1000 Genomes data tutorial at ASHG

Structural variants

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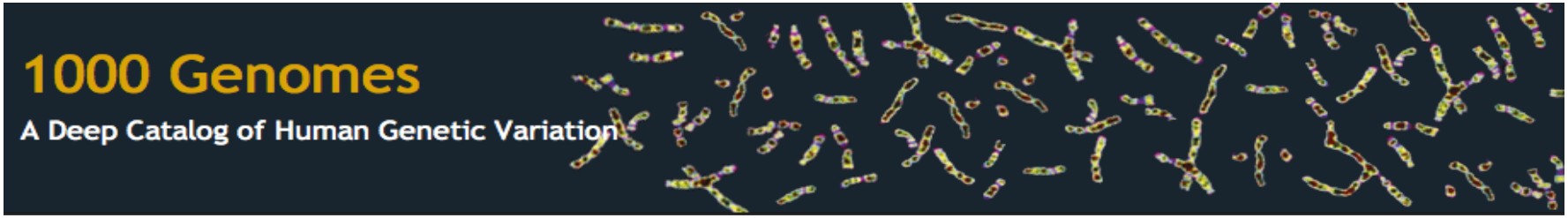
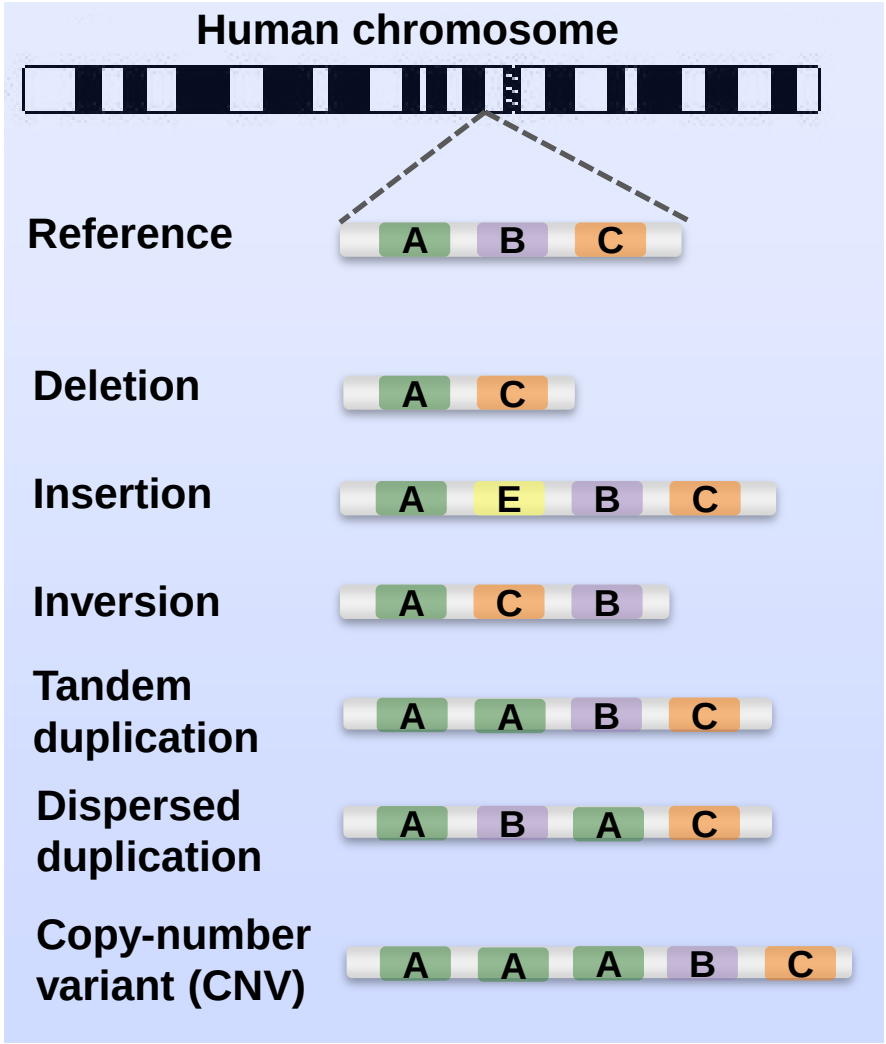


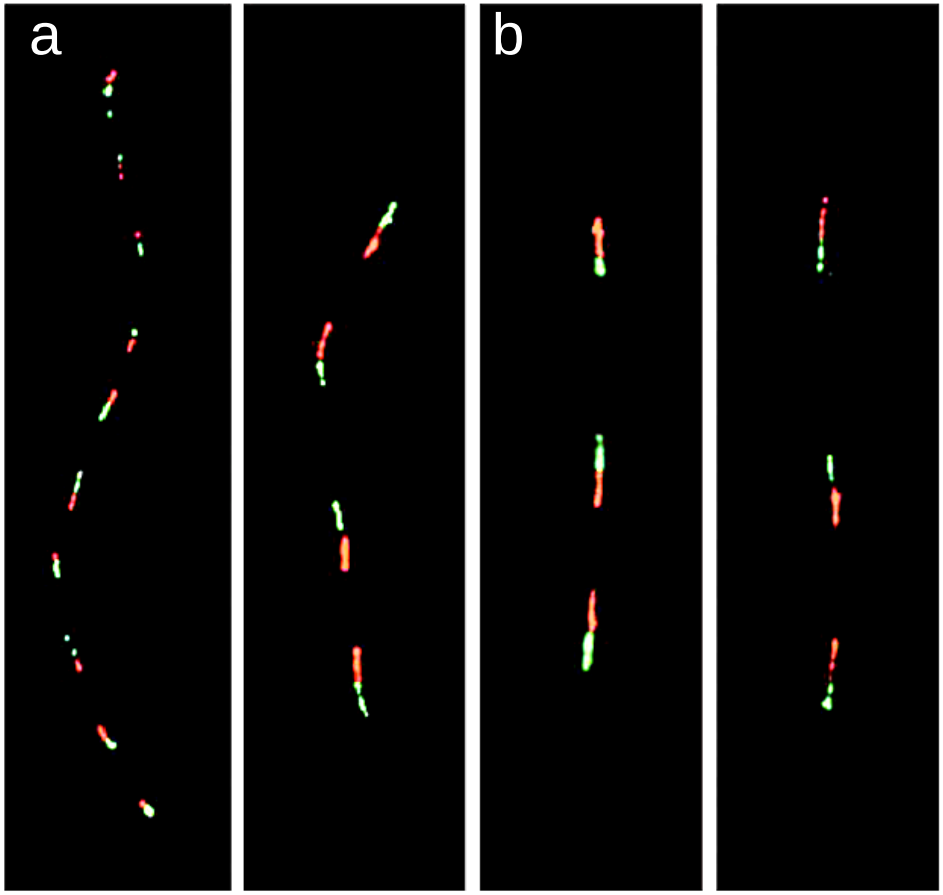
Structural variants (SVs) in the genome

[polymorphic rearrangements of the genome of 50bp up to hundreds of kb in size]



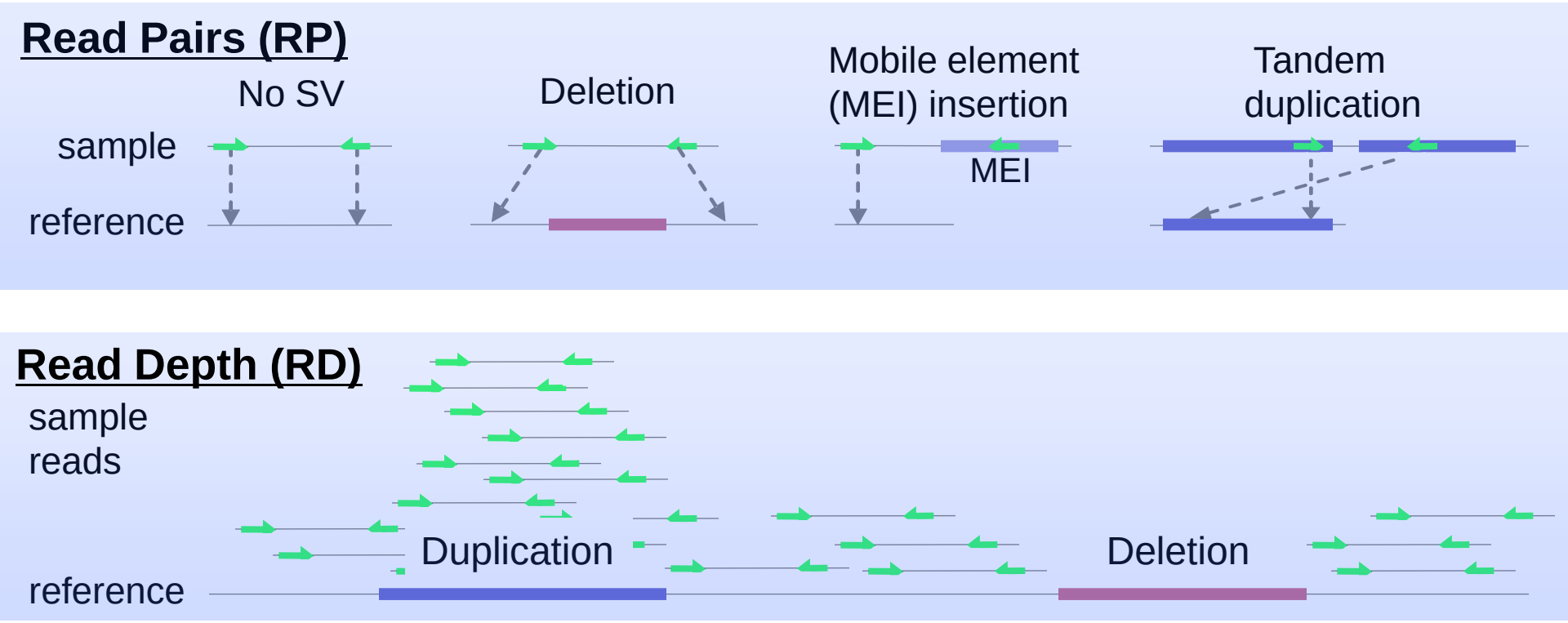
~0.5% of the genome affected by SVs in each individual

Example: *AMY1* copy-number variation

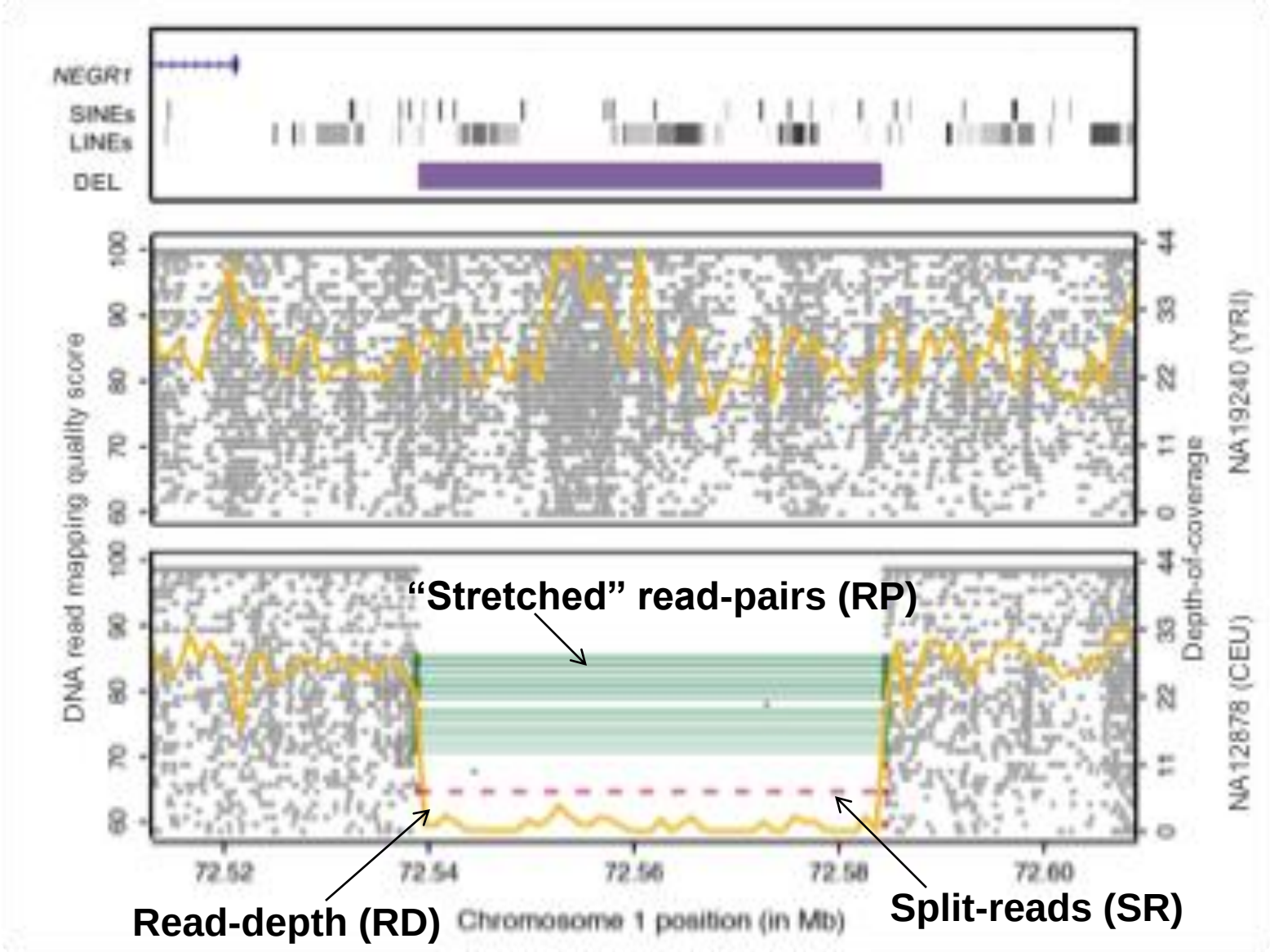


a, Japanese; **b**, African (Biaka) individual
[Perry *et al.*, *Nat. Genet.* 2007]

SV discovery considering evidence from multiple sources

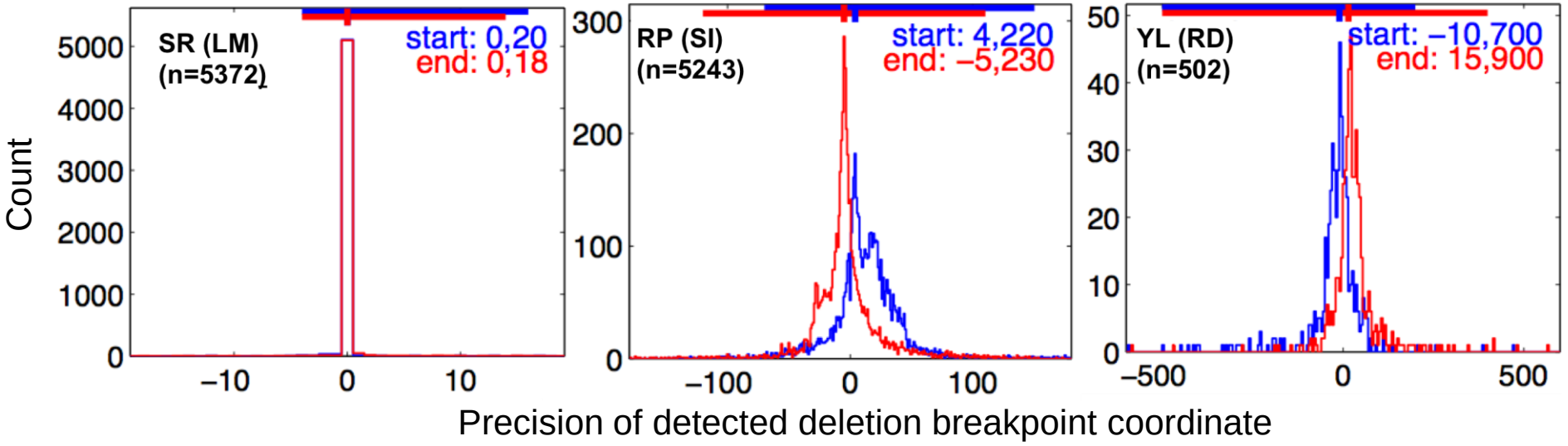


A deletion simultaneously detected by paired-end mapping (PEM), read-depth analysis, and split reads



Klaudia Walter

Ascertainment differences among deletion discovery methods: SV breakpoint precision



Blue and red histograms: breakpoint residuals for predicted start/end coordinates relative to assembled coordinates. Horizontal lines at the top of each plot mark the 98% (2.3 sigma) confidence intervals.

Chip Stewart

Individuals analyzed in the pilot 1 (low-coverage) and pilot 2 (trio) studies of the 1000 Genomes Project

	Trios	Low coverage
Samples	6	179
Raw data	1.08Tbp	2.22Tbp
Deletions	11,248	15,893
Mobile element insertions	2,531	4,775
Tandem Duplications	256	407
Novel sequence insertions	174	-
SV breakpts	6,169	9,092

Deletion genotypes from the 1000 Genomes Project

- 13,826 deletion polymorphisms (48 bp – 960 kb) genotyped in 156 genomes using Genome STRiP (Handsaker et al., manuscript submitted)
- Concordance with array-based genotypes: **99.1%** (for 1,970 deletions from Conrad et al., 2009)

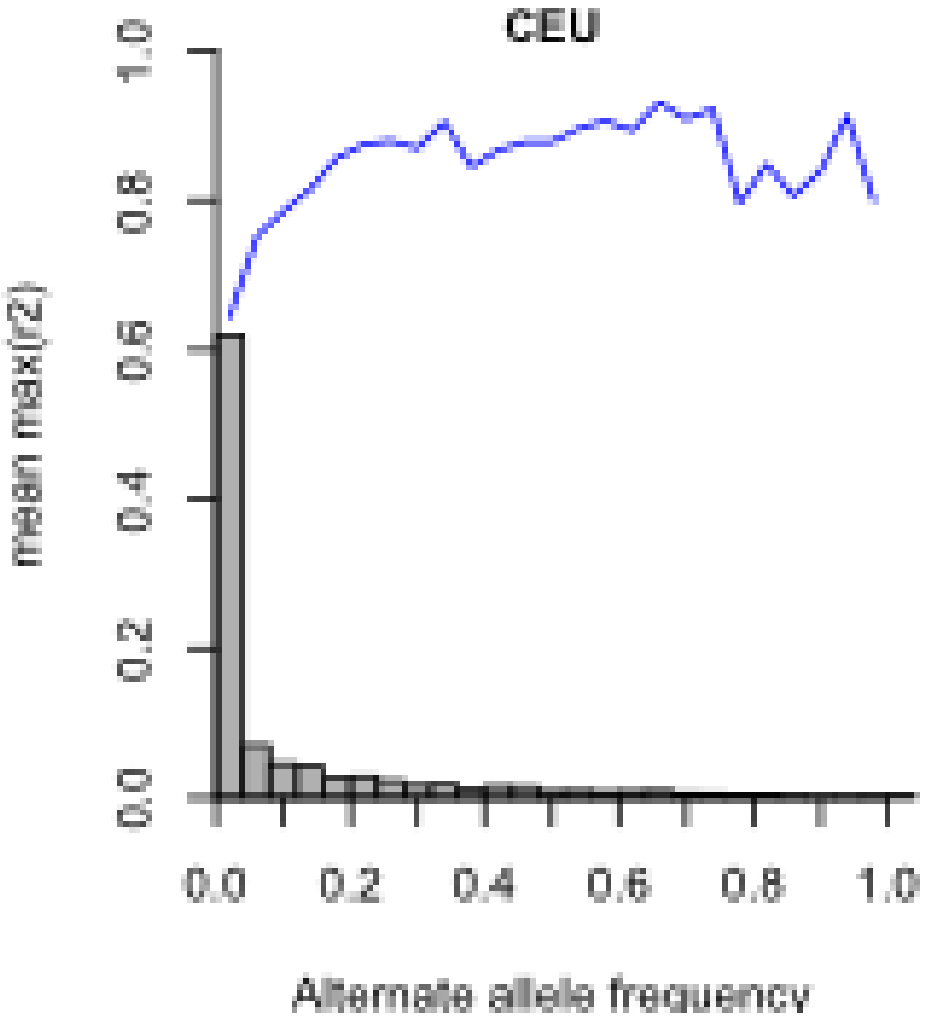
Genome STRiP integrates multiple features of sequencing data

- Read depth
- Read pairs
- Split reads

Bob Handsaker

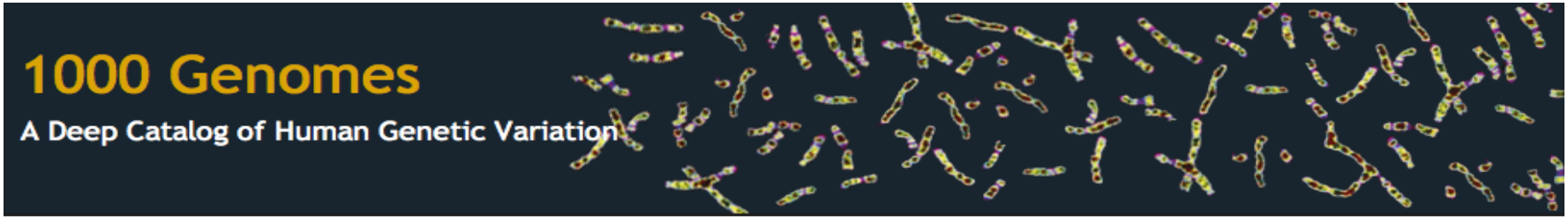
Deletions and SNPs on shared haplotypes

LD between 1000 Genomes deletions and HapMap3 SNPs



81% of common deletions are tagged by one or more HapMap SNPs ($r^2 > 0.8$)

Bob Handsaker



Data formats

SV Pilot Paper Data Release

(for 1000 Genomes Project pilot 1 [low-coverage] and pilot 2 [trios])

- **SV data is available as different formats, providing different levels of detail**

  (1) Variant Call Format (VCF) – Primary
  - Contains SV discovery (release) set and deletion genotypes
  - Standardized format (version 4.0)

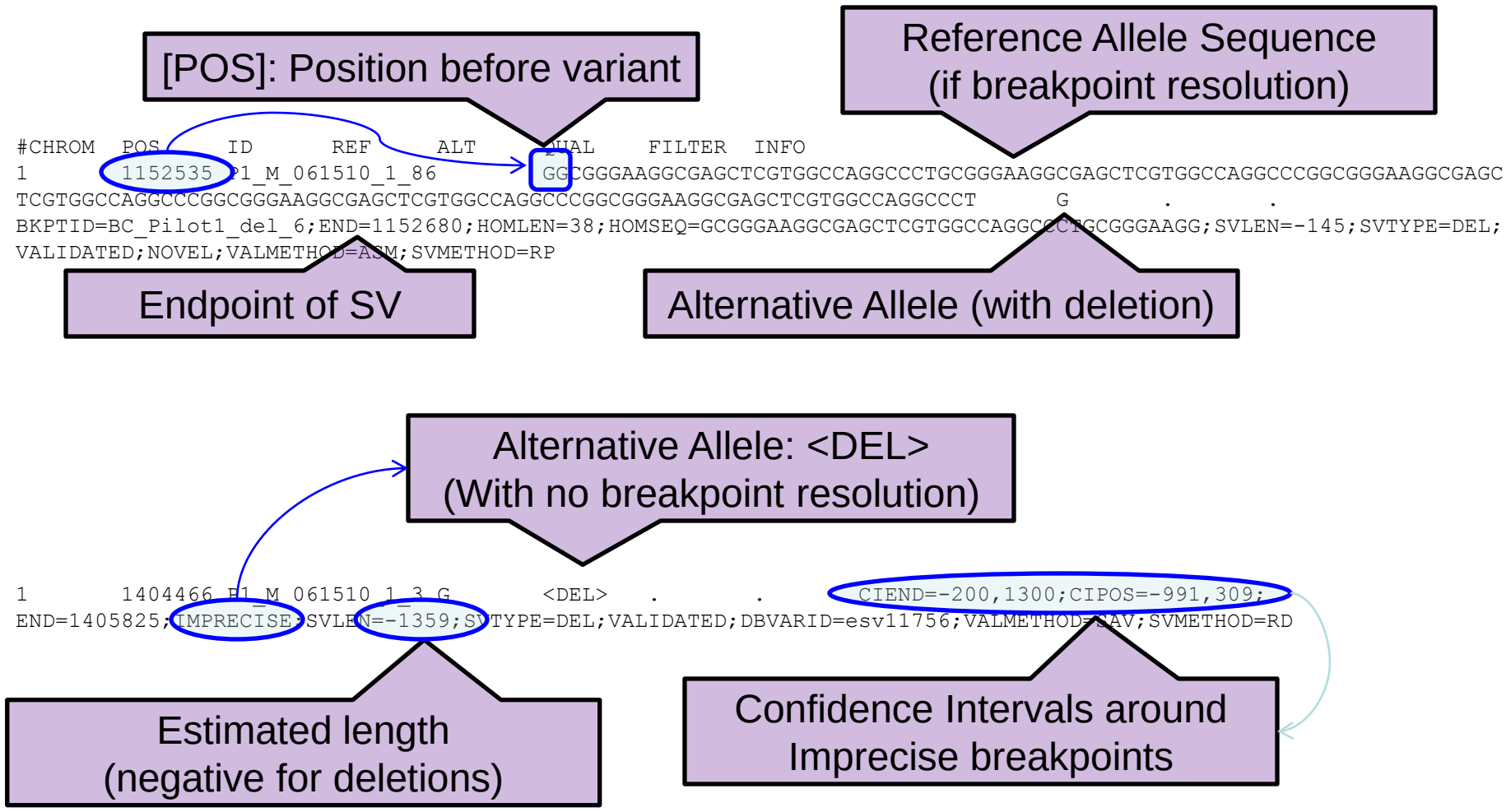
  (2) Master Validation Format (MVT) – Auxiliary
  - Raw data from individual SV discovery methods
  - Includes additional information regarding validation and original SV coordinate predictions

  (3) SV breakpoint information available as textfiles – Auxiliary
  - SV breakpoint junctions generated by the TIGRA targeted assembly algorithm (FASTA format)
  - BreakSeq annotations available (mechanism & ancestral state for SVs with assembled breakpoints) in GFF format

SV discovery set as VCF format

- Accessible as tab-delimited files
  - These can be converted into **Excel** spreadsheets
  - They can also be processed with **vcftools**: http://vcftools.sourceforge.net/
  - **PERL** module (Vcf.pm), also available through vcftools
- Format
  - #CHROM POS ID REF ALT QUAL FILTER INFO
  - [POS] is the position ***before*** the variant
  - [ID] links the variant to the original SV discovery method and callset (SV master validation tables)
  - `[REF]` and `[ALT]` show exact sequence if breakpoints are known, otherwise a variant-specific tag is usd: (<DEL>, <DUP:TANDEM>, <INS:ME:ALU>, <INS:ME:L1>, <INS:ME:SVA>)
  - [INFO] contains various information including [END] as the SV end coordinate

Example VCF Records for SVs

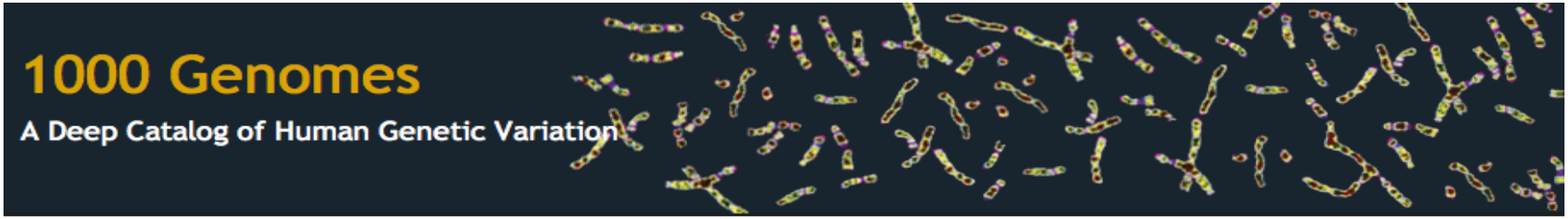


Master Validation Tables

- Contains all reported SVs in standardized format for each individual algorithm
  - May find SVs in particular regions of interest which may be real but did not meet our stringent criteria (FDR <10%) for inclusion in release set.
- Reports specific validation results for each call
  - e.g., whether a call was validated by PCR, arrays, or sequence assembly
- Contains other meta information not found in VCF files
  - e.g. sequence technology and mapping algorithm used, assembled breakpoint sequences
- Particular fields of interest (see readme for more information)
  - [SAMPLES]: Which samples SV was originally discovered in
  - [SEQUENCE_TECHNOLOGY]: Sequencing platform used to make call
  - [MAPPING_ALGORITHM]: Mapping algorithm used to map reads to reference
  - [*_VALIDATION_*]: Results from various validation experiments

Master Validation Table Format

- Complete call sets with validation information
  - Tab-delimited Files
    - MasterValidation.Pilot1.all.leftmost.061510.txt
    - MasterValidation.Pilot2.all.leftmost.061510.txt
  - Assembled Breakpoints (if available)
    - MasterValidation.Pilot1.deletion.061510a.assembly.fasta
    - MasterValidation.Pilot2.deletion.061510a.assembly.fasta
- Merged call sets with refined breakpoint information
  - Similar format as complete call set files
  - [MERGED_ID] consistent with VCF [ID] field
  - [ID] column links back to complete call set files
  - MasterValidation.Pilot1.deletion.leftmost.061510a_mergedValPlus.txt
  - MasterValidation.Pilot2.deletion.leftmost.061510a_mergedValPlus.txt



Information on ancestral state of SVs and of formational mechanism involved

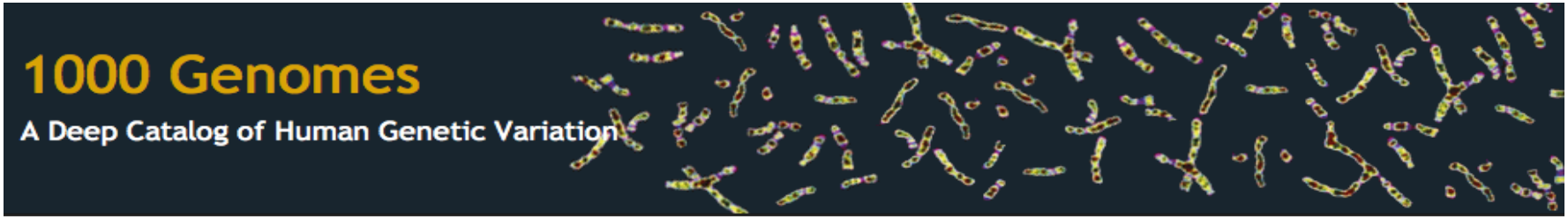
[inferred with the BreakSeq algorithm; Lam *et al.*, *Nat. Biotechnol.,* 2010]

BreakSeq's GFF Format

#	Column	Description	Example
1	**seqname**	Chromosome	chrX
2	**source**	Source name	Yale
3	**feature**	Event type (Insertion/Deletion/Inversion)	Insertion
4	**start**	Start coordinate	13330
5	**end**	End coordinate	13331
6	**score**	<EMPTY>	.
7	**strand**	<EMPTY>	.
8	**frame**	<EMPTY>	.
9	**additional attributes**	Inserted sequence* ID Mechanism (e.g., non-allelic homologous recombination) Ancestral State of SVs (discriminates deletions from insertions)	Iseq “AATTGGGGCCTATAGTCCA”; Id “LIB000001”; Mech “NAHR”; Ancestral “Deletion”; etc

* for insertion; inserted sequences can be stored in a separate FASTA file

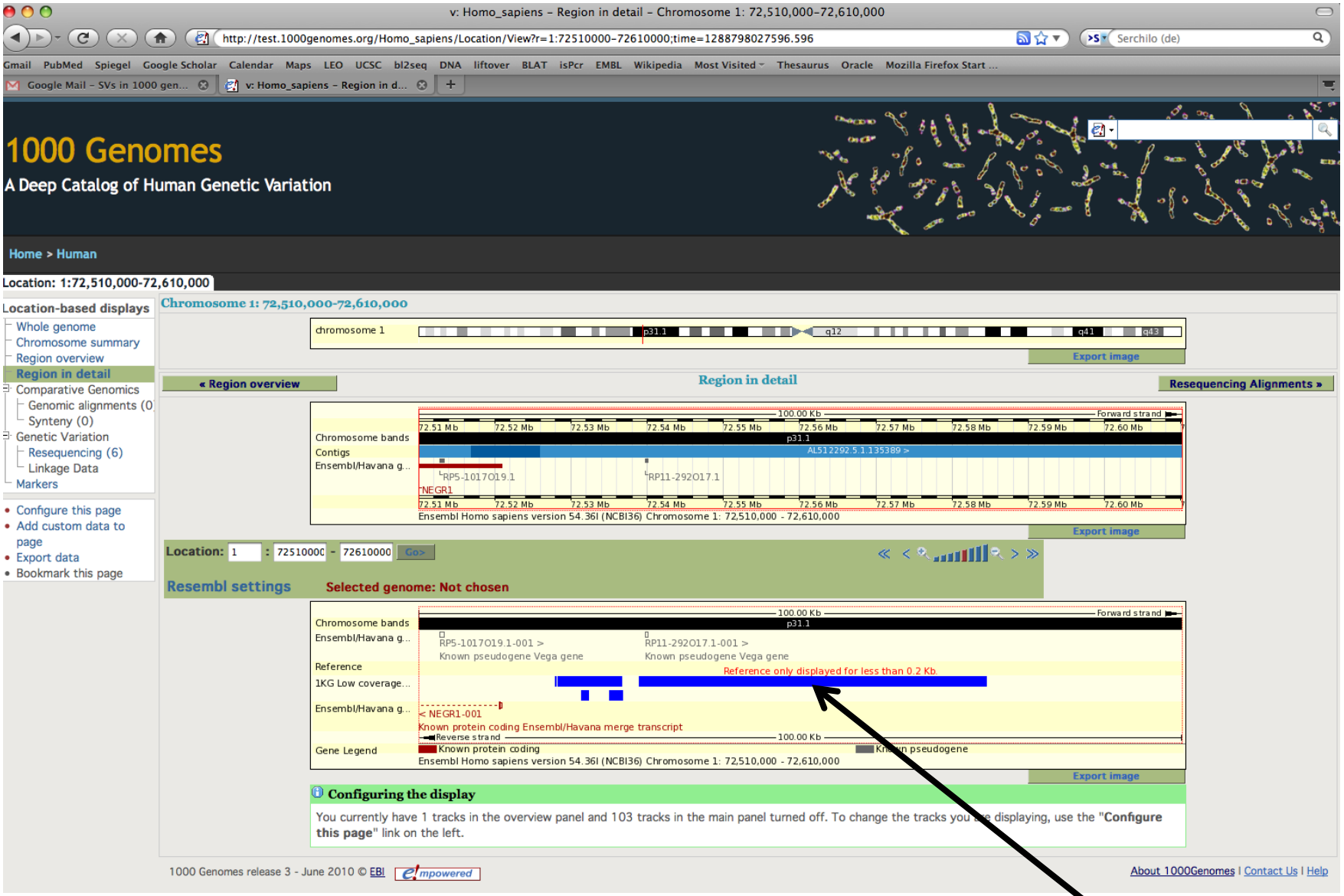
Hugo Lam, Jasmine Mu



Displaying SVs in the 1000 Genomes Browser

[presently available for deletions]

Example: deletion displayed on the 1000 Genomes Browser



Imputing deletions into GWAS

- These deletions can be imputed into GWAS using existing tools (Beagle, MACH, etc.)
- Data availability

  http://www.1000genomes.org

  - Genotypes in VCF file format (Danacek *et al.*, manuscript submitted)
  - Genotype calls at 95% confidence
  - Genotype likelihoods for imputation

Further Information & Data Links

- 1000 Genomes Pilot Project SV Release Data & Readme files (links)
  - ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/paper_data_sets/a_map_of_human_variation/low_coverage/sv/
  - ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/paper_data_sets/a_map_of_human_variation/trio/sv/

- Link to auxiliary master validation tables & breakpoint assembly/analysis tables
  - ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/pilot_data/technical/working/20100916_paper_data/companion_papers/mapping_structural_variation/

→ FTP directories contain readme files

- More information on the description of SVs in the VCF format
  - http://vcftools.sourceforge.net/specs.html
- More information on bgzip and tabix (compression and coordinate indexing)
  - http://samtools.sourceforge.net/tabix.shtml

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Leiden Univ – Kai Ye

Co-chairs: Matthew Hurles, Evan Eichler, Charles Lee

1000 Genomes Project Structural Variation Glossary

- Structural variant (SV): deletion, duplication, or insertion (≥50bp) relative to the reference genome (NCBI build36).
- Ancestral state: inferred SV class (deletion, duplication, insertion) relative to likely ancestral genome.
- SV genotype: allelic state determination of SVs in each genome (e.g., homozygous reference allele, homozygous SV allele, heterozygous SV allele).
- SV breakpoint: boundary (start- and end-coordinate) of SV, in case of breakpoint assembly and/or split-read analysis available at nucleotide resolution.