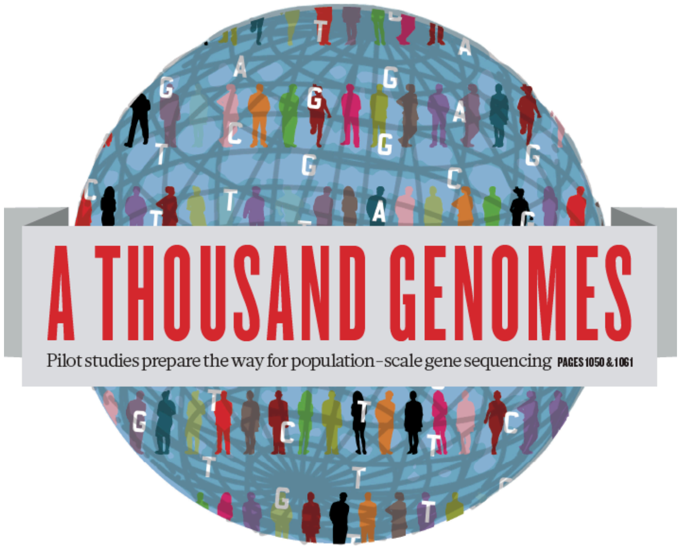

A THOUSAND GENOMES
Pilot studies prepare the way for population-scale gene sequencing PAGES 1050 & 1061

The 1000 Genomes Project Tutorial

ICHG 2011

Montreal, Quebec, Canada

October 13, 2011

- International project to construct a foundational data set for human genetics
  - Discover virtually all common human variations by investigating many genomes at the base pair level
  - Consortium with multiple centers, platforms, funders
- Aims
  - Discover population level human genetic variations of all types (95% of variation > 1% frequency)
  - Define haplotype structure in the human genome
  - Develop sequence analysis methods, tools, and other reagents that can be transferred to other sequencing projects

Agenda

Time	Topic	Presenter	Presenter affiliation
7:30	Description of 1000 Genomes data	Gabor Marth, D.Sc.	Boston College, Boston, MA
7:55	How to access the data	Paul Flicek, D.Sc.	EMBL European Bioinformatics Inst., Hinxton, Cambridge, UK
8:20	Lessons in variant calling and genotyping	Hyun Min Kang, Ph.D.	Univ. of Michigan, Ann Arbor, MI
8:40	Structural variants	Ryan Mills, Ph.D.	Brigham and Women's Hospital, Boston, MA
9:00	Imputation in GWAS studies	Bryan Howie, Ph.D.	Univ. of Chicago, Chicago, IL
9:20	Q&A	-	-

The 1000 Genomes Project Datasets

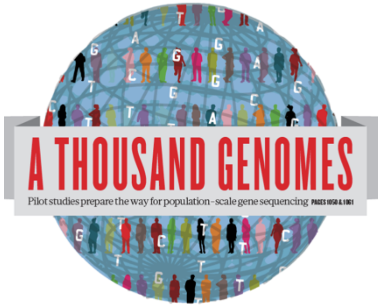
Gabor T. Marth

Boston College Biology Department

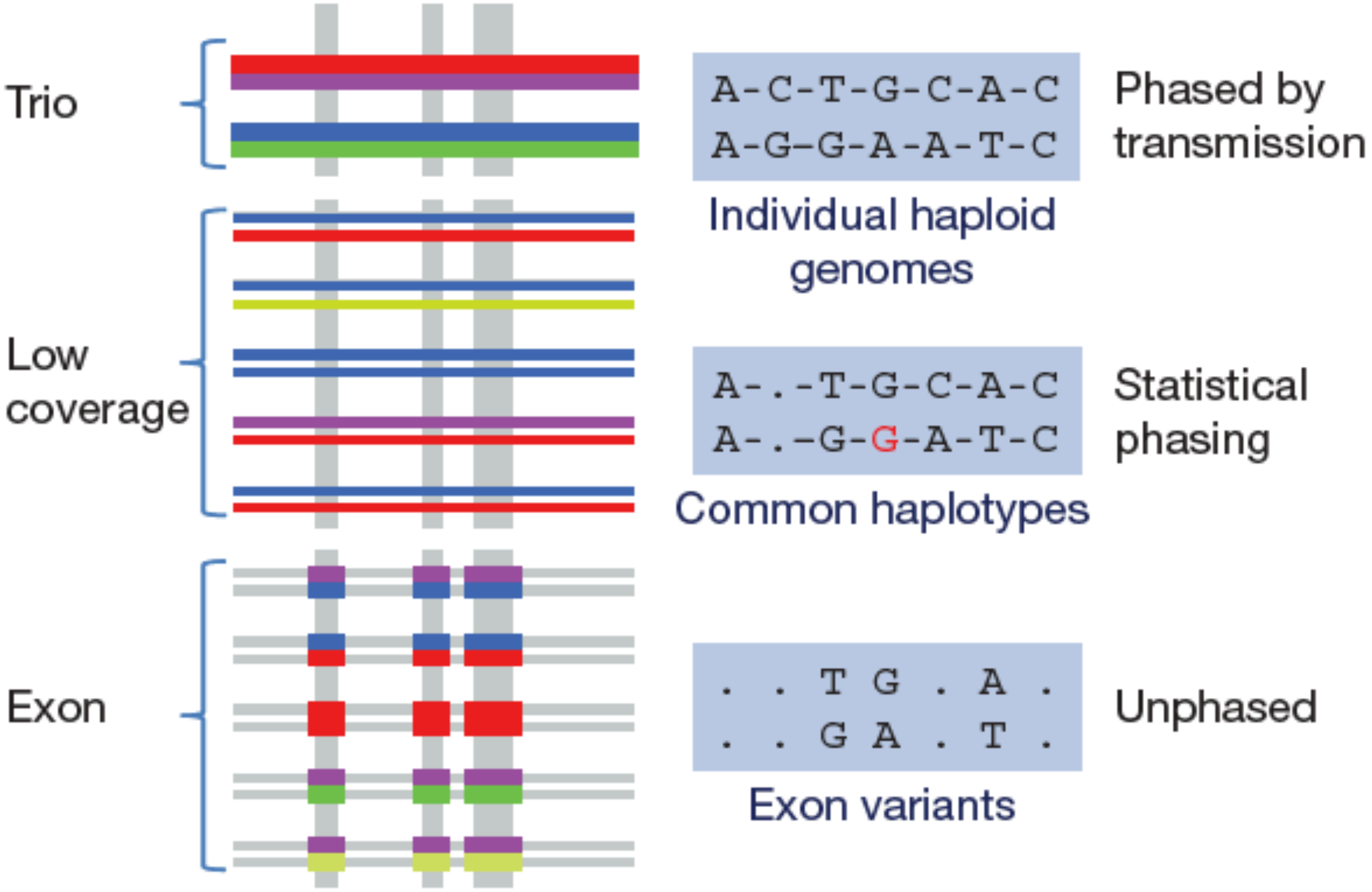
1000 Genomes Project Tutorial

Montreal, Quebec, Canada

October 13, 2011


A THOUSAND GENOMES
Pilot studies prepare the way for population-scale gene sequencing PAGES 1050 & 1061

3 pilot coverage strategies



Trio

A-C-T-G-C-A-C
A-G-G-A-A-T-C

Phased by transmission

Individual haploid genomes

Low coverage

A-.-T-G-C-A-C
A-.-G-G-A-T-C

Statistical phasing

Common haplotypes

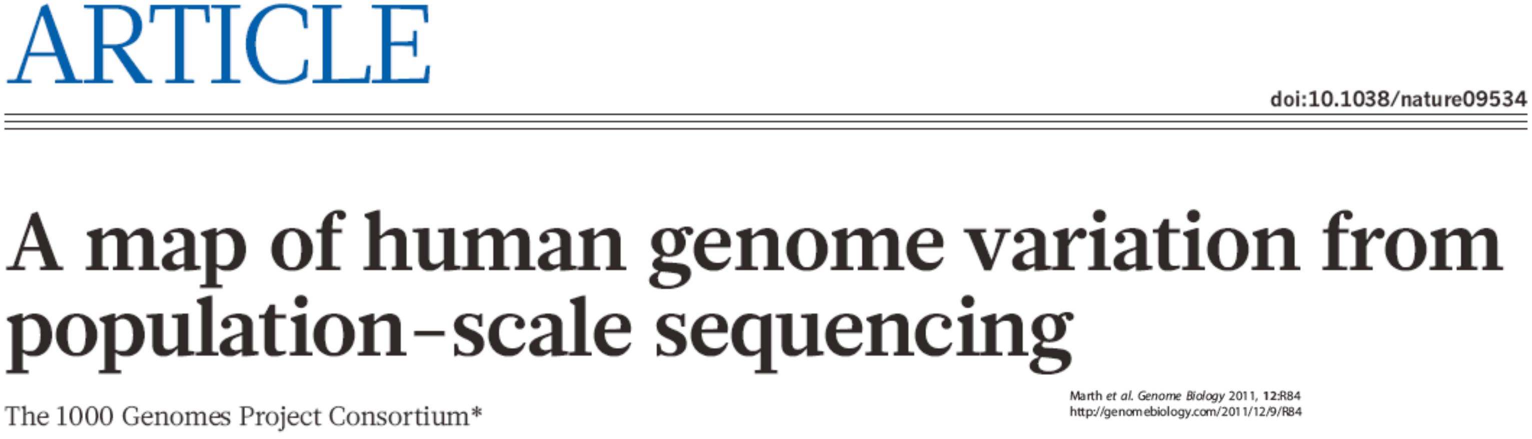
Exon

. . T G . A .
. . G A . T .

Unphased

Exon variants

Pilot results published



ARTICLE

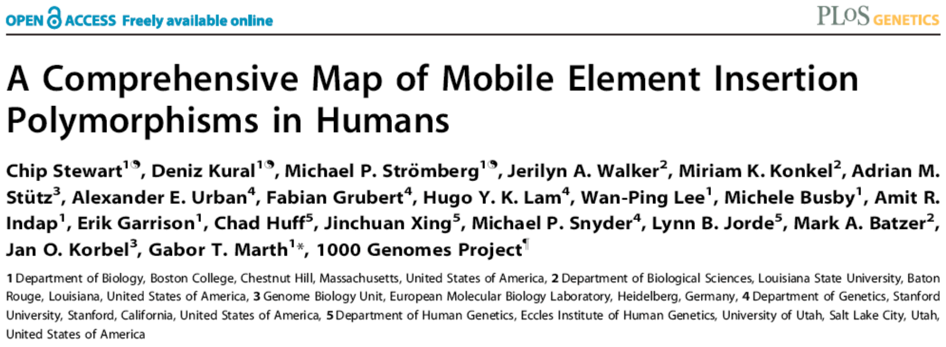
doi:10.1038/nature09534

A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium*

Marth *et al. Genome Biology* 2011, 12:R84
http://genomebiology.com/2011/12/9/R84

Genome Biology



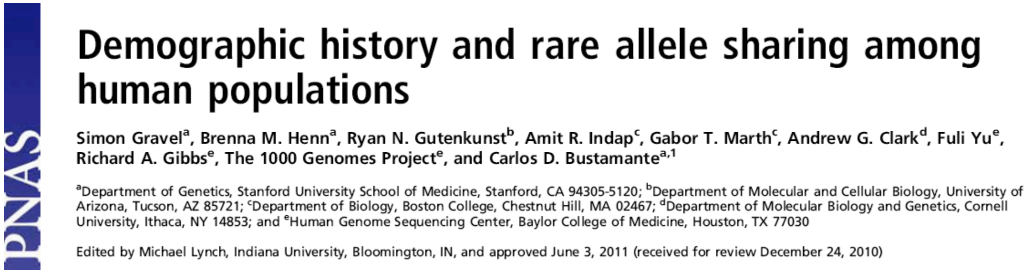
OPEN ACCESS Freely available online

PLoS GENETICS

A Comprehensive Map of Mobile Element Insertion Polymorphisms in Humans

Chip Stewart[1☯], Deniz Kural[1☯], Michael P. Strömberg[1☯], Jerilyn A. Walker[2], Miriam K. Konkel[2], Adrian M. Stütz[3], Alexander E. Urban[4], Fabian Grubert[4], Hugo Y. K. Lam[4], Wan-Ping Lee[1], Michele Busby[1], Amit R. Indap[1], Erik Garrison[1], Chad Huff[5], Jinchuan Xing[5], Michael P. Snyder[4], Lynn B. Jorde[5], Mark A. Batzer[2], Jan O. Korbel[3], Gabor T. Marth[1*], 1000 Genomes Project[¶]

1 Department of Biology, Boston College, Chestnut Hill, Massachusetts, United States of America, 2 Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, United States of America, 3 Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany, 4 Department of Genetics, Stanford University, Stanford, California, United States of America, 5 Department of Human Genetics, Eccles Institute of Human Genetics, University of Utah, Salt Lake City, Utah, United States of America



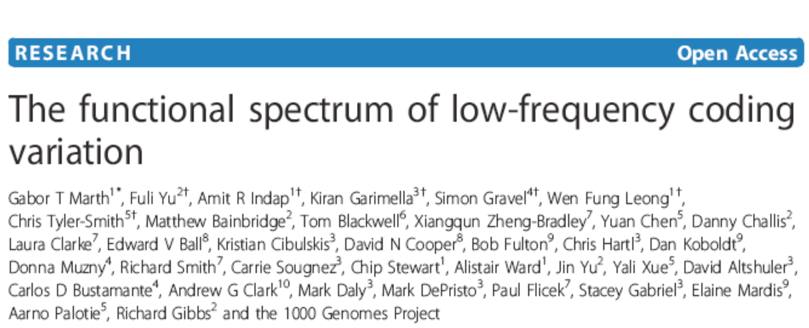
PNAS

Demographic history and rare allele sharing among human populations

Simon Gravel[a], Brenna M. Henn[a], Ryan N. Gutenkunst[b], Amit R. Indap[c], Gabor T. Marth[c], Andrew G. Clark[d], Fuli Yu[e], Richard A. Gibbs[e], The 1000 Genomes Project[e], and Carlos D. Bustamante[a,1]

[a]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305-5120; [b]Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721; [c]Department of Biology, Boston College, Chestnut Hill, MA 02467; [d]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853; and [e]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030

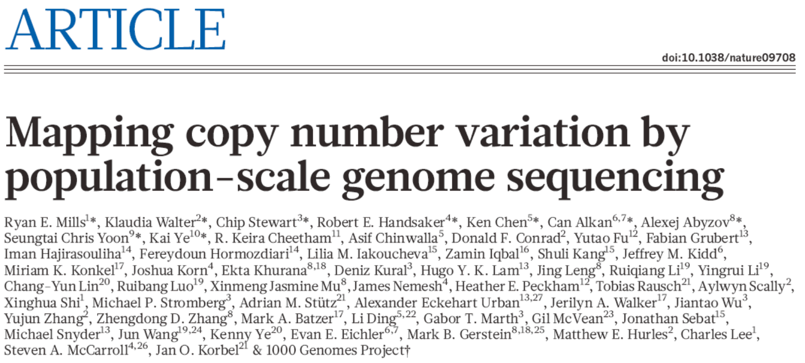
Edited by Michael Lynch, Indiana University, Bloomington, IN, and approved June 3, 2011 (received for review December 24, 2010)



RESEARCH Open Access

The functional spectrum of low-frequency coding variation

Gabor T Marth[1*], Fuli Yu[2†], Amit R Indap[1†], Kiran Garimella[3†], Simon Gravel[4†], Wen Fung Leong[1†], Chris Tyler-Smith[5†], Matthew Bainbridge[2], Tom Blackwell[6], Xiangqun Zheng-Bradley[7], Yuan Chen[5], Danny Challis[2], Laura Clarke[7], Edward V Ball[8], Kristian Cibulskis[3], David N Cooper[8], Bob Fulton[9], Chris Hartl[3], Dan Koboldt[9], Donna Muzny[4], Richard Smith[7], Carrie Sougnez[3], Chip Stewart[1], Alistair Ward[1], Jin Yu[2], Yali Xue[5], David Altshuler[3], Carlos D Bustamante[4], Andrew G Clark[10], Mark Daly[3], Mark DePristo[3], Paul Flicek[7], Stacey Gabriel[3], Elaine Mardis[9], Aarno Palotie[5], Richard Gibbs[2] and the 1000 Genomes Project



ARTICLE

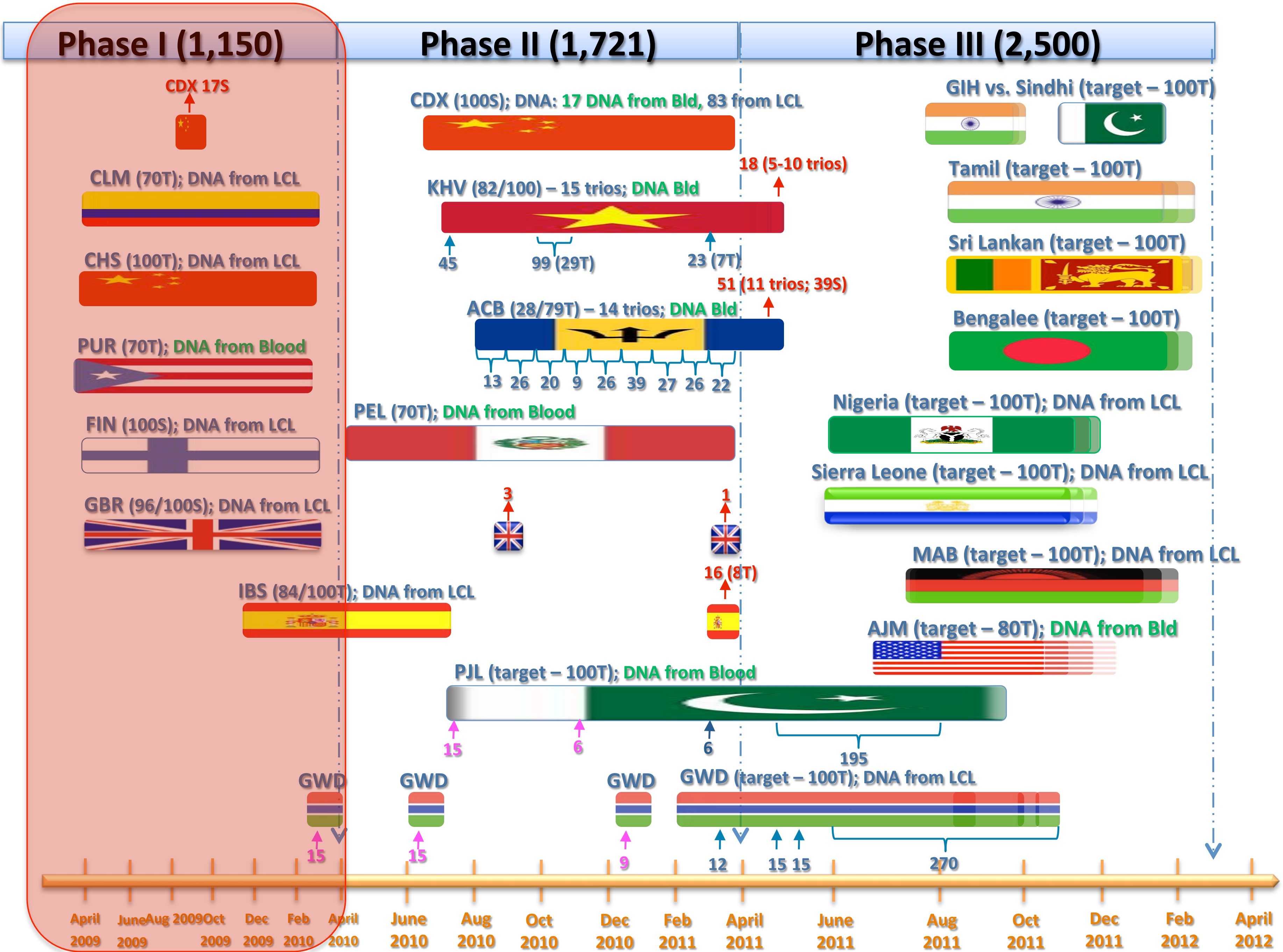
doi:10.1038/nature09708

Mapping copy number variation by population-scale genome sequencing

Ryan E. Mills[1]*, Klaudia Walter[2]*, Chip Stewart[3]*, Robert E. Handsaker[4]*, Ken Chen[5]*, Can Alkan[6,7]*, Alexej Abyzov[8]*, Seungtai Chris Yoon[9]*, Kai Ye[10]*, R. Keira Cheetham[11], Asif Chinwalla[5], Donald F. Conrad[2], Yutao Fu[12], Fabian Grubert[13], Iman Hajirasouliha[14], Fereydoun Hormozdiari[14], Lilia M. Iakoucheva[15], Zamin Iqbal[16], Shuli Kang[15], Jeffrey M. Kidd[6], Miriam K. Konkel[17], Joshua Korn[4], Ekta Khurana[8,18], Deniz Kural[3], Hugo Y. K. Lam[13], Jing Leng[8], Ruiqiang Li[19], Yingrui Li[19], Chang-Yun Lin[20], Ruibang Luo[19], Xinmeng Jasmine Mu[8], James Nemesh[4], Heather E. Peckham[12], Tobias Rausch[21], Aylwyn Scally[2], Xinghua Shi[1], Michael P. Stromberg[3], Adrian M. Stütz[21], Alexander Eckehart Urban[13,27], Jerilyn A. Walker[17], Jiantao Wu[3], Yujun Zhang[2], Zhengdong D. Zhang[8], Mark A. Batzer[17], Li Ding[5,22], Gabor T. Marth[3], Gil McVean[23], Jonathan Sebat[15], Michael Snyder[13], Jun Wang[19,24], Kenny Ye[20], Evan E. Eichler[6,7], Mark B. Gerstein[8,18,25], Matthew E. Hurles[2], Charles Lee[1], Steven A. McCarroll[4,26], Jan O. Korbel[21] & 1000 Genomes Project†

Finalized project design

- Based on the result of the pilot project, we decided to collect data on 2,500 samples from 5 continental groupings
  - Whole-genome low coverage data (>4x)
  - Full exome data at deep coverage (>50x)
  - A number of deep coverage genomes to be sequenced, with details to be decided
  - Hi-density genotyping at subsets of sites
- Moved from the Pilot into Phase 1 of the project



Phase I (1,150)

Phase II (1,721)

Phase III (2,500)

CDX 17S

CLM (70T); DNA from LCL

CHS (100T); DNA from LCL

PUR (70T); DNA from Blood

FIN (100S); DNA from LCL

GBR (96/100S); DNA from LCL

IBS (84/100T); DNA from LCL

GWD

15

CDX (100S); DNA: 17 DNA from Bld, 83 from LCL

KHV (82/100) – 15 trios; DNA Bld

45

99 (29T)

23 (7T)

18 (5-10 trios)

ACB (28/79T) – 14 trios; DNA Bld

13 26 20 9 26 39 27 26 22

51 (11 trios; 39S)

PEL (70T); DNA from Blood

3

1

16 (8T)

PJL (target – 100T); DNA from Blood

15

6

6

195

GWD

15

GWD

9

GWD (target – 100T); DNA from LCL

12

15 15

270

GIH vs. Sindhi (target – 100T)

Tamil (target – 100T)

Sri Lankan (target – 100T)

Bengalee (target – 100T)

Nigeria (target – 100T); DNA from LCL

Sierra Leone (target – 100T); DNA from LCL

MAB (target – 100T); DNA from LCL

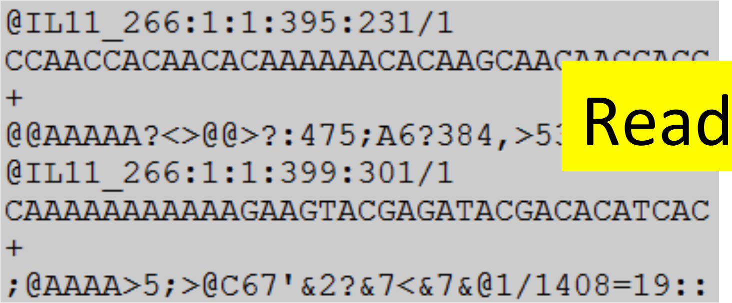
AJM (target – 80T); DNA from Bld

Phase I data

- Samples from 14 populations: ASW, CEU, CHB, CHS, CLM, FIN, GBR, IBS, JPT, LWK, MXL, PRU, TSI, YRI

Dataset	Low coverage whole genome	Deep coverage whole exome
# samples	1,094	1,128
Sequencing technologies	Illumina, SOLiD, 454	Illumina, SOLiD
Primary alignments (BAMs)	BWA, BFAST	MOSAIK, BFAST
Second alignments (BAMs)	MOSAIK	BWA, MOSAIK
Read coverage	4-8X per sample	≥70% of targets with ≥20X coverage in every sample

Raw data & read alignment delivery



```
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CCAACCACAACACAAAAAACACAAGCAACAACCACC
+
@@AAAAA?<>@@>?:475;A6?384,>53
@IL11_266:1:1:399:301/1
CAAAAAAAAAAAGAAGTACGAGATACGACACATCAC
+
;@AAAA>5;>@C67'&2?&7<&7&@1/1408=19::
```
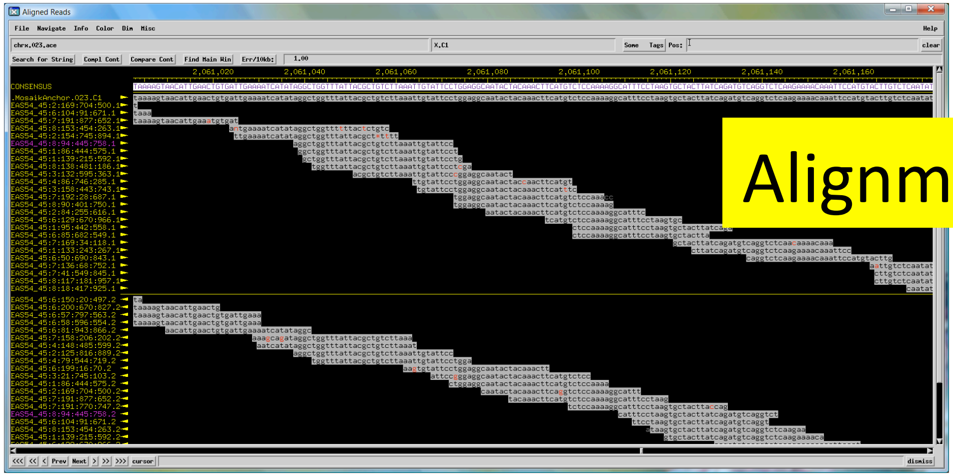

Reads: FASTQ

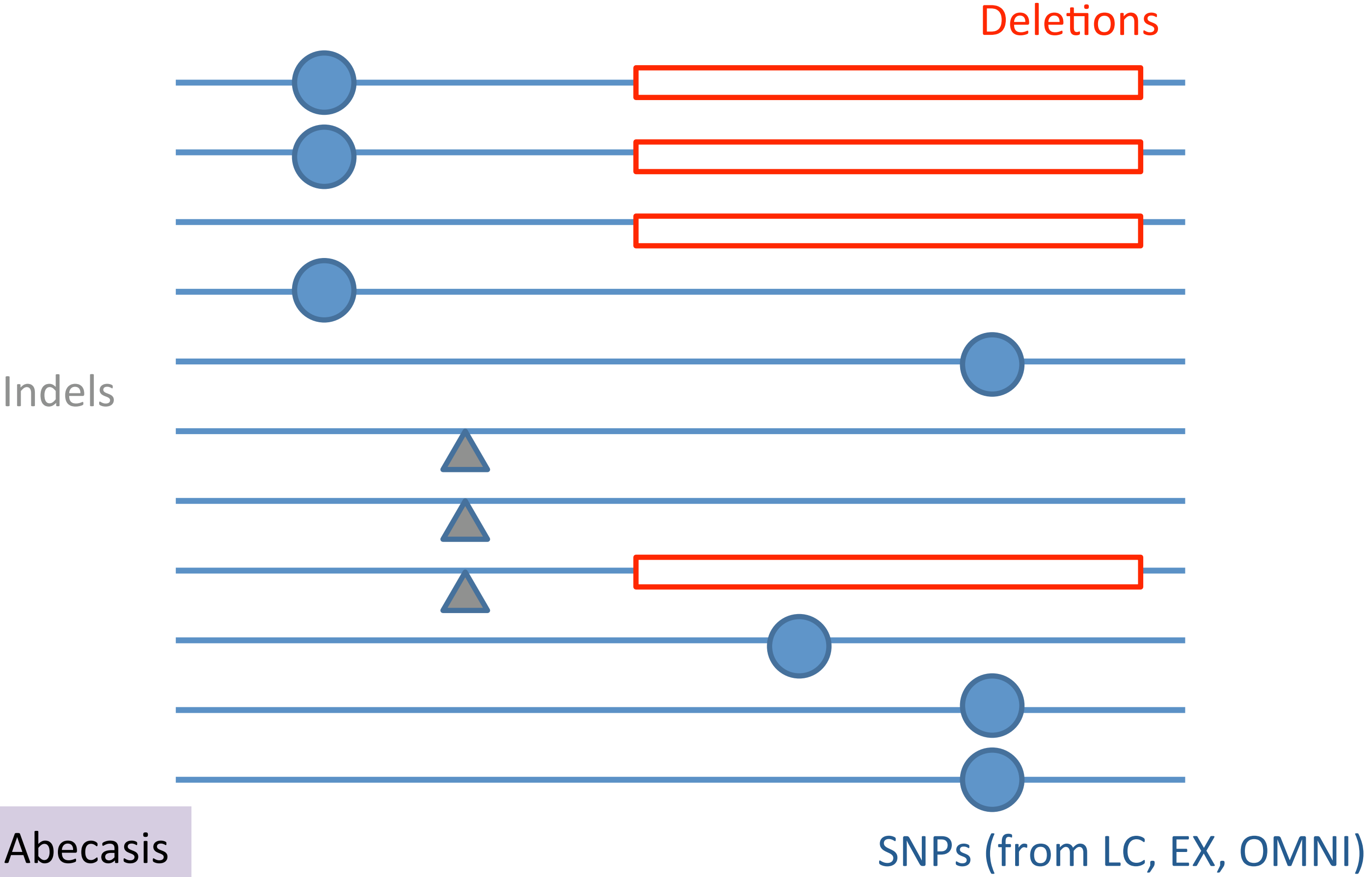


Alignments: BAM

ftp://ftp.1000genomes.ebi.ac.uk

Phase 1 analysis goal: an **integrated view of human variations**

- Reconstruct haplotypes including all variant types, using all datasets

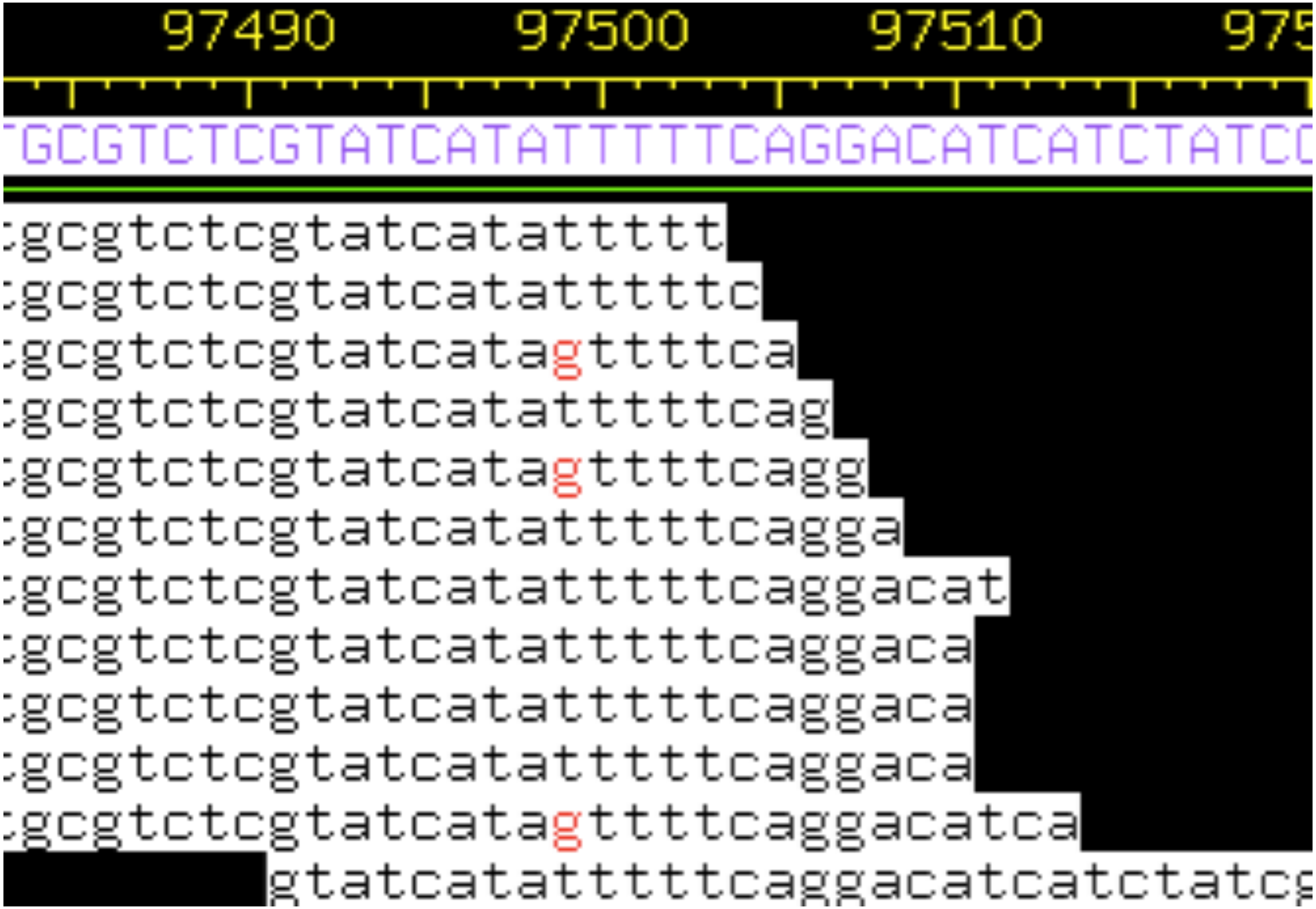


Goncalo Abecasis

Pipelines for data processing and variant calling

- Tens of analysis groups have contributed
- Individual pipelines and component tools vary
- Typical main steps:
  - Read mapping
  - Duplicate filtering
  - Base quality value recalibration
  - INDEL realignment
  - Variant calling (sites)
  - Sample genotype calling (sometime part of variant calling)
  - Variant filtering / call set refinement
  - Variant reporting

SNPs



```
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gcgtctcgtatcatattttt
gcgtctcgtatcatatttttc
gcgtctcgtatcatagttttca
gcgtctcgtatcatatttttcag
gcgtctcgtatcatagttttcagg
gcgtctcgtatcatatttttcagga
gcgtctcgtatcatatttttcaggacat
gcgtctcgtatcatatttttcaggaca
gcgtctcgtatcatatttttcaggaca
gcgtctcgtatcatatttttcaggaca
gcgtctcgtatcatagttttcaggacatca
       gtatcatatttttcaggacatcatctatcg
```
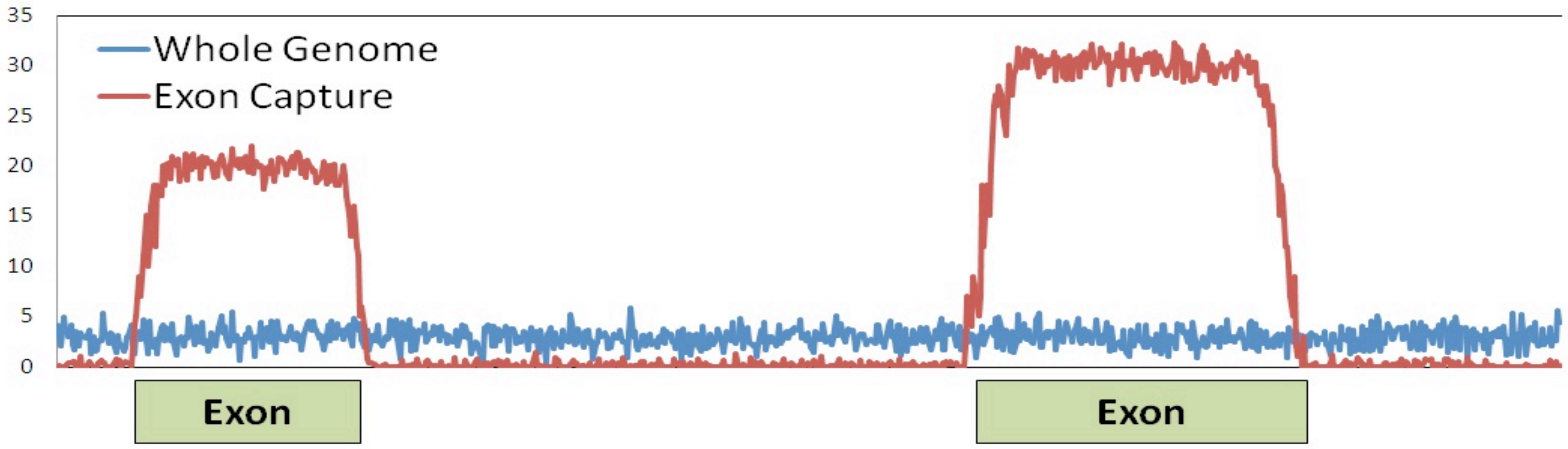
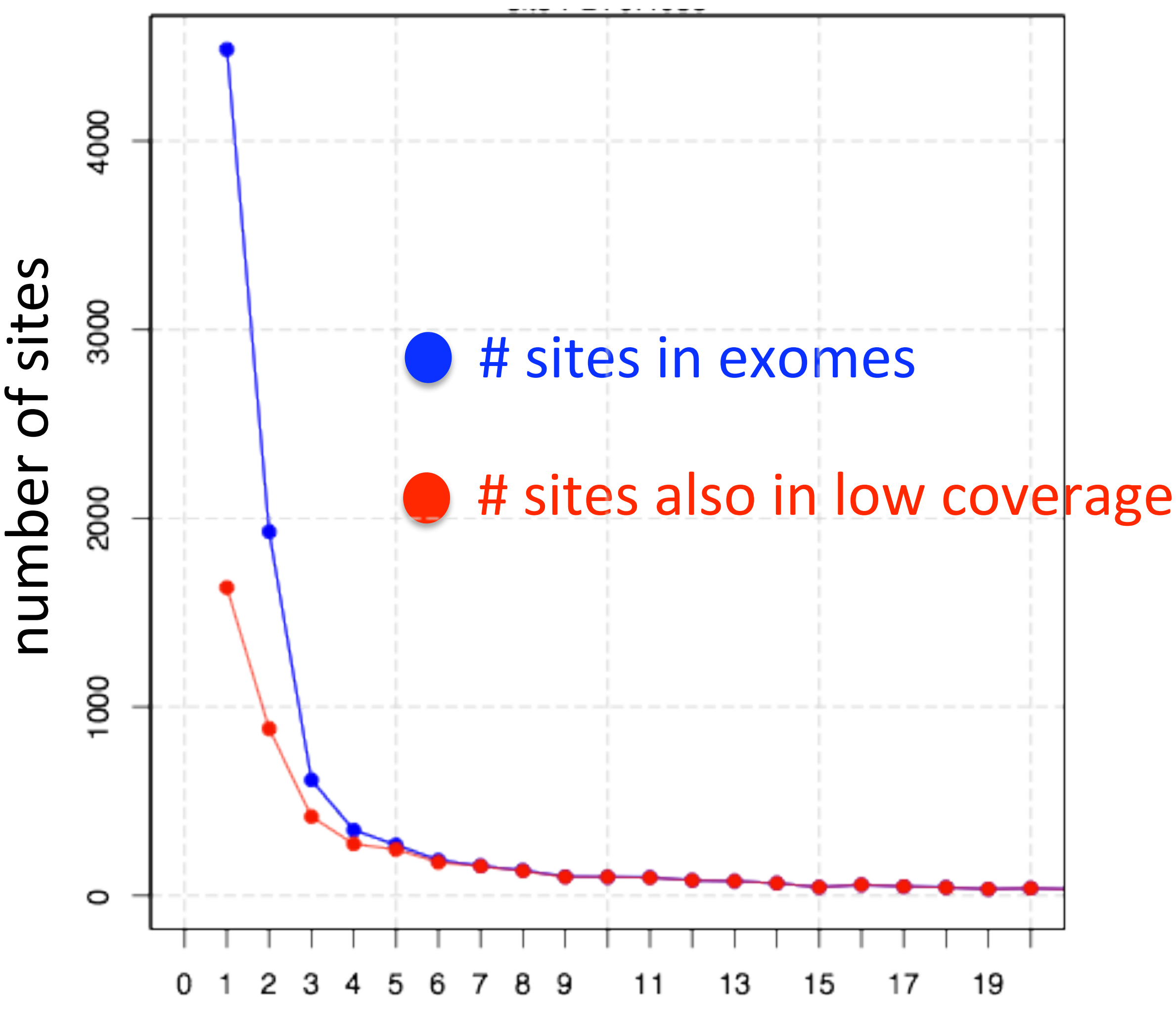

SNP calls

Dataset	Contributing datasets	Consensus method	#SNPs	# Novel SNPs	Novel Ts/Tv	%ONMI poly (sensitivity)	%OMNI mono (FDR)
Low coverage	BC, BCM, BI, NCBI, UM	VQSR	**37.9M**	**29.65M**	2.16	98.4	1.80
Exome/ Illumina	BC, BCM, BI, Cornell, UM	SVM	**598K**	**468K**	2.74	98.01	1.97
Exome/SOLiD	BC, BCM, UM	SVM	**356K**	**243K**	2.91	90.67	1.29



Deep coverage exome data is more sensitive to low-frequency variants

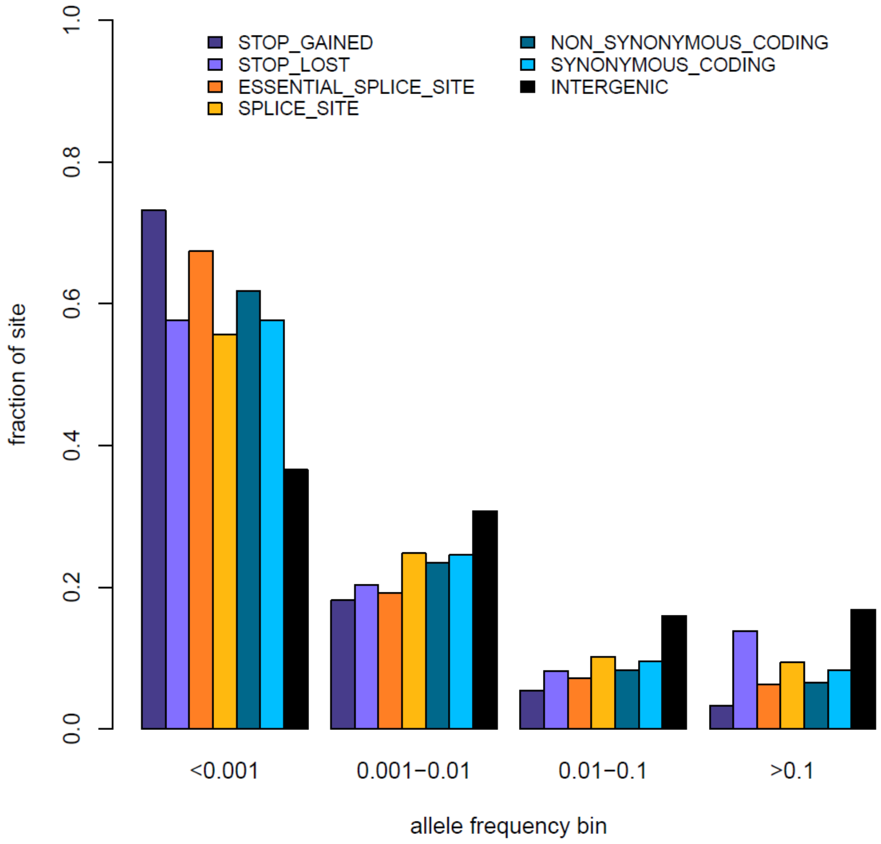


Allele count in 766 exomes (chr. 20, exons only)

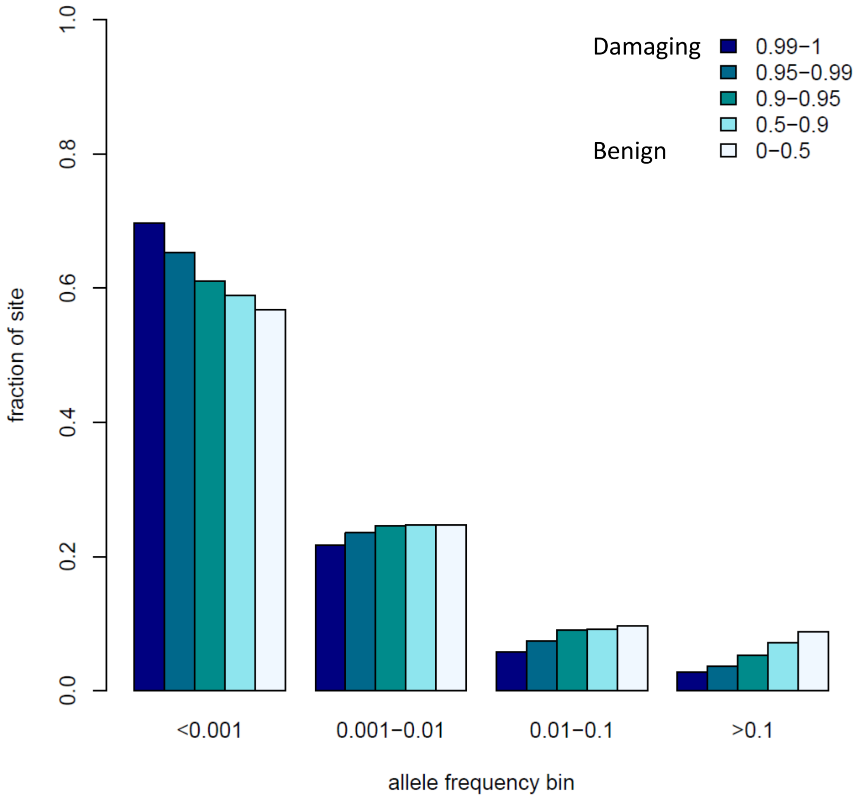
Erik Garrison

Newly discovered SNPs are mostly at low frequency and enriched for functional variants

Functional category



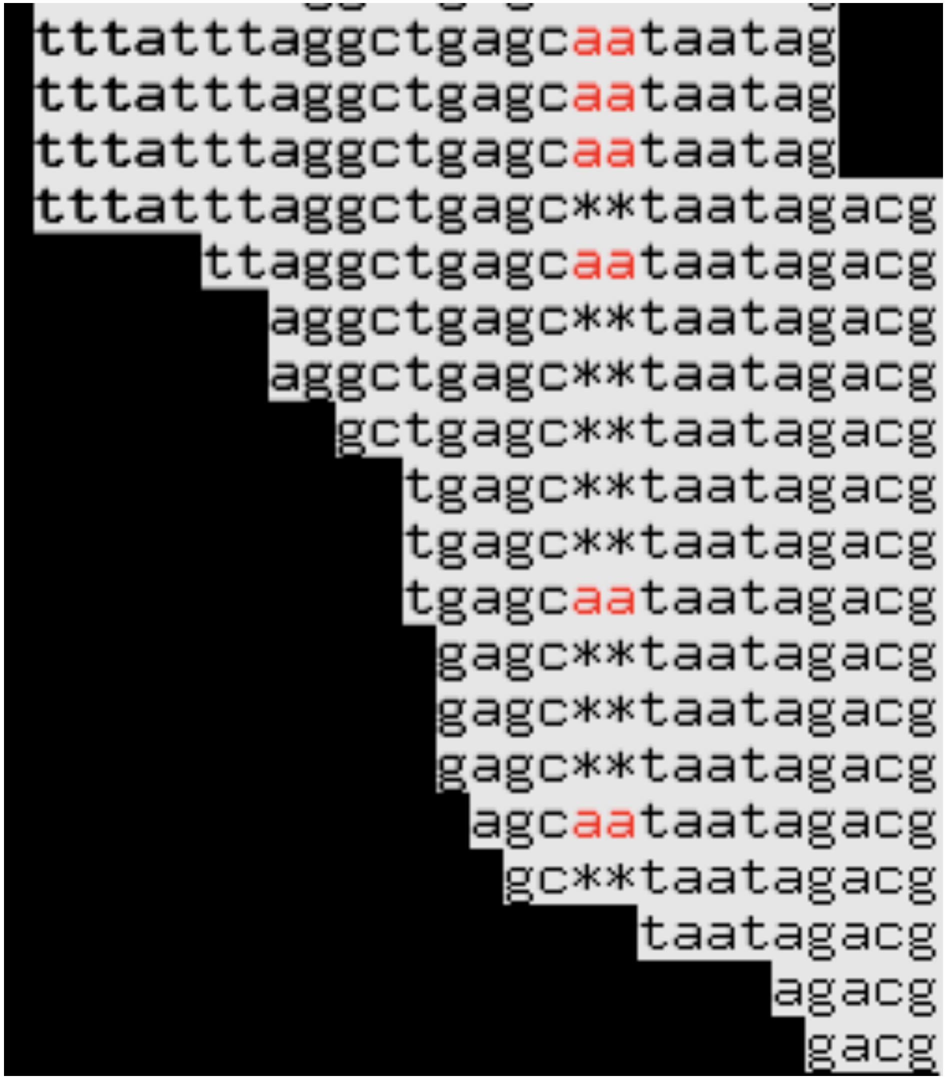
Non-synonymous: Condel score



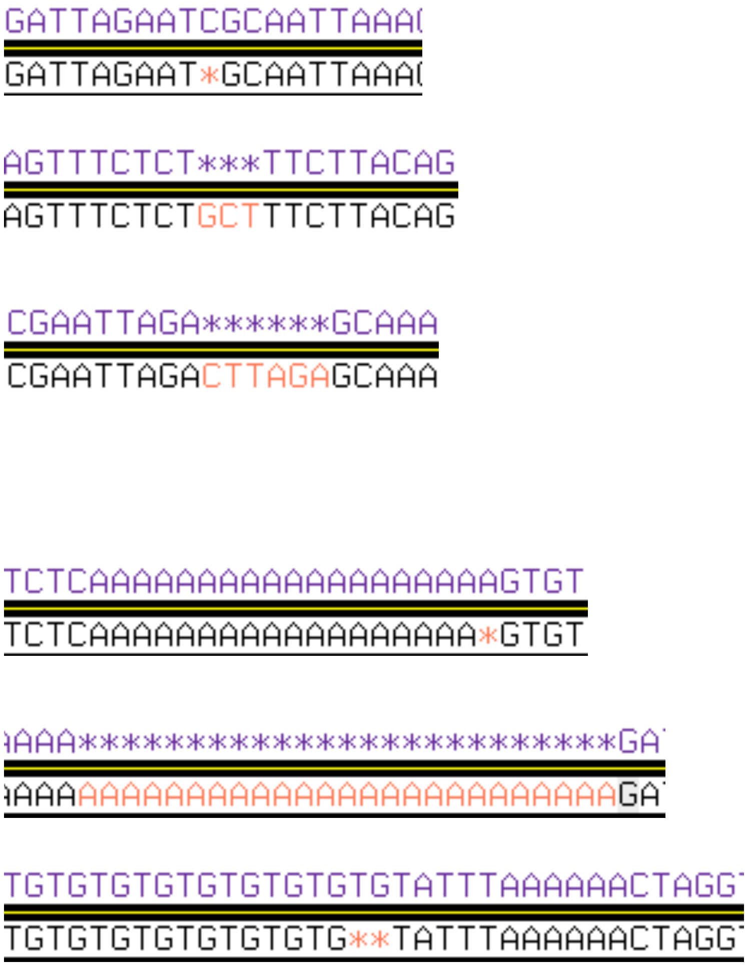
Presentation on using the data for GWAS by Brian Howie

Enza Colonna, Yuan Chen, Yali Xue

INDELs



```
tttatttaggctgagcaataatag
tttatttaggctgagcaataatag
tttatttaggctgagcaataatag
tttatttaggctgagc**taatagacg
     ttaggctgagcaataatagacg
       aggctgagc**taatagacg
       aggctgagc**taatagacg
         gctgagc**taatagacg
           tgagc**taatagacg
           tgagc**taatagacg
           tgagcaataatagacg
            gagc**taatagacg
            gagc**taatagacg
            gagc**taatagacg
             agcaataatagacg
              gc**taatagacg
                  taatagacg
                      agacg
                       gacg
```



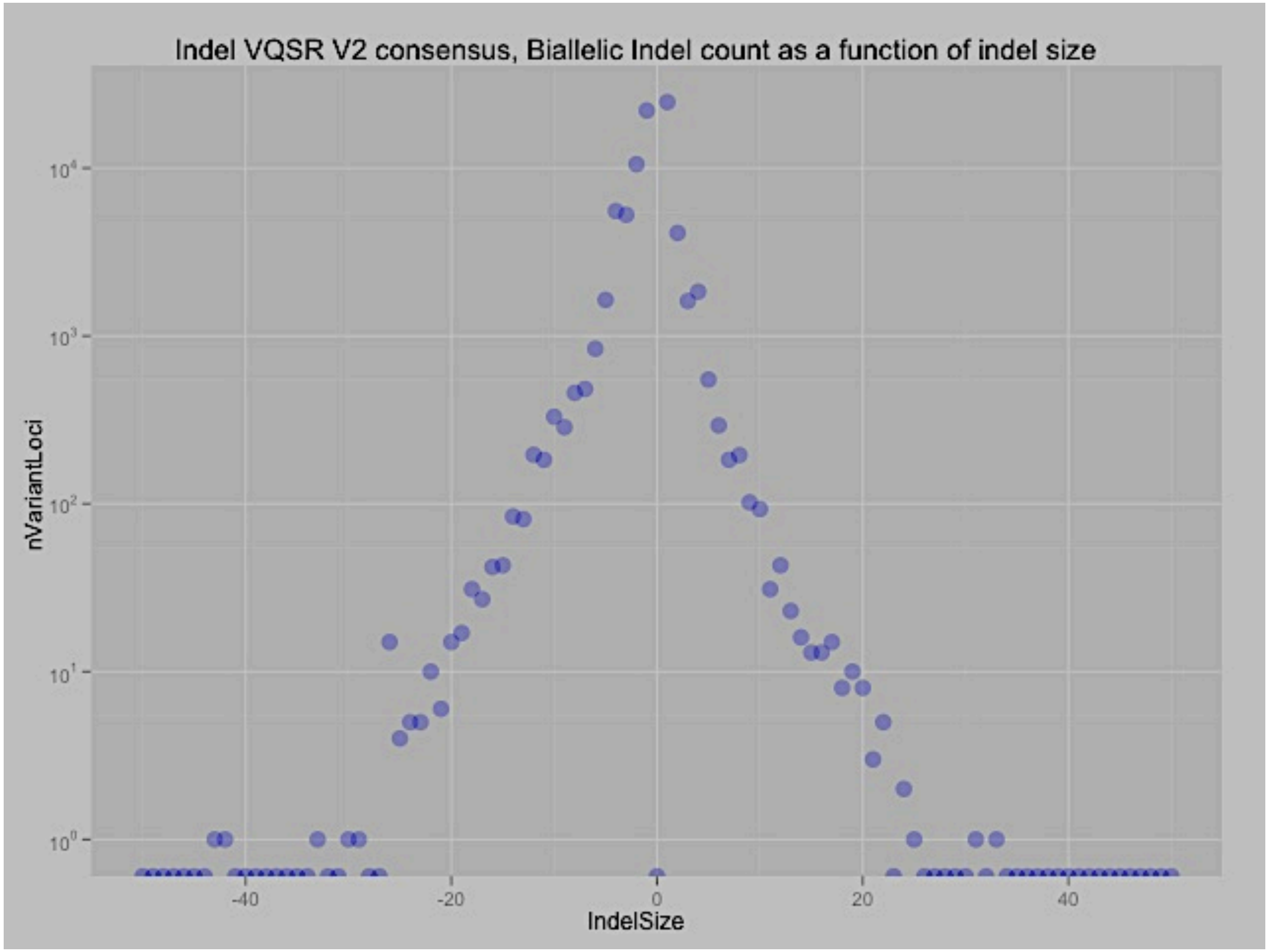
```
GATTAGAATCGCAATTAAA
GATTAGAAT*GCAATTAAA

AGTTTCTCT***TTCTTACAG
AGTTTCTCTGCTTTCTTACAG

CGAATTAGA******GCAAA
CGAATTAGACTTAGAGCAAA

TCTCAAAAAAAAAAAAAAAAAAAGTGT
TCTCAAAAAAAAAAAAAAAAAA*GTGT

AAA*************************GA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAGA

TGTGTGTGTGTGTGTGTGTATTTAAAAAACTAGG
TGTGTGTGTGTGTGTG**TATTTAAAAAACTAGG
```

INDEL calls

Dataset	Contributing datasets	Consensus method	#INDELs
Low coverage	BC, BI, DI, OX, SI	VQSR	**5.5M**
Exome/Illumina	BC, BCM, BI	N.A.	**6.5 – 10.2K**
Exome/SOLiD	BCM	N.A.	**4.2 – 5.0K**

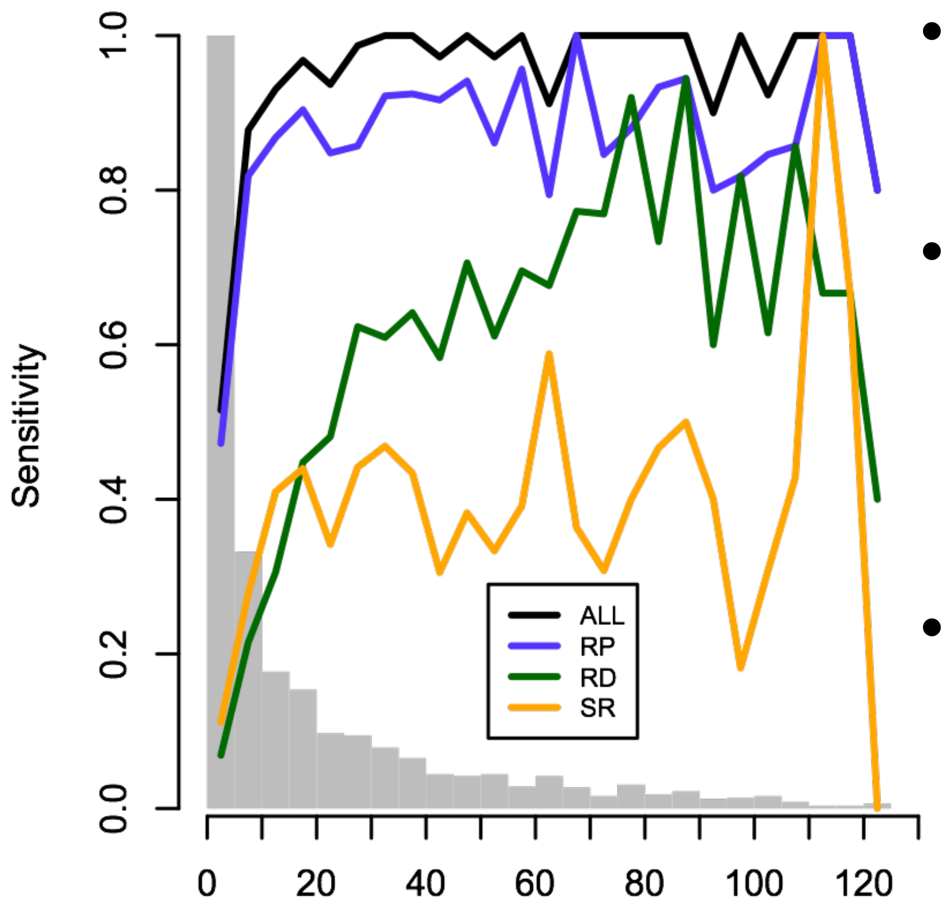
Guillermo Angel

INDEL length



Guillermo Angel

Finding structural variants



- Discovery with a number of different methods
- Several types (e.g. deletions, tandem duplications, mobile element insertions) now detectable with high accuracy
- We are pulling in new types for the Phase I data (inversions, *de novo* insertions, translocations)

Presentation on structural variations by Ryan Mills

SNP validations (low coverage data)

	Total	Polymorphic	Monomorphic	No Call	Confirmation Rate	Failure Rate
All Sites	**300**	**282**	**12**	**6**	**0.959**	**0.020**
Called in Validation Samples	**287**	**276**	**5**	**6**	**0.982**	**0.021**
Singletons	70	65	3	2	0.956	0.029
MAF<0.01*	134	131	2	1	0.985	0.007
0.01<MAF<0.05	33	33	0	0	1.000	0.000
MAF>0.05	50	47	0	3	1.000	0.060

*Excludes singletons

Danny Challis, Eric Banks

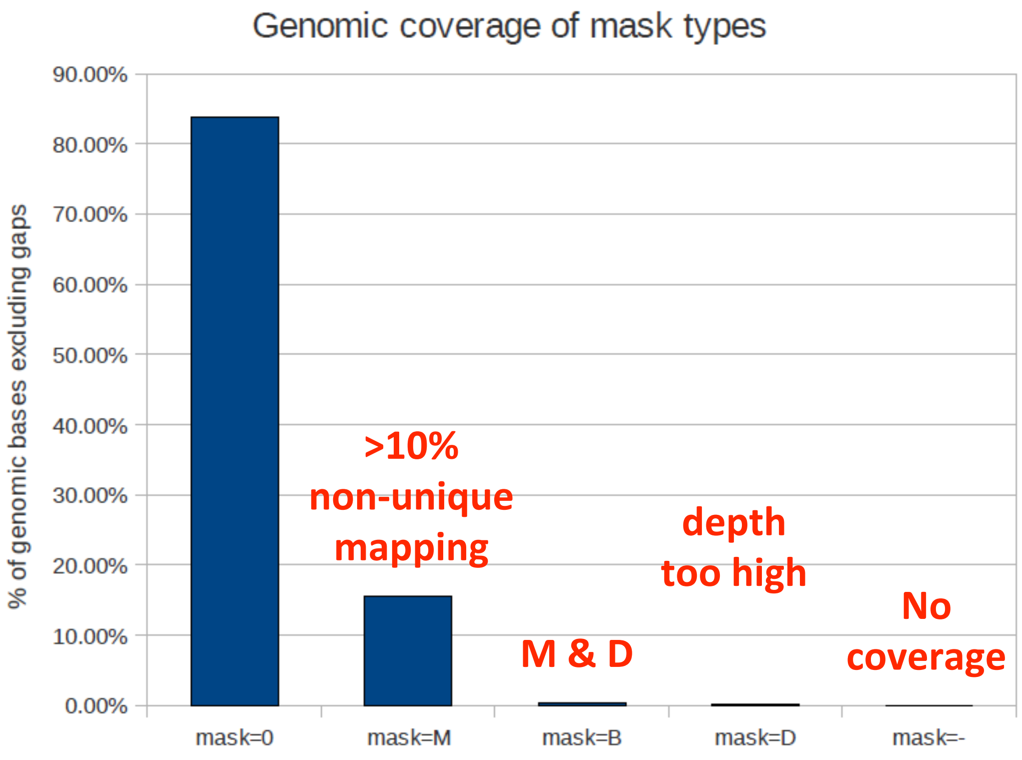
Genotypes are accurate

- Average low coverage depth is ~5x
- We obtain genotypes by sharing data between samples (using imputation-related methods)

Genotype	HomRef	Het	HomAlt	Overall
Error rate	0.16%	0.76%	0.39%	0.37%

- Genotypes are expected to be even more accurate after integration of multiple variant sources

Accessible fraction of genome

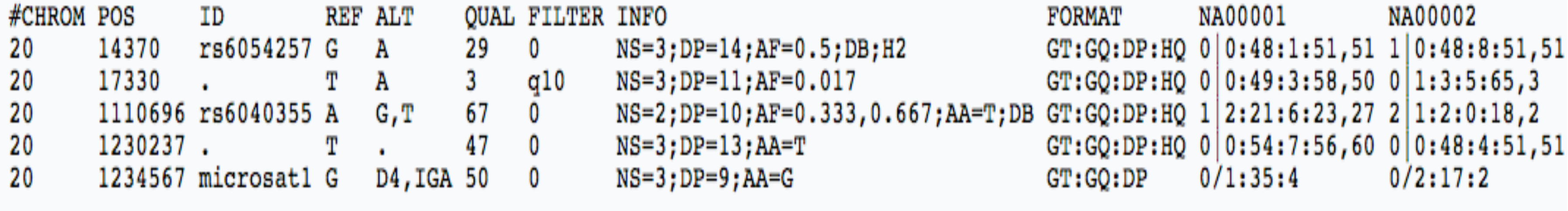


- In the Pilot data, we found that >80% of the human genome reference was accessible for SNP variant calling
- We are currently re-evaluating this fraction for the Phase 1 data (which used longer reads)
- We are developing methods to estimate the fraction for other variants (especially INDELs)

Goncalo Abecasis

Variant call delivery

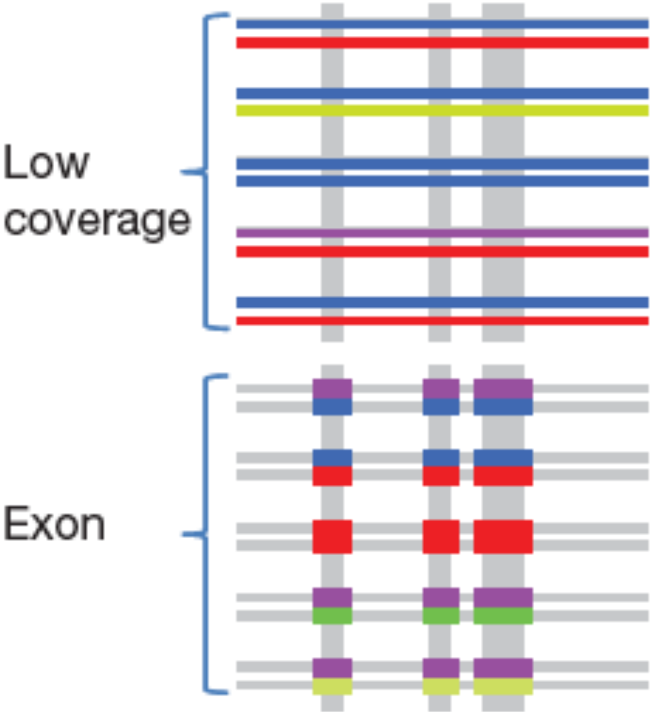
Format: VCF



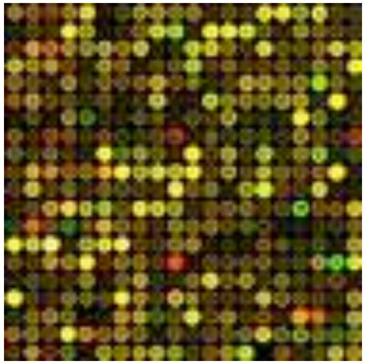
```
#CHROM POS     ID        REF ALT    QUAL FILTER INFO                              FORMAT      NA00001        NA00002
20     14370   rs6054257 G   A      29   0      NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51
20     17330   .         T   A      3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3
20     1110696 rs6040355 A   G,T    67   0      NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2
20     1230237 .         T   .      47   0      NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51
20     1234567 microsat1 G   D4,IGA 50   0      NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2
```

ftp://ftp.1000genomes.ebi.ac.uk

Datasets & variant types



SNP array data

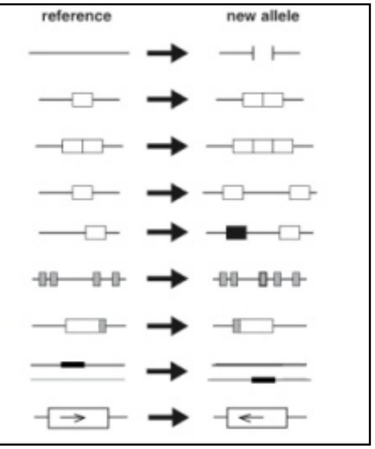


CTGAG
ATGAG

SNP

CCTGAG
--TGAG

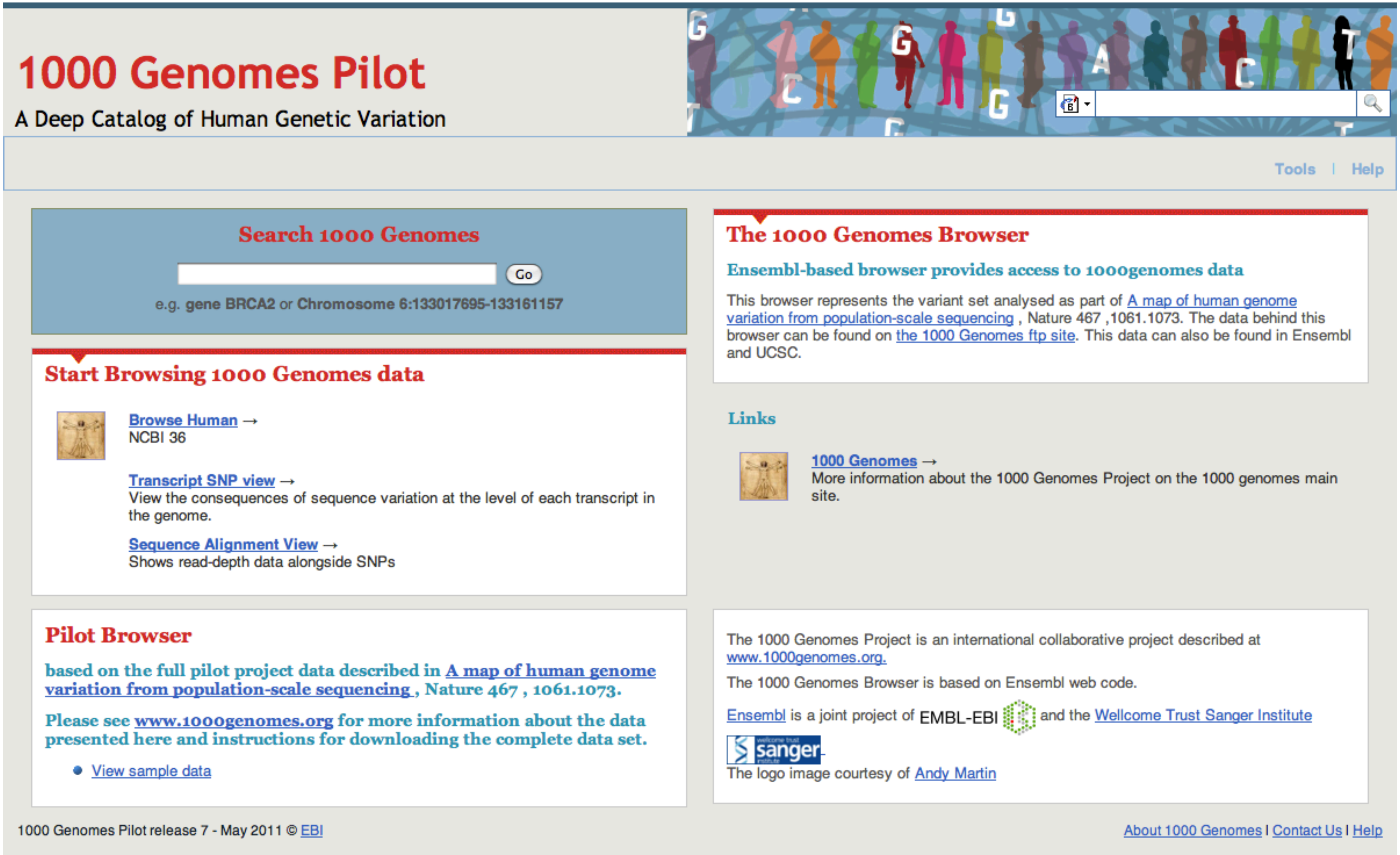
INDEL



SV

Presentation on integration by Hyun Min Kang

Data delivery



1000 Genomes Pilot

A Deep Catalog of Human Genetic Variation

Search 1000 Genomes

e.g. gene BRCA2 or Chromosome 6:133017695-133161157

Start Browsing 1000 Genomes data

Browse Human →
NCBI 36

Transcript SNP view →
View the consequences of sequence variation at the level of each transcript in the genome.

Sequence Alignment View →
Shows read-depth data alongside SNPs

Pilot Browser

based on the full pilot project data described in A map of human genome variation from population-scale sequencing , Nature 467 , 1061.1073.

Please see www.1000genomes.org for more information about the data presented here and instructions for downloading the complete data set.

- View sample data

The 1000 Genomes Browser

Ensembl-based browser provides access to 1000genomes data

This browser represents the variant set analysed as part of A map of human genome variation from population-scale sequencing , Nature 467 ,1061.1073. The data behind this browser can be found on the 1000 Genomes ftp site. This data can also be found in Ensembl and UCSC.

Links

1000 Genomes →
More information about the 1000 Genomes Project on the 1000 genomes main site.

The 1000 Genomes Project is an international collaborative project described at www.1000genomes.org.

The 1000 Genomes Browser is based on Ensembl web code.

Ensembl is a joint project of EMBL-EBI and the Wellcome Trust Sanger Institute

The logo image courtesy of Andy Martin

1000 Genomes Pilot release 7 - May 2011 © EBI

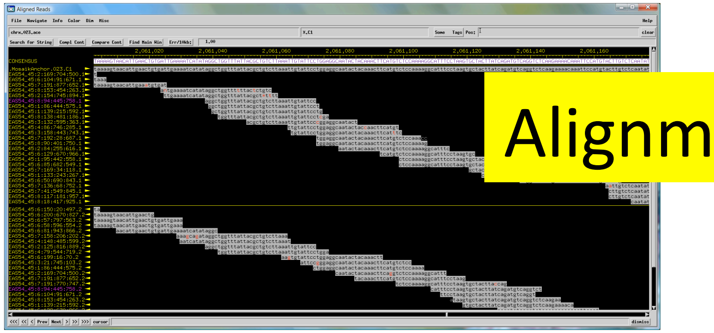
About 1000 Genomes | Contact Us | Help

Presentation on data access by Paul Flicek

The 1000GP is a driver for method and tool development

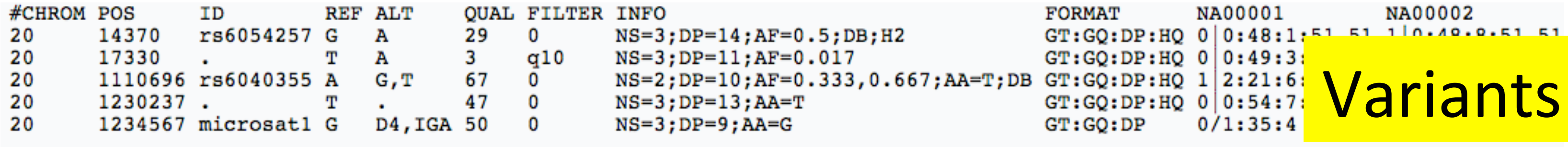
- New data formats (SAM/BAM, VCF) developed by the 1000GP are now adopted by the entire genomics community
- Tools (read mappers e.g. BWA, MOSAIK, etc; variant callers including those for SVs)
- Data processing protocols (BQ recalibration, duplicate read removal, etc.)
- Imputation and haplotype phasing methods

Tools for analyzing & manipulating 1000G data



Alignments: SAM/BAM

- samtools: http://samtools.sourceforge.net/
- BamTools: http://sourceforge.net/projects/bamtools/
- GATK: http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit



```
#CHROM POS     ID        REF ALT    QUAL FILTER INFO                              FORMAT      NA00001        NA00002
20     14370   rs6054257 G   A      29   0      NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51
20     17330   .         T   A      3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:
20     1110696 rs6040355 A   G,T    67   0      NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:
20     1230237 .         T   .      47   0      NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:
20     1234567 microsat1 G   D4,IGA 50   0      NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4
```

Variants: VCF

- VCFTools: http://vcftools.sourceforge.net/
- VcfCTools: https://github.com/AlistairNWard/vcfCTools

Project timeframe (approximate)

- Phase 1
  - Raw data, alignments available
  - Integrated variant set available
  - Phase 1 analysis paper by end of 2011
- Phase 2
  - Raw data mid-December 2011
  - Read mapping, variant calling early 2012
- Phase 3
  - Samples end March 2012
  - Data Summer 2012
  - Call sets end of 2012, Final paper 2013?
- End of the project

Richard Durbin

Fraction of variant sites present in an individual that are NOT already represented in dbSNP

Date	Fraction not in dbSNP
February, 2000	98%
February, 2001	80%
April, 2008	10%
February, 2011	2%
October 2011 (now)	<1%

Ryan Poplin, David Altshuler