Studying Genetic Variation II: Computational Techniques

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Science
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Some points from other lectures

- Population Genetics: Practical Applications
  Lynn Jorde
  - Described patterns of human genetic variation among and within populations, linkage disequilibrium and HapMap and how all this relates to the search for complex disease genes.
- Linkage Analysis and Complex Traits
  Elaine Ostrander
  - Linkage based approaches to finding disease susceptibility genes.
- Studying Genetic Variation I: Laboratory Techniques
  Karen Mohlke
  - Types of sequence polymorphisms and genotyping methods.

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Genetic Variation Discovery

The primary method for discovering sequence variation is by sequencing DNA and comparing the sequences

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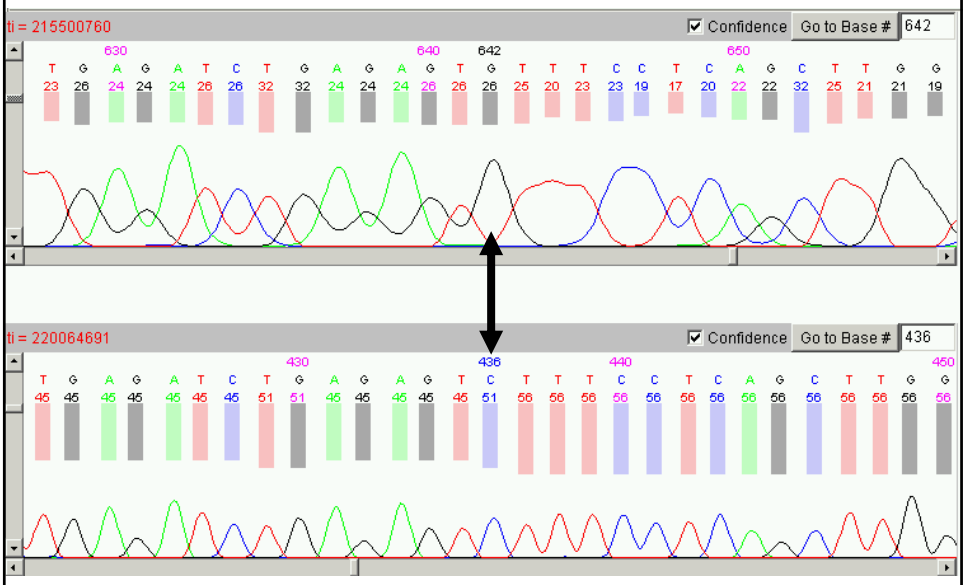

Trace Archive Search query:
trace_name='S213P602260RC9.T0' OR trace_name='50766946'

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Overview of Topics

- Review of genetic variation discovery
- Database of SNPs, dbSNP
- Other types of genetic variation
- Medical sequencing
- Next-generation sequencing and SNPs
- Targeted Genomic Selection

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A few definitions

- Alleles
  - Alternate forms of a gene or chromosomal locus that differ in DNA sequence
- Single Nucleotide Polymorphism (SNP)
  - The most common form of genetic variation in the genome: a single-base substitution
- Minor Allele Frequency (MAF)
  - Proportion of the less common of 2 alleles in a population
- Polymorphic
  - Usually implies a MAF of at least 1%

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NCBI dbSNP database of genetic variation

- http://www.ncbi.nlm.nih.gov/SNP/
- This is the main repository of publicly available genetic variation data.
- You'll also find information on allele frequencies, populations, genotype assays and much more.

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Review of Genetic Variation Discovery Efforts

- Expressed sequence tag (EST) mining
- Clone overlap
- The SNP Consortium (TSC)
- Haplotype Map Project (HapMap)
- Chip based sequencing arrays
- Human Genome Structural Variation (HGSV)
- Personal Genomes (available from NCBI trace archive)
  - Craig Venter (*PLoS Biology* Vol. 5, No. 10, e254)
  - Jim Watson (http://jimwatsonsequence.cshl.edu/cgi-perl/gbrowse/jwsequence/)

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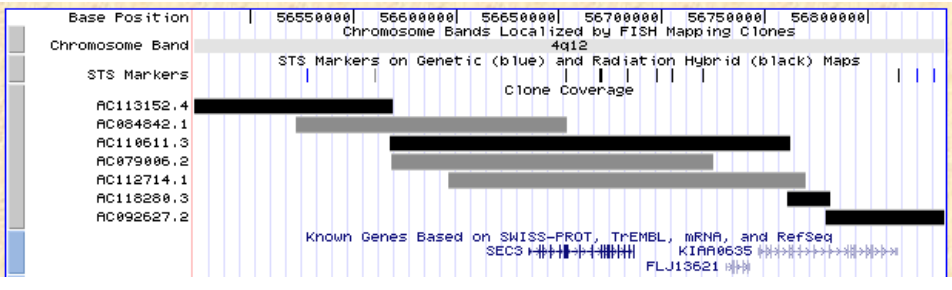
Clone Overlap

- The human genome was sequenced from BAC clones (containing about 150kb of sequence each).
- These overlapped to various levels, and within the overlap regions, high quality base differences indicated the position and alleles of SNPs.



Clone Overlap

- About 1.3M SNPs in dbSNP come from mining of clone overlaps.
- Special care was required to insure that the overlapping clones came from different haploids. (see references)
- This can be accomplished by
  - looking at the source DNA for the two clones to see that it originated from different individuals, or
  - if from the same individual, that the variation rate within the overlapping regions indicated that the DNA was from different haploids of one individual.

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The SNP Consortium

- A two year effort (1999-2001) funded by the Wellcome Trust and 11 pharmaceutical and technology companies to discover 300,000 SNPs randomly distributed across the human genome.
- The SNPs were developed from a pool of DNA samples obtained from 24 individuals representing several ethnic groups.
- The initial target of 300,000 SNP was passed quickly, and now the sequence generated from that project contributes over 1.3M SNPs to the public archives.

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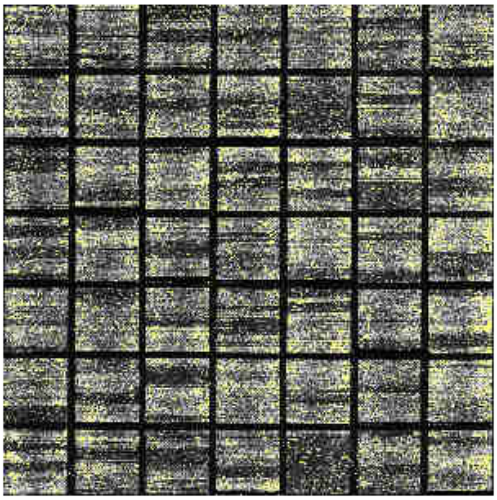
Perlegen used Affymetrix's chip design process to place 60M probes on a 5x5" chip. From 20 single haploid chromosome 21 chromosomes, they discovered 36k SNPs.



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http://www.perlegen.com/

More SNPs for HapMap Project

- This project required many more SNPs than were available when it started in October 2002, which totaled about 2M.
- Additional random shotgun sequencing has brought this to 8.2M SNPs for the HapMap Project.
- It has been estimated that there are perhaps 10M common SNPs (> 5% MAF), so there are many more SNPs yet to discover.

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Vol 449|18 October 2007|doi:10.1038/nature06258 nature

ARTICLES

A second generation human haplotype map of over 3.1 million SNPs

The International HapMap Consortium*

We describe the Phase II HapMap, which characterizes over 3.1 million human single nucleotide polymorphisms (SNPs) genotyped in 270 individuals from four geographically diverse populations and includes 25–35% of common SNP variation in the populations surveyed. The map is estimated to capture untyped common variation with an average maximum r^2 of between 0.9 and 0.96 depending on population. We demonstrate that the current generation of commercial genome-wide genotyping products captures common Phase II SNPs with an average maximum r^2 of up to 0.8 in African and up to 0.95 in non-African populations, and that potential gains in power in association studies can be obtained through imputation. These data also reveal novel aspects of the structure of linkage disequilibrium. We show that 10–30% of pairs of individuals within a population share at least one region of extended genetic identity arising from recent ancestry and that up to 1% of all common variants are untaggable, primarily because they lie within recombination hotspots. We show that recombination rates vary systematically around genes and between genes of different function. Finally, we demonstrate increased differentiation at non-synonymous, compared to synonymous, SNPs, resulting from systematic differences in the strength or efficacy of natural selection between populations.

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Table 2. Estimated coverage of the Phase II HapMap in the ten HapMap ENCODE regions

Panel	MAF bin	Pairwise linkage disequilibrium	
		$r^2 \geq 0.8$ (%)	Mean maximum r^2
YRI	≥0.05	82	0.90
	<0.05	61	0.76
	0.05–0.10	81	0.89
	0.10–0.25	90	0.94
	0.25–0.50	87	0.93
CEU	≥0.05	93	0.96
	<0.05	70	0.79
	0.05–0.10	87	0.92
	0.10–0.25	94	0.96
	0.25–0.50	95	0.97
CHB+JPT	≥0.05	92	0.95
	<0.05	65	0.74
	0.05–0.10	81	0.89
	0.10–0.25	90	0.94
	0.25–0.50	94	0.96

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Table 4 | Estimated coverage of commercially available fixed marker arrays

Platform*	YRI		CEU	
	$r^2 \geq 0.8$ (%)	Mean maximum r^2	$r^2 \geq 0.8$ (%)	Mean maximum r^2
Affymetrix GeneChip 500K	46	0.66	68	0.81
Affymetrix SNP Array 6.0	66	0.80	82	0.90
Illumina HumanHap300	33	0.56	77	0.86
Illumina HumanHap550	55	0.73	88	0.92
Illumina HumanHap650Y	66	0.80	89	0.93
Perlegen 600K	47	0.68	92	0.94

* Assuming all SNPs on the product are informative and pass QC; in practice these numbers are overestimates.

Platform*	CHB+JPT	
	$r^2 \geq 0.8$ (%)	Mean maximum r^2
Affymetrix GeneChip 500K	67	0.80
Affymetrix SNP Array 6.0	81	0.89
Illumina HumanHap300	63	0.78
Illumina HumanHap550	83	0.89
Illumina HumanHap650Y	84	0.90
Perlegen 600K	84	0.90

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Genome-Wide Association Studies

- Enabled by the HapMap project and spinoff SNP genotyping chips
- Availability of large, well studied sample cohorts
- Funded internationally
  - Genetic Association Information Network (GAIN, a public-private partnership)
    - http://www.fnih.org/GAIN2/home_new.shtml
  - Genes, Environment and Health Initiative (GEI)
    - http://www.genesandenvironment.nih.gov/
  - Wellcome Trust Case Control Consortium (WTCCC)
    - http://www.wtccc.org.uk/

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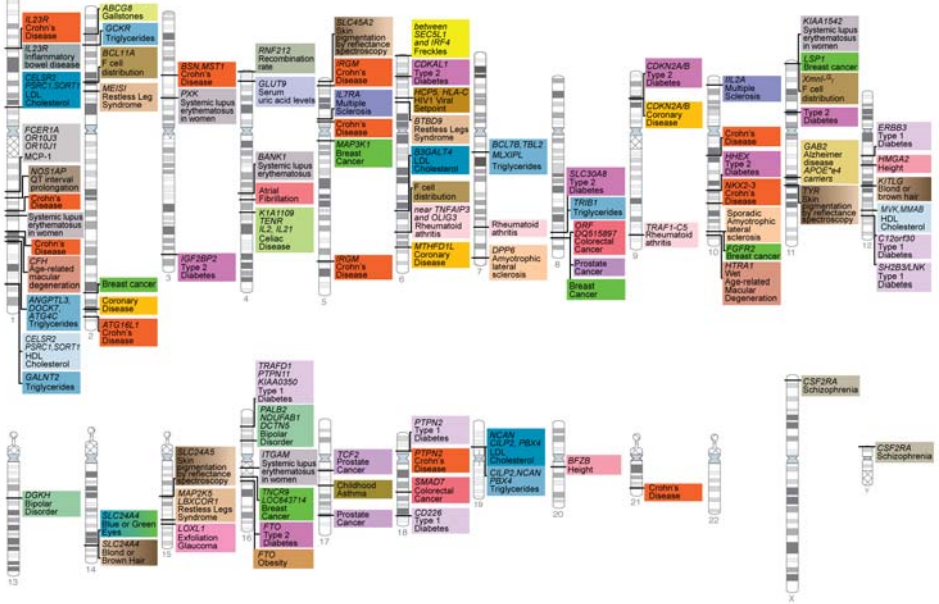
February 2008



Manolio, Brooks, Collins, J. Clin. Invest., in press.

A Catalog of Published Genome-Wide Association Studies

- http://www.genome.gov/26525384

First Author/Date/ Journal/Study	Disease/Trait	Initial Sample Size	Replication Sample Size	Platform [SNPs passing QC]
Gold March 11, 2008 *PNAS* Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33	Breast cancer	249 cases, 299 controls	1,193 cases, 1,166 controls	Affymetrix [391,467]
Kirov March 11, 2008 *Mol Psychiatry* A genome-wide association study in 574 schizophrenia trios using DNA pooling	Schizophrenia	605 controls 574 cases, 1148 parents of cases	NR	Affymetrix [~550,000] (pooled)
Doring March 09, 2008 *Nat Genet* SLC2A9 influences uric acid concentrations with pronounced sex-specific effects	Uric acid	1,644 individuals	9,947 individuals	Affymetrix [335,152]
Vitart March 09, 2008 *Nat Genet* SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout	Serum urate	794 individuals	706 individuals	Illumina [308,140]
Liu March 05, 2008 *Hum Mol Genet* Genome-wide association scans identified CTNNBL1 as a novel gene for obesity	Obesity	1,000 individuals	896 obese individuals, 2,916 lean individuals	Affymetrix [379,319]
Sklar March 04, 2008 *Mol Psychiatry* Whole-genome association study of bipolar disorder	Bipolar disorder	1,461 cases, 2,008 controls	409 trios, 365 cases, 351 controls	Affymetrix [372,193]

And 130 more entries…

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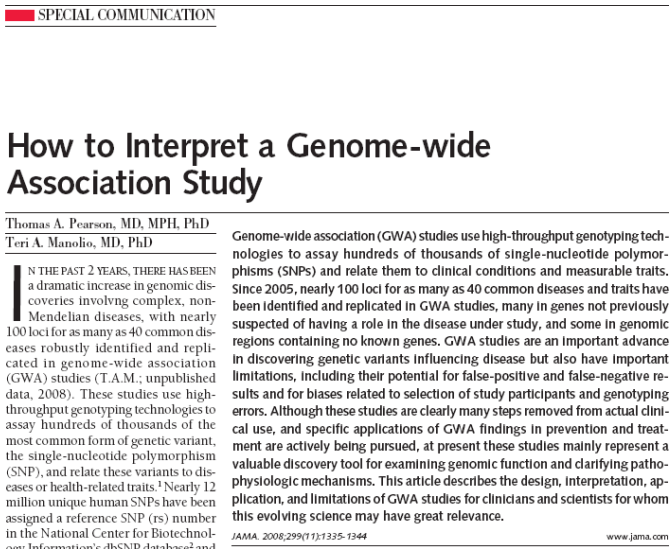

SPECIAL COMMUNICATION

How to Interpret a Genome-wide Association Study

Thomas A. Pearson, MD, MPH, PhD
Teri A. Manolio, MD, PhD

Genome-wide association (GWA) studies use high-throughput genotyping technologies to assay hundreds of thousands of single-nucleotide polymorphisms (SNPs) and relate them to clinical conditions and measurable traits. Since 2005, nearly 100 loci for as many as 40 common diseases and traits have been identified and replicated in GWA studies, many in genes not previously suspected of having a role in the disease under study, and some in genomic regions containing no known genes. GWA studies are an important advance in discovering genetic variants influencing disease but also have important limitations, including their potential for false-positive and false-negative results and for biases related to selection of study participants and genotyping errors. Although these studies are clearly many steps removed from actual clinical use, and specific applications of GWA findings in prevention and treatment are actively being pursued, at present these studies mainly represent a valuable discovery tool for examining genomic function and clarifying pathophysiologic mechanisms. This article describes the design, interpretation, application, and limitations of GWA studies for clinicians and scientists for whom this evolving science may have great relevance.

JAMA. 2008;299(11):1335-1344 www.jama.com

JAMA. 2008;299(11):1335-1344.

dbGaP

- http://www.ncbi.nlm.nih.gov/entrez/query/Gap/gap_tmpl/about.html
- The **d**ata**b**ase of **G**enotype **a**nd **P**henotype (dbGaP) was developed to archive and distribute the results of studies that have investigated the interaction of genotype and phenotype.
- http://www.ncbi.nlm.nih.gov/entrez/query/Gap/gap_tmpl/dbGaP_HowTo.pdf

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- Next-generation sequencing and SNPs
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What's recorded in dbSNP

- From their main web page, they have extensive information on how to submit SNPs, genotypes, validation experiments, population frequencies, etc., for any species.
- SNPs that you submit are called Submitter SNPs and get ssIDs.
- If there is a reference sequence available for the species submitted, they will map SNPs to this reference using the flank information you provide.
- SNPs that cluster at the same locus, are merged into Reference SNPs which have unique rsIDs.

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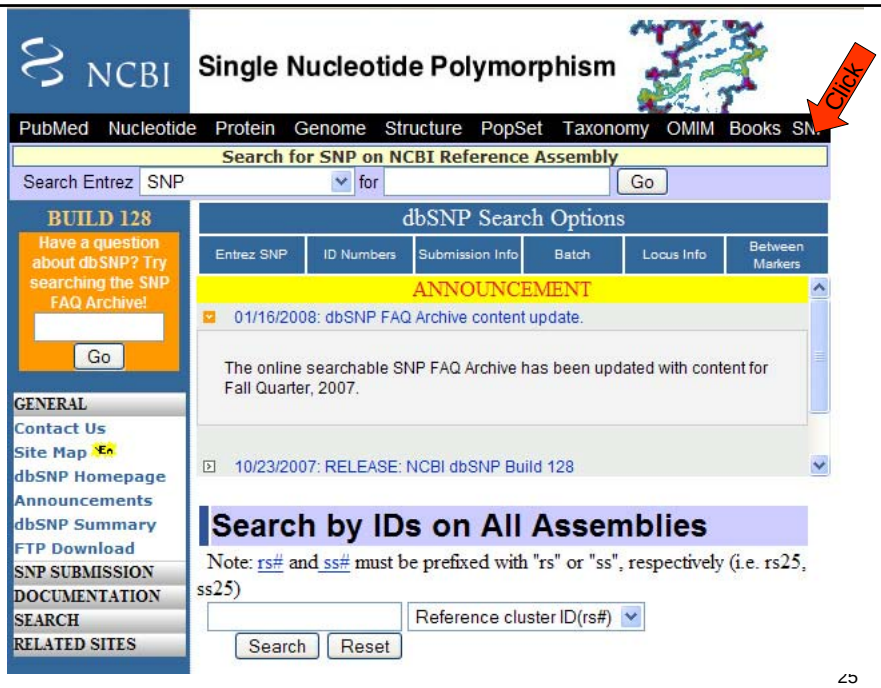

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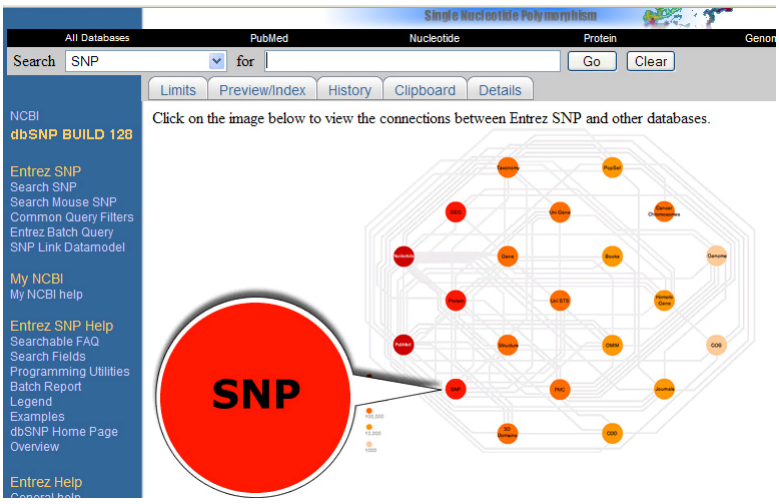
http://www.ncbi.nlm.nih.gov/SNP/index.html



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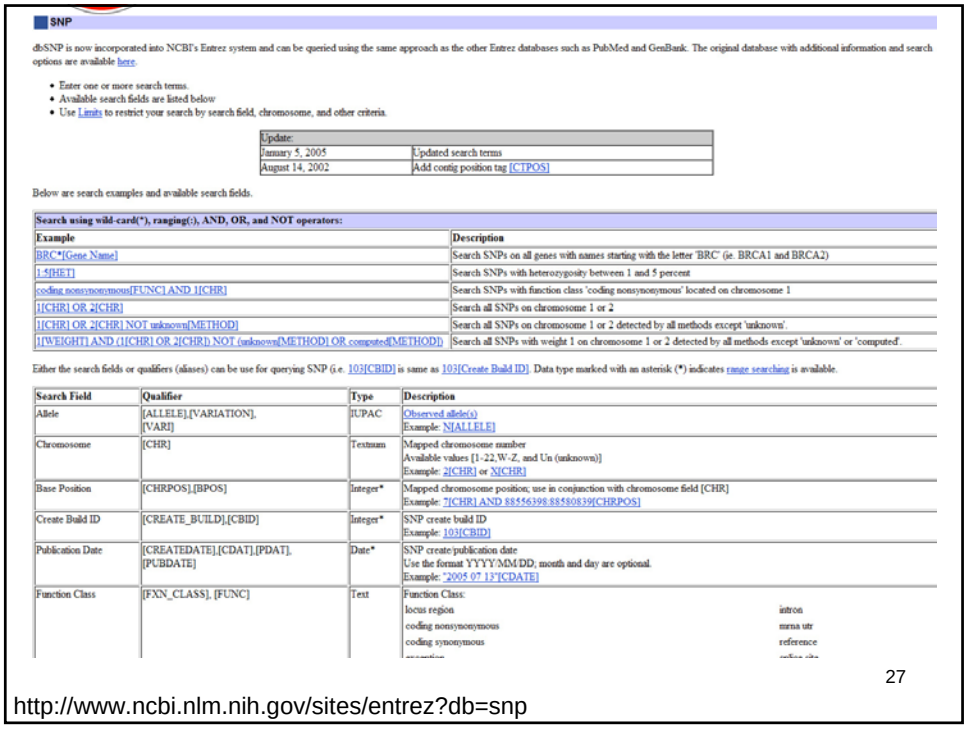
http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp



SNP

dbSNP is now incorporated into NCBI's Entrez system and can be queried using the same approach as the other Entrez databases such as PubMed and GenBank. The original database with additional information and search options are available here.

- Enter one or more search terms.
- Available search fields are listed below
- Use Limits to restrict your search by search field, chromosome, and other criteria.

Update:	
January 5, 2005	Updated search terms
August 14, 2002	Add contig position tag [CTPOS]

Below are search examples and available search fields.

Search using wild-card(*), ranging(:), AND, OR, and NOT operators:	
Example	**Description**
BRC*[Gene Name]	Search SNPs on all genes with names starting with the letter 'BRC' (ie. BRCA1 and BRCA2)
1:5[HET]	Search SNPs with heterozygosity between 1 and 5 percent
coding nonsynonymous[FUNC] AND 1[CHR]	Search SNPs with function class 'coding nonsynonymous' located on chromosome 1
1[CHR] OR 2[CHR]	Search all SNPs on chromosome 1 or 2
1[CHR] OR 2[CHR] NOT unknown[METHOD]	Search all SNPs on chromosome 1 or 2 detected by all methods except 'unknown'.
1[WEIGHT] AND (1[CHR] OR 2[CHR]) NOT (unknown[METHOD] OR computed[METHOD])	Search all SNPs with weight 1 on chromosome 1 or 2 detected by all methods except 'unknown' or 'computed'.

Either the search fields or qualifiers (aliases) can be use for querying SNP (i.e. 103[CBID] is same as 103[Create Build ID]. Data type marked with an asterisk (*) indicates range searching is available.

Search Field	Qualifier	Type	Description
Allele	[ALLELE],[VARIATION], [VARI]	IUPAC	Observed allele(s) Example: N[ALLELE]
Chromosome	[CHR]	Textnum	Mapped chromosome number Available values [1-22,W,Z, and Un (unknown)] Example: 2[CHR] or X[CHR]
Base Position	[CHRPOS],[BPOS]	Integer*	Mapped chromosome position; use in conjunction with chromosome field [CHR] Example: 7[CHR] AND 88556398:88580839[CHRPOS]
Create Build ID	[CREATE_BUILD],[CBID]	Integer*	SNP create build ID Example: 103[CBID]
Publication Date	[CREATEDATE],[CDAT],[PDAT], [PUBDATE]	Date*	SNP create/publication date Use the format YYYY/MM/DD, month and day are optional. Example: "2005 07 13"[CDATE]
Function Class	[FXN_CLASS], [FUNC]	Text	Function Class: locus region — intron coding nonsynonymous — mrna utr coding synonymous — reference

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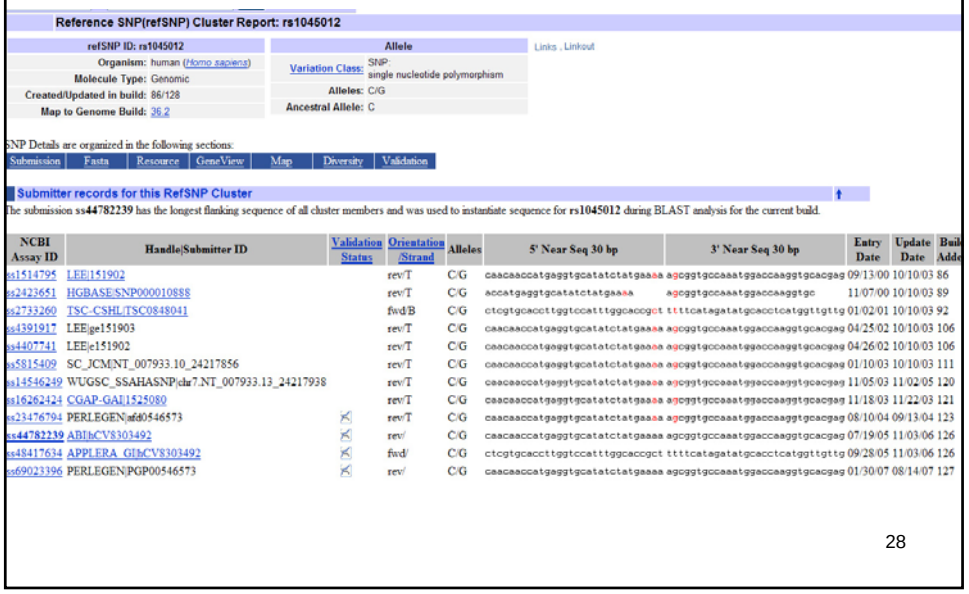
http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp

dbSNP record for rs1045012



Reference SNP(refSNP) Cluster Report: rs1045012

refSNP ID: rs1045012	
Organism:	human (Homo sapiens)
Molecule Type:	Genomic
Created/Updated in build:	86/128
Map to Genome Build:	36.2

Allele	
Variation Class:	SNP: single nucleotide polymorphism
Alleles:	C/G
Ancestral Allele:	C

Links . Linkout

SNP Details are organized in the following sections:

Submission | Fasta | Resource | GeneView | Map | Diversity | Validation

Submitter records for this RefSNP Cluster

The submission **ss44782239** has the longest flanking sequence of all cluster members and was used to instantiate sequence for **rs1045012** during BLAST analysis for the current build.

NCBI Assay ID	Handle|Submitter ID	Validation Status	Orientation /Strand	Alleles	5' Near Seq 30 bp	3' Near Seq 30 bp	Entry Date	Update Date	Build Added
ss1514795	LEE|151902		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	09/13/00	10/10/03	86
ss2423651	HGBASE:SNP000010888		rev/T	C/G	accatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgc	11/07/00	10/10/03	89
ss2733260	TSC-CSHL|TSC0848041		fwd/B	C/G	ctcgtgcaccttggtccatttggcaccgct	ttttcatagatatgcacctcatggttgttg	01/02/01	10/10/03	92
ss4391917	LEE|ge151903		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	04/25/02	10/10/03	106
ss4407741	LEE|e151902		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	04/26/02	10/10/03	106
ss5815409	SC_JCM|NT_007933.10_24217856		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	01/10/03	10/10/03	111
ss14546249	WUGSC_SSAHASNP|chr7.NT_007933.13_24217938		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	11/05/03	11/02/05	120
ss16262424	CGAP-GAI|1525080		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	11/18/03	11/22/03	121
ss23476794	PERLEGEN|afd0546573		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	08/10/04	09/13/04	123
ss44782239	ABI|hCV8303492		rev/	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	07/19/05	11/03/06	126
ss48417634	APPLERA_GI|hCV8303492		fwd/	C/G	ctcgtgcaccttggtccatttggcaccgct	ttttcatagatatgcacctcatggttgttg	09/28/05	11/03/06	126
ss69023396	PERLEGEN|PGP00546573		rev/	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	01/30/07	08/14/07	127

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Fasta sequence (Legend)

>gnl|dbSNP|rs1045012|allelePos=301|totalLen=601|taxid=9606|snpclass=1|alleles='C/G'|mol=Genomic|build=126

```
GCAGAAAAGA TGGGTTCTTG GTCATGTGGA GCTGCTGGAT CAAGCCTCTC CTGAAGCCCT
CAACCCTGTG AGTTTTTGGT AACATGAGCC AACACAGTCC CCTTAAAATT GAAGCCAGTT
TGAATCCGGG TTTCACGGTG AGTGGGCAGA TGCTCCACAA TGAGTGGCCA TGCCCTGCCT
TGCACCACCC CCCCAACCCA CCACCTCCTT TCAGGACGGT GGTCCCAGCC ACCCTGACAT
ACCTGTCACC TGCCCGTTGT GCTCCTTGAG CTCGTGCACC TTGGTCCATT TGGCACCGCT
S
TTTTCATAGA TATGCACCTC ATGGTTGTTG GGGCAGATGG CAATCTCTGA AGGGGAGATG
GAGGGAGATT GAGGGGCCCT CTCCATGACT GCCCTCTGCC AGGACACACT ACACAGTGCA
CCTAGGCAAC AACACCTCAC CTTTCATGAC TCAGTCTCTC CTCTTCTGCC TTGCAGGGGC
CCCCTGAAGT CCTTCAGGCC CTGCTAGGCC ACCCTGTCTT CTCCTGGAAC TGGCTGTCCT
TTACTCGCAG CAATGAACCC TGGGACCTCT CCCCACCCTA TTGCTCTGGC CAACCAGGAA
```

GeneView

GeneView via analysis of contig annotation: ARPC1B actin related protein 2/3 complex, subunit 1B, 41kDa

Click to see [all] [cSNP] [has frequency] [double hit] [haplotye tagged] variations associated with this gene.

Group Label	Contig->mRNA	Gene Model (contig mRNA transcript) Color Legend
reference	NT_007933->NM_005720 sv function	
Celera	NW_923574->NM_005720 sv function	
CRA_TCAGchr7v2	NT_079595->NM_005720 sv function	

Group label	Contig-->mRNA-->Protein	Contig position	mRNA orientation	mRNA pos	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
reference	NT_007933->NM_005720->NP_005711	24218630	forward	200	nonsynonymous	C	Asn [N]	3	37
					contig reference	G	Lys [K]	3	37
Celera	NW_923574->NM_005720->NP_005711	22257590	forward	200	nonsynonymous	C	Asn [N]	3	37
					contig reference	G	Lys [K]	3	37
CRA_TCAGchr7v2	NT_079595->NM_005720->NP_005711	24245339	forward	200	nonsynonymous	C	Asn [N]	3	37
					contig reference	G	Lys [K]	3	37

Integrated Maps:

NCBI MapViewer: rs1045012 maps exactly once on NCBI human chromosome 7

Chromosome	Contig accession	Contig position	Chromosome position	Hit orientation	Contig Allele	Assembly Type	Group label	Contig label	Neighbor SNP	SNP_flank position
7	NW_923574.1	22257590	93718553	minus	G	alt_assembly_1	Celera	Celera	view	300
7	NT_079595.2	24245339	98344127	minus	G	alt_assembly_2	CRA_TCAGchr7v2	CRA_TCAGchr7v2	view	300
7	NT_007933.14	24218630	98822290	minus	G	ref_assembly	reference	reference	view	300

NCBI Resource Links

Submitter-Referenced	dbSNP Blast Analysis	UniGene Cluster ID	3D structure mapping
GenBank	GenBank HTGS Finished:	489284	NP_005711
T74087 BM803458 Hs.11538	AC004922.2 NC_000007.12		

Population Diversity

	Sample Assertainment					Genotypes			Alleles		
ss#	Population	Individual Group	Sample (2N)	Founder (N)	Source	C/C	C/G	HWP	C	G	Het. +/-std err
ss23476794	AFD_EUR_PANEL	European	48	24	IG	0.917	0.083	1.000	0.958	0.042	
	AFD_AFR_PANEL	African American	46	23	IG	0.739	0.261	0.479	0.870	0.130	
	AFD_CHN_PANEL	Asian	48	24	IG	0.958	0.042	1.000	0.979	0.021	
ss44782239	AoD_African_American		90		AF				0.880	0.120	

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	AGD_Caucasian			92	AF				0.990	0.010	
ss48417634	AGI_ASP population	African American		78	IG	0.795	0.205	0.479	0.897	0.103	
ss69023396	HapMap-CEU	European		120	GF	0.917	0.083		0.958	0.042	
	HapMap-HCB	Asian		90	GF	0.956	0.044		0.978	0.022	
	HapMap-JPT	Asian		90	GF	0.956	0.044		0.978	0.022	
	HapMap-YRI	Sub-Saharan African		120	GF	0.650	0.300	0.050	0.800	0.200	

Concordant Genotype	Total Sample	C/C	C/G	G/G
ss23476794	71		9	62
ss44782239	269	5	37	224
ss48417634	39	31	8	
ss69023396	269	5	37	227

RefSNP Genotype Summary	Total Individual	C/C	C/G	G/G
rs1045012	371	36	53	281

Discordant Genotypes:

Individual SampleID	SubSNP(ss)	Genotype	Population Handle	Submitter Population	Submitter SampleID	SampleID Alias	Submission Batch
5291	ss44782239	G/G	CSHL-HAPMAP	HapMap-YRI	NA19207	YOR051.03	rel21a_chr7_YRI_BROAD_BEADARRAY
5291	ss69023396	C/G	CSHL-HAPMAP	HapMap-YRI	NA19207	YOR051.03	chr7-HapMap-YRI

Genotype data submitted for 380 **samples from 371 individuals** **Individual with multiple genotypes submission:** 270

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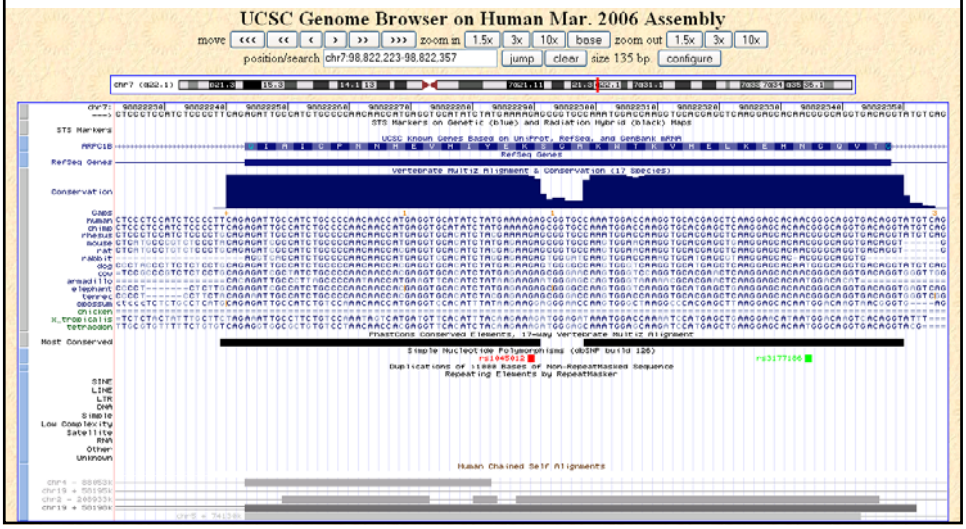
Viewing SNPs in Browsers

NCBI Ensembl UCSC



Overview of Topics

- Review of genetic variation discovery
- Database of SNPs, dbSNP
- Other types of genetic variation
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- Targeted Genomic Selection

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Other Types of Sequence Variation

- Deletion/Insertion Polymorphisms (DIPs)
  - Also called indels, sizes from 1base to ~1kb
  - More difficult to detect and automatically type
  - Occur at less frequent intervals; about 8 times less frequent compared to SNPs
    - 2.1M DIPs and 9.3M SNPs
    - More difficult to cluster, e.g. rs34505627 and rs10581774:

```
atttatttatttattt reference
attt----atttattt rs10581774
atttattta----ttt rs34505627
```

- Structural Variation
- Copy Number Variation

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Definition of Terms: Larger Scale Variation

Table 1. Selected terms in the CNV literature

Term	Definition	Reference
Structural variant	A genomic alteration (e.g., a CNV, an inversion) that involves segments of DNA >1 kb	Feuk et al. (2006a)
Copy number variant (CNV)	A duplication or deletion event involving >1 kb of DNA	
Duplicon	A duplicated genomic segment >1 kb in length with >90% similarity between copies	
Indel	Variation from insertion or deletion event involving <1 kb of DNA	
Intermediate-sized structural variant (ISV)	A structural variant that is ~8 kb to 40 kb in size. This can refer to a CNV or a balanced structural rearrangement (e.g., an inversion)	Tuzun et al. (2005)
Low copy repeat (LCR)	Similar to segmental duplication	Lupski (1998)
Multisite variant (MSV)	Complex polymorphic variation that is neither a PSV nor a SNP	Fredman et al. (2004)
Paralogous sequence variant (PSV)	Sequence difference between duplicated copies (paralogs)	Eichler (2001)
Segmental duplication	Duplicated region ranging from 1 kb upward with a sequence identity of >90%	Eichler (2001)
Interchromosomal	Duplications distributed among nonhomologous chromosomes	
Intrachromosomal	Duplications restricted to a single chromosome	
Single nucleotide polymorphism (SNP)	Base substitution involving only a single nucleotide; ~10 million are thought to be present in the human genome at >1%, leading to an average of one SNP difference per 1250 bases between randomly chosen individuals	The International HapMap Consortium (2003)

Genome Res. 2006 16: 949-961

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Human Genome Structural Variation Project

- NHGRI funded initiative
- A sequence-based survey of human structural variation aims to characterize common structural variants that are larger than (>5 kb)
- Types include multi-kilobase deletions, insertions, inversions, translocations, and duplications
- The approach entails sequencing the ends of fosmids and BACs from multiple individuals

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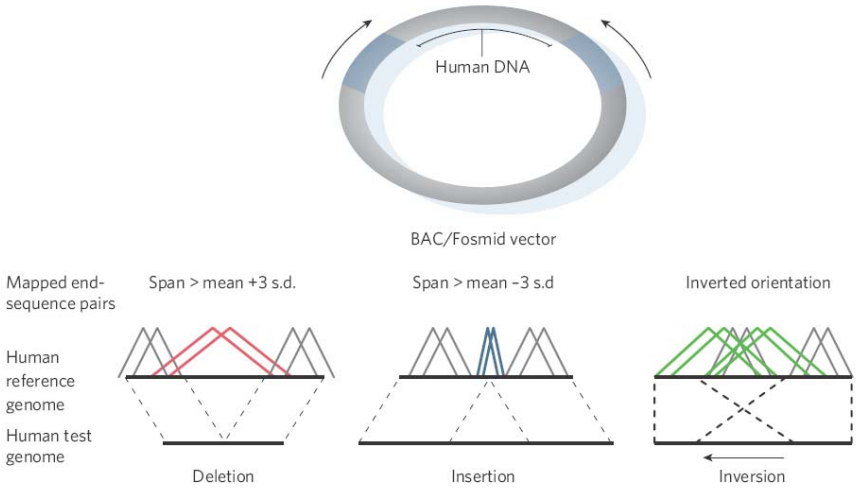
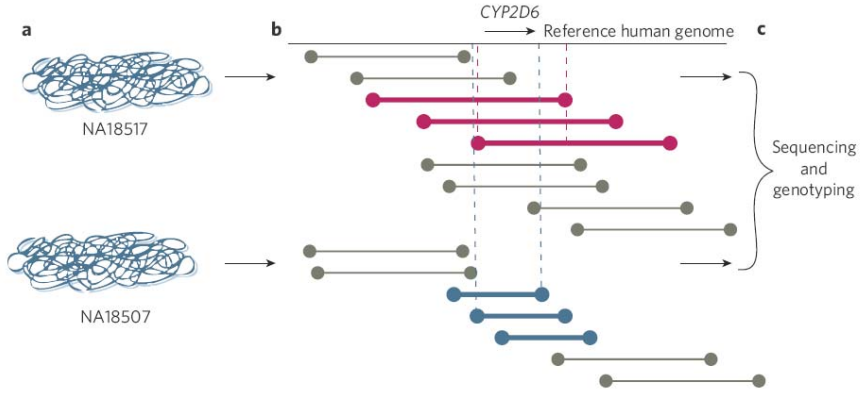


Nature. 2007 May 10;447(7141):161-5.

37

- Sequence from 1M fosmid ends (2M sequence reads) covers the genome to about 12X template coverage
- Or about 6X coverage across each of an individuals 2 haploids
- This is enough coverage to detect deletions and insertion as small as about 5kb



Nature. 2007 May 10;447(7141):161-5.

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Table 1 | Common structural polymorphisms and disease

Gene	Type	Locus	Size (kb)	Phenotype	Copy number variation
UGT2B17	Deletion	4q13	150	Variable testosterone levels, risk of prostate cancer	0–2
DEFB4	VNTR	8p23.1	20	Colonic Crohn's disease	2–10
FCGR3	Deletion	1q23.3	>5	Glomerulonephritis, systemic lupus erythematosus	0–14
OPN1LW/OPN1MW	VNTR	Xq28	13-15	Red/green colour blindness	0–4/0–7
LPA	VNTR	6q25.3	5.5	Altered coronary heart disease risk	2–38
CCL3L1/CCL4L1	VNTR	17q12	Not known*	Reduced HIV infection; reduced AIDS susceptibilty	0–14
RHD	Deletion	1p36.11	60	Rhesus blood group sensitivity	0–2
CYP2A6	Deletion	19q13.2	7	Altered nicotine metabolism	2–3

*Precise boundaries of the copy-number variant are not known.
VNTR, variable number tandem repeats.

Nature. 2007 May 10;447(7141):161-5.

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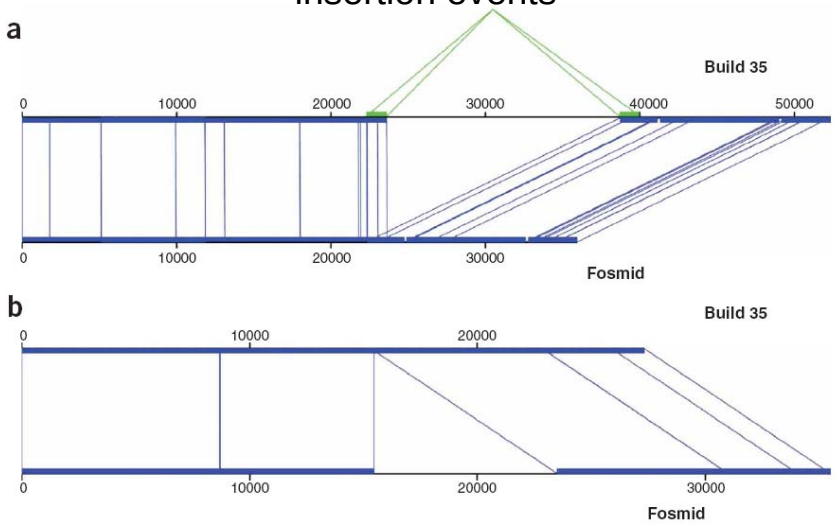
Sequence level identification of deletion and insertion events



Nature Genetics **37**, 727 - 732 (2005)

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Structural Variation Project Goals

- Generate fosmid and BAC end sequence data for up to 48 HapMap individuals
- Sequence for 9 individuals are available
- Twelve more are “ongoing”
- Mine the data for common and rare structural variants
- Mine the trace data for SNPs and DIPs

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http://www.genome.gov/25521748

Copy Number Variation

- This is structural variation, however the methods used to detect CNVs do not give precise local structural information
- Typically detected using an array-based technology, e.g.
  - SNP genotyping chips
  - Oligonucleotide arrays

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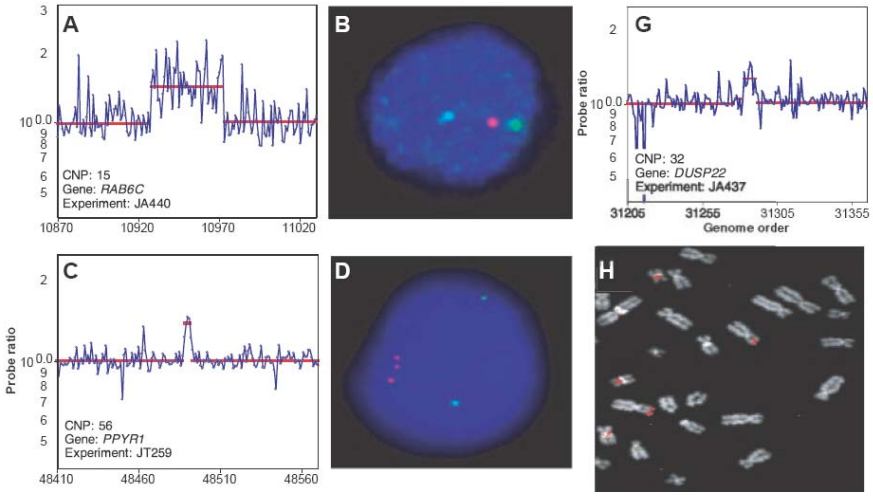
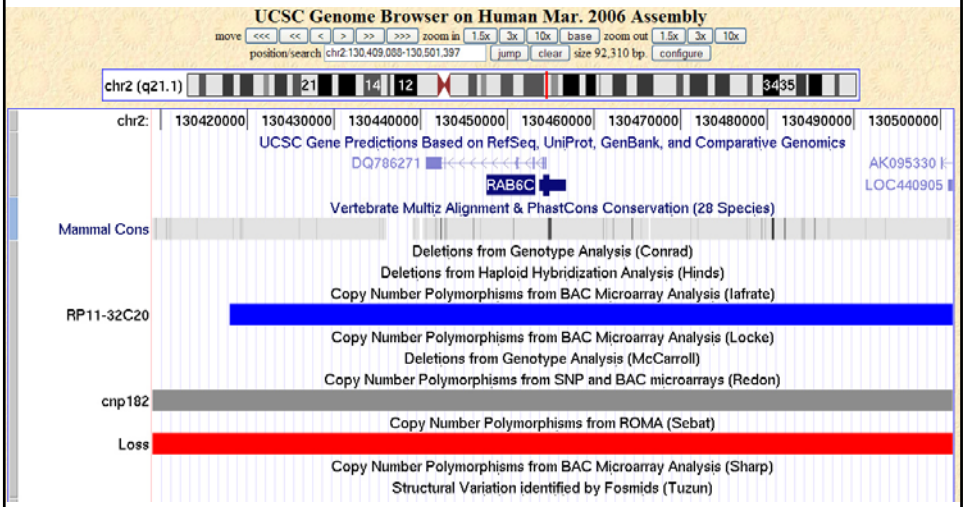
Copy number variation detected using representational oligonucleotide microarray analysis (ROMA)



Science. 2004 July 23;305(5683):525-8



Future of CNV detection

- New SNP chips are being designed to include more features to detect CNVs at a higher resolution across the genome
- These new chips will be applied to many more samples

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Overview of Topics

- Review of genetic variation discovery
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Medical Sequencing Project Initiatives

- Mapped Autosomal Mendelian Disorders
- Allelic Spectrum in Common Disease

  http://www.genome.gov/20019648
- Tumor Sequencing Project

  http://www.genome.gov/19517442
- The Cancer Genome Atlas Project
  - NCI GRAND ROUNDS Lecture by Dr. Collins

    http://videocast.nih.gov/Summary.asp?File=14383

    http://cancergenome.nih.gov/about/index.asp

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Mendelian Initiative:
- mapped Mendelian disorders to intervals of about 10 Mb or less

Allelic Spectrum Initiative:
- sequencing genes implicated in common disorders in large, well-phenotyped cohorts

Active Medical Sequencing Projects

Initiative	Disorder	Contributing Investigator	OMIM Number	Center	Status
Mendelian	Lymphedema-Cholestasis Syndrome (LCS; Aagenaes Syndrome)	Laura Bull	214900	WUGSC	Assigned
Mendelian	Joubert Syndrome (JBTS1)	Joseph Gleeson	213300	BI-MIT	Assigned
Mendelian	Dominant Restrictive Cardiomyopathy	Margart Wallace	609578	NISC	Assigned
Mendelian	Thoracic Aortic Aneurysms and Dissection (TAAD1)	Dianna Milewicz	607087	NISC	Assigned
Mendelian	Paroxysmal Kinesigenic Dyskinesia (PKD)	Louis Ptacek	118800	WUGSC	Assigned
Mendelian	Atrial Fibrillation, Dominant (ATFB3)	Calum MacRae	608988	BI-MIT	Assigned
Allelic Spectrum	Age-Related Macular Degenration	Goncalo Abecasis			Not Assigned
Allelic Spectrum	Diabetes	Michael Boehnke		NISC	Assigned
Allelic Spectrum	Cardiovascular Disease/Diabetes	Eric Boerwinkle			Not Assigned
Allelic Spectrum	Metabolic Syndrome	Nelson Freimer		WUGSC	Assigned
Allelic Spectrum	Early Onset Stroke	Steven Kittner			Not Assigned
Allelic Spectrum	Neural Tube Defects	Jasper Rine			Not Assigned
Allelic Spectrum	Cardiovascular Disease	Christine Seidman		BI-MIT	Assigned
Allelic Spectrum	Tetralogy of Fallot	Christine Seidman			Not Assigned
Allelic Spectrum	Schizophrenia	Patrick Sullivan		BCM-HGSC	Assigned

http://www.genome.gov/20019648

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Medical Sequencing

- This is accomplished using PCR amplification of selected targets followed by Sanger sequencing
  - Regions of interest (ROI) are defined, e.g. all coding exons in a suspected disease gene
  - PCR primer pairs designed to cover ROIs
  - PCR amplification and sequencing
  - Sequence variant detection

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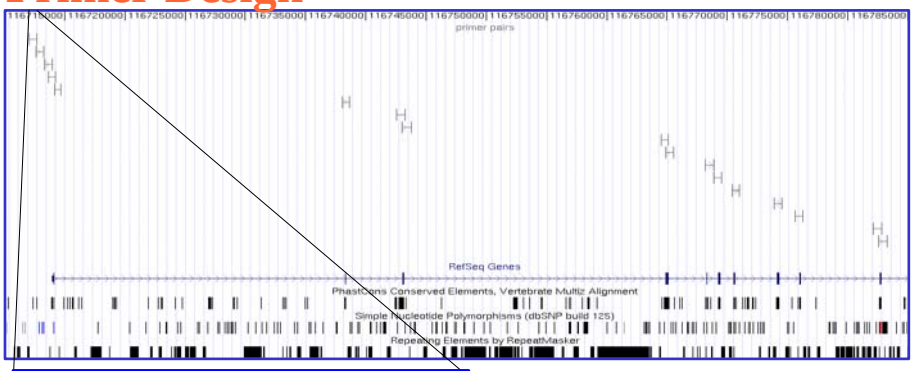
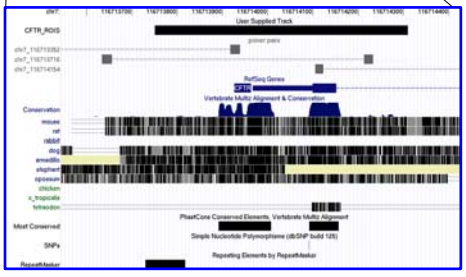
Primer Design

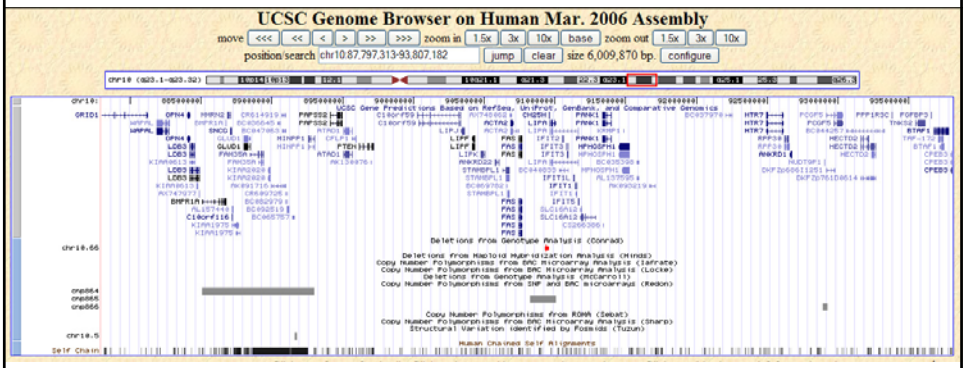




Choice of Genomic Regions

The regions of interest (ROIs) are typically defined by their biological context (coding, conservation, regulatory function, known variation). When features are in close proximity, the number of amplimers is automatically reduced, maintaining optimal coverage.

Watch out for segmental duplications or CNVs



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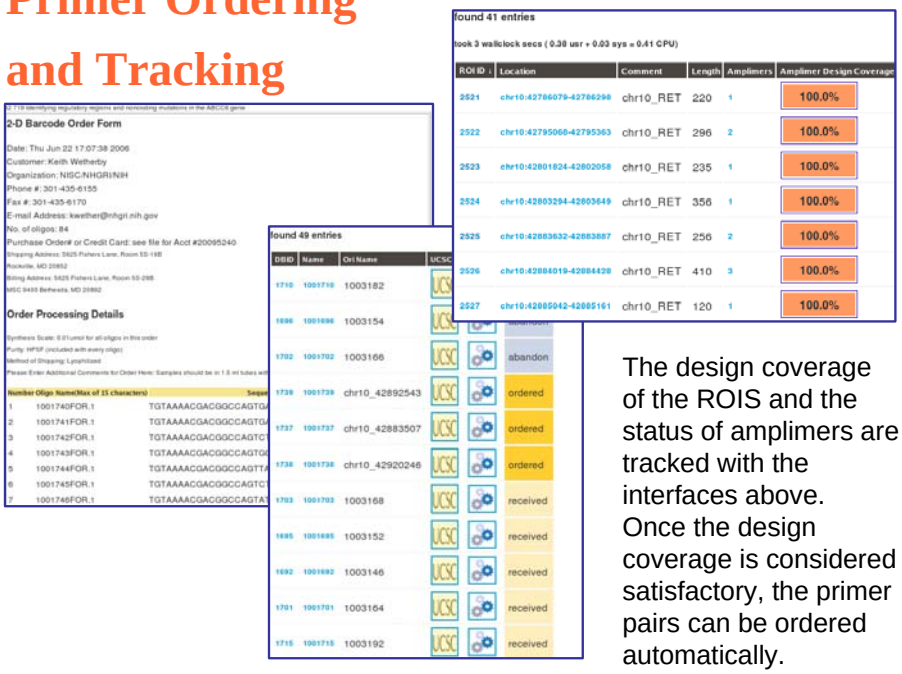
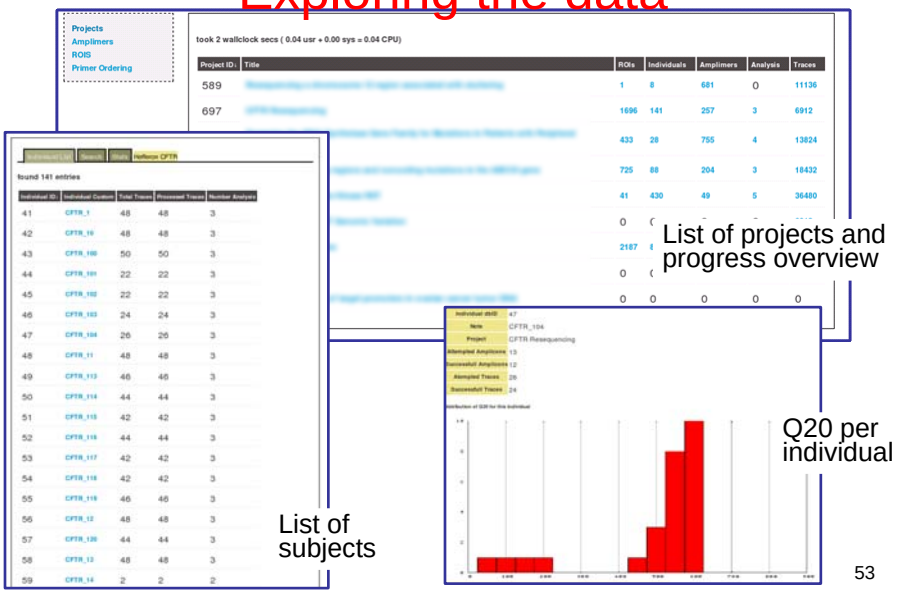
Primer Ordering and Tracking

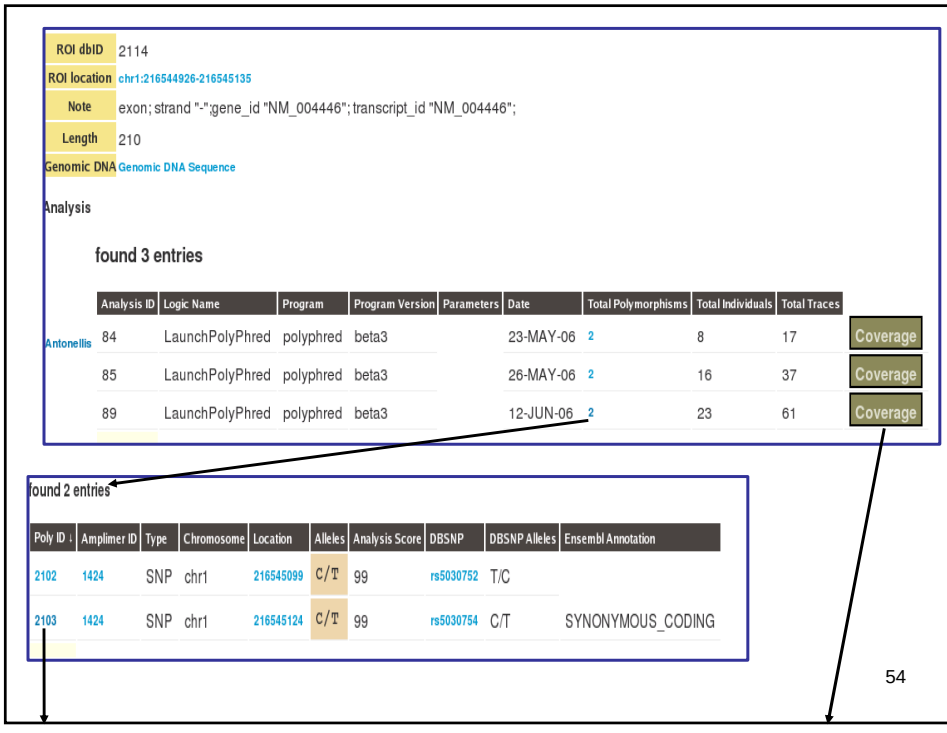


The design coverage of the ROIS and the status of amplimers are tracked with the interfaces above. Once the design coverage is considered satisfactory, the primer pairs can be ordered automatically.

Exploring the data



53



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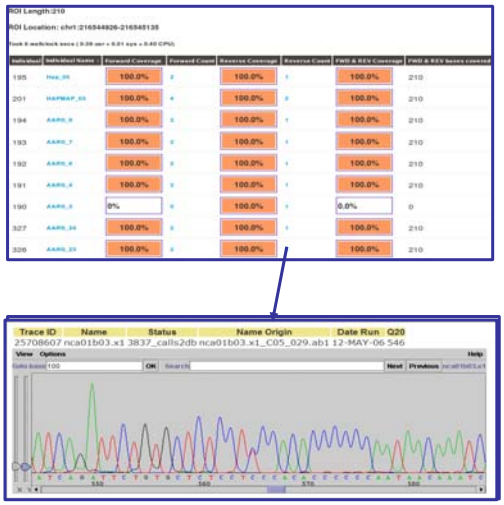
found 40 entries

Individual ↑	Alleles	Score	Trace	Trace Info	Strand
Hap_05	C/C	99	25822169	53129	-1
Hap_05	C/C	99	25821785	53137	1
HAPMAP_03	C/C	99	26204656	53153	-1
HAPMAP_03	C/C	99	25938327	53169	-1
HAPMAP_03	C/C	99	25936695	53127	1
HAPMAP_03	C/C	99	26202832	53134	1
AARS_8	C/C	99	25938363	53163	-1
AARS_8	C/C	99	25936731	53130	1
AARS_7	C/C	99	25936719	53161	1
AARS_7	C/C	99	25938351	53128	-1
AARS_6	C/T	99	25936707	53159	1
AARS_6	C/T	99	25938339	53126	-1
AARS_4	C/T	99	25936683	53141	1
AARS_4	C/T	99	25938315	53143	-1



The system keeps track of analysis performed on the data and coverage attained for each ROI. It also allows a user to browse the detected genotypes.

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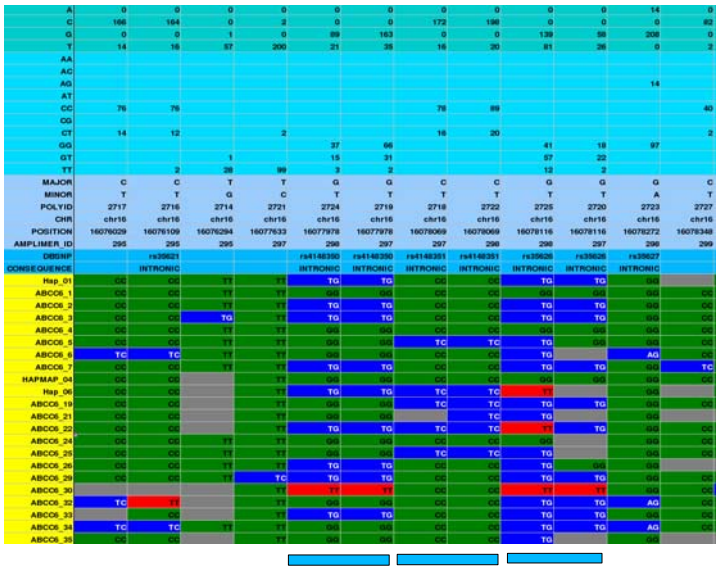

We are developing interfaces that allow exploring the results and identify interesting results as well flag problems.

Three examples of same SNP detected in overlapping amplimers. This information is used to assess accuracy of the detection.

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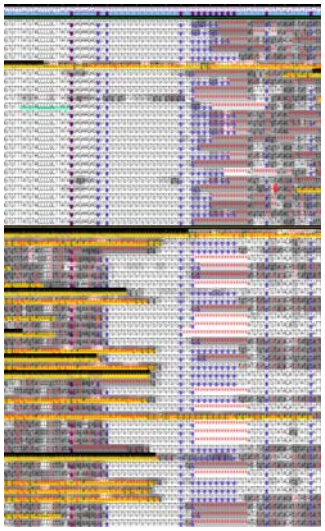
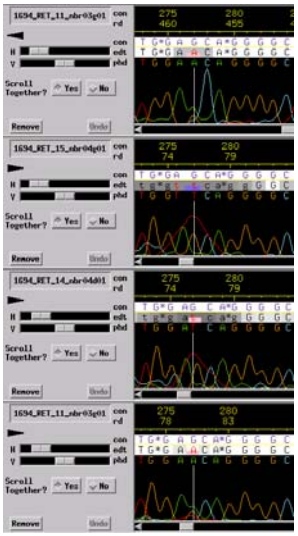
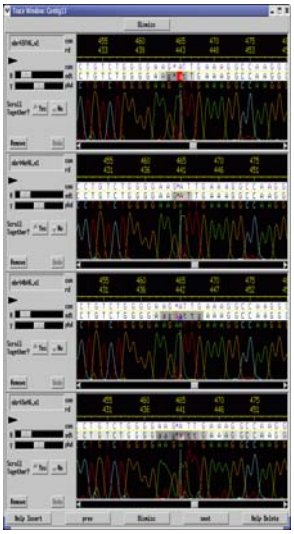
Some of the challenges of variation detection



Heterozygous DIPs



"Dye blob"



Detection saturation

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Future of Medical Sequencing

- Many sequencing centers have medical sequencing pipelines in operation
- Next-generation sequencing platforms will radically change this approach

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Overview of Topics

- Review of genetic variation discovery
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Next-gen Sequencing

- Introduced by Dr. Margulies in an earlier CTGA lecture
- How these can be used for variation detection and genotyping
- Techniques for targeted genomic capture in combination with next-gen sequencing
- Large scale efforts for greatly expanding the list of known variants in the genome

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454 FLX

Pyrosequencing

Genome Analyzer (Solexa)
Sequencing by synthesis

SOLiD

Ligation-based extension

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Platform Comparisons

Criterion	ABI 3730	Roche 454	Illumina	AB Solid
Sequencing chemistry	Big dye ddNTPs	Pyrosequencing	Sequencing by synthesis	Ligation-based sequencing
Amplification approach	Linear PCR	Emulsion PCR	Bridge amplification	Emulsion PCR
Paired ends/ separation	Yes/ variable	Yes/3kb	Yes/200bp	Yes/3kb
Time/run (bases/run)	1hr (65kb)	7hr (100Mb)	4d/8d (2000 Mb)	4d/10d (4000 Mb)
Read length	+650 bp	~230 bp	36 bp	35 bp

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Next-Gen Sequencing to Detect SNPs from Diploid DNA

- 454-FLX, Solexa and SOLiD generate sequence from clonal substrates
- If one would like to know both alleles at each base, sequence coverage must be high, e.g. over 10X
- To sequence an individual's diploid genome, therefore, would require at least 30Gb of sequence
  - 300 454-FLX runs (100 machine-days)
  - 15 Solexa runs (120 machine-days)
  - 8 SOLiD runs (80 machine-days)

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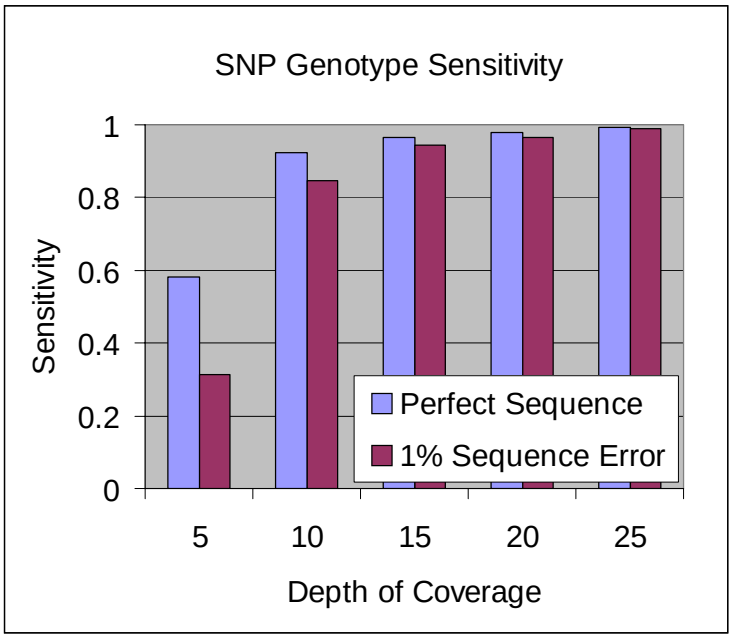

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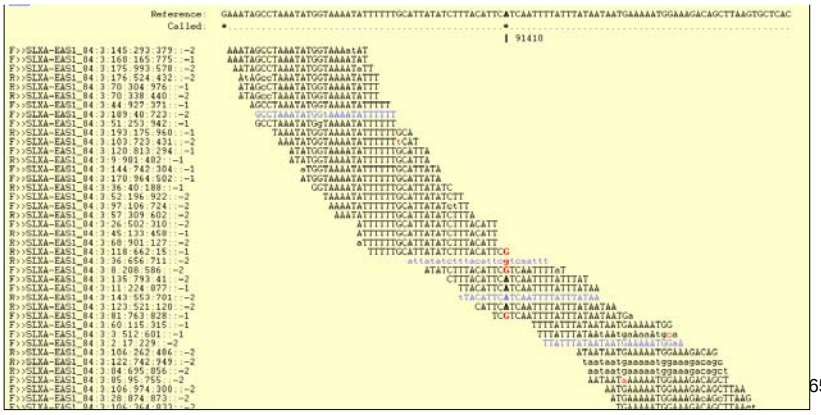
Example of short read sequence alignment



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SNP/Genotype Calling

- Alleles at each base with aligned data called using a Bayesian based method
  - ten possible genotypes, four homozygous and 6 heterozygous
  - Non-reference genotype prior probability is 0.001, sequencing error rate is 1.7%
  - Score is the difference between the log-odds of the most probable genotype and the second most probable genotype

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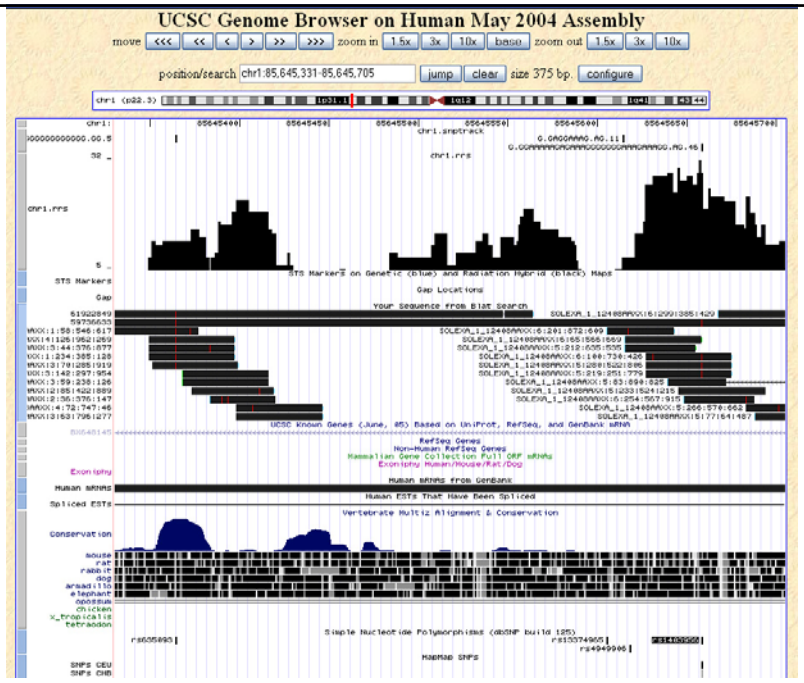

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Targeted Genomic Selection

- Multiplex PCR
  - Expensive to cover large regions
- Reduced representation using restriction enzymes
  - Inexpensive, but cannot be targeted
- Long Range PCR
  - Difficult to design, suffers from allelic dropout

- Hybridization capture
- Molecular Inversion Probe capture

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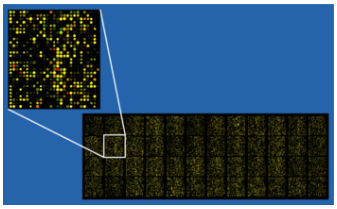
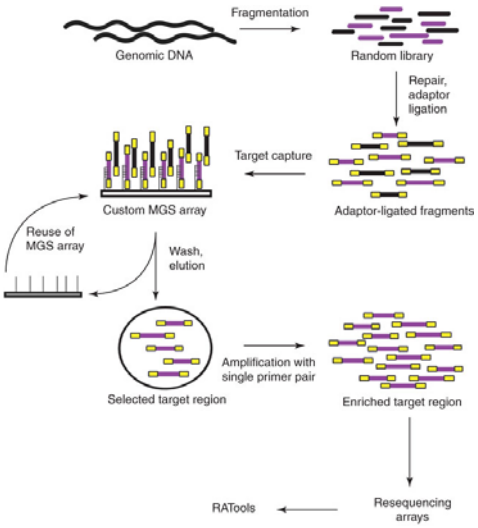
Microarray Direct Capture

- **385k features / chip (6MB seq.)**
- **24-30k exons / chip**
- **55-85% specificty (seq in ROI)**
- **12-50% total ROI seq coverage***
- **Exon coverage 40-78% (22-60% dropout)**
- **Non-uniform seq. depth**
- **20 ug DNA input**

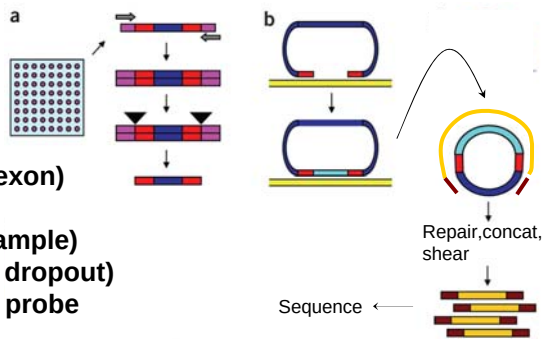




Hodges etal. *Nature Genetics* **39**, 1522-1527 (2007)
Okou etal *Nature Methods* **4**, 907-909 (2007)

70

Molecular Inversion Probe Capture



- 55,000 capture oligos (1 / exon)
- 6.7 Mb total seq.
- Specifity = 98.6% (small sample)
- Exon coverage = 91% (9% dropout)
- Each exon targeted with 1 probe
- 750 ng - 1.5 ug DNA input
- Highly non-uniform seq. coverage (several logs) - but consistent
- Het calls - 96% sens.

Porreca etal. *Nature Methods* **4**, 931-936 (2007)

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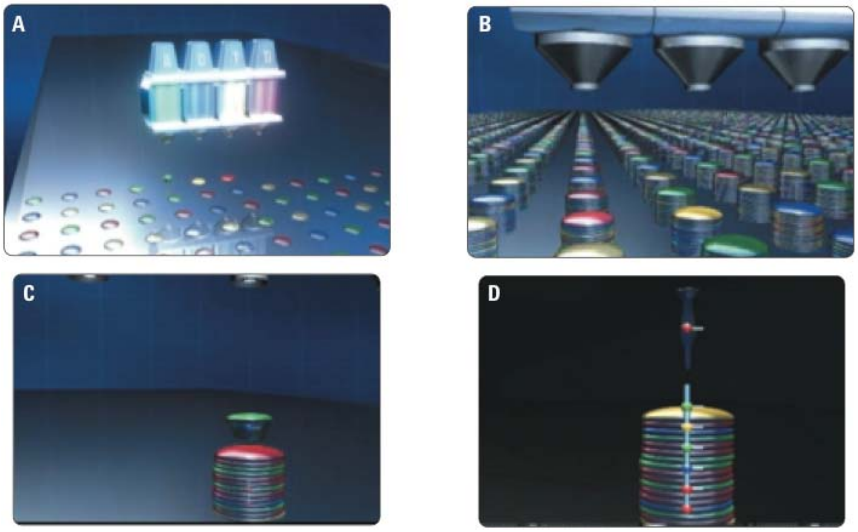
High Throughput Synthesis Of Long Oligo Libraries



Agilent Technologies

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Molecular Inversion Probe Capture

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Experimental Design - Workflow

Design Primers	3-4 days
↓	
Probe Amp	4 days
↓	
MI Capture	3-4 days
↓	
Library Prep	1-2 days
↓	
Sequencing	3 days
↓	
Analysis	

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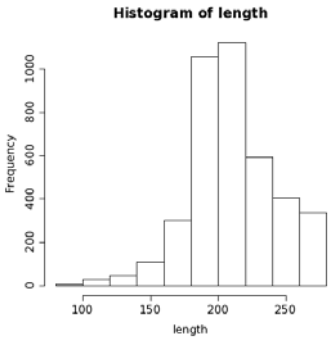
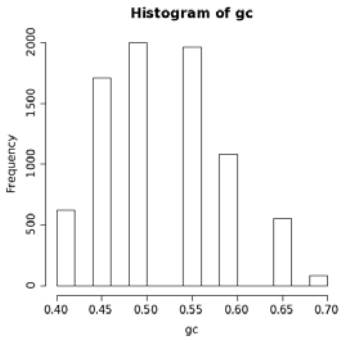
Experimental Design - Primers

- 4000 probes - (492kbp)
- Use primer_tile to design - similar to PCR primers
- Capture Region Criteria
  - length of region: 90-280bp, 200 optimal
  - GC% in targeting pairs: 45-65%, then 40-70%
  - minimize non-specific targeting pairs using ePCR
  - no Nt.AlwI or Nb.BsrDI restriction sites in targeting arms
  - no SNPs in targeting arms





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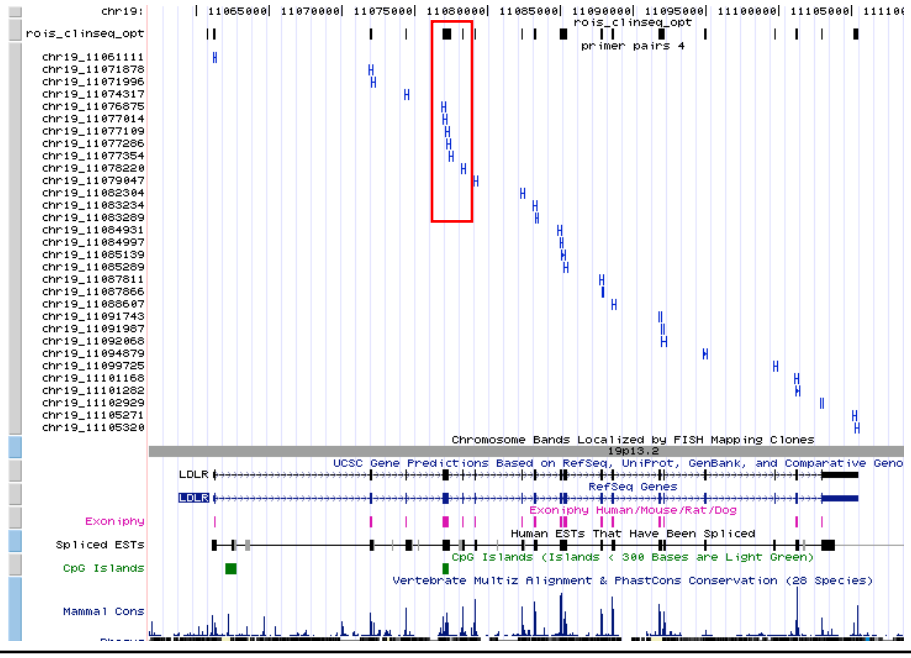
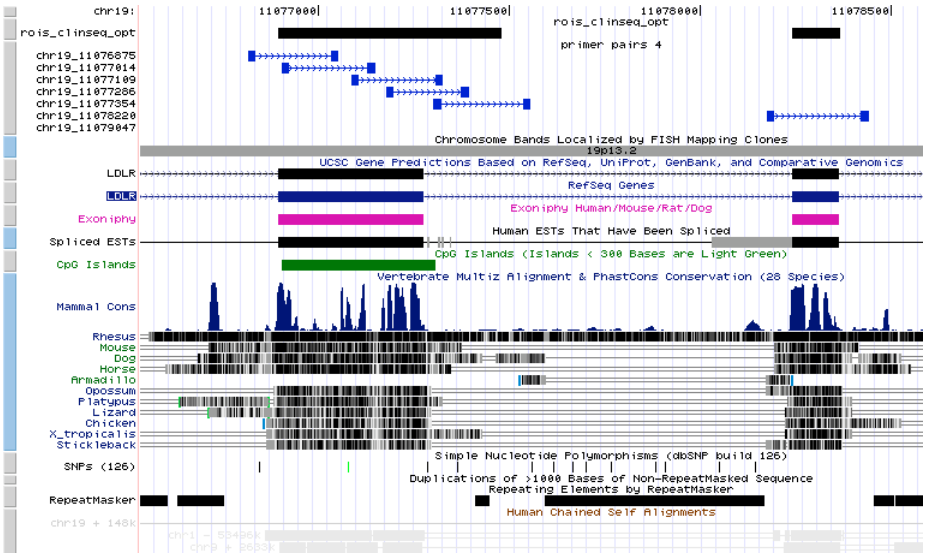
Example Region



Example Zoomed In



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Which technology to use depends on the scale of the project

- PCR with Sanger based sequencing
  - 10s of exons
  - 250 amplicons
- Targeted genomic selection and next-gen sequencing
  - Over 2Mb of sequence
  - Entire exome
  - Part of a chromosome

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The 1000 Genomes Project

- An international research consortium launched in January 2008
- With funding from
  - The Wellcome Trust Sanger Institute, UK
  - Beijing Genomics Institute, China
  - NHGRI, USA
- Sequence at least 1000 people from around the world
  - Vastly improve the genome-wide map of variation
  - Allow discovery of nearly all SNPs with MAFs down to 1%
  - Assist confirmation of rare variants
- http://www.1000genomes.org/

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Concluding remarks

- Along with the emergence of the human genome, we also have a growing database of variations that are critical to the overall value of the human genome sequence.
- These variations are what make us all (phenotypically) different, and impart different levels of resistance and susceptibility to disease.
- The collection of human sequence variation as well as that for other species will continue to evolve rapidly.

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References

EST SNPs

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