GOLDEN HELIX

Enabling Precision Medicine

Annotating and Cataloging CNVs in VarSeq

Dr. Nathan Fortier – Director of Research

CIOReview

20 most promising Biotech Technology Providers

pharma TECH OUTLOOK

Top 10 Analytics Solution Providers

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Hype Cycle for Life sciences

Q & A

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- PI is Dr. Andreas Scherer, CEO Golden Helix.
- The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Golden Helix – Who We Are

Golden Helix is a global bioinformatics company founded in 1998.

GlaxoSmithKline

varSEQ®

- Variant Calling
- Filtering and Annotation
- Variant Interpretation
- Clinical Reports
- CNV Analysis
- Pipeline: Run Workflows

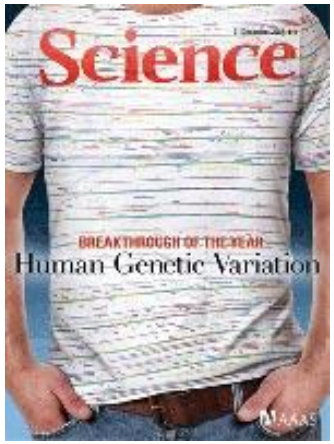
VARIANT WAREHOUSE

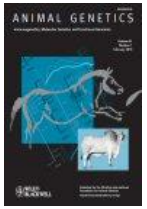
- Variant Warehouse
- Centralized Annotations
- Hosted Reports
- Sharing and Integration

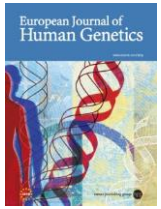
SNP & VARIATION SUITE

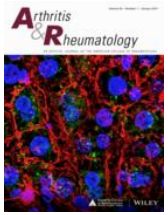
- CNV Analysis
- GWAS
- Genomic Prediction
- Large-N-Population Studies
- RNA-Seq
- Large-N CNV Analysis

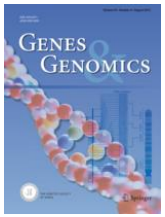
Cited in over 1300 peer-reviewed publications











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Over 400 customers globally

SickKids THE HOSPITAL FOR SICK CHILDREN

Cincinnati Children's Hospital Medical Center

The Children's Hospital of Philadelphia

Stanford University

UBC

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The Feinstein Institute for Medical Research North Shore-Long Island Jewish Health System

North Shore LIJ

PREVENTION GENETICS DISEASE PREVENTION THROUGH GENETIC TESTING

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MOFFITT CANCER CENTER

Dartmouth-Hitchcock NORRIS COTTON CANCER CENTER

BAYER

Lilly

abbvie

TEVA

NATIONAL CANCER INSTITUTE

USDA Agricultural Research Service

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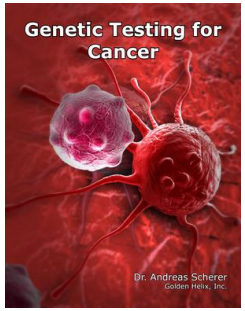
Golden Helix – Who We Are

When you choose a Golden Helix solution, you get more than just software

- REPUTATION
- TRUST
- EXPERIENCE



- INDUSTRY FOCUS
- THOUGHT LEADERSHIP
- COMMUNITY

- TRAINING
- SUPPORT
- RESPONSIVENESS

- INNOVATION and SPEED

GENE PANEL | EXOME | GENOME

SEQUENCER

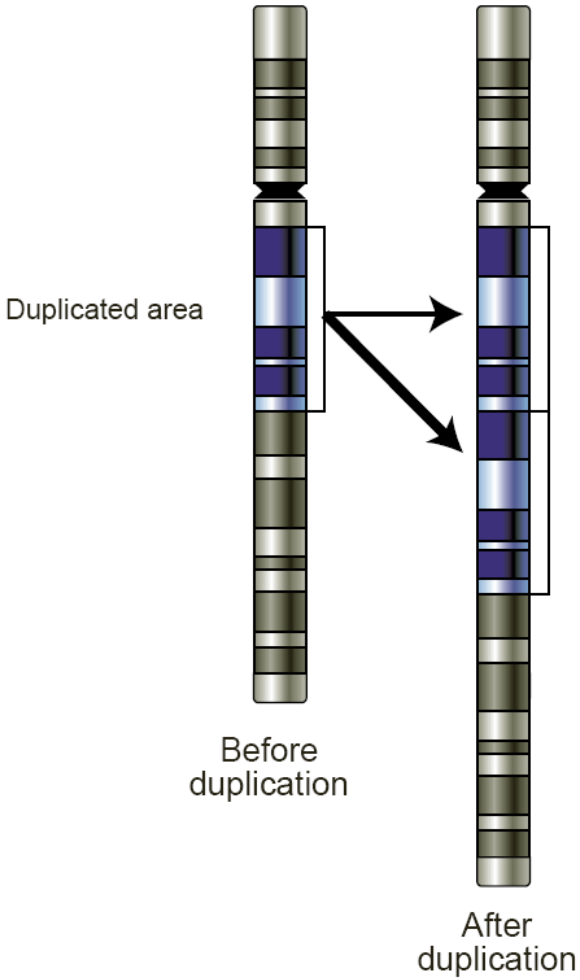
PRODUCTS	BIOINFORMATICS PIPELINE	FUNCTION
DNAseq (Sentieon) TNseq (Sentieon) VS-CNV	FASTQ BAM VCF	▸ Single nucleotide variation ▸ Copy number variation & loss of heterozygosity ▸ Chromosomal aberration
Annotations	Annotated VCF	▸ Public & commercial annotations to enrich genomic data sets
VarSeq VSReports VSPipeline	Clinical Report	▸ Annotate & filter ▸ Visually inspect alignments ▸ Variant prioritization ▸ Clinical assessment
VSClinical	Automated ACMG Guidelines	▸ Clinical variant interpretation in concordance with ACMG Guidelines
VSWarehouse	Data Warehousing Web-Enabled Interface + Powerful API: JSON, XML, TSV, CSV, SQL, FHIR	▸ Clinical assessment catalog ▸ Advanced data querying ▸ Versioning ▸ Interoperability ▸ Compliance with HIPPA, CLIA & CAP data discovery

CNVs in Clinical Testing

- **Critical evidence needed for many diagnostic tests**
- **Common driver specific cancers, causal hereditary variation**
  - EGFR Exon 19 deletion common in lung cancer
  - PIK3CA Amplification in breast cancer
- **Large events**
  - Chromosome 13 deletion common in melanoma
  - Autism Spectrum Disorder (ASD)
  - Developmental Delay (DD)
  - Intellectual Delay (ID)

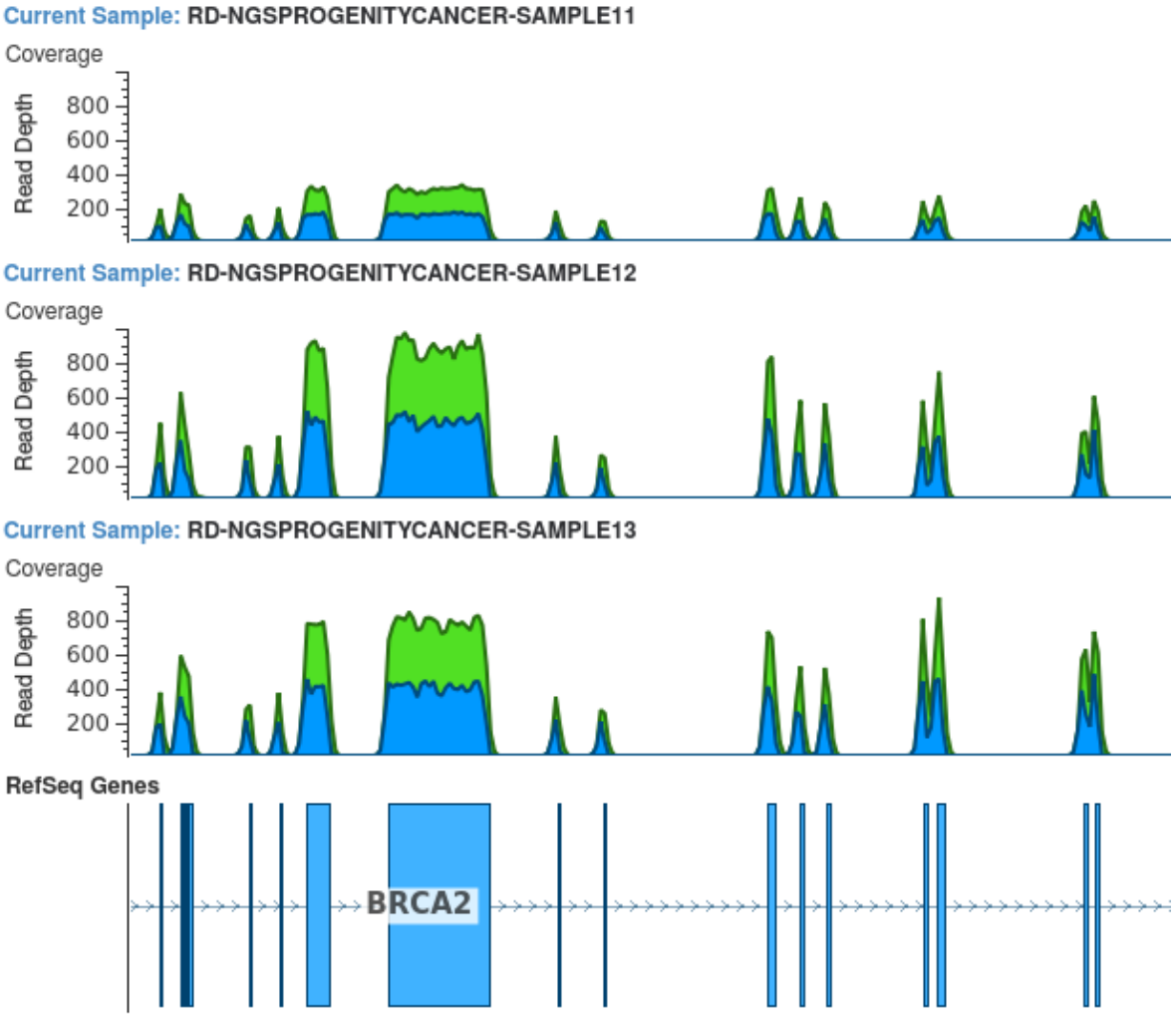
CNV Detection

- **Chromosomal microarray**
  - Current best practice
  - Slow
  - Additional expense
  - Only detects large events
- **CNV calling from NGS data**
  - Calls from existing coverage data
  - Detects small single-exon events
  - Provides faster results, simplified clinical workflow



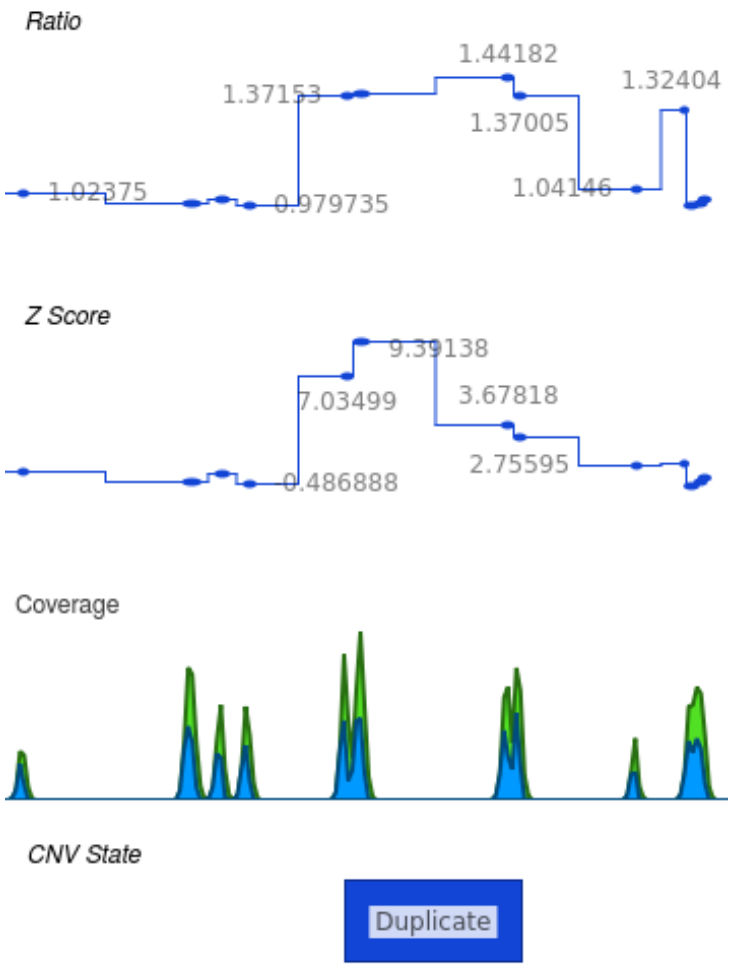
CNV Detection via NGS

- **CNVs are called from coverage data**
- **Challenges**
  - Coverage varies between samples
  - Coverage fluctuates between targets
  - Systematic biases impact coverage
- **Solutions**
  - Data Normalization
  - Reference Sample Comparison



CNV calling in VarSeq

- **Reference samples used for normalization**
- **Metrics**
  - Z-score: number of standard deviations from reference sample mean
  - Ratio: sample coverage divided by reference sample mean
  - VAF: Variant Allele Frequency
- **For Gene Panels and Exomes**
  - Probabilistic model used to call CNVs
  - Segmentation identifies large cytogenetic events
- **For Whole Genome Data**
  - Targets segmented using Z-scores
  - Events called based on Z-score and Ratio thresholds



QC Events

- **Low quality events can be flagged if**
  - Event targets have low coverage
  - There is high variation between samples at event targets
  - Event cannot be differentiated from noise at a region

- **Samples can be flagged if**
  - The sample does not match the references
  - The sample has extremely low coverage
  - There is high variance across the target regions

- **Filtering flagged events improves precision**

Reference Samples

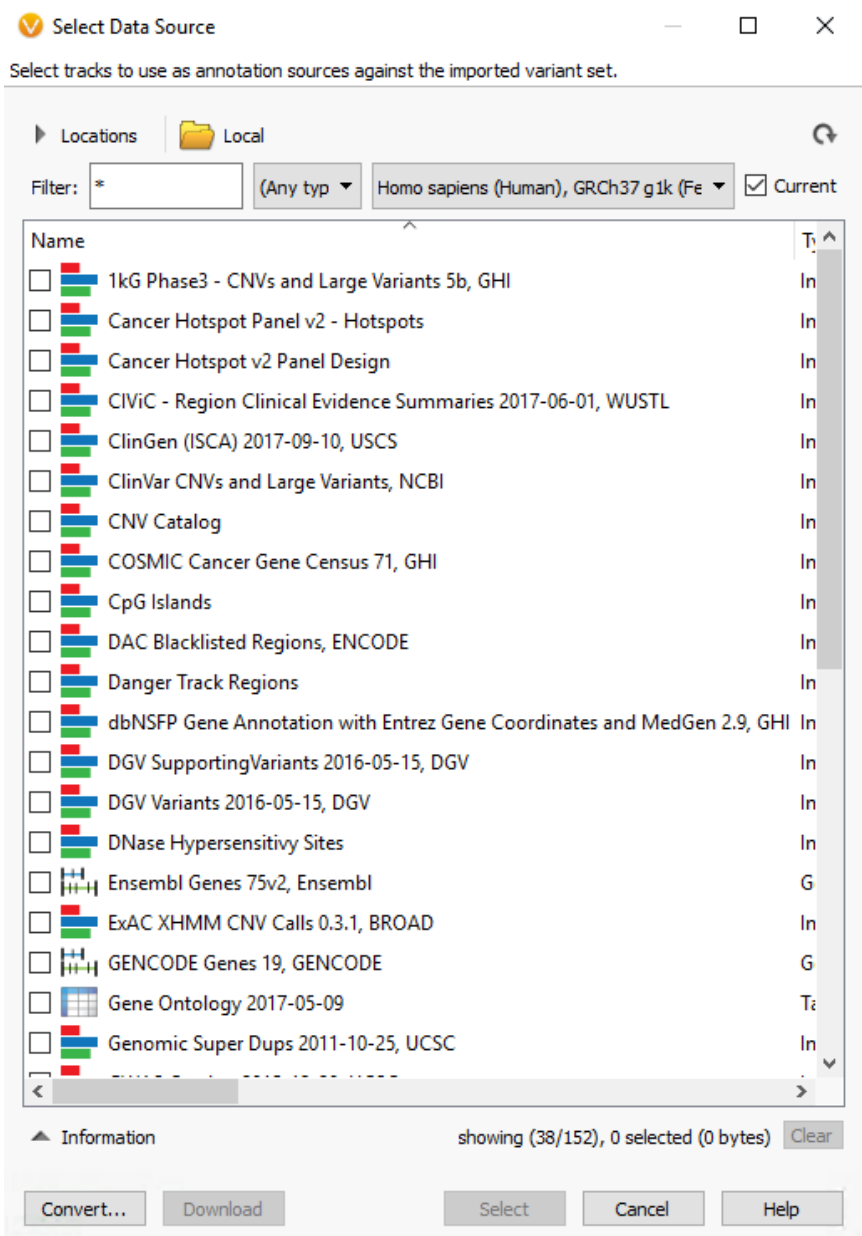
- Match references are chosen for each sample
- Samples with lowest percent difference chosen
- Performance affected if controls don’t have matching coverage profile
- Samples are flagged if the average percent difference is above 20%

Requirements

- **100x Coverage**
- **Reference samples**
  - Recommend at least 30 references
  - From same platform and library preparation
  - Automatic gender matched references for non-autosomal calls

Sources for Annotating CNVs

- **CNV calls in Populations:**
  - 1000 Genomes Phase3 Large Variants
  - ExAC per-sample CNV calls
  - DGV large-cohort studies
- **Clinical Interpretations:**
  - ClinVar Large Variants
  - ClinGen (Previously ISCA)
- **Genes**
  - Gene track, which transcripts/exons
  - Special considerations considering large sizes
- **Regions**
  - Genomic Superdups (Large Scale)
  - Low Complexity Regions (Smaller Scale)



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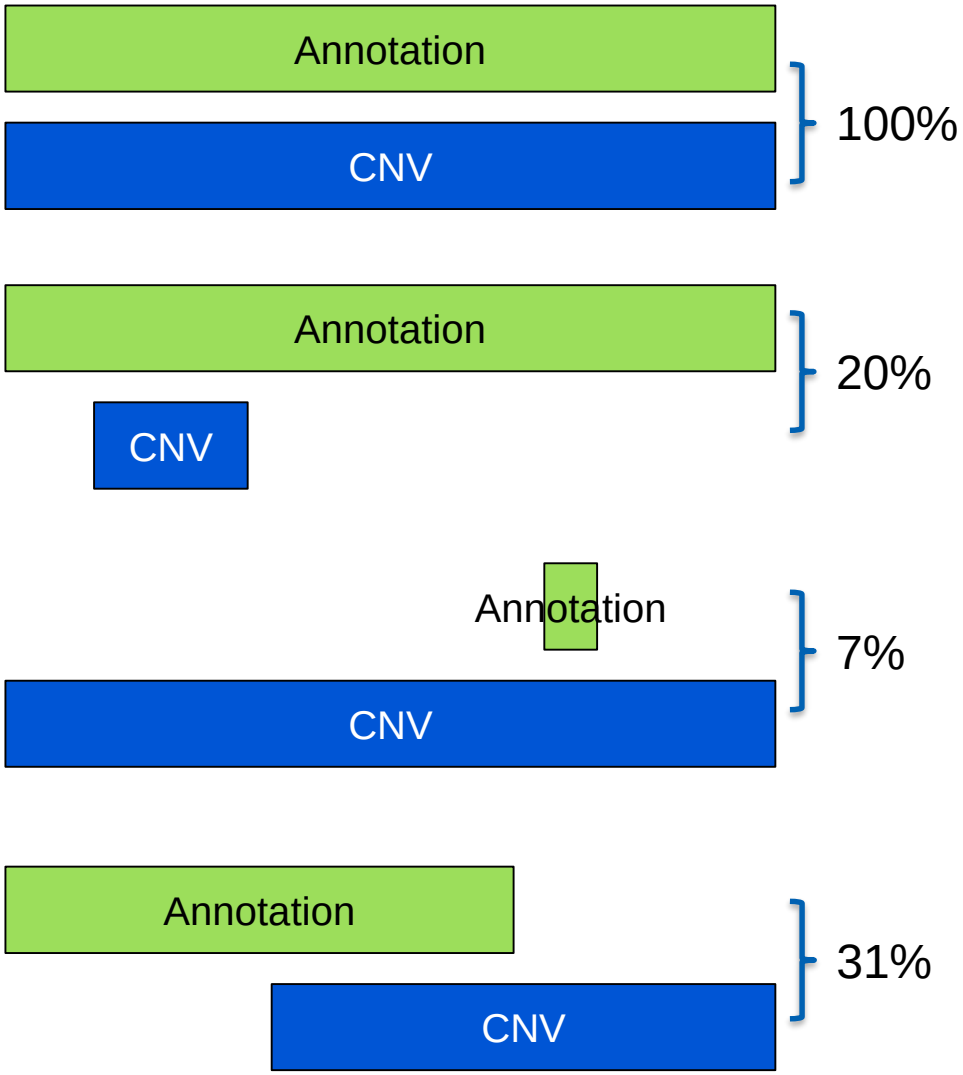
Annotation Algorithms: Overlapping Regions

- **Not expect exact matches**
- **Percent overlap not correct metric**
- **Need metric of "sameness"**
- **Jaccard index:**
  - "similarity coefficient"

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}$$

  - For fully overlapped regions, the percent overlap of the smaller to the larger
  - Default value of 20% for annotations
  - If set to 0%, then any overlap matches
  - If set to 100%, then exact matches



VarSeq Demonstration

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Q & A

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