CNV, GWAS and Clinical Analysis Advancements in SVS

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- **PI is Dr. Andreas Scherer, CEO Golden Helix.**
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Q & A

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

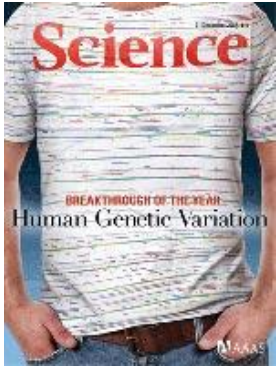
VARIANT WAREHOUSE

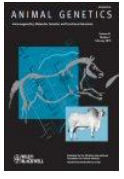
- Variant Warehouse
- Centralized Annotations
- Hosted Reports
- Sharing and Integration

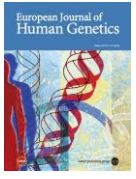
SNP & VARIATION SUITE

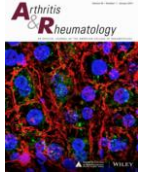
- GWAS
- Genomic Prediction
- Large-N-Population Studies
- RNA-Seq
- Large-N CNV-Analysis

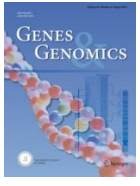
Cited in over 1,300 peer-reviewed publications

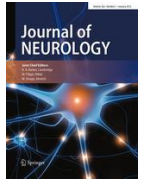












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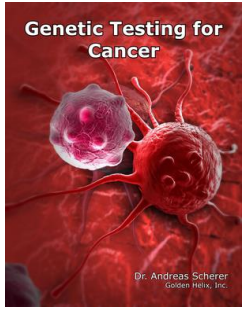

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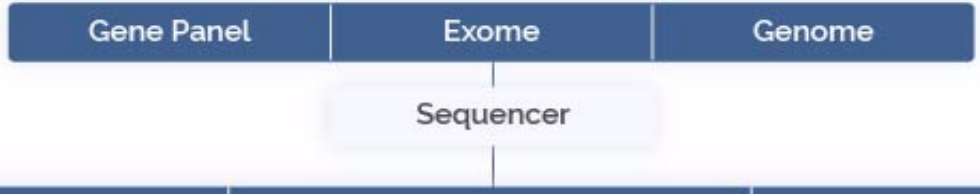


Products	Bioinformatics Pipeline	Function
DNAseq (Sentieon) TNseq (Sentieon) VS-CNV	FASTQ BAM VCF	▸ Single nucleotide variation ▸ Copy number variation & loss of heterozygosity ▸ Chromosomal aberration
Annotations	Annotated VCF	▸ Public & commercial annotations to enrich genomic data sets
VarSeq VSReports 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

Agenda

Dr. Eli Sward (FAS)

- Background on detecting CNVs
- Targeted vs. Binned Region approach
- Software demonstration of VarSeq
- Software demonstration in SVS
- Downstream applications of CNV analysis in SVS

Gabe Rudy (VP of Product)

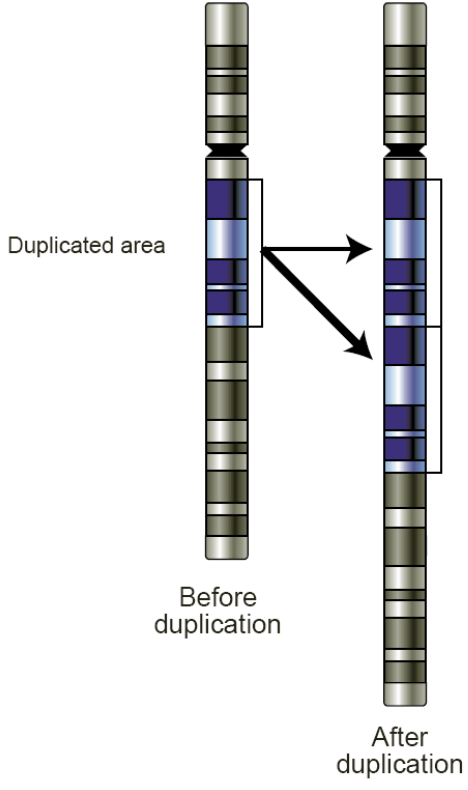
- GWAS improvement and explanations
- SVS-Clinical Variant Scoring
- Splice Site Predictions
- Functional Predictions & Conservation
- Other updates to SVS

CNVs in Clinical and Research settings

- **Clinical – Diagnostic testing**
  - Common drivers in specific cancers and causal agents in hereditary variation
    - EGFR Exon 19 deletion (J Clin Oncol. 2011 May 20; 29(15): 2066–2070.)
    - PIK3CA Amplification breast cancer (Mol Cytogenet. 2018; 11: 5.)
  - Large Events commonly seen in disorders
    - Autism Spectrum Disorder
    - Developmental Delay
- **Research – Large population based discovery**
  - Large microdeletions associated with schizophrenia (Nature. 2008 Sep 11; 455(7210): 232–236.)
  - Genome wide CNV analysis for growth rates *Bos indicus* (BMC Genomics. 2016 Jun 1;17:419.)
  - Discovery leads to testing for association

Detecting CNVs

- **Chromosomal microarray**
  - Current best practice
  - Slow
  - Additional expense
  - Only detects large events
- **CNV calling from NGS data**
  - Calls CNVs from existing coverage data
  - Detects large and small single-exon events
  - Can be applied to both **Clinical** and **Research** settings
    - Implement a targeted approach (exomes and gene panels)
    - Or binned region approach (whole genome data)



Targeted vs. Binned Region Approach

Targeted (BED file)

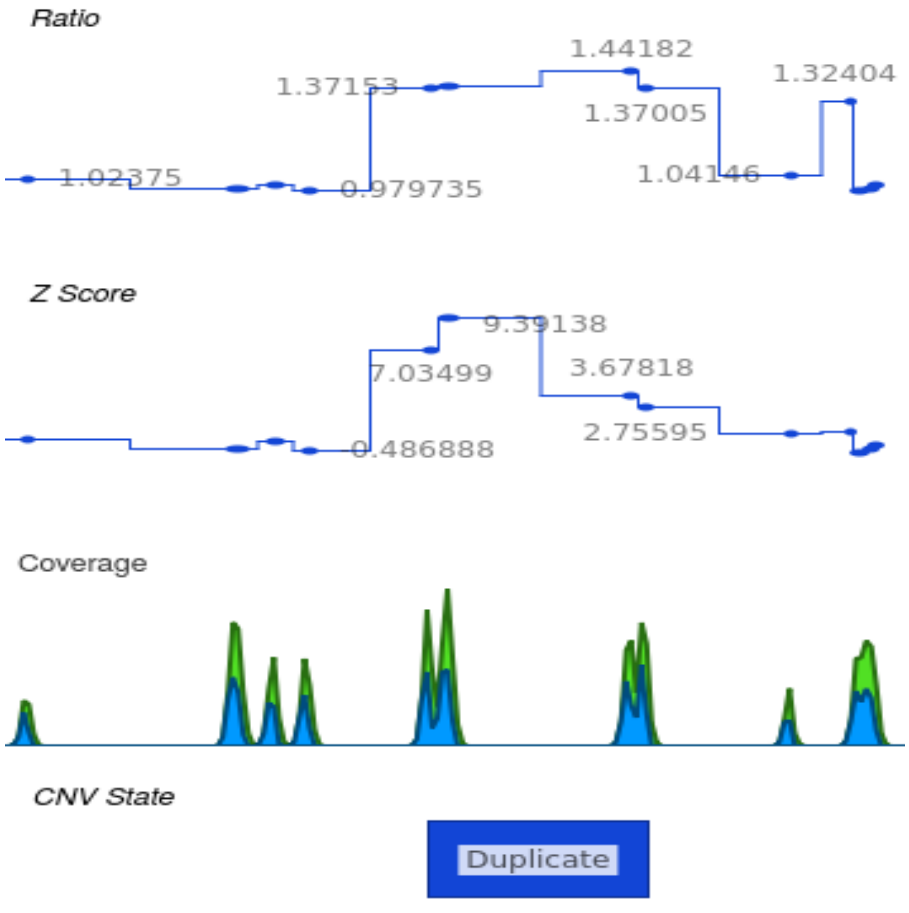
- Gene Panels, Whole Exome Sequencing
- 30+ Samples (same library and preparation)
- Normalization
- ≥ 100X coverage
- ≥ Single exon level
- Metrics (Z-score, Ratio, p-value)

Binned (Binned widths)

- Shallow Whole Genomes
- 30+ Samples (same library and preparation)
- Normalization
- ≥ 0.5X Coverage
- ≥ 100Kbp CNV events
- Metrics (Z-score, Ratio, p-value)

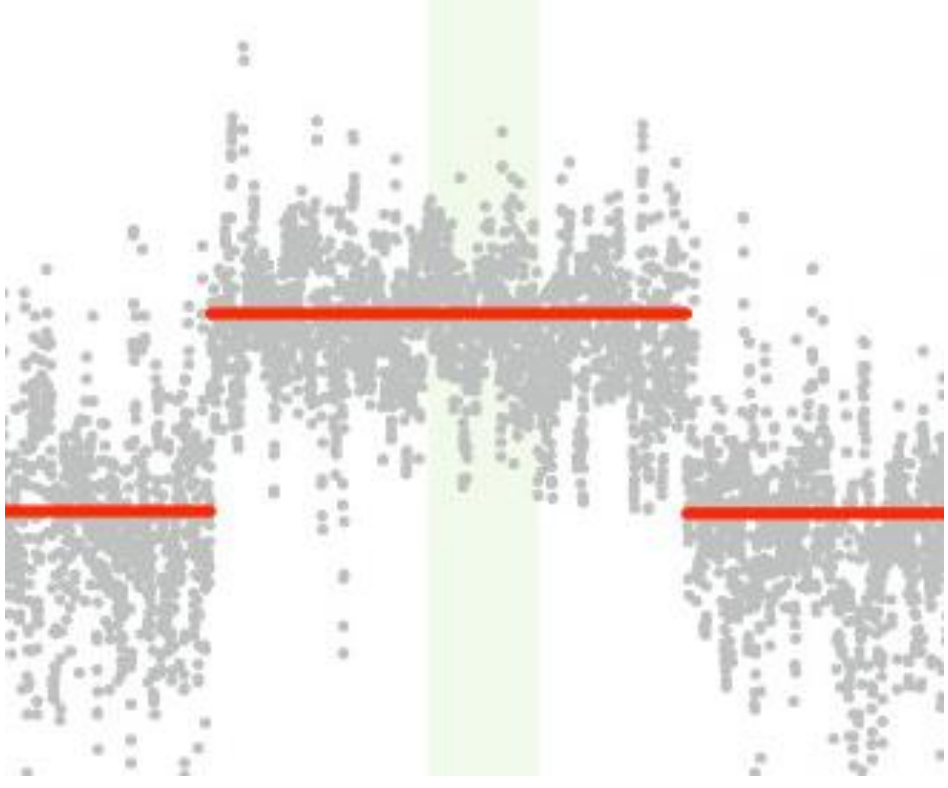
CNV Calling in VarSeq and SVS

- **Metrics**
  - Z-score number of s.d from reference sample mean
  - Ratio sample coverage/reference sample mean
  - Variant allele frequency (supporting metric)
- **Flags**
  - CNV events
    - Low coverage, high variation, noise
  - Samples
    - Mismatch to reference, low coverage, high variance across regions
- **Improves precision**



Calling CNVs from WGS implements segmentation

- **Metrics are noisy over large regions**
- **Outliers cause large events to be called as many small events**
- **Address this using CNAM Optimal Segmentation**
  - Regions containing many events are segmented
  - Small events sharing a segmented region are merged



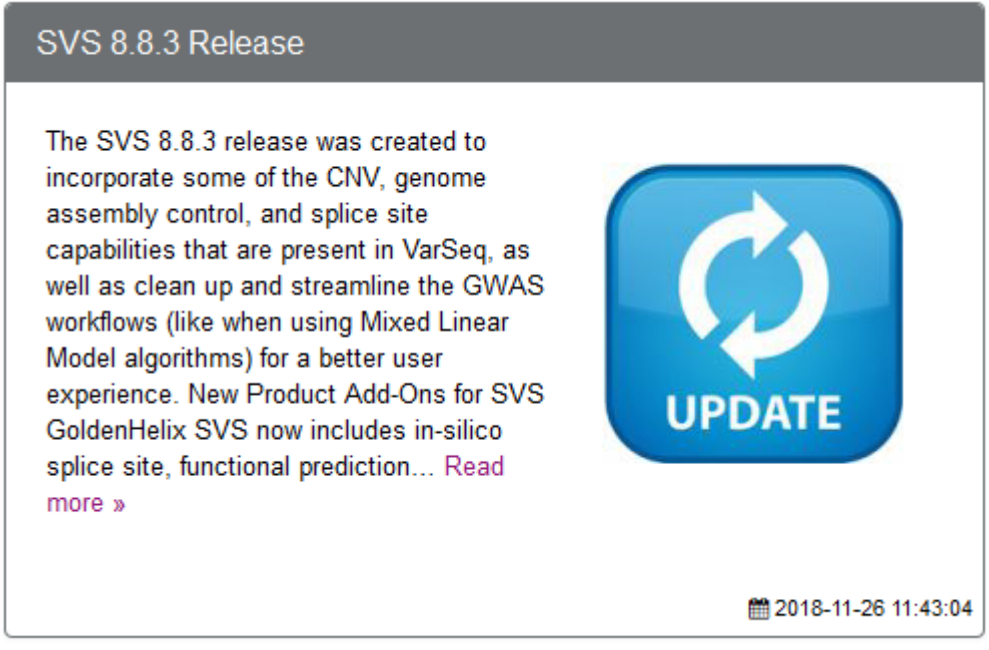
Workflow and Demonstration

- Filter CNVs from WGS
- Annotate
- Visualize > Sample specific
- Catalog

- Filter CNVs from WGS
- Annotate
- Visualize > Cohort
- Discuss downstream analyses

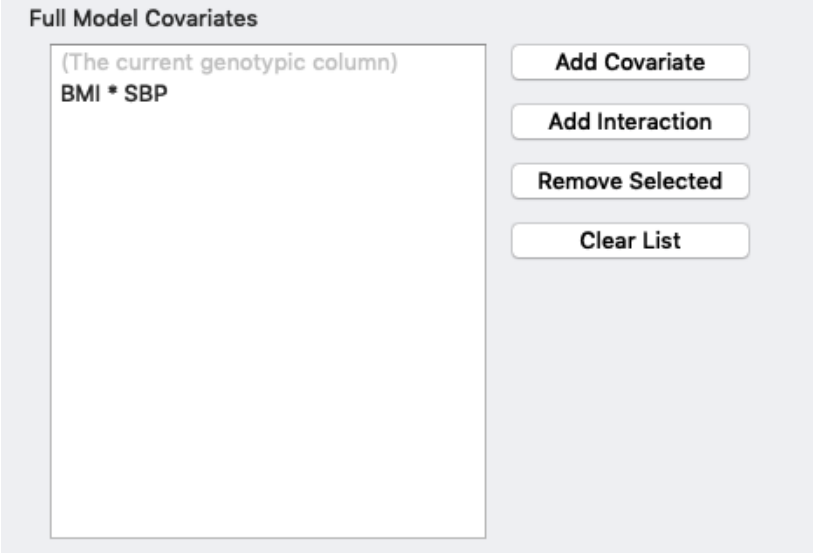
Highlights of Latest SVS Release

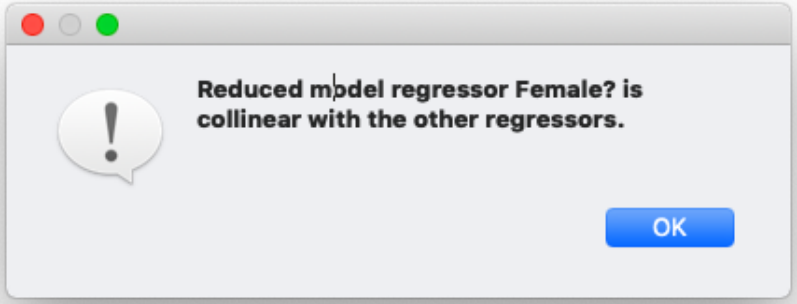
- **Whole Genome CNV Calling**
- **GWAS and Mixed Model Improvements**
- **Clinical Variant Scoring Module**



SVS: GWAS Improvements

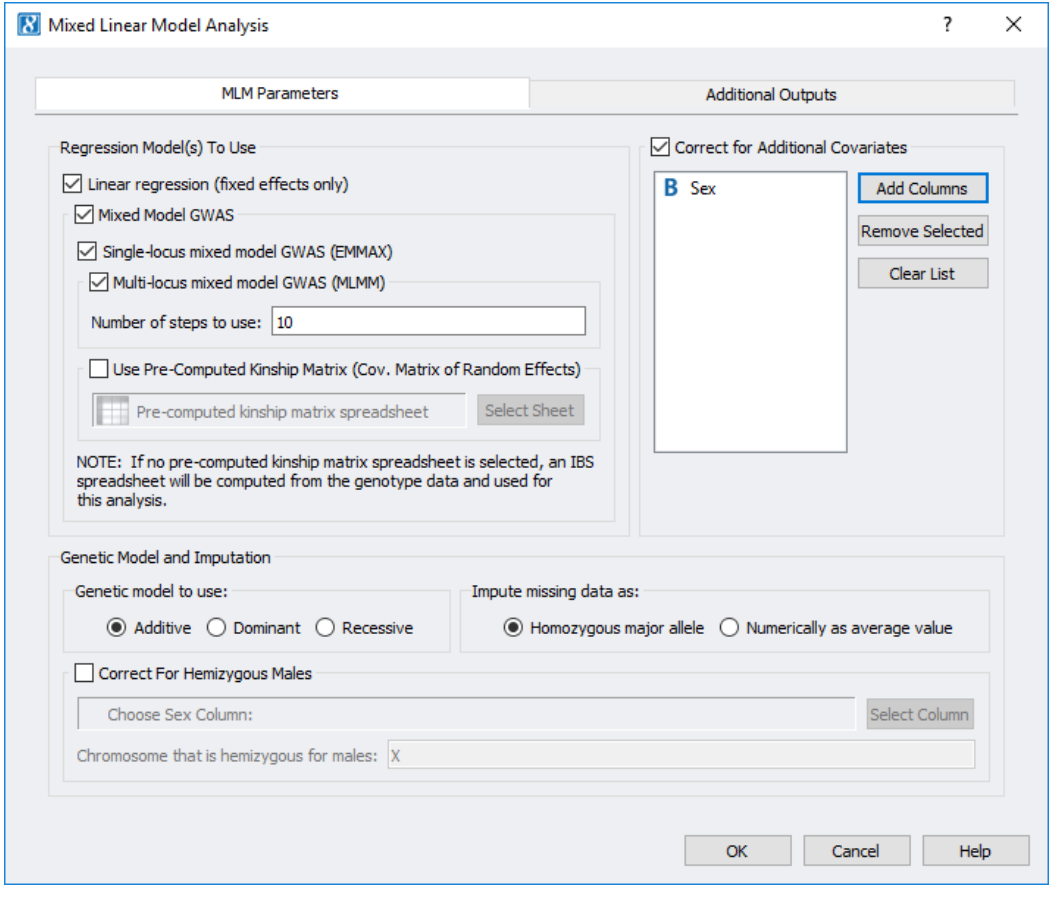
- **Genotype Regression**
  - Does not require recoding genotypes to numeric
  - Includes marker statistics
- **Improved Regression:**
  - Display of “implicit” full & reduced model terms
  - Collinear detection and warnings
  - Don’t allow double-adding terms
- **Output Improvements:**
  - Covariate term names in column headers for Beta / Beta-Standard-Error columns





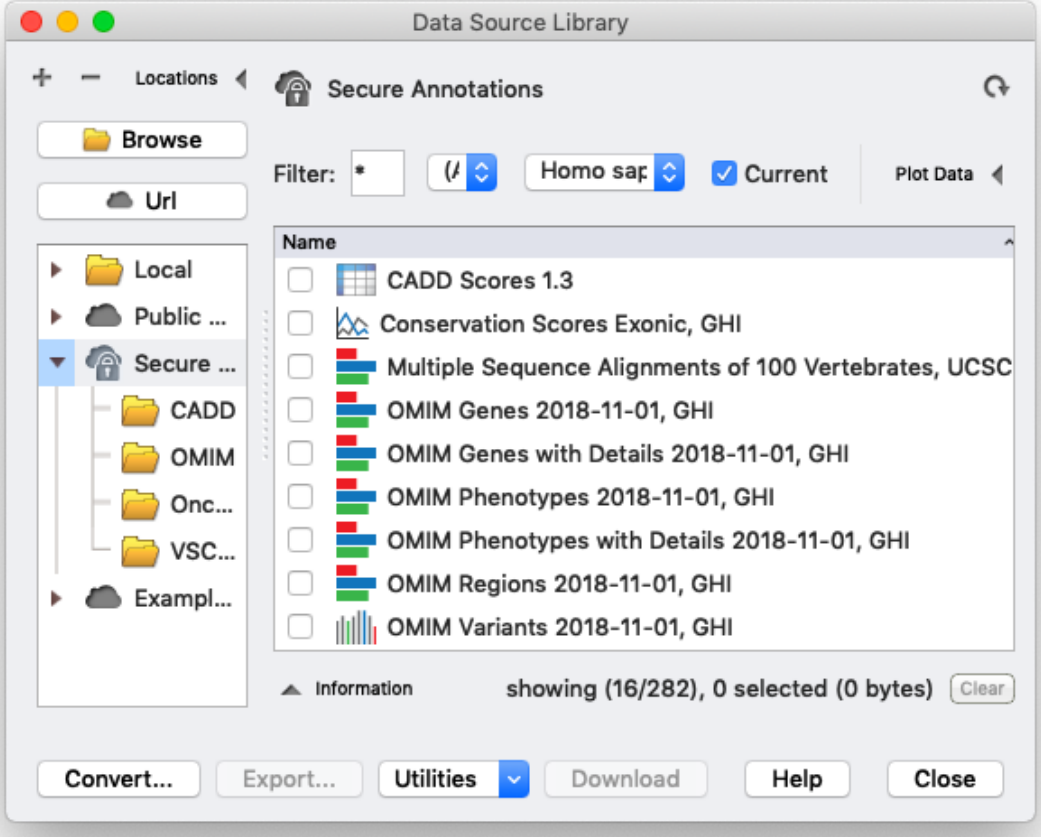
SVS: GWAS Mixed Model Improvements

- **GBLUP Improvements**
  - General speedup (2-5X)
  - Precision tightened from .0001 to .000001
- **Mixed Model Analysis:**
  - Collinear detection with informative diagnosis
  - All Beta values and their Standard Error output
  - Multi-allelic genotypes supported
  - Various numerical ”edge cases” now supported disorders



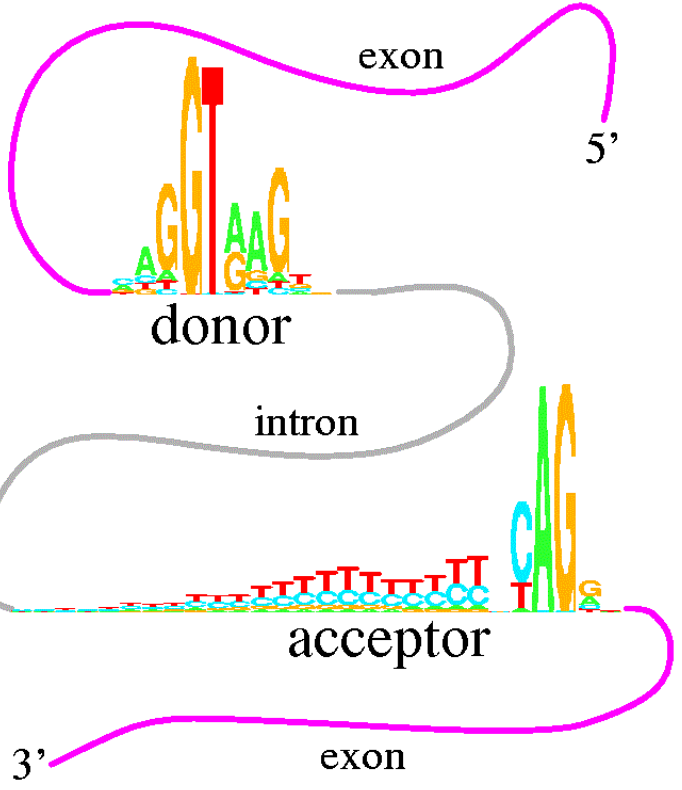
SVS: Clinical Variant Scoring Module

- **Premium Annotations for Clinical Workflows**
  - Splice Site Predictions
  - Multiple Sequence Alignment SIFT/Polyphen2
  - Conservation Scores: GERP++/PhyloP
- **Other Premium SVS Annotations:**
  - **CADD**: Best option for annotating whole genomes and indels. Updated 1.4 coming soon!
  - **OMIM**: Variants and gene annotations for genomic disorders



Splice Sites

- **Introns have distinct nucleotide pairs at each end**
  - GT at the 5’ end (Donor Site)
  - AG at the 3’ end (Acceptor Site)
- **Sequences around splice sites are highly variable**
- **Machine learning and probabilistic methods are used to identify sites**

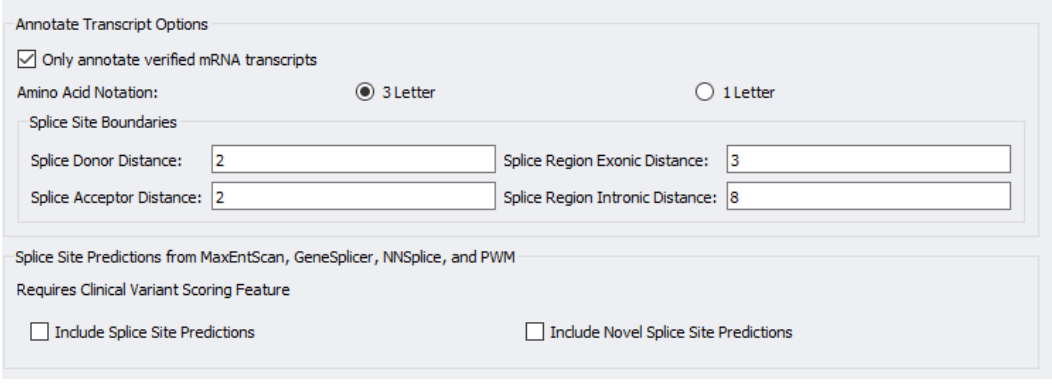


Splice Site Algorithms

- **VSClinical supports four splice site prediction algorithms**
  - PWM: Uses position weight matrix similar to SpliceSiteFinder and Human Splice Finder
  - MaxEntScan: Approximates sequence motifs using Maximum Entropy Distribution
  - NNSplice: Identifies splice sites using neural networks
  - GeneSplicer: Uses Markov models combined with maximal dependence decomposition

Splice Site Algorithms in SVS

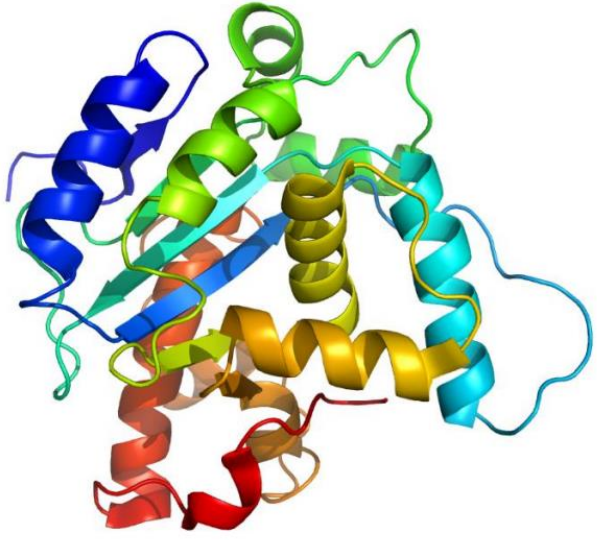
- **Runs Transcript Annotation**
- **Two Modes:**
  - Predicted Splicing Disrupted
  - Novel Splice Site Detection



C 11	C 12	C 13	I 14
Novel Splice Site	Novel Splice Type	N of 4 Predicted Novel Splice Site	Distance to Novel Splice Site
1:17301791	Donor	1 of 4 Predicted Splicing Disrupted	-11
1:40555086	Donor	4 of 4 Predicted Splicing Disrupted	-1
1:41284287	Donor	4 of 4 Predicted Splicing Disrupted	-5
1:116283389	Donor	4 of 4 Predicted Splicing Disrupted	-1
1:155207936	Donor	3 of 4 Predicted Splicing Disrupted	0
1:158645957	Donor	4 of 4 Predicted Splicing Disrupted	0
1:161276536	Donor	4 of 4 Predicted Splicing Disrupted	-1
1:209791295	Donor	4 of 4 Predicted Splicing Disrupted	-1
1:216498839	Donor	3 of 4 Predicted Splicing Disrupted	2
2:166895936	Donor	1 of 4 Predicted Splicing Disrupted	0
2:169791747	Donor	4 of 4 Predicted Splicing Disrupted	0
2:219674478	Donor	2 of 4 Predicted Splicing Disrupted	-1
2:219677824	Donor	1 of 4 Predicted Splicing Disrupted	5
3:15495354	Donor	2 of 4 Predicted Splicing Disrupted	-1
3:48618331	Donor	4 of 4 Predicted Splicing Disrupted	-1

Functional Prediction Algorithms

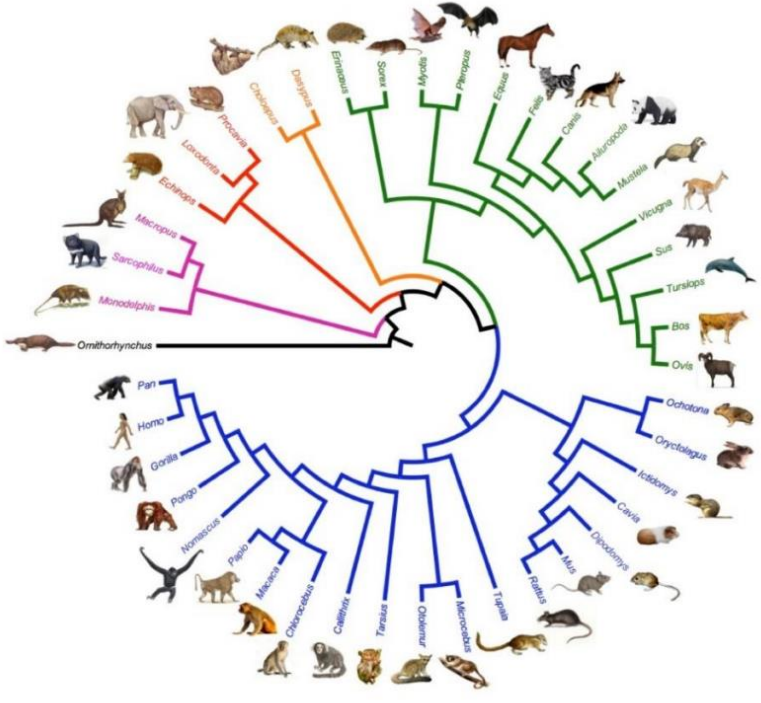
- **SIFT**
  - Uses matrix to encode the probability of each amino acid at each position of the protein
  - Probabilities computed from protein sequence alignment
  - Other Premium SVS Annotations:
- **PolyPhen2**
  - Naïve Bayes multi-evidence approach
  - Uses similar protein alignment-based probability score called PSIC
  - Incorporates nine other metrics



Functional Prediction Algorithms

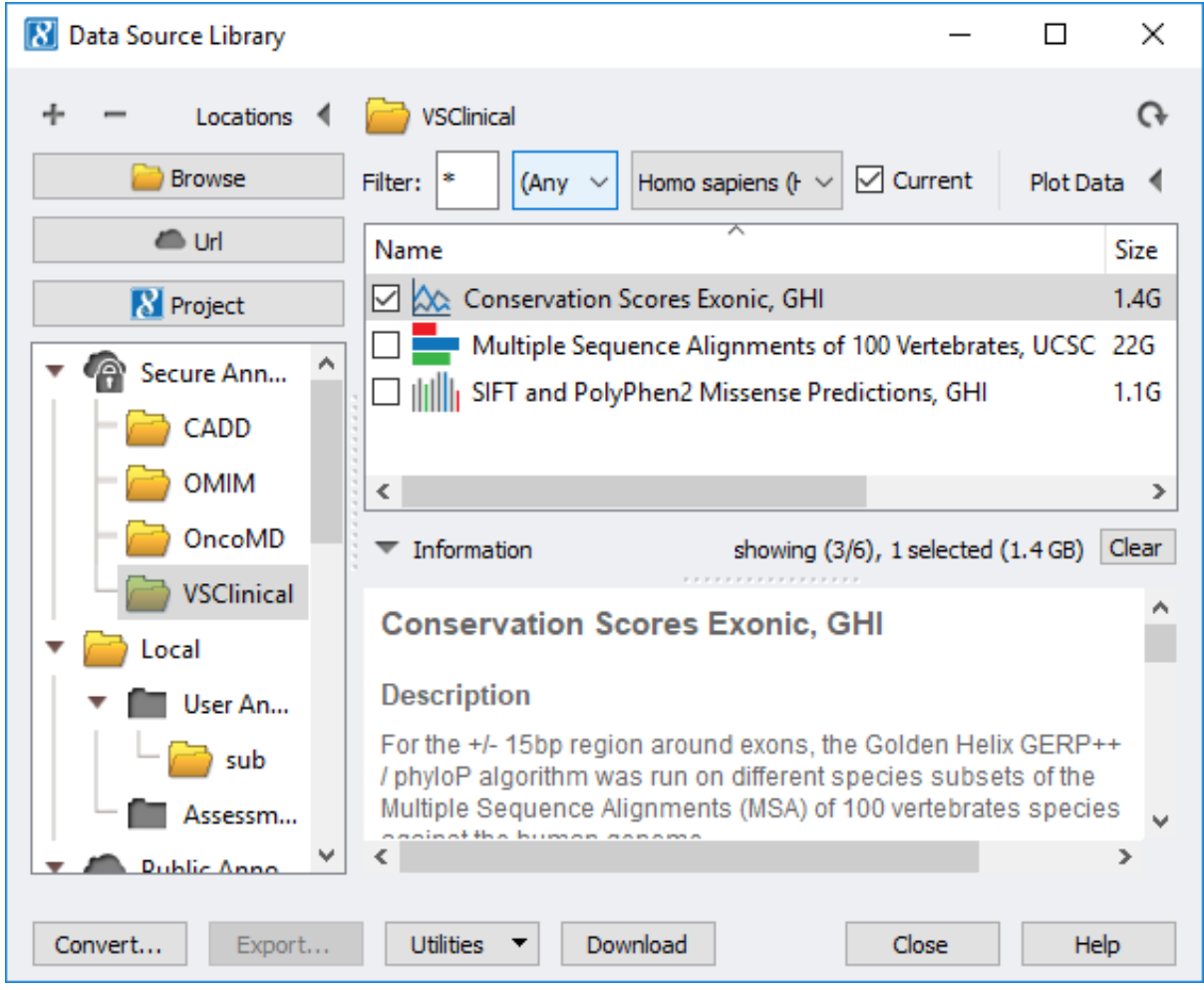
- **Conservation Scores**
  - Use phylogenetic models
  - Find maximum likelihood scaling factor for model given an alignment
- **GERP++**
  - Uses rejected substitutions (RS) as test statistic
  - RS value is computed from the neutral rate **n** and the maximum likelihood scaling factor **θ**

    RS = n(1- θ)
- **PhyloP LR**
  - Two times the difference in log likelihood between
    - null hypothesis (no scaling factor)
    - alternative hypothesis (maximum likelihood scaling factor)



Conservation Scores in SVS

- **Precomputed Scores for Exon Regions**
  - RefSeq exons +/- 15bp
  - Conservation Scores Exonic track
- **Annotate Any Variant (Non-Exonic)**
  - Uses Multiple Sequence Alignment to compute conservation
  - Download the (large) MSA for performant annotation



Demo

SNP & VARIATION SUITE

[DEMONSTRATION]

Q & A

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Thank you!

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- 4 remain ~~(5)~~ SNP & Variation Suite (SVS) w/ CNV & VarSeq w/ CNV | 1-Seat License - $9,995
- 2 remain ~~(3)~~ SNP & Variation Suite (SVS) Imputation Module | 2-Seat License - $7,995
- 2 remain ~~(3)~~ VarSeq w/ CADD & OMIM | 3-Seat License - $8,995
- 3 remain ~~(5)~~ VarSeq CNV PowerPack & Sentieon Tier One | 2-Seat License - $17,495
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