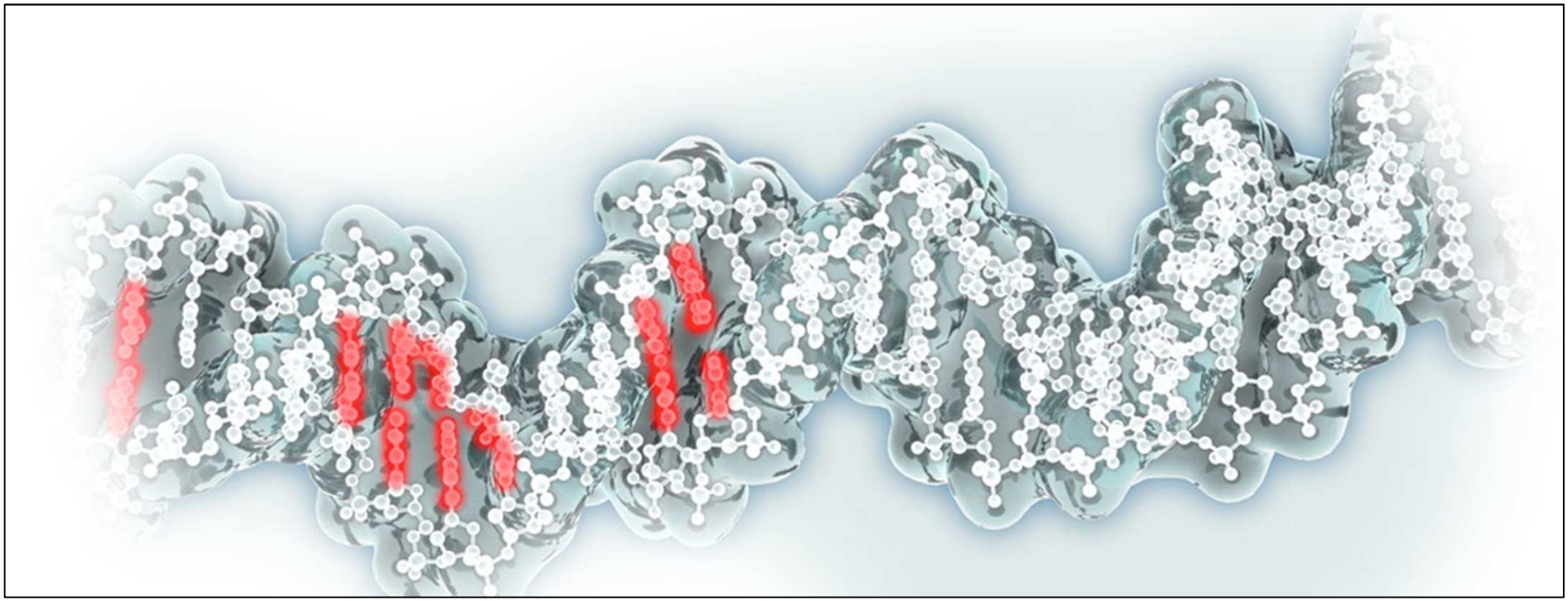
Edge effects in calling variants from targeted amplicon sequencing

Ravi Vijaya Satya* and John DiCarlo

The whole truth about BRCA1/2

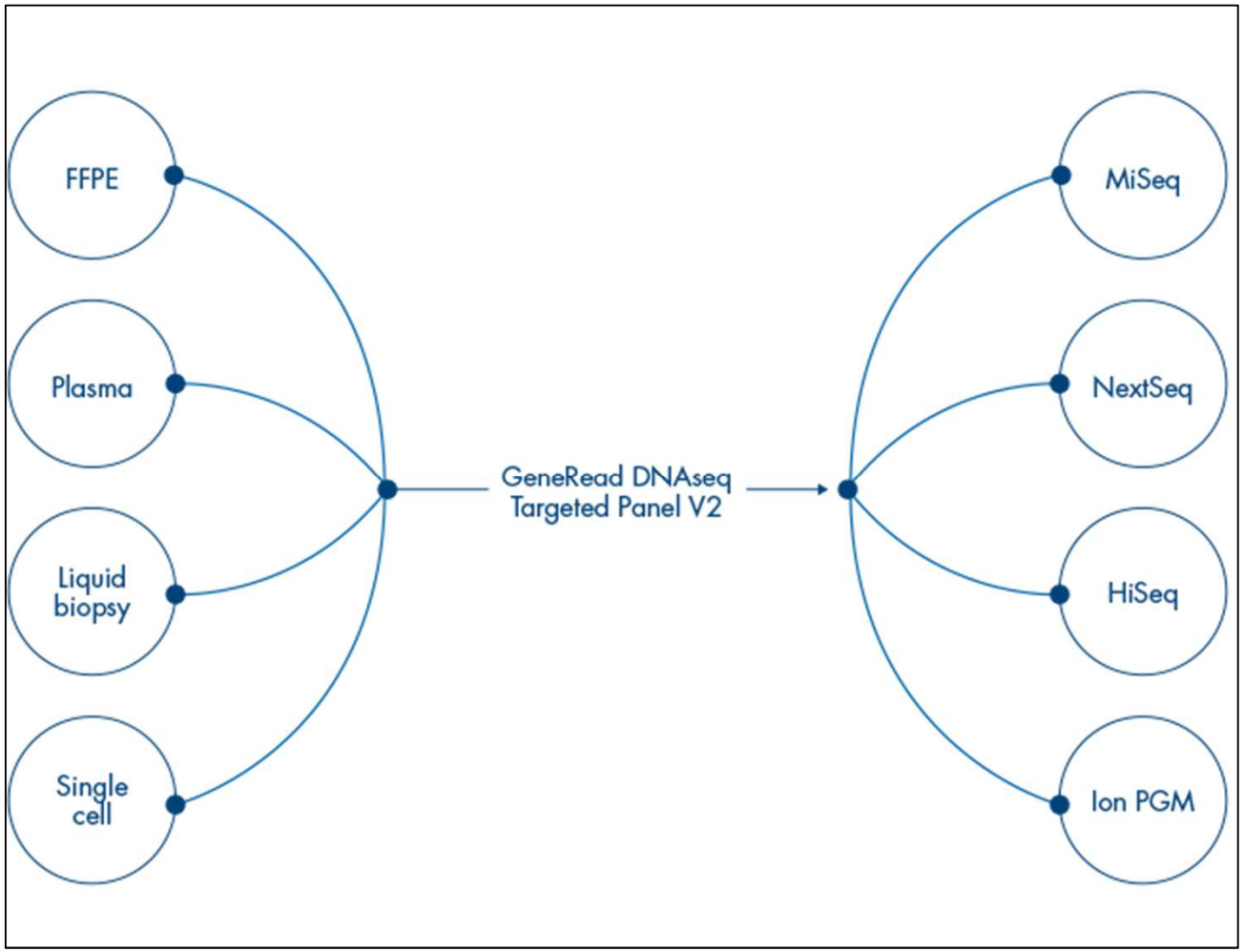
“To fight cancer, nothing less than 100% truth is good enough”

- BRCA1 and BRCA2 are tumor suppressor genes
- Every base of BRCA1 and BRCA2 is important to your cancer research.
- GeneRead DNAseq BRCA1/2 Panels give you 100% coding region and intron-exon junction coverage.



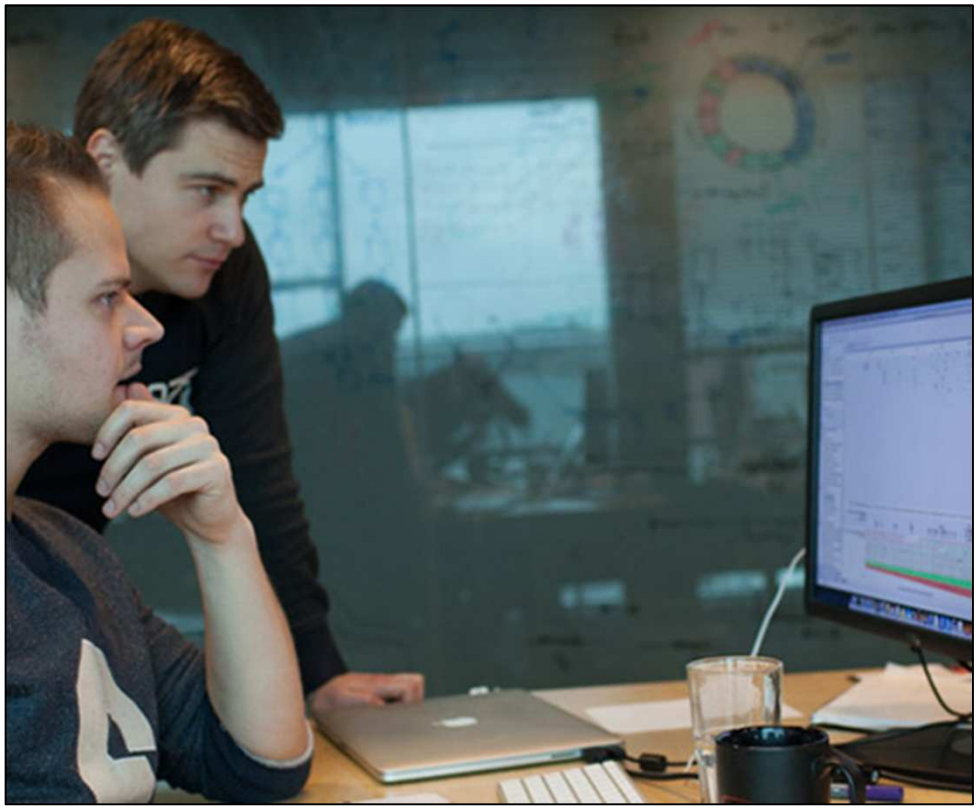
A Universal Panel

Work with any sample on any sequencing platform



True insight into BRCA1 and BRCA2 genes

Don’t just settle for FASTQ files

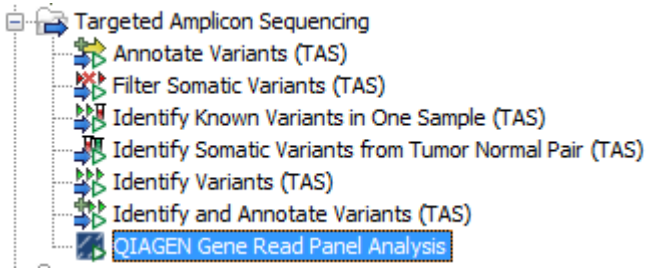


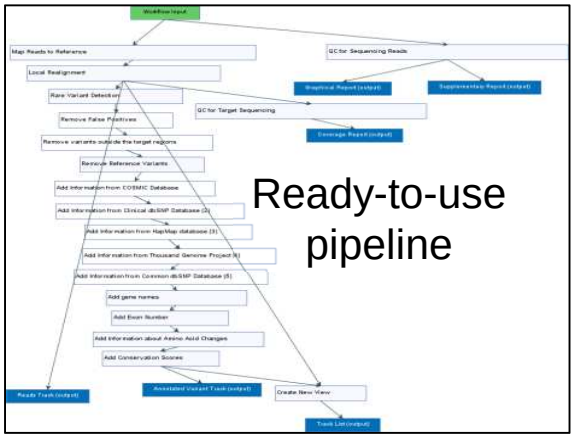
BRCA1/2 Panel comes with integrated data analysis and biological interpretation “user friendly” solutions

- CLC Cancer Research Workbench
- Ingenuity Variant Analysis

Easy-to-use and end-to-end solution

QIAGEN GeneRead DNAseq Panel Analysis Plugin

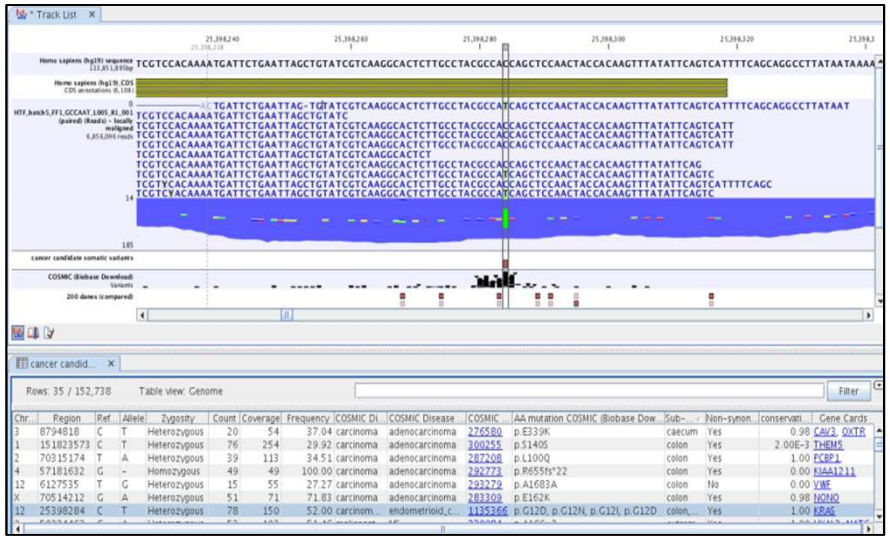




Ready-to-use pipeline

QC reports

Visualization

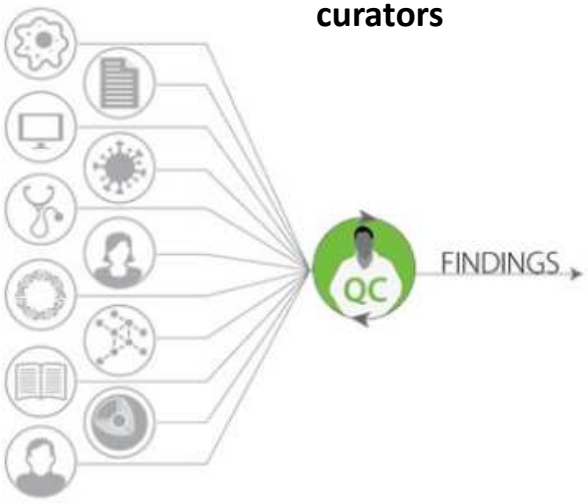


A knowledgebase that's 15 years in the making

Unprecedented Access to Literature Knowledge

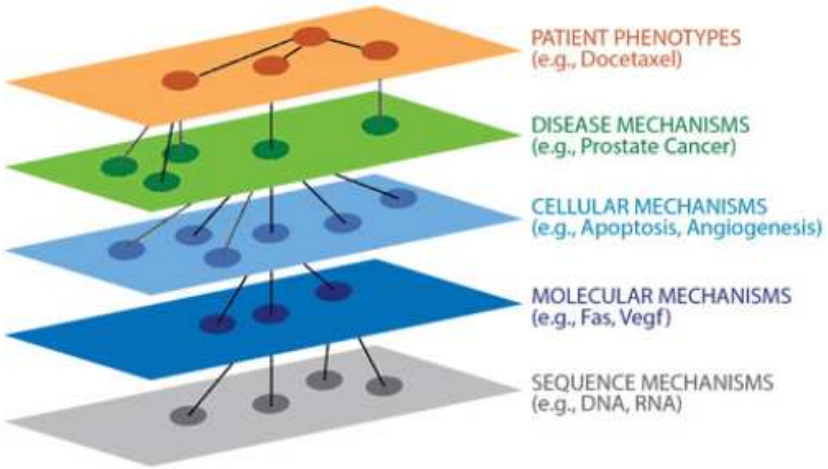
Literature findings

MD/PhD level curators



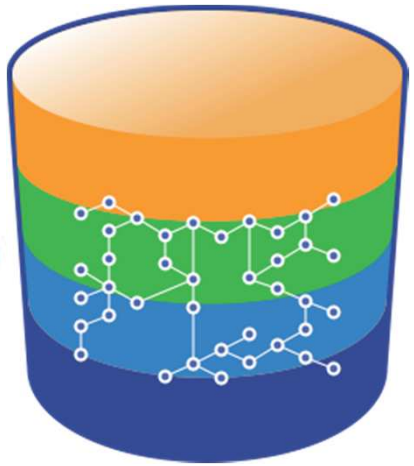
Content Acquisition

Biomedical Ontology



Ingenuity Ontology

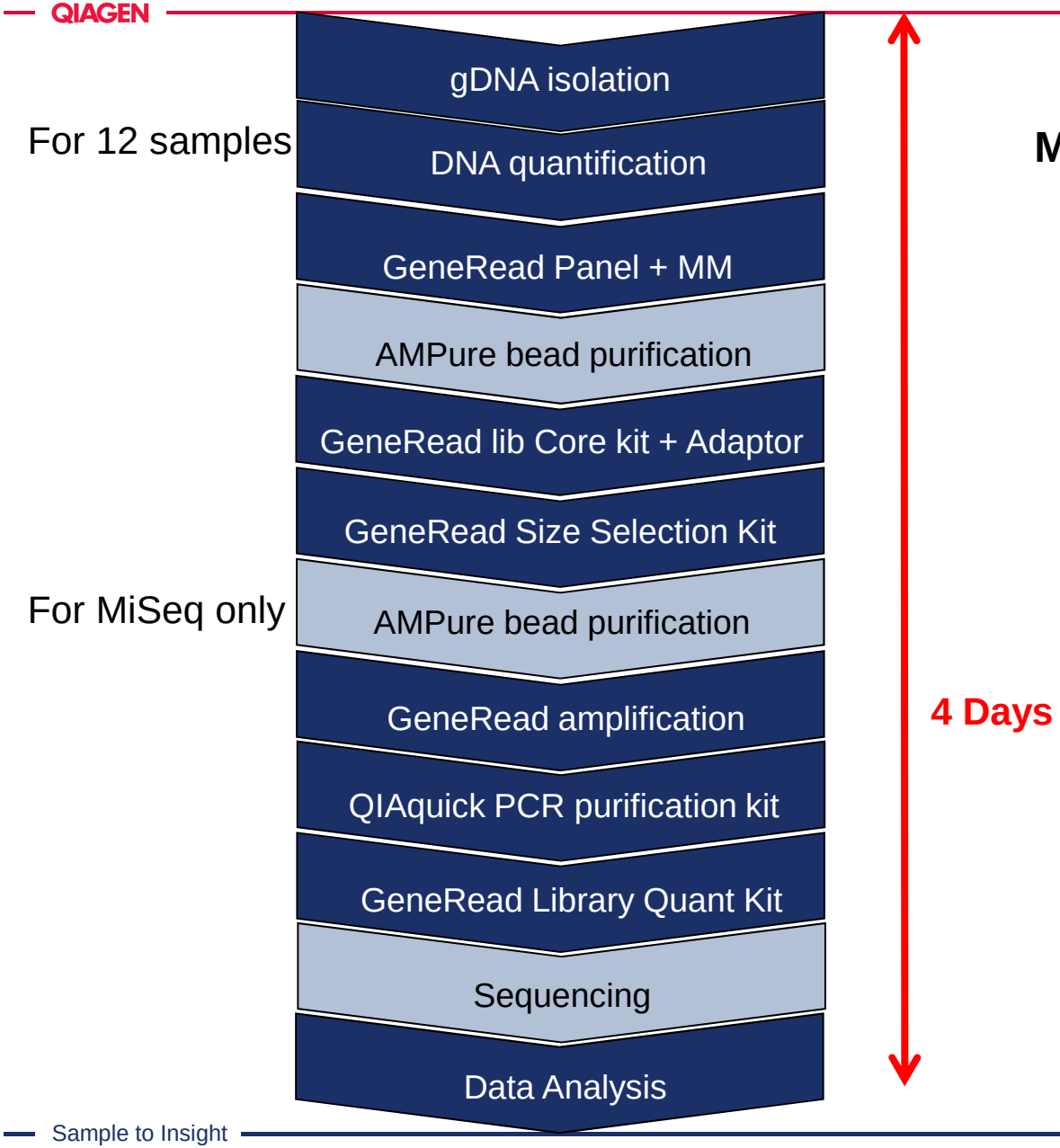
The Ingenuity Knowledge Base



HGMD®

POWERED BY INGENUITY®

Workflow



Make the most of your resources

- Need just 40 ng of DNA/sample
- Takes only 3 hours for target enrichment
- One Library prep per sample

Outline

BRCA1 and BRCA2 Genes

QIAGEN “Sample-to-Insight” Solution for Next Generation Sequencing challenges

Performance and Application Data

- Dr. Nicola Normanno,
  Chief of the Laboratory Of Pharmacogenomics, Centro Ricerche Oncologiche, Italy
- Dr. Reinhard Büttner
  Director, Institute of Pathology, Cologne University Hospital, Germany

Summary

Study 1

Goal:

Verification of BRCA1 and BRCA2 panel performance with blood samples from hereditary breast cancer cases

Experimental design

- Six previously analyzed samples (5 blood sample + 1 control gDNA) were enriched for BRCA1 and BRCA2 using the GeneRead panel and subjected to PGM run using QIAGEN workflow

Dr. Nicola Normanno, MD

Chief

Laboratory of Pharmacogenomics

Centro Ricerche Oncologiche Mercogliano, Italy

Excellent Sequencing metrics

Sequencing metric	Value (average of 6 runs)
Specificity	99%
Mean read depth	1501
Uniformity (0.2x median)	98%
% of bases covered at >= 100x	100%
% of bases callable	100%

GeneRead DNAseq BRCA1 and BRCA2 panel

100% concordance with Sanger sequencing

Barcode (sample)	Method of prior detection	Expected Mutation	GeneRead Panel
001	Sanger Sequencing	BRCA2: c.9401delG; p.Gly3134Alafs chr13: 32968970	
002	Sanger Sequencing	BRCA1 c.4964_4982del19 (p.Ser1655Tyrfs) chr17: 41,222,949 - 41,222,967	
003	Sanger Sequencing	BRCA EX20 1c.5277+1G>A chr17:41209068	
004	Sanger Sequencing	BRCA1 EX11 c.843_846del p.(Ser282fs) chr17: 41246702	
005	Sanger Sequencing	BRCA2 EX11 c.6275_6276delTT p.(Leu2092fs) chr13:32914766	

GeneRead DNAseq BRCA1 and BRCA2 panel

100% concordance with Sanger sequencing

Barcode (sample)	Method of prior detection	Expected Mutation	GeneRead Panel	AmpliSeq Panel
001	Sanger Sequencing	BRCA2: c.9401delG; p.Gly3134Alafs chr13: 32968970	✓	✗
002	Sanger Sequencing	BRCA1 c.4964_4982del19 (p.Ser1655Tyrfs) chr17: 41,222,949 - 41,222,967	✓	✗
003	Sanger Sequencing	BRCA EX20 1c.5277+1G>A chr17:41209068	✓	✓
004	Sanger Sequencing	BRCA1 EX11 c.843_846del p.(Ser282fs) chr17: 41246702	✓	✗
005	Sanger Sequencing	BRCA2 EX11 c.6275_6276delTT p.(Leu2092fs) chr13:32914766	✓	✓

GeneRead outperforms AmpliSeq

Close the gaps

"The complete coverage provided by the GeneRead DNAseq BRCA1 and BRCA2 panel allowed us to detect mutations that were missed by other targeted enrichment technologies. Complete coverage is a required feature for the mutational profiling of BRCA1 and BRCA2 by NGS for breast and ovarian cancers"

Study 2

Goal:

Verification of BRCA1 and BRCA2 panel performance with FFPE samples from sporadic breast cancer and sporadic epithelial ovarian cancer cases

Experimental design

- FFPE samples from 11 sporadic breast cancer and 18 sporadic epithelial ovarian cancer cases were enriched for BRCA1 and BRCA2 using the GeneRead panel and subjected to MiSeq run using QIAGEN workflow

Dr. R. Büttner, Dr. M. Odenthal and Dr. C. Vollbrecht

Director, Institute of Pathology,

Cologne University Hospital, Germany

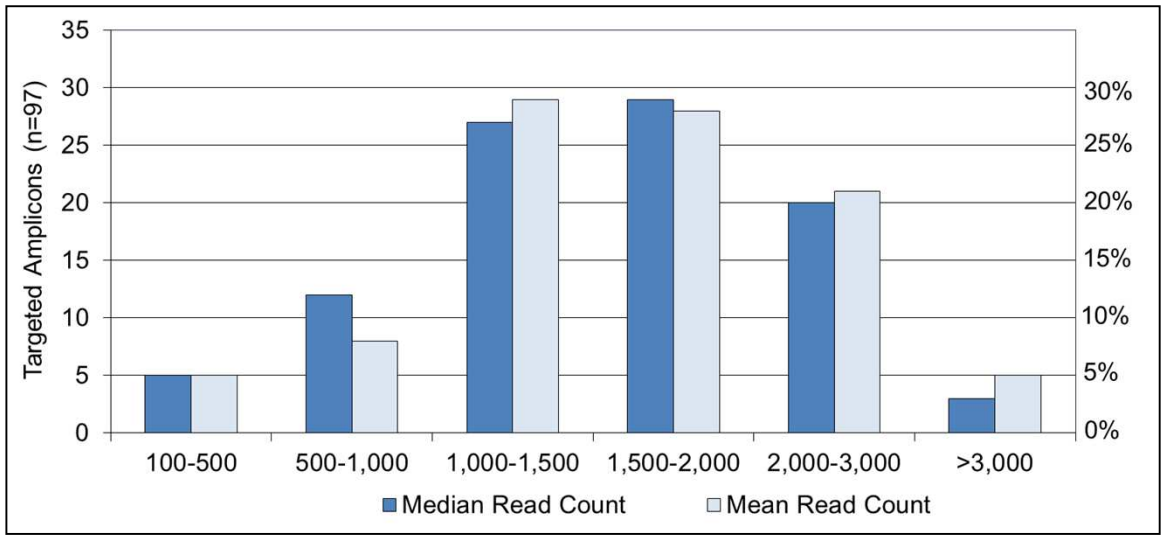
Run 1/2 (AmpliSeq) versus Run 4 (GeneRead)

44 samples (35x ovarian, 9x mammary carcinoma)

	Run 1/2	Run 4 (Repeat of run 1 and 2)
Concordance with variants detected in an external institut*		
BRCA1	100% (60/60)	100% (60/60)
BRCA2	81% (17/21)	100% (21/21)
* 60 *BRCA1* variants in 25 patients and 21 *BRCA2* variants in 19 patients		

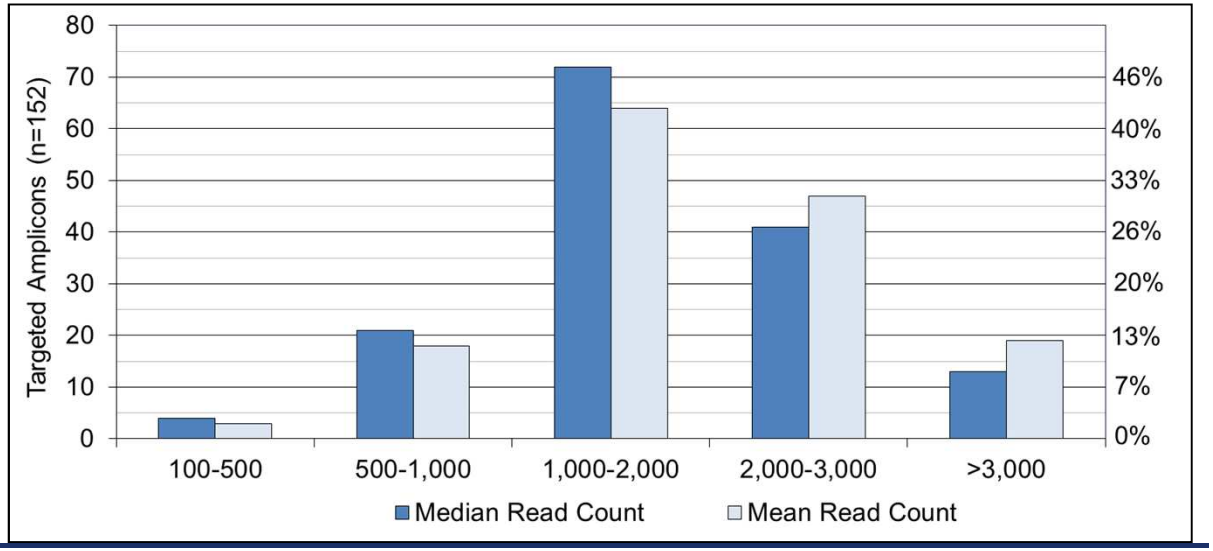
High Uniformity

BRCA1 (reads: min 85, max. 7,224)



>70% of the amplicons show >1,000 reads

BRCA2 (reads: min. 114, max. 9,809)



>75% of the amplicons show >1,000 reads

Variants detected in Sporadic Mamma Carcinoma (n=11)

Variants as low as 5% can be detected, with high coverage

Gene	Exon	cDNA change	AA change	Count	Allele Frequency	Coverage
BRCA1	Exon 16	c.4900A>G	p.S1634G	9	16-100%	2012-4905
	Exon 10	c.3548A>G	p.K1183R	9	19-100%	1879-3894
		c.3113A>G	p.E1038G	9	17-100%	294-1635
		c.2612C>T	p.P871L	9	17-100%	607-1133
		c.2077G>A	p.D693N	1	10%	1326
		c.1877_1878insTAGT	p.V626fs	1	85%	2121
BRCA2	Exon 10	c.865A>C	p.N289H	2	62-82%	612-711
		c.1114A>C	p.N372H	5	29-100%	1031-1415
	Exon 11	c.2971A>G	p.N991D	2	59-83%	3909-5151
	Exon 14	c.7397T>C	p.V2466A	11	100%	1053-4139
	Exon 23	c.9090delA	p.T3030fs	1	5%	1848

Variants in Sporadic Ovarian Carcinoma (n=18)

Gene	Exon	cDNA change	AA change	Count	Allele Frequency	Coverage
BRCA1	Exon 20	c.5329_5330insC	p.Q1777fs	1	95%	2025
	Exon 16	c.5019G>A	p.M1673I	1	83%	3020
		c.4900A>G	p.S1634G	8	20-99%	1967-3674
	Exon 15	c.4598G>T	p.S1533I	1	39%	2775
	Exon 10	c.3889_3890insT	p.S1297fs	1	62%	1143
		c.3548A>G	p.K1183R	8	24-99%	1652-3243
		c.3113A>G	p.E1038G	8	18-100%	361-1143
		c.2612C>T	p.P871L	8	22-99%	420-997
		c.2077G>A	p.D693N	2	27-99%	790-1291
		c.1067A>G	p.Q356R	3	20-77%	558-847
		c.1016delA	p.K339fs	1	71%	2398
BRCA2	Exon 3	c.125A>G	p.Y42C	1	41%	2258
	Exon 10	c.837C>A	p.C279*	1	61%	1412
		c.865A>C	p.N289H	1	61%	534
		c.1114A>C	p.N372H	6	37-77%	883-2235
		c.1514T>C	p.I505T	1	21%	2753
	Exon 11	c.2971A>G	p.N991D	1	62%	4473
		c.5070A>C	p.K1690N	1	36%	2368
		c.5455C>T	p.P1819S	1	36%	1025
		c.5744C>T	p.T1915M	2	50%	2970-4564
	Exon 14	c.7397T>C	p.V2466A	18	100%	1725-4184

Outline

BRCA1 and BRCA2 Genes

QIAGEN “Sample-to-Insight” Solution for Next Generation Sequencing challenges

Performance and Application Data

Dr. Nicola Normanno,
Chief of the Laboratory Of Pharmacogenomics, Centro Ricerche Oncologiche, Italy

Dr. Reinhard Büttner
Director, Institute of Pathology, Cologne University Hospital, Germany

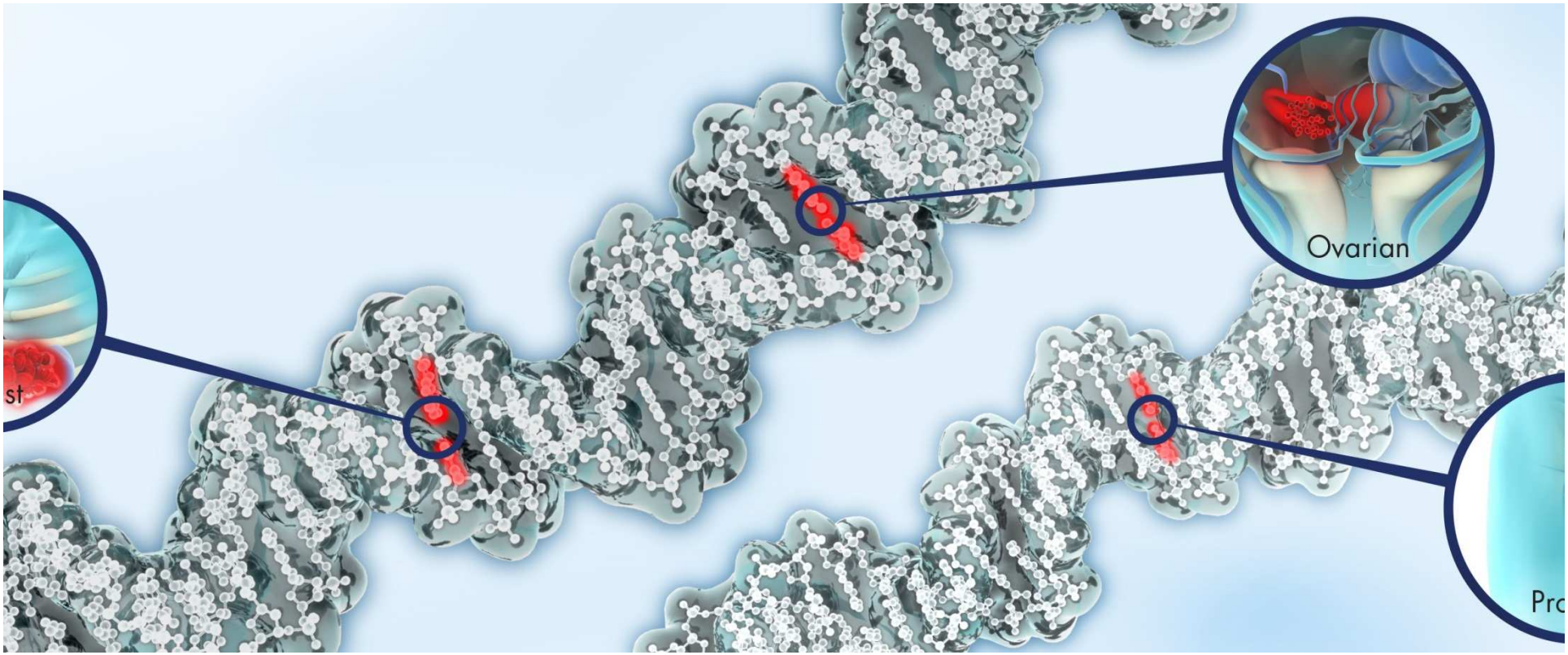
Summary

Summary

- The BRCA1 and BRCA2 panel delivers
  - 100% coverage of all bases
  - Outstanding sequencing metrics
    - Specificity (99%)
    - Uniformity (98% bases are covered >0.2X of mean)
  - Streamlined workflow with integrated data analysis and biological interpretation

- The BRCA1 and BRCA2 panel is ideal for the detection of both hereditary and somatic mutations in different sample types including blood and FFPE samples with different sequencing platform

Questions



Because every base counts: sample-to-insight NGS workflow for BRCA1 and BRCA2 testing

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