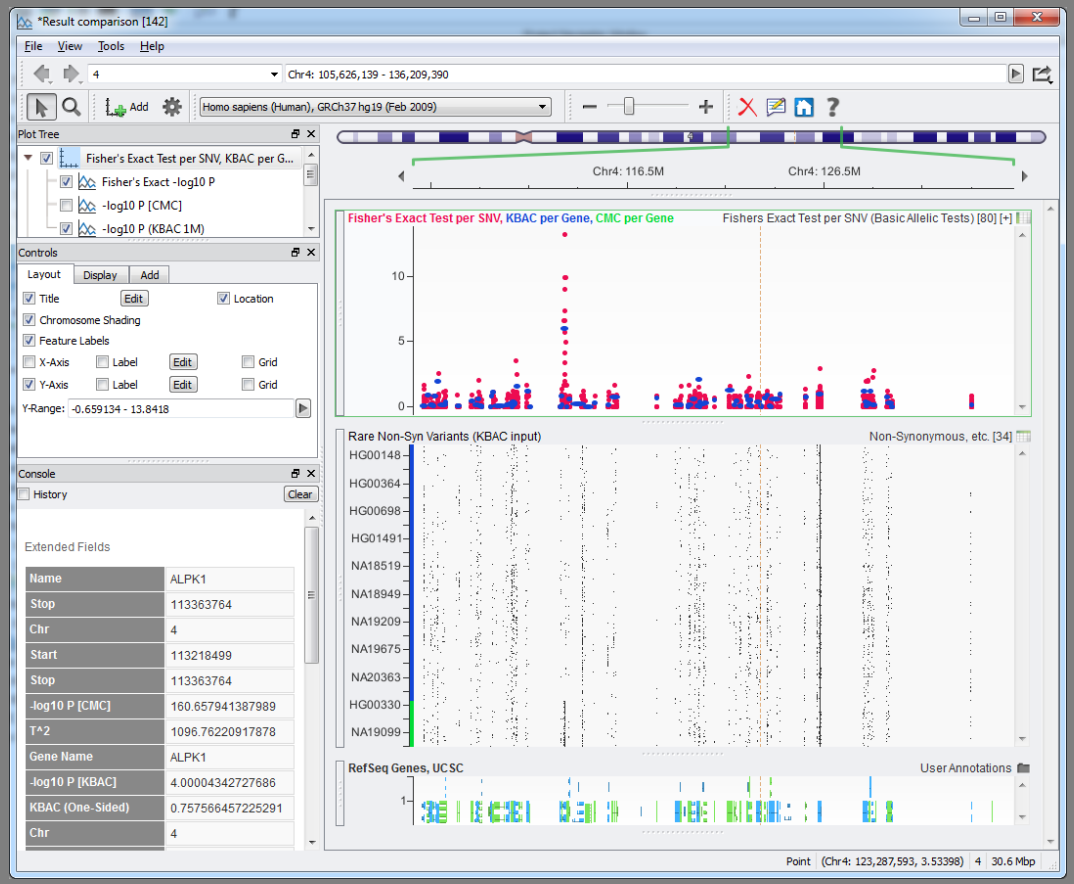

Rare Variant Analysis Workflows: Analyzing NGS Data in Large Cohorts

Nov 13, 2013

Bryce Christensen
Statistical Geneticist / Director of Services

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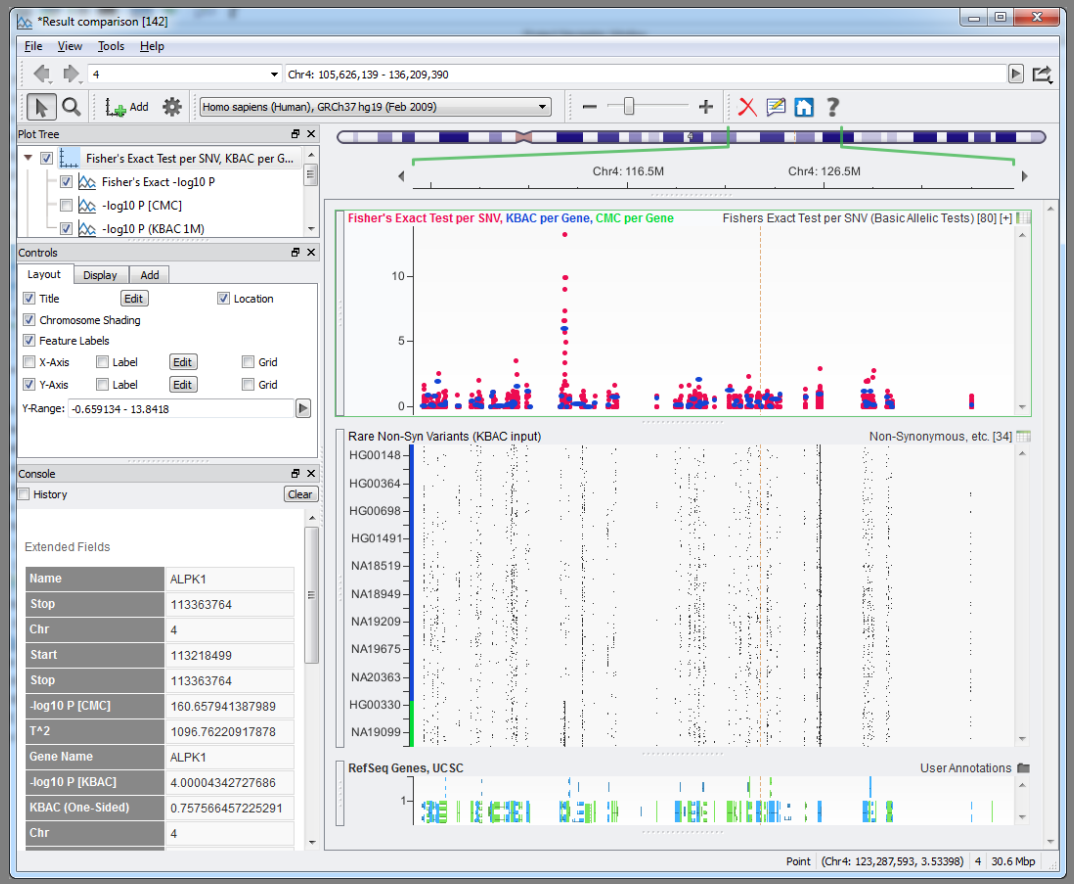

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Questions during the presentation

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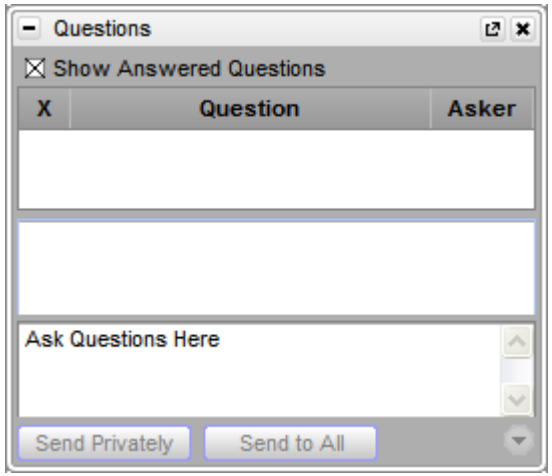

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About Golden Helix

Leaders in Genetic Analytics

- Founded in 1998
- Multi-disciplinary: computer science, bioinformatics, statistics, genetics
- Software and analytic services

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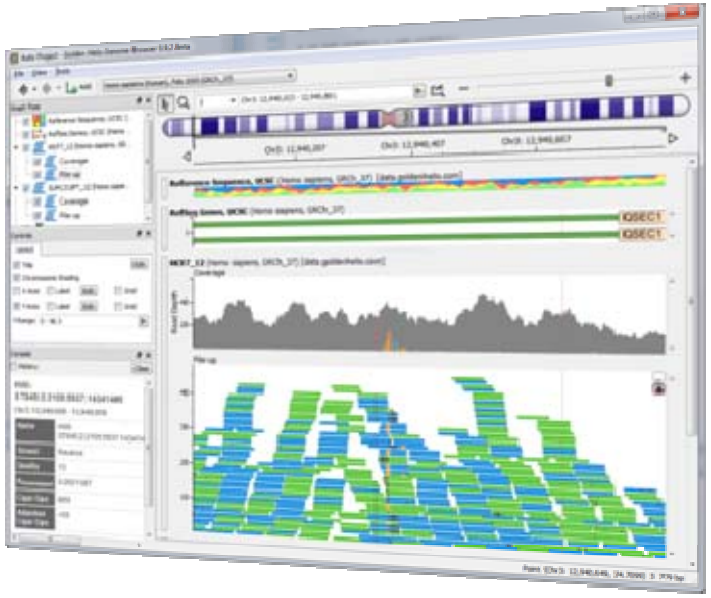
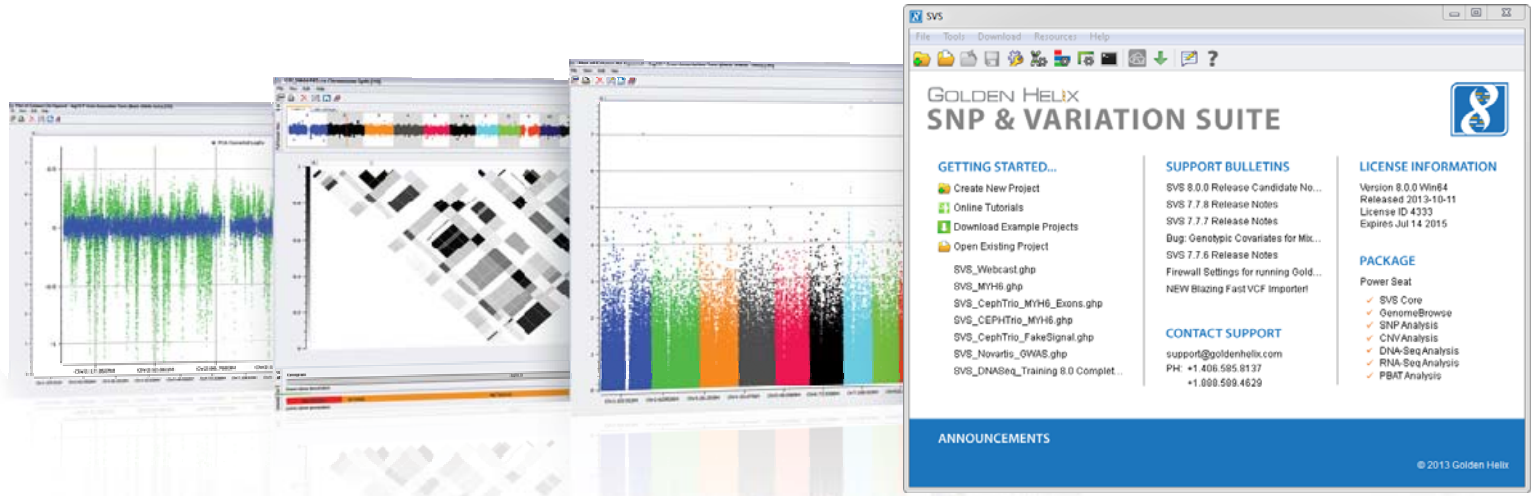
GenomeBrowse

- Free sequencing visualization tool
- Launched in 2011
- Makes the process of exploring DNA-seq and RNA-seq pile-up and coverage data intuitive and powerful
- Stream public annotations via the cloud
- Use it to validate variant calls, trio exploration, de Novo discovery, and more

Golden Helix GenomeBrowse™



SNP & Variation Suite (SVS)



Core Features

- Powerful Data Management
- Rich Visualizations
- Robust Statistics
- Flexible
- Easy-to-use

Applications

- Genotype Analysis
- DNA sequence analysis
- CNV Analysis
- RNA-seq differential expression
- Family Based Association

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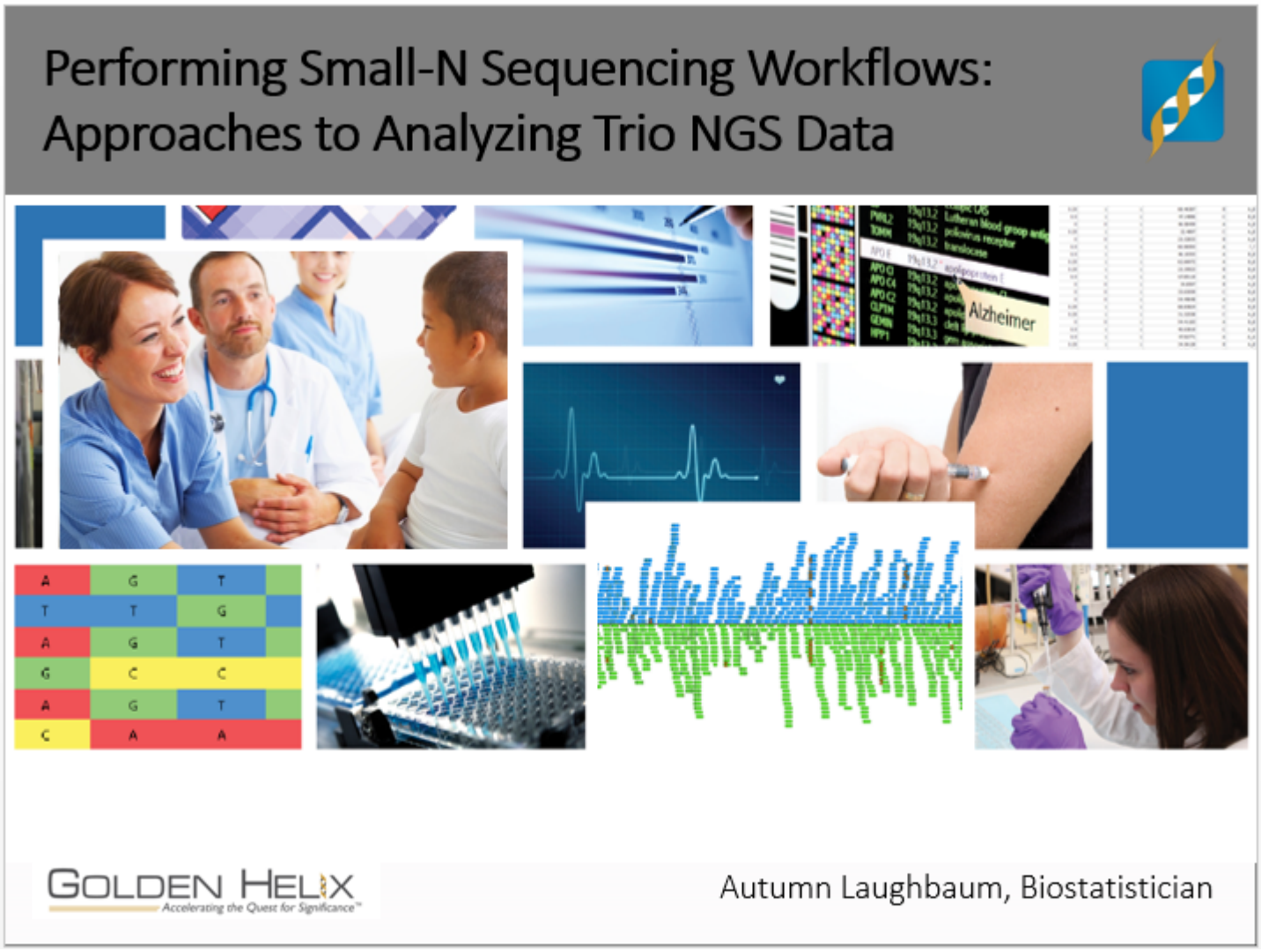
Merging of Two Great Products

Previous Webcast Recording Available



Agenda

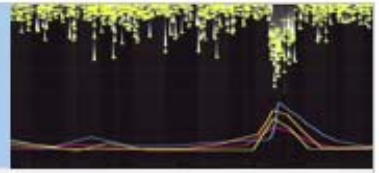
1. Define the problem: What is rare variant (RV) analysis?
2. Brief review of upstream and QC considerations
3. Overview of RV analysis approaches
4. NGS workflow design in SVS
5. Interactive software demo
   - GenomeBrowse
   - SVS 8: Exploratory tools, Analysis workflows
6. What about exome chips?

The Problem

- Array-based GWAS has been the primary technology for gene-finding research for most of the past decade
  - Common variant – common disease hypothesis
- NGS technology, particularly whole-exome sequencing, makes it possible to include rare variants (RVs) in the analysis
- Individual RVs lack statistical power for standard GWAS approaches
  - How do we utilize that information?
- Proposed solution: combine RVs into logical groups and analyze them as a single unit
  - AKA “Collapsing” or “Burden” tests.

From the Vault:
January 2011 Slide on RV Analysis

Analysis of Sequence Data



What have we learned since then?

Sequence annotations are very important

NGS Analysis

Primary Analysis	▪ Analysis of hardware generated data, on-machine real-time stats. ▪ Production of sequence reads and quality scores ▪ Typical product is "**FASTQ**" file
Secondary Analysis	▪ Recalibrating, de-duplication, QA and clipping/filtering reads ▪ Alignment/Assembly of reads ▪ Variant calling on aligned reads ▪ Typical products are "**BAM**" and/or "**VCF**" files
Tertiary Analysis **"Sense Making"**	▪ QA and filtering of variant calls ▪ Annotation and filtering of variants ▪ Multi-sample integration ▪ Visualization of variants in genomic context ▪ Experiment-specific inheritance/population analysis ▪ "Small-N" and "Large-N" approaches

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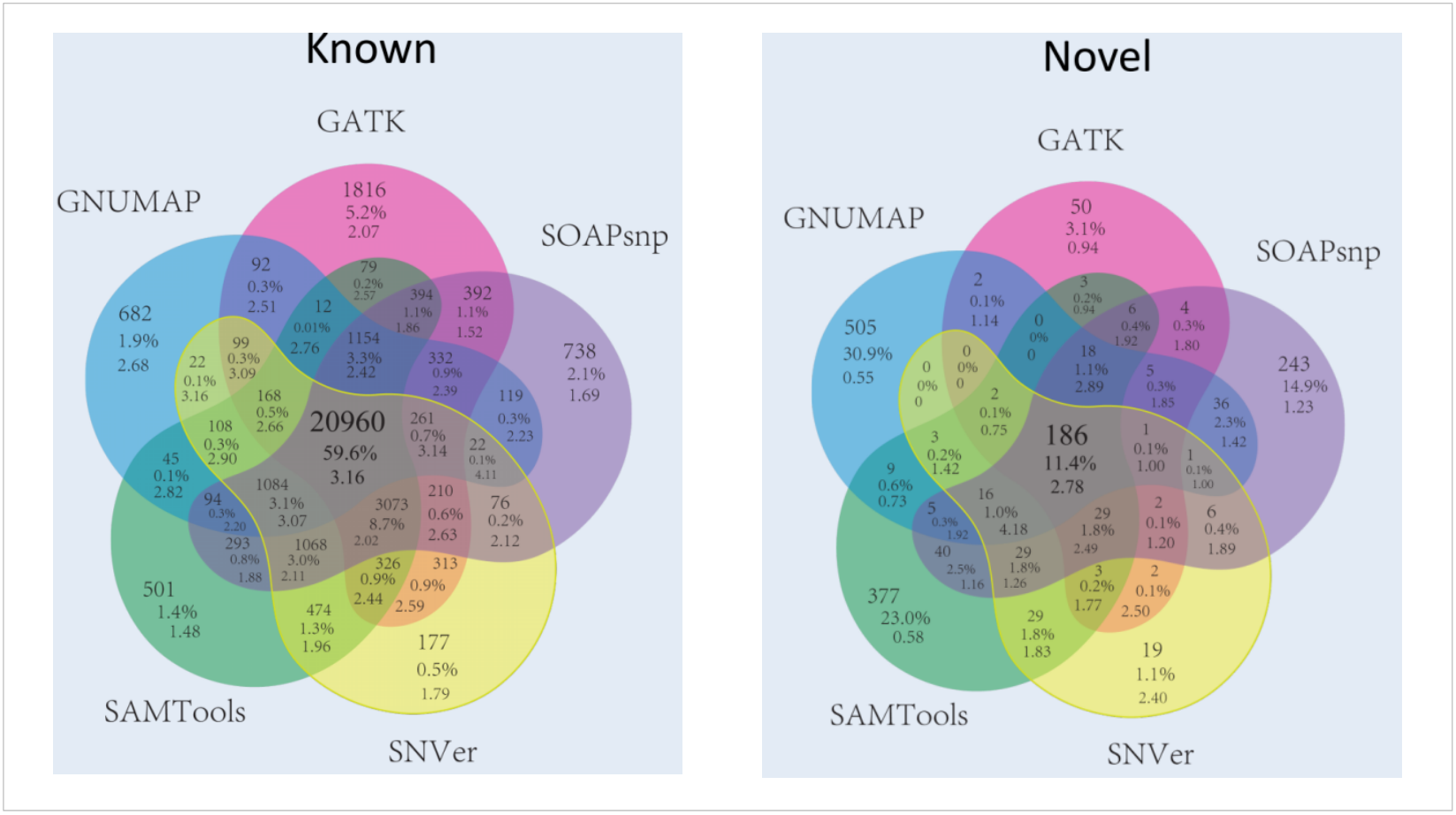
Most Importantly: Be Consistent!



Gholson Lyon, 2012

Things That Can Confound Your Experiment

Library preparation errors	Sequencing errors	Analysis errors
▪ PCR amplification point mutations (e.g. TruSeq protocol, amplicons) ▪ Emulsion PCR amplification point mutations (454, Ion Torrent and SOLiD) ▪ Bridge amplification errors (Illumina) ▪ Chimera generation (particularly during amplicon protocols) ▪ Sample contamination ▪ Amplification errors associated with high or low GC content ▪ PCR duplicates	▪ Base miscalls due to low signal ▪ InDel errors (particular PacBio) ▪ Short homopolymer associated InDels (Ion Torrent PGM) ▪ Post-homopolymeric tract SNPs (Illumina) and/or read-through problems ▪ Associated with inverted repeats (Illumina) ▪ Specific motifs particularly with older Illumina chemistry	▪ **Calling variants without sufficient reads mapping** ▪ Bad mapping (incorrectly placed read) ▪ **Correctly placed read but InDels misaligned** ▪ **Multi-mapping to paralogous regions** ▪ Sequence contamination e.g. adaptors ▪ Error in reference sequence ▪ Alignment to ends of contigs in draft assemblies ▪ Incorrect trimming of reads, aligning adaptors ▪ Inclusion of PCR duplicates

Nick Loman: Sequencing data: I want the truth! (You can't handle the truth!)
Qual et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genomics. 2012 Jul

Secondary Analysis and QC Considerations

- **What did we do in GWAS?**
  - Call rate
  - HWE
  - MAF
  - But those aren't really applicable for NGS/RV analysis…
- **What do we use for NGS?**
  - Coverage depth
  - Singleton counts
  - Ts/Tv ratios
  - Quality scores per variant and per genotype call
  - Mappability of the region

NGS Analysis

Primary Analysis

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Tertiary Analysis

"Sense Making"

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- Visualization of variants in genomic context
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- "Small-N" and "Large-N" approaches

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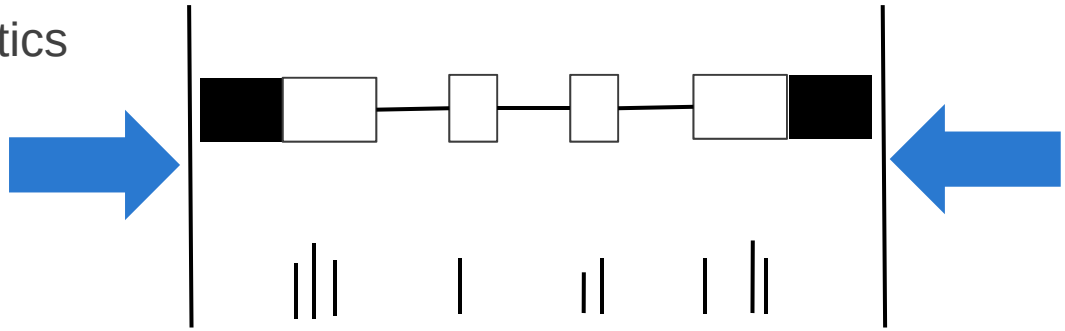
Two Primary Approaches

- **Direct search for susceptibility variants**
  - Assume highly penetrant variant and/or Mendelian disease
  - Extensive reliance on bioinformatics for variant annotation and filtering
  - Sample sizes usually small—from single case up to nuclear families
- **Rare Variant (RV) "collapsing" methods**
  - More common in complex disease research
    - May require very large sample sizes!
  - Assume that any of several LOF variants in a susceptibility gene may lead to same disease or trait
  - Many statistical tests available
  - Also relies heavily on bioinformatics



Families of Collapsing Tests

- **Burden Tests**
  - Combine minor alleles across multiple variant sites…
    - Without weighting (**CMC**, CAST, CMAT)
    - With fixed weights based on allele frequency (WSS, RWAS)
    - With data-adaptive weights (Lin/Tang, **KBAC**)
    - With data-adaptive thresholds (Step-Up, VT)
    - With extensions to allow for effects in either direction (Ionita-Laza/Lange, C-alpha)
- **Kernel Tests**
  - Allow for individual variant effects in either direction and permit covariate adjustment based on kernel regression
    - Kwee et al., *AJHG,* 2008
    - SKAT
    - SKAT-O

Credit: Schaid et al., *Genet Epi*, 2013

CMC: Combined Multivariate and Collapsing

- Multivariate test: simultaneous test for association of common and rare variants in gene
- Flexibility in variant frequency bin definition
- Testing methods include Hotelling T^2 and Regression
- Regression method allows for covariate correction
- Li and Leal, *AJHG*, 2008

KBAC: Kernel Based Adaptive Clustering

- Per-gene tests models the risk associated with multi-locus genotypes at a per-gene level
- Adaptive weighting procedure that gives higher weights to genotypes with higher sample risks
  - Meant to attain good balance between classification accuracy and the number of estimated parameters
- SVS implementation includes option for 1- or 2-tailed test
  - But most powerful when all variants in gene have unidirectional effect
- Permutation testing or regression options
  - Regression allows for covariate correction
- Liu and Leal, *PLoS Genetics*, 2010

SKAT: Sequence Kernel Association Test

- Utilizes kernel machine methods
- Aggregates test statistics of SNPS over gene region to compute region level p-values
- Many extensions of the method
- “This method can be more powerful when causal variants have bidirectional effects and/or a large proportion of the variants within gene region are non-causal.”
- “SKAT is less powerful than burden tests when causal variant effects are unidirectional.”
  - Liu and Leal, *PLoS Genetics,* 2012

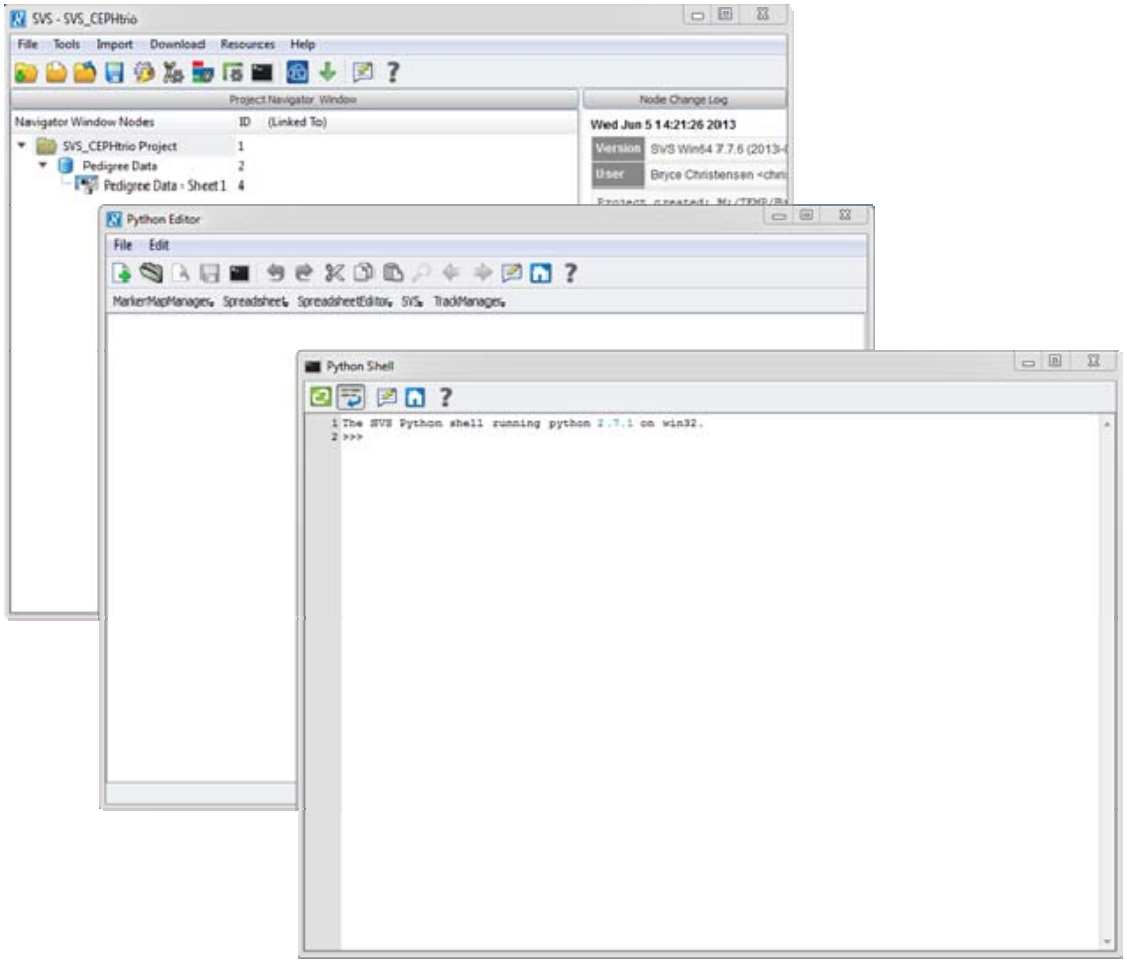
Bioinformatic Filtering and Annotation

- The genomics community has spent years producing vast resources of data about DNA sequence variants
  - Some data is observational, like variant frequencies from the 1000 genomes project or the NHLBI Exome Sequencing Project
  - Other data is based on predictive algorithms, like PolyPhen or SIFT.
  - Even “simple” annotations, like mapping data for genes, segmental duplications and other sequence features are extremely valuable for analytic workflows.
- These data sources can be used to annotate variants identified in an NGS experiment
  - Annotations may be used for both QC and analysis purposes.
- Once annotated, variants may be filtered, sorted, and prioritized to help us identify disease-causing mutations

NGS Analysis Workflow Development in SVS

- SVS is very flexible in workflow design.
- SVS includes a broad range of tools for data manipulation and variant annotation and visualization that can be used together to guide us on an interactive exploration of the data.
- We begin by defining the final goal and the steps needed to help us reach that goal:
  - Are we looking for a very rare, non-synonymous variant that causes a dominant Mendelian trait?
  - Are we looking for a gene with excess rare variation in cases vs controls?
- Once we know what we are looking for, we can identify the available annotation sources that will help us answer the question.

Python Integration in SVS



- Allows rapid development and iteration of new functions
- API access to most SVS functions
- Access to extensive Python analytic libraries
- Fully documented in manual

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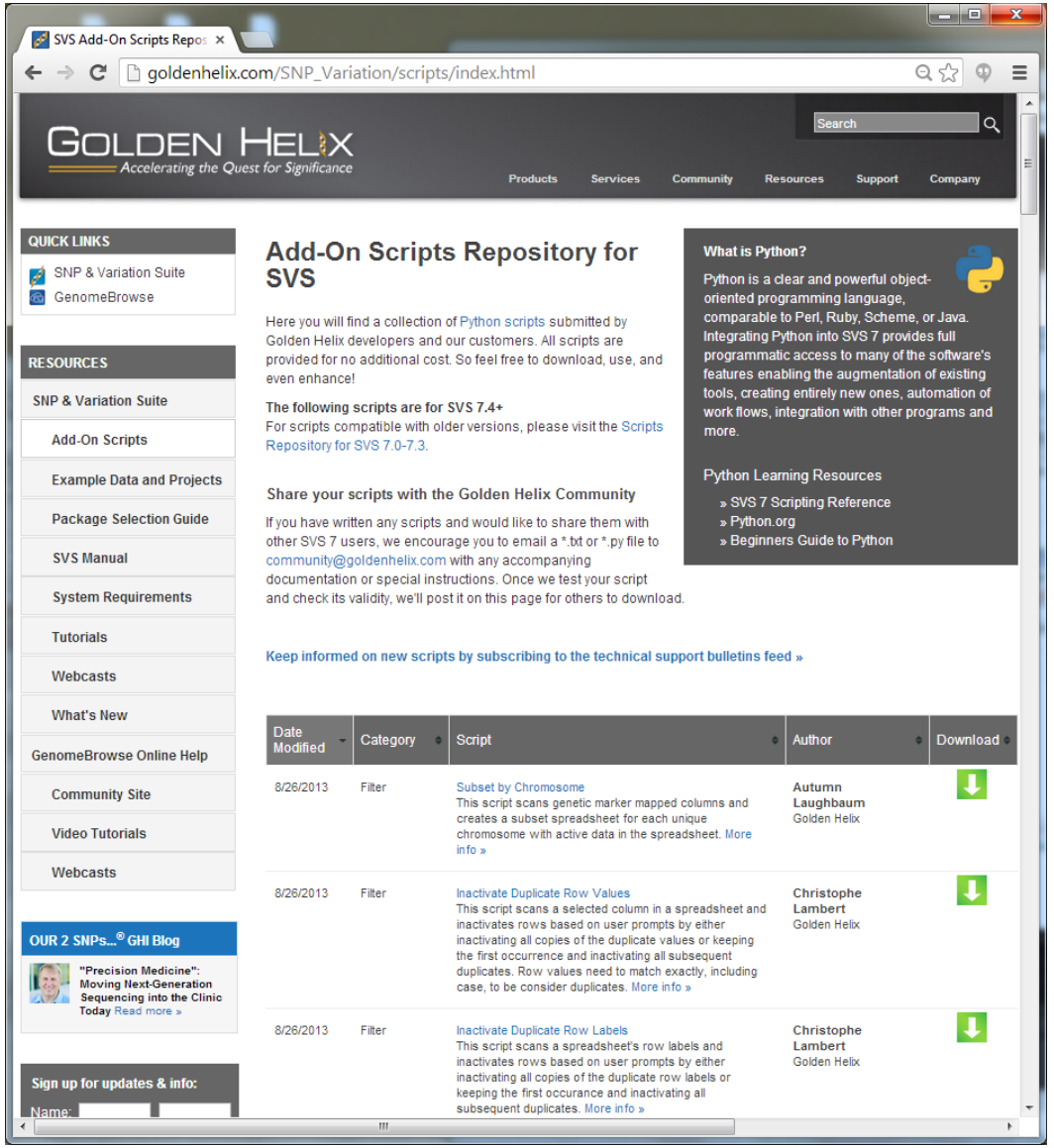
SVS Online Scripts Repository

- Downloadable add-on functions for a variety of analysis and data management tasks
- “Plug-and-play”
- Some contributed by customers
- Popular scripts often get adopted into the “shipped” version of SVS.
- Scripts in repository are forward compatible to SVS 8.0



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Today's Featured Scripts

- **Activate Variants by Genotype Count Threshold**
  - Identify variants that occur with a specified frequency in one or several groups
- **Filter by Marker Map Field**
  - Variant-level "INFO" fields from VCF files are imported to the SVS marker map
  - This script allows you to filter markers based on those variables
- **Many more useful scripts to take a look at:**
  - Add Annotation Data to Marker Map from Spreadsheet
  - Nonparametric association tests
  - Import Unsorted VCF Files
  - Build Variant Spreadsheet
  - Many, many more

Interactive Demonstration

- **GenomeBrowse**
  - Exploring multi-sample VCF files in our free genome viewer software
- **SVS 8.0**
  - Exploratory analysis workflow
    - Using downloaded scripts
    - Using basic analysis tools to create advanced workflows
    - Simulate the development of a burden test
  - RV association testing workflow
    - KBAC
    - CMC
    - Data visualization

SVS Demo

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What about Exome Chips?

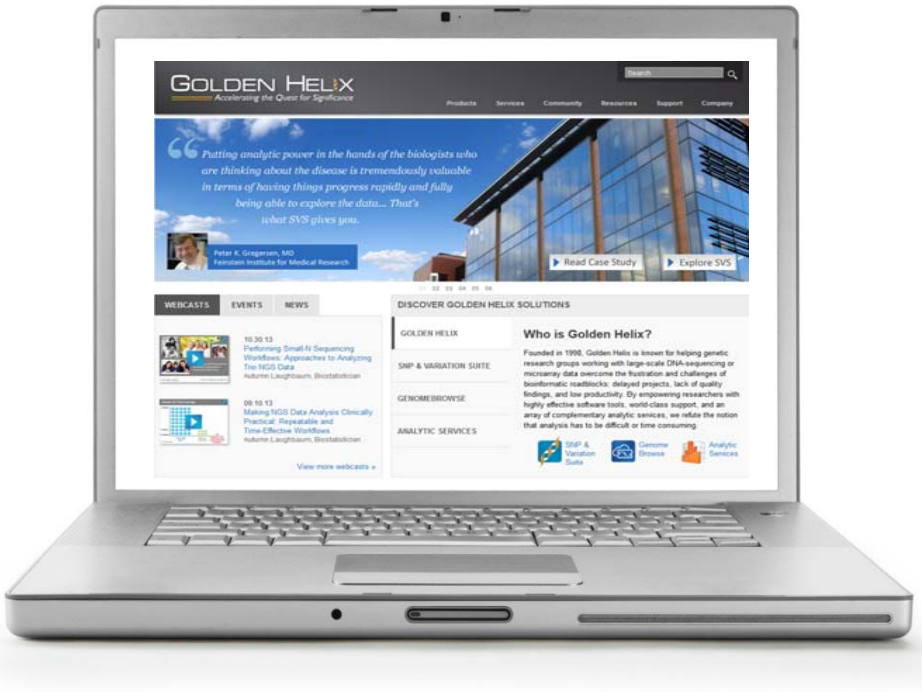
- Exome chips CAN be used with RV association tests
- Exome chips include both common and rare variants
- Remember: Exome chips don’t capture all rare variants.
- Exome chips are thus less powerful than WES for RV associations, but also significantly cheaper.

A Note about Exome Chips

- **Exome chips are not GWAS chips**
  - GWAS chips focus on common SNPs, have uniform spacing, minimal LD and are designed to capture population variability
  - Exome chips include rare variants and the content is anything but uniform
- **Most GWAS functions can be used with exome chips, but require some workflow adjustments**
  - Gender checking
  - IBD estimation
  - Principal components
- **Not unlike other chips with custom/targeted content**
  - Cardio-MetaboChip
  - ImmunoChip

Questions or more info:

- info@goldenhelix.com
- Request a copy of SVS at www.goldenhelix.com
- Download GenomeBrowse for free at www.GenomeBrowse.com



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Any Questions?

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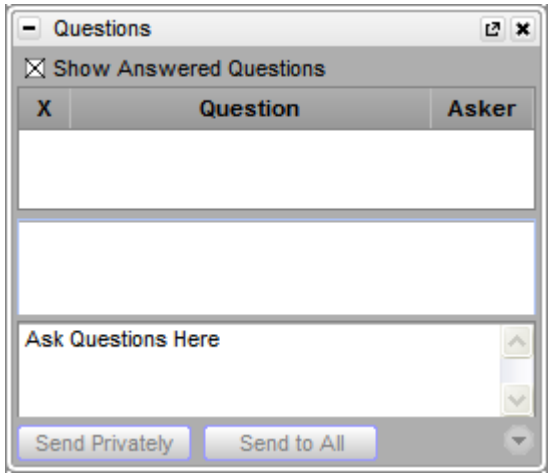

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