Next Generation Sequencing: An Introduction to Applications and Technologies
Welcome to the NGS webinar series

- **Webinar 1**
  Next-Generation Sequencing, an Introduction to Technology and Applications

- **Webinar 2**
  Addressing the challenges of NGS workflow: sample preparation, quality control and automation

- **Webinar 3**
  Targeted Enrichment Technology in Cancer Research

- **Webinar 4**
  Advanced NGS Data Analysis and Interpretation: Biomedical Genomics Workbench and Ingenuity Variant Analysis
QIAGEN products shown here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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.
Agenda

- Next Generation Sequencing
  - Background
  - Technologies
  - Applications
  - Workflow

- Targeted Enrichment
  - Methodologies
  - Data analysis
DNA Sequencing – Machine Output

Rapid Decrease in Cost

Cost per Genome

Moore's Law

NIH National Human Genome Research Institute
genome.gov/sequencingcosts
Almost the $1000 genome….

Have your human genome samples sequenced at a minimum of 30X coverage for the low price of $1,325* (USD) until May 31st. Send your samples of 1-2 μg of purified DNA, and you will receive the data in less than 8 weeks.

The Ramaciotti Centre in Sydney, Australia will sequence your samples on the Illumina HiSeq X™Ten platform and send the FASTQ sequence files back to you via a secure online portal (free) or hard drive (additional $25 USD/sample).

To take advantage of this offer, simply complete the request quote form at Science Exchange, pay a 50% deposit and send your samples to the Ramaciotti Centre by May 31st.

REQUEST QUOTE

*additional charges apply for orders of less than 10 samples
Automated Sanger Sequencing
one residue at a time

DNA is fragmented

Cloned to a plasmid vector
Or single PCR fragment

Cyclic sequencing reaction

Separation by electrophoresis
Readout with fluorescent tags
What is Next-Generation Sequencing?

**Automated Sanger Sequencing**
- DNA fragmentation
- Cloned to a plasmid vector
- Or single PCR fragment
- Cyclic sequencing reaction
- Separation by electrophoresis
- Readout with fluorescent tags

**NGS: Massive Parallel Sequencing**
- DNA is fragmented
- **Adaptors** ligated to fragments
- Clonal amplification of fragments on a solid surface (Bridge PCR or Emulsion PCR)
- Direct step-by-step detection of each nucleotide base incorporated during the sequencing reaction
DNA fragments are flanked with adaptors (Library)

A solid surface is coated with primers complementary to the two adaptor sequences

PCR Amplification, with one end of each ‘bridge’ tethered to the surface

Clusters of DNA molecules are generated on the chip. Each cluster is originated from a single DNA fragment, and is thus a clonal population.

Used by Illumina
Illumina HiSeq/MiSeq

- Run time 1- 10 days
- Produces 2 - 600 Gb of sequence
- Read length 2X100 bp – 2X250bp (paired-end)
- Cost: $0.05 - $0.4/Mb
Illumina Single-End vs. Paired-End

- **Single-end reading (SE):**
  - Sequencer reads a fragment from only one primer binding site

- **Paired-end reading (PE):**
  - Sequencer reads both ends of the same fragment
  - More sequencing information, reads can be more accurately placed (“mapped”)
  - May not be required for all experiments, more expensive and time-consuming
  - Required for high-order multiplexing of samples (indexes on both sides)
Emulsion PCR: Ion, Solid, 454

- Fragments with adaptors (the library) are PCR amplified within a water drop in oil
- One PCR primer is attached to the surface of a bead
- DNA molecules are synthesized on the beads in the water droplet. Each bead bears clonal DNA originated from a single DNA fragment
- Beads (with attached DNA) are then deposit into the wells of sequencing chips, one well one bead
- Used by Roche 454, IonTorrent and SOLiD
Run time 3 hrs – no termination and deprotection steps

Read length 100-300 bp; homopolymer can be an issue

Throughput determined by chip size (pH meter array): 10Mb – 5 Gb

Cost: $1 - $20/Mb
Multiple samples with different indices can be combined and sequenced together.

Depending on the application, may not need to generate many reads per sample (e.g. SNP).

Save money on sequencing costs (more samples per run), optimize use of read budget.
NGS Applications

Next Generation Sequencing

Genomics

Transcriptomics

Epi-genomics

Meta-genomics
NGS Applications

Next Generation Sequencing

Genomics

Transcript-omics

Epi-genomics

Meta-genomics

DNA-Seq

Mutation,
SNVs,
Indels,
CNVs,
Translocation
Next Generation Sequencing

Genomics
- DNA-Seq
- Mutation, SNVs, Indels, CNVs, Translocation

Transcriptomics
- RNA-Seq
- Expression level, Novel transcripts, Fusion transcripts, Splice variants

Epi-genomics

Meta-genomics
NGS Applications

Next Generation Sequencing

Genomics
- DNA-Seq
  - Mutation, SNVs, Indels, CNVs, Translocation

Transcript-omics
- RNA-Seq
  - Expression level, Novel transcripts, Fusion transcript, Splice variants

Epi-genomics
- ChIP-Seq, Methyl-Seq
  - Global mapping of DNA-protein interactions, DNA methylation, histone modification

Meta-genomics
NGS Applications

Next Generation Sequencing

Genomics
- DNA-Seq
  - Mutation, SNVs, Indels, CNVs, Translocation

Transcriptomics
- RNA-Seq
  - Expression level, Novel transcripts, Fusion transcript, Splice variants

Epi-genomics
- ChIP-Seq, Methyl-Seq
  - Global mapping of DNA-protein interactions, DNA methylation, histone modification

Meta-genomics
- Microbial-Seq
  - Microbial genome Sequence, Microbial ID, Microbiome Sequencing
Considerations of NGS experiments: Read Budget

Read Budget is platform specific and determines multiplexing capacity

Read budget requirements differ for different applications

- Genome
- Methylome
- Whole transcriptome
- Exome
- Targeted gene expression
- Fusion transcripts
- Somatic mutation detection
- Structural variants
- Germline SNP

Reads needed

Multiplexing possibility
Sample preparation
- Isolate samples (DNA/RNA)
- Qualify and quantify samples
- Several hours to days

Library construction
- Prepare NGS library
- Qualify and quantify library
- ~4-8 hours

Sequencing
- Perform sequencing run
- 8 hours to several days

Data analysis
- Primary, secondary data analysis
- Several hours to days to...
QIAGEN Solution for NGS Workflow

Sample preparation
- GeneRead® DNAseq NGS Panel System V2
- MagAttract® HMW DNA Kit
- REPLI-g® Single Cell Kit
- GeneRead™ rRNA Depletion Kit
- GeneRead™ DNA QuantiMIZE

Library construction
- GeneRead™ DNA Library Prep
- GeneRead Targeted Gene Panels
- GeneRead™ Size Selection Kit
- GeneRead™ Library Quant Kits

Sequencing

Data analysis
- GeneRead DNAseq data analysis
- Ingenuity Variant Analysis

Result validation
- RT² Profiler PCR Arrays
- Somatic Mutation PCR Arrays
- PyroMark Pyrosequencing
- CNA/CNV PCR Arrays
- EpiTect ChIP PCR Arrays
Agenda

- Next Generation Sequencing
  - Background
  - Technologies
  - Applications
  - Workflow

- Targeted Enrichment
  - Methodology
  - Data analysis
  - ???
The Problem with Patient Samples

- **Low purity**
  - Cancerous cells may be a minor fraction of total sample

- **Heterogeneity**
  - Multiple sub-clones of cancer may be present in one tumor sample

- **Deep sequencing**
  - 1000X coverage is required to get >90% sensitivity to detect ~5% mutation frequency

Whole genome / exome sequencing is expensive and may not yield sufficient coverage
What is targeted sequencing?
- Sequencing a region or subset of the genome or transcriptome

Why targeted sequencing?
- Not all regions of the genome or transcripts are of interest or relevant to a specific study
  - Exome Sequencing: sequencing *most* of the coding regions of the genome (exome). The protein-coding region constitutes less than 2% of the entire genome
  - Focused panel/hot spot sequencing: focused on the genes or regions of interest e.g. Clinical relevance – tumor suppressor genes, inherited mutations

What are the advantages of targeted panel sequencing?
- More coverage per sample, more sensitive mutation detection
  - 1 gene copy ~ 3 pg, 3000 copies in 10 ng
  - Heterogeneous sample 1% tumor cell = 30 copies in 10ng
  - Every base not covered equally in typical NGS experiment
  - Typically read 1000 reads per locus for somatic mutation
- More samples per run, lower cost per sample
Hybridization capture

- High input requirement (1 ug)
- Long processing time (2-3 days)
- Heterogeneous, lower specificity
- Cover large regions (exome)
**Multiplex PCR**

- Small DNA input (< 100 ng)
- No processing prior to enrichment
- Short library prep time (<8 hrs)
- Relatively small target region (KB - MB region)
QIAGEN GeneRead DNAseq Panel System V2

- **Focused:**
  - Biologically relevant content selection enables deep sequencing on relevant genes and identification of rare mutations

- **Flexible:**
  - Mix and match any gene of interest
  - Fully customizable panels available

- **NGS platform agnostic:**
  - Functionally validated for Ion Torrent, MiSeq/HiSeq

- **Integrated controls:**
  - Enabling quality control of prepared library before sequencing

- **Free, complete and easy of use data analysis tool**
# GeneRead DNAseq V2 Panel Specifications

<table>
<thead>
<tr>
<th>Application</th>
<th>Panel name</th>
<th># genes</th>
<th>Target region (bases)</th>
<th>Coverage (%)</th>
<th>Specificity (%)</th>
<th>Uniformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid tumors</td>
<td>Tumor Actionable Mutations</td>
<td>8</td>
<td>7,104</td>
<td>100.0</td>
<td>98.2</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Clinically Relevant Tumor</td>
<td>24</td>
<td>39,603</td>
<td>98.1</td>
<td>95.3</td>
<td>90</td>
</tr>
<tr>
<td>Hematologic malignancies</td>
<td>Myeloid Neoplasms</td>
<td>50</td>
<td>236,319</td>
<td>98.1</td>
<td>97.4</td>
<td>94</td>
</tr>
<tr>
<td>Disease-specific</td>
<td>Breast Cancer</td>
<td>44</td>
<td>268,621</td>
<td>98.2</td>
<td>96.8</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Colorectal Cancer</td>
<td>38</td>
<td>182,851</td>
<td>98.7</td>
<td>98.3</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Liver Cancer</td>
<td>33</td>
<td>191,170</td>
<td>99.0</td>
<td>96.4</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Lung Cancer</td>
<td>45</td>
<td>332,999</td>
<td>97.5</td>
<td>98.1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Ovarian Cancer</td>
<td>32</td>
<td>189,058</td>
<td>98.9</td>
<td>96.6</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Prostate Cancer</td>
<td>32</td>
<td>167,195</td>
<td>98.4</td>
<td>97.3</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Gastric Cancer</td>
<td>29</td>
<td>222,333</td>
<td>98.1</td>
<td>98.5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy</td>
<td>58</td>
<td>249,727</td>
<td>96.3</td>
<td>96.7</td>
<td>87</td>
</tr>
<tr>
<td>Comprehensive</td>
<td>Carrier Testing</td>
<td>157</td>
<td>664,735</td>
<td>97.5</td>
<td>97.9</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Cancer Predisposition</td>
<td>143</td>
<td>620,318</td>
<td>98.3</td>
<td>96.8</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Comprehensive Cancer</td>
<td>160</td>
<td>744,835</td>
<td>98.0</td>
<td>97.7</td>
<td>92</td>
</tr>
</tbody>
</table>

Panel optimization results in outstanding experimental performance metrics.
GeneRead BRCA1 and BRCA2 custom panel

Design and specifications

- Overlapping amplicon design
- 100% coverage

<table>
<thead>
<tr>
<th>Experimentally-verified 100% coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regions targeted</strong></td>
</tr>
<tr>
<td><strong># of bases targeted</strong></td>
</tr>
<tr>
<td><strong>Average amplicon length</strong></td>
</tr>
<tr>
<td><strong>Total input DNA</strong></td>
</tr>
<tr>
<td><strong>Number of amplicons</strong></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
</tr>
<tr>
<td><strong>Uniformity (0.2x mean)</strong></td>
</tr>
<tr>
<td><strong>% of bases callable</strong></td>
</tr>
</tbody>
</table>
GeneRead DNAseq Custom Panel Builder

Build customized NGS panels in three simple steps:

1. List targets
2. Design for genomic coordinates
3. View & Order

Choose amplicon size:
- 150 (Ideal for exquisitely targeted DNA, e.g., FFPE samples)
- 225 (Ideal for panels requiring high sensitivity)

Design for gene:
- GeneGlobe cat#: MNGHS-00237X-235
- Number of PCR primer tubes/pools: 4
- Amplicon size: 150
- QC primers: Yes
- Date designed: February 19, 2015 – 07:49 PM CET
- Download
- Re-design with Relaxed specificity (this option could increase breadth of coverage, but may result in lower specificity). Relaxed specificity will change the size of certain amplicons. Amplicon size under this specificity is up to 225 bp.
- Specificity: High
- Download primer file of this panel (downloading primer files is available only for purchased panels).

add to cart
Quick start guide

Enter targets

Gene names

Enter or upload your genomic locations:

Chr Start Stop

Amplicon size: 150/225
Flanking bases: 10 (default)

Choose specs

Add more gene(s) or special regions

Choose amplicon size

Choose exon flanking bases (for gene targets)

Specify a name for this panel

View design & Order

Download primer file of this panel (downloading primer files is available only after creating a panel)
NGS Data Analysis

- **Base calling**
  - From raw data to DNA sequences: generate sequencing reads

- **Mapping reads to a reference**
  - Align the reads to reference sequence, e.g. GRCh37
  - Similar to a BLAST search: compares millions of reads against a reference database

- **Variants identification**
  - Identify the differences between sample DNA and reference DNA

- **Variant prioritization/filtering/validation/interpretation**

- **Downstream validation**
  - Typically PCR based, e.g. Somatic Mutation PCR analysis, methylation analysis, allele specific amplification.
NGS Data Analysis: Alignment

GRCh37 reference genome

Sequencing reads
**Coverage depth (or depth of coverage):** how many times each base has been sequenced. Unlike Sanger sequencing, in which each sample is sequenced 1-3 times to be confident of its nucleotide identity, NGS generally needs to cover each position many times to make a confident base call, due to relatively high error rate (0.1 - 1% vs 0.001 – 0.01%).

Increasing coverage depth is also helpful to identify low frequent mutation in heterogeneous samples such as identifying a somatic mutation in a heterogeneous cancer sample.
**Specificity**: the percentage of sequences that map to the intended targets region of interest

\[
\text{number of on-target reads / total number of reads}
\]
**Specificity**: the percentage of sequences that map to the intended targets region of interest

\[
\text{number of on-target reads / total number of reads}
\]
**Coverage uniformity**: measure the evenness of the coverage depth across target region

- Calculate coverage depth of each position
- Calculate the median coverage depth
- Set the lower boundary of the coverage depth relative to median depth
  - (e.g. 0.2 X median coverage depth in PCR panels)
- Calculate the percentage of the target region covered to the depth of or deeper than the lower boundary

GRCh37 reference

NGS reads
NGS Data Analysis: Uniformity

- **Coverage uniformity**: measure the evenness of the coverage depth across target region
  - Calculate coverage depth of each position
  - Calculate the median coverage depth
  - Set the lower boundary of the coverage depth relative to median depth
    - (e.g., 0.2 X median coverage depth in PCR panels)
  - Calculate the percentage of the target region covered to the depth of or deeper than the lower boundary

![Diagram of NGS reads and GRCh37 reference]

- GRCh37 reference
- NGS reads

- coverage depth = 10
- coverage depth = 3
- coverage depth = 2

Sample to Insight
GeneRead Data Analysis Solution

Data analysis for the non-bioinformaticians among us

Input

- FASTQ file
- Panel ID
- Job type
  - Single
  - Matched Tumor/Normal
- Analysis mode
  - Somatic
  - Germline

Output

- Bam (sequence) and VCF (variant) files
  - Sequencing metrics
  - Variants detected, confidence
  - Copy number alterations
- QIAGEN Advanced Bioinformatics (webinar IV)

Comprehensive and easy-to-use data analysis

Note: Acceptable file extensions are ".fastq" or ".fastq.gz" for Illumina reads, and ".basecaller.bam" for Ion reads. Please submit only unaligned base-caller bam files generated by Torrent Server 3.4.1 or higher. Please do not submit aligned bam files. Please DO NOT refresh the browser or navigate to other pages while uploading files.
## NGS Data Analysis Metrics

### Run Summary
- Specificity
- Coverage
- Uniformity
- Numbers of SNPs and Indels

### Summary By Gene
- Specificity
- Coverage
- Uniformity
- # of SNPs and Indels

<table>
<thead>
<tr>
<th>Gene</th>
<th>Bases covered by amplicon</th>
<th>% of bases covered &gt;=1x</th>
<th>% of bases covered &gt;=30x</th>
<th>% of bases covered &gt;=100x</th>
<th>% of bases covered at &gt;=0.1 median</th>
<th>Median coverage</th>
<th>SNP/MNP count</th>
<th>INDEL count</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2</td>
<td>5237</td>
<td>100</td>
<td>99</td>
<td>95</td>
<td>89</td>
<td>1718</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PTGS2</td>
<td>4334</td>
<td>100</td>
<td>93</td>
<td>81</td>
<td>92</td>
<td>554</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>7577</td>
<td>100</td>
<td>99</td>
<td>91</td>
<td>90</td>
<td>1146</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>1595</td>
<td>95</td>
<td>90</td>
<td>81</td>
<td>81</td>
<td>1301</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>TPS3</td>
<td>3197</td>
<td>98</td>
<td>94</td>
<td>87</td>
<td>93</td>
<td>598</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ALK</td>
<td>7697</td>
<td>98</td>
<td>95</td>
<td>93</td>
<td>91</td>
<td>1363</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>AKT1</td>
<td>3686</td>
<td>100</td>
<td>97</td>
<td>90</td>
<td>85</td>
<td>1388</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>4688</td>
<td>97</td>
<td>94</td>
<td>82</td>
<td>90</td>
<td>446</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>HRAS</td>
<td>1423</td>
<td>100</td>
<td>90</td>
<td>85</td>
<td>85</td>
<td>915</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>STK11</td>
<td>3154</td>
<td>93</td>
<td>82</td>
<td>80</td>
<td>77</td>
<td>1421</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NRAS</td>
<td>4534</td>
<td>99</td>
<td>95</td>
<td>84</td>
<td>94</td>
<td>362</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>RB1</td>
<td>5355</td>
<td>100</td>
<td>95</td>
<td>81</td>
<td>95</td>
<td>242</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>KIT</td>
<td>6085</td>
<td>100</td>
<td>99</td>
<td>97</td>
<td>97</td>
<td>349</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>4342</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>99</td>
<td>1029</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PTEN</td>
<td>5576</td>
<td>95</td>
<td>86</td>
<td>77</td>
<td>84</td>
<td>393</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>MTOR</td>
<td>11640</td>
<td>100</td>
<td>99</td>
<td>98</td>
<td>94</td>
<td>1703</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>BRAF</td>
<td>3812</td>
<td>100</td>
<td>100</td>
<td>91</td>
<td>100</td>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EGFR</td>
<td>7579</td>
<td>100</td>
<td>99</td>
<td>95</td>
<td>92</td>
<td>1231</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>MET</td>
<td>7793</td>
<td>98</td>
<td>95</td>
<td>91</td>
<td>92</td>
<td>830</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>KRAS</td>
<td>4577</td>
<td>100</td>
<td>99</td>
<td>76</td>
<td>95</td>
<td>499</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>
Features of Variant Report (Qiagen)

- SNP detection
- Indel detection
- dbSNP and COSMIC ID (hyperlink)
- Predicted amino acid change

### Example Table

<table>
<thead>
<tr>
<th>Chrom</th>
<th>Pos</th>
<th>Ref</th>
<th>Alt</th>
<th>Gene Name</th>
<th>Mutation type</th>
<th>Codon Change</th>
<th>AA Change</th>
<th>Filtered Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>116436097</td>
<td>rs41737</td>
<td>COSM150</td>
<td>G</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>24</td>
</tr>
<tr>
<td>27</td>
<td>116437606</td>
<td>rs1621</td>
<td></td>
<td>MET</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>125</td>
</tr>
<tr>
<td>28</td>
<td>21968199</td>
<td>rs11515</td>
<td>COSM142</td>
<td>C</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>121</td>
</tr>
<tr>
<td>29</td>
<td>105236287</td>
<td>rs1621</td>
<td></td>
<td>KRAS</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>31</td>
</tr>
<tr>
<td>30</td>
<td>105236666</td>
<td>rs712</td>
<td></td>
<td>KRAS</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>121</td>
</tr>
<tr>
<td>31</td>
<td>105236966</td>
<td>rs4362222</td>
<td></td>
<td>KRAS</td>
<td>SNP</td>
<td>c.4146G&gt;A</td>
<td>p.P1382</td>
<td>48</td>
</tr>
<tr>
<td>32</td>
<td>7577121</td>
<td>rs121913343</td>
<td>COSM1</td>
<td>G</td>
<td>SNP</td>
<td>c.817G&gt;A</td>
<td>p.R273C</td>
<td>403</td>
</tr>
</tbody>
</table>

### Example Diagram

- Effect of SNP
- snpeff.sourceforge.net
- Impact of SNP
- Link to qPCR somatic mutation assay
Confirm Variants of Your NGS Runs

qBiomarker Somatic Mutation ARMS-PCR Assays and Arrays

Greek Symbol = Gene
# = Mutation

• For Normalization
• Assays detect non-variable region (not ARMS-based design)
GeneRead DNA NGS Solutions from Sample to Insight

Sample

Sample Isolation

Targeted Enrichment

Library Construction

NGS Run

Data Analysis

Insight

Variant Confirmation

Interpretation

Sample QC

Library QC

GeneRead DNA FFPE Kit

GeneRead DNA QuantMIZE Kit

GeneRead DNAseq BRCA 1/2 V2

GeneRead Library Prep Kits

Any Sequencer

Miseq

NextSeq

HiSeq

Ion PGM

GeneRead DNAseq PCR Kit V2

GeneRead Size Selection Kit

GeneRead DNAseq Library Quant Array

CLC Cancer Research Workbench

Ingenuity Variant Analysis

Eric Lader NGS
Thank You For Attending

Questions?

Contact QIAGEN
Call: 1-800-426-8157
Email:
  techservice-na@qiagen.com
  BRCsupport@QIAGEN.com
  QIAwebinars@QIAGEN.com