Studying Genetic Variation I: Computational Techniques

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NHGRI

Some points from other lectures

- Population Genetics: Practical Applications by 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.
- Identification of Cancer Susceptibility Genes by Elaine Ostrander
  - Genome wide scans to find cancer susceptibility genes and apply haplotype analyses to identify founder haplotypes.
- Genetic Variation II: Laboratory Techniques by Karen Mohlke
  - Focusing primarily on SNP genotyping methods

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Overview of Topics

- Genome variation origins
- Types of polymorphisms
- Polymorphism discovery methods
- Access to genetic variation data
- How to find SNPs in a region of interest
- Haplotype Map project
- Extra topics, time permitting

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Genome variation origins

- Mutations are fundamentally produced by errors in DNA replication.
- DNA is replicated in the production of the egg and sperm cells.
- Thus, a child does not receive exact copies of information from mother and father.

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Types of polymorphisms

- Single Nucleotide Polymorphisms (SNPs) are single base changes and occur at a rate of about 30 - 60 sites per genome per generation.

ACTCCTCTTATCCCTGC
ACTCCTCTCATCCCTGC

ACTCCTCT[C/T]ATCCCTGC

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Types of polymorphisms

- Short Tandem Repeats (STRs) are specific repeated segments of sequence.

```
GGTTTTTGCC------TATATATATAAGTAGGA
GGTTTTTGCC----TATATATATATAAGTAGGA
GGTTTTTGCC--TATATATATATATAAGTAGGA
GGTTTTTGCCTATATATATATATATAAGTAGGA

TTGCC[(TA)5/(TA)6/(TA)7/(TA)8]AGT
```

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Types of polymorphisms

- Deletion/Insertion Polymorphisms (DIPs) are deletions or insertions of 1 base to as large as a few kilobases.

```
CATAAAAAAAGAACAAAATC
CATAAAAAAA-AACAAAATC

CATAAAAAAA[G/-]AACAAAATC
```

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Beyond polymorphisms

- When a mutational event is sufficiently large, these events are classified as chromosomal rearrangements.
- There are many examples of these as seen in karyotypes.
- These larger scale rearrangements, duplications or deletions are often associated with various diseases and severe abnormalities.

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Discovery methods

- The primary method for discovering polymorphisms is by sequencing DNA and comparing the sequences.

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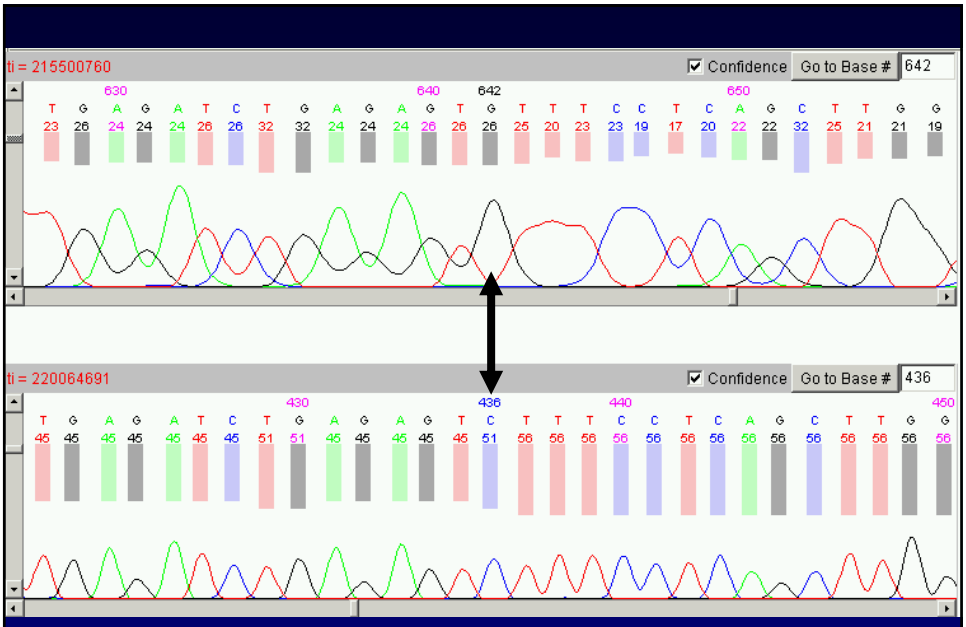

Trace Archive Search query:
trace_name='S213P602260RC9.T0' OR trace_name='50766946'

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Mining SNPs from sequence

- EST mining
- Clone overlap
- The SNP Consortium (TSC)
- Targeted resequencing
- Haplotype Map Project (HapMap)
- Chip based sequencing arrays

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Expressed Sequence Tag Mining

- These sequences are primarily associated with coding regions of genes.
- By clustering these sequences, selected differences are identified as SNPs.
- There are over 100,000 SNPs in dbSNP from a variety of species detected from clustered ESTs.
- The following example is from the CGAP SNP project (see refs).

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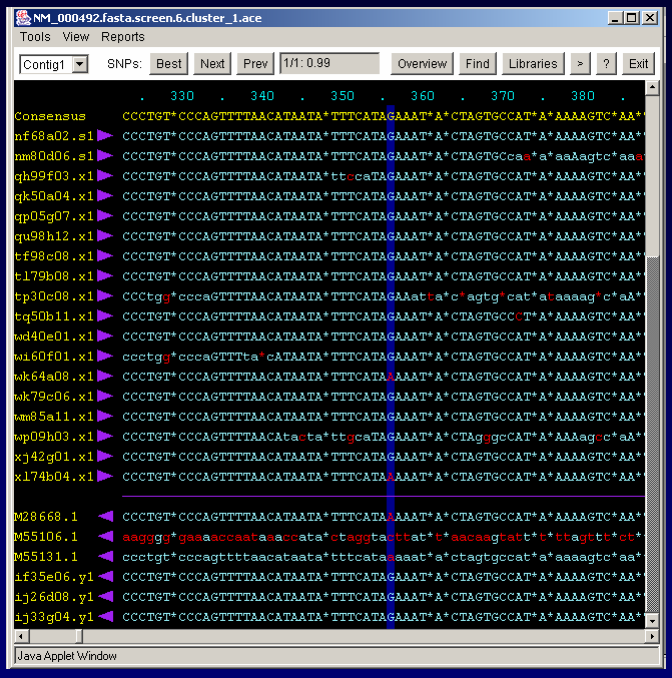

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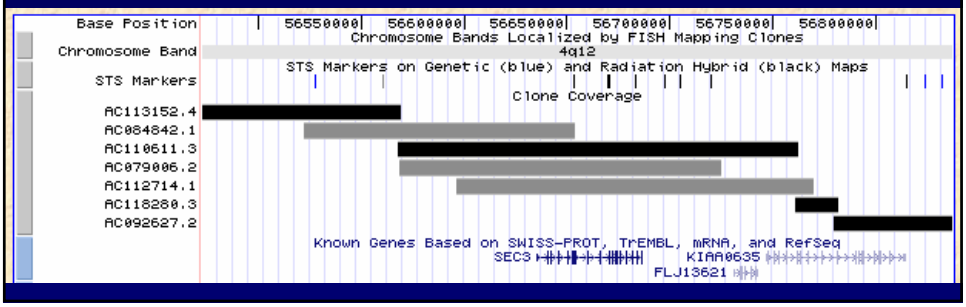
Clone Overlap

- 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 funded by the Wellcome Trust and 11 pharmaceutical and technological companies to discover 300,000 SNPs randomly distributed across the human genome.
- At its initiation in April 1999, the genome was only 10% finished and 20% in draft form.
- The SNPs were developed from a pool of DNA samples obtained from 24 individuals representing several ethnic groups.

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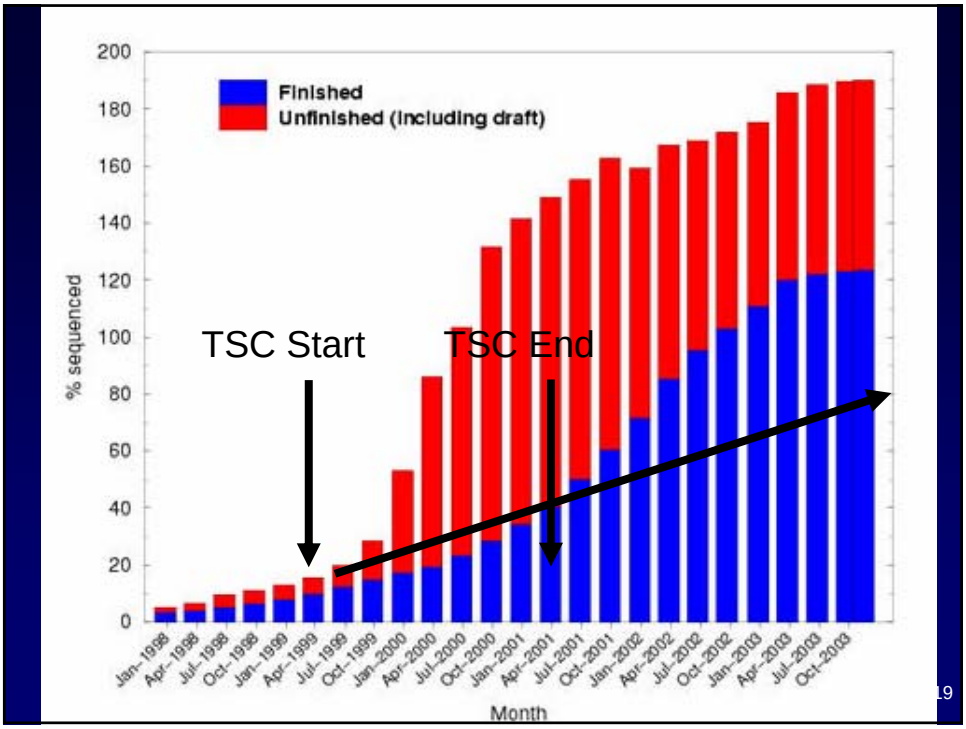

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The SNP Consortium

- With the rapid increase in genome coverage from the public Human Genome Project, the strategies changed to take full advantage of the draft and finished sequence.
- 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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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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Targeted Resequencing (Medical Sequencing)

- Any region of the genome can be targeted for resequencing. From the finished sequence, PCR primers can be designed to amplify a target followed by sequencing.
- This method generally works from a 1:1 mixture of an individuals two haploids, so the special case of heterozygous base positions must be properly processed.

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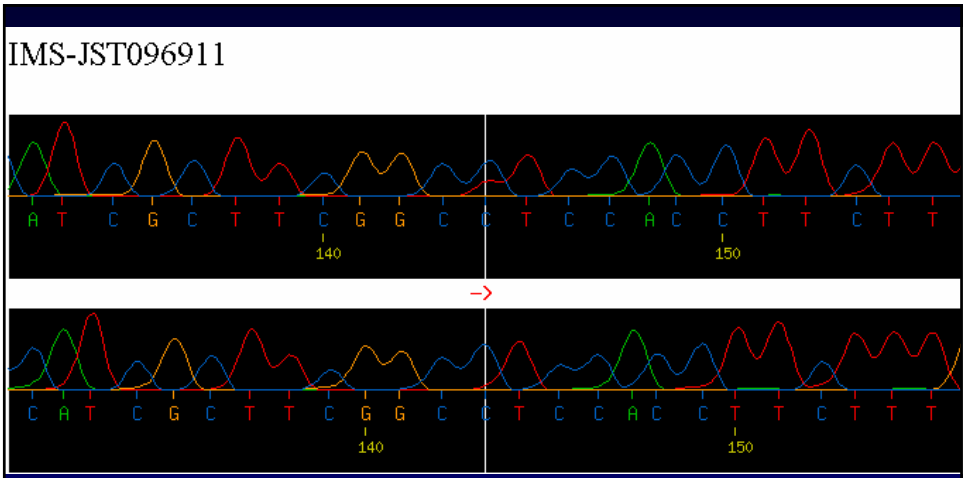

http://snp.ims.u-tokyo.ac.jp/

Chr 19 PTGER1 gcC/gcT A/A

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Targeted Resequencing

- JSNP database contains 190,562 SNPs detected from resequencing genomic regions containing genes in DNA from 24 Japanese individuals.
- Many groups use this technique for either SNP discovery in their region of interest, or as a way to validate SNPs.
- PolyPhred (see web links) is commonly used for analyzing resequencing traces.

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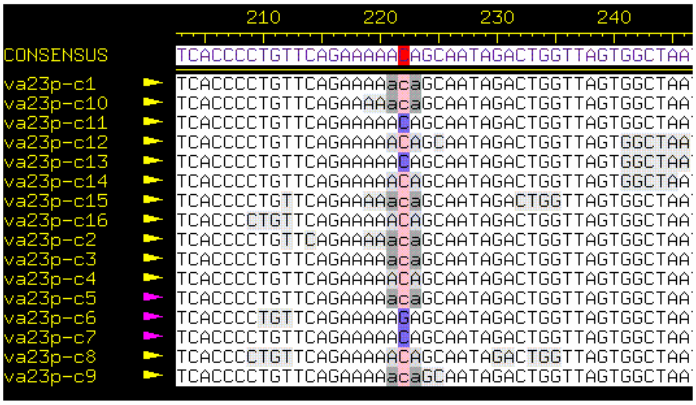
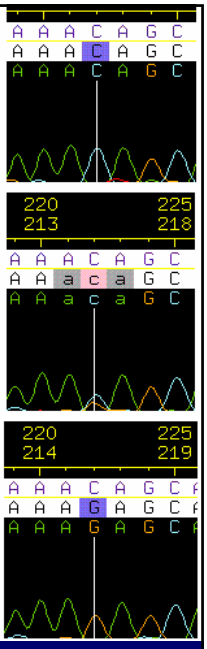
SNP detection by PolyPhred. View of a Consed window with a tag (red=highest ranking SNP tag) marking the consensus position of the SNP in the traces and genotype tags marking each of the samples below (purple=homozygote, pink=heterozygote). On the right trace windows for alternate homozygoes (C/C (top) and G/G (bottom>> and a heterozygoe (C/G) middle).





PolyPhred example from their web site.

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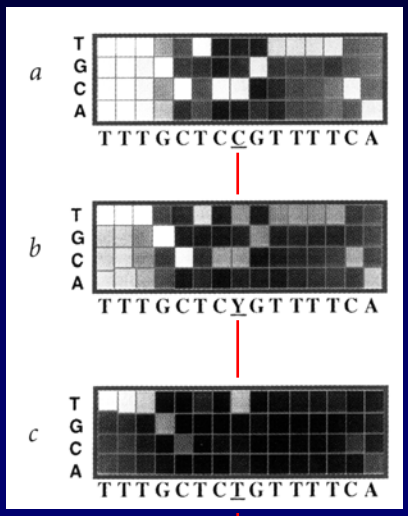
Sequencing Chips

...GCTCCGTTT...
...GCTCTGTTT...



The Sanger Institute

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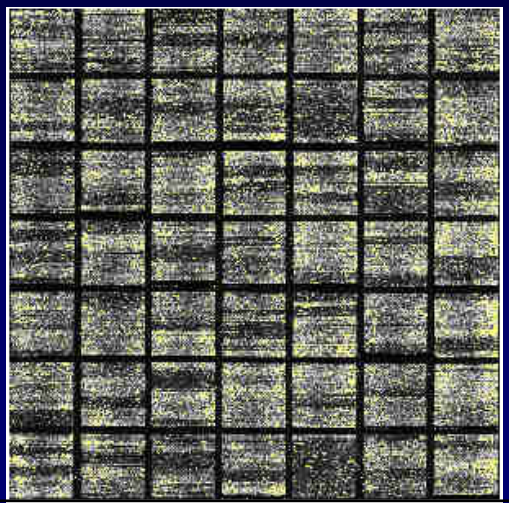
Perlegen used Affymetrix's chip design process to place 60M probes on a 5x5" chip. From 20 single haploid chromosome 21 chromosomes, they discovered 36k SNPs.



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Distribution properties

- EST mining
  - Locates SNPs primarily within coding regions.
- Clone overlap
  - High density of SNPs within overlap regions, absent elsewhere.
- The SNP Consortium (TSC)
  - Randomly distributed across the genome, however, total sequence only covers 50% of the genome

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Distribution properties

- Haplotype Map Project (HapMap)
  - Random, like TSC, for first phase that reached 2X coverage
  - Chromosome sorted phase increased coverage from 1X-6X
- Targeted resequencing
  - Focused discovery that has been applied to 100s of individuals
- Chip based resequencing
  - Repetitive elements in the genome are masked

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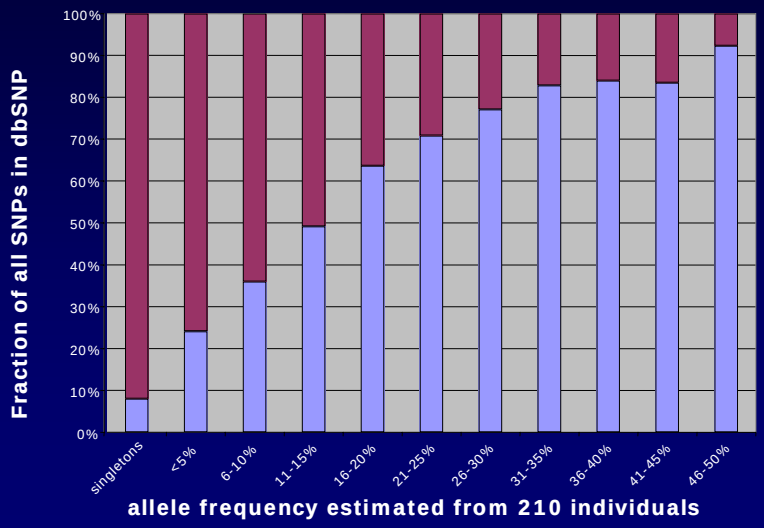
SNPs detected from 48 HapMap individuals gives an estimate dbSNP build 121 completeness



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NCBI dbSNP database of genetic variation

- This is the main repository of publicly available polymorphisms.
- You'll also find information on allele frequencies, populations, genotypes assays and much more.
- Most groups submit SNPs to dbSNP and only a few maintain web access to their SNPs.

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Submitting SNPs to 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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refSNP ID: rs1045012

Organism: human (*Homo sapiens*)
Molecule Type: Genomic
Created/Updated in build: 86/126
Map to Genome Build: 36.1

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
ss1514795	LEE|151902		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	09/13/00
ss2423651	HGBASE|SNP000010888		rev/T	C/G	accatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgc	11/07/00
ss2733260	TSC-CSHL|TSC0848041		fwd/B	C/G	ctcgtgcaccttggtccatttggcaccgct	ttttcatagatatgcacctcatggttgttg	01/02/01
ss4391917	LEE|ge151903		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	04/25/02
ss4407741	LEE|e151902		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	04/26/02
ss5815409	SC_JCM|NT_007933.10_24217856		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	01/10/03
ss14546249	WUGSC_SSAHASNP|chr7 NT_007933.13_24217938		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	11/05/03
ss16262424	CGAP-GAI|1525080		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	11/18/03
ss23476794	PERLEGEN|af60546573		rev/T	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	08/10/04
ss44782239	ABI|hCV8303492		rev/	C/G	caacaaccatgaggtgcatatctatgaaaa	agcggtgccaaatggaccaaggtgcacgag	07/19/05
ss48417634	APPLERA_GI|hCV8303492		fwd/	C/G	ctcgtgcaccttggtccatttggcaccgct	ttttcatagatatgcacctcatggttgttg	09/28/05

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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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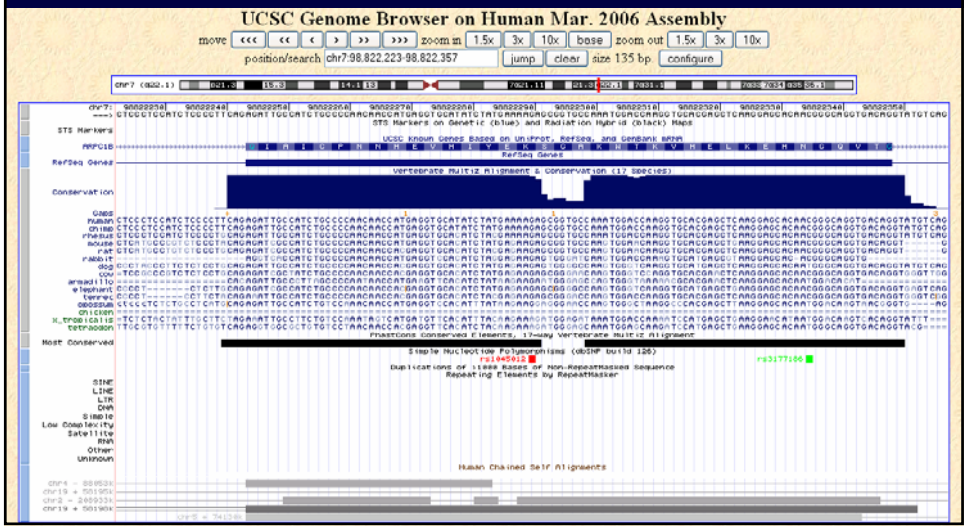
Viewing SNPs in Browsers

NCBI Ensembl UCSC



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How to find SNPs in a region of interest

- Gene based example
- A 2 Mbp region
- From a list of candidate genes

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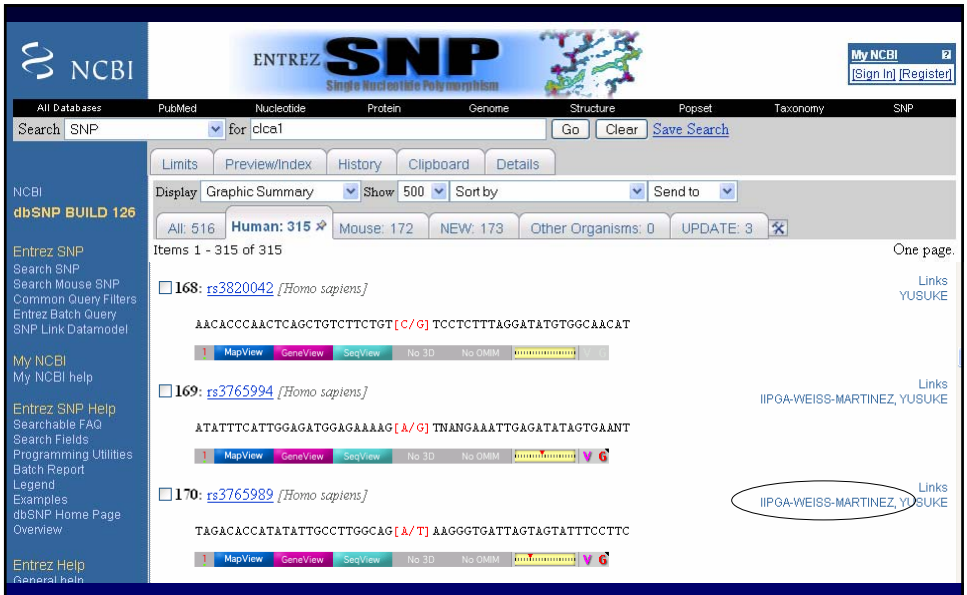

http://www.ncbi.nlm.nih.gov/SNP/index.html

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Graphic Summary :

- MapView — Mapped to chromosome shown with map weight 1 (single green bar), linkout to MapViewer
- MapView — Mapped to chromosome shown with map weight greater than 1 (two or more green bar)
- no Map — Mapped to multiple chromosomes
- MapView — Unknown, not on chromosome
- GeneView — SNP in locus region, linkout to Gene View in dbSNP
- SeqView — SNP in coding region (Non-synonymous)
- SeqView — SNP in coding region (synonymous)
- SeqView — SNP in other mRNA regions (intron, UTR, etc.)
- Not on mRNA — SNP not on mRNA
- Protein 3D — Structure neighbor available (Cn3D), linkout to structure mapping summary
- OMIM — linkout to Omim record
- V — Validated
- G — Genotype data available

Actual percentage (1-100) heterozygosity indicated by the red arrow (ie. 9%)and actual success rate indicated by the blue arrow (ie. 95%).

http://www.ncbi.nlm.nih.gov/entrez/query/Snp/EntrezSNPlegend.html

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IIPGA

Innate Immunity in Heart, Lung and Blood Disease
Programs for Genomic Applications

Home | Genes | Tools | Pubs | FAQ | Links | About Us Search: – Select a Gene – Go!

User: **Anonymous User** (Login | Register)

CLCA1

The following information is based on the unmasked version of the consensus sequence. We have also generated data for the masked version of the assembly. There is also an Introduction available if you are looking for a place to get started.

Information	
Name	chloride channel, calcium activated, family member 1
Source	InnateImmunity
Chromosome	chr1 (+) (chr1:86646072-86677963)
Accession	NM_001285
SNPs	203
Indels	0
Populations	2
Subjects	0
Links	[SNPper] [GoldenPath] [Gene Image] [LocusLink] [Omim] [PubMed]
Biological Significance	(See Omim for more ...)

http://innateimmunity.net/IIPGA/PGAs/InnateImmunity/CLCA1

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Gene Model (mRNA alignment) information from genome sequence

Total gene model (contig mRNA transcript):				2		
mrna	transcript	protein	mrna orientation	Contig	Contig Label	snp list
NM_001285	plus strand	NP_001276	forward	NT_032977	reference	currently shown
NM_001285	plus strand	NP_001276	forward	NW_921795	Celera	view

in gene region | cSNP | has frequency | double hit | haplotype tagged | refresh

gene model (contig mRNA transcript):	Contig Label	Contig	mrna	protein	mrna orientation	transcript	snp count
	reference	NT_032977	NM_001285	NP_001276	forward	plus strand 18, coding	

Color Legend

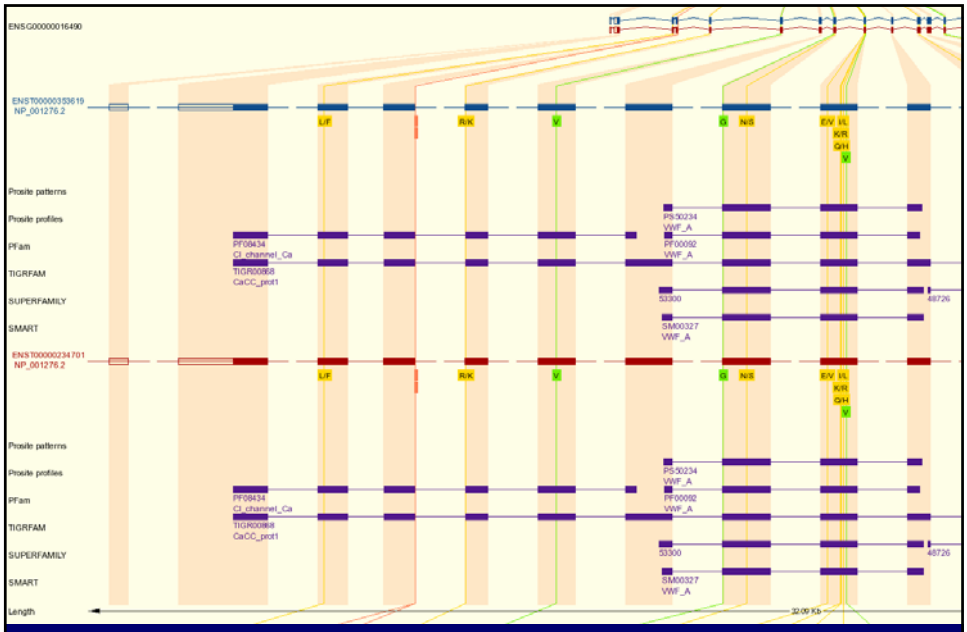
Region	Contig position	mRNA pos	dbSNP rs# cluster id	Hetero-zygosity	Validation	3D	OMIM	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
exon_3	56911049	544	rs2145412	0.148				nonsynonymous	T	Phe [F]	1	65
				0.148				contig reference	C	Leu [L]	1	65
exon_5	56914053	806	rs2753386	N.D.				nonsynonymous	A	Lys [K]	2	152
				N.D.				contig reference	G	Arg [R]	2	152
exon_6	56919894	996	rs1321694	0.484				synonymous	T	Val [V]	3	215
				0.484				contig reference	A	Val [V]	3	215
exon_8	56924133	1311	rs4630108	N.D.				synonymous	C	Gly [G]	3	320
				N.D.				contig reference	T	Gly [G]	3	320

Ensembl Gene Variation Report for ENSG00000016490

Gene	CLCA1 (HGNC Symbol) To view all Ensembl genes linked to the name click here. This gene is a member of the human CCDS set: CCDS709
Ensembl Gene ID	ENSG00000016490
Genomic Location	This gene can be found on Chromosome 1 at location 86,706,639-86,738,532. The start of this gene is located in Contig AL122002.16.1.113764.
Description	calcium activated chloride channel 1 precursor Source: RefSeq_peptide NP_001276

SNPs and variations in region of gene ENSG00000016490

http://www.ensembl.org/Homo_sapiens



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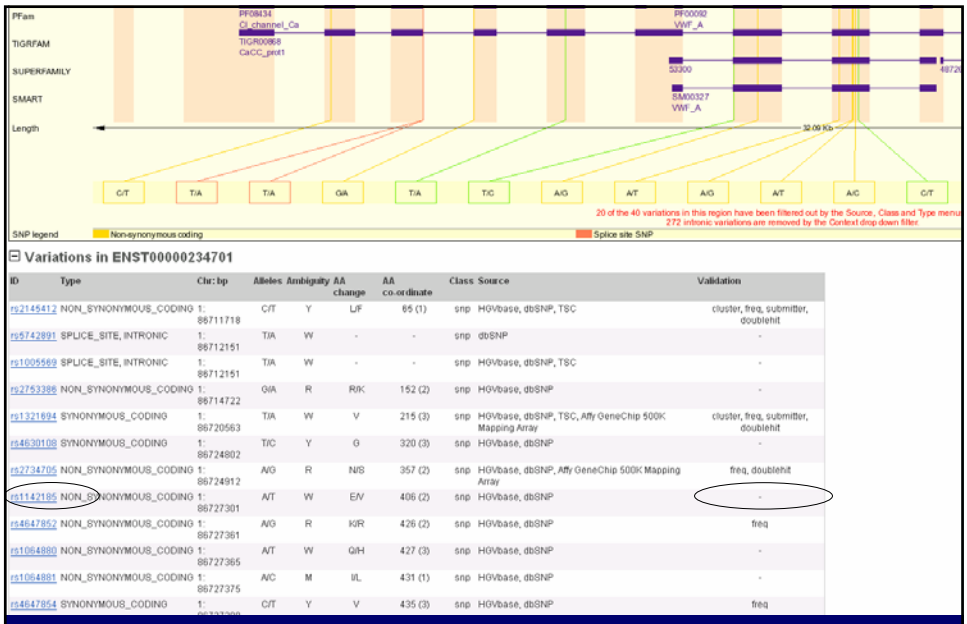
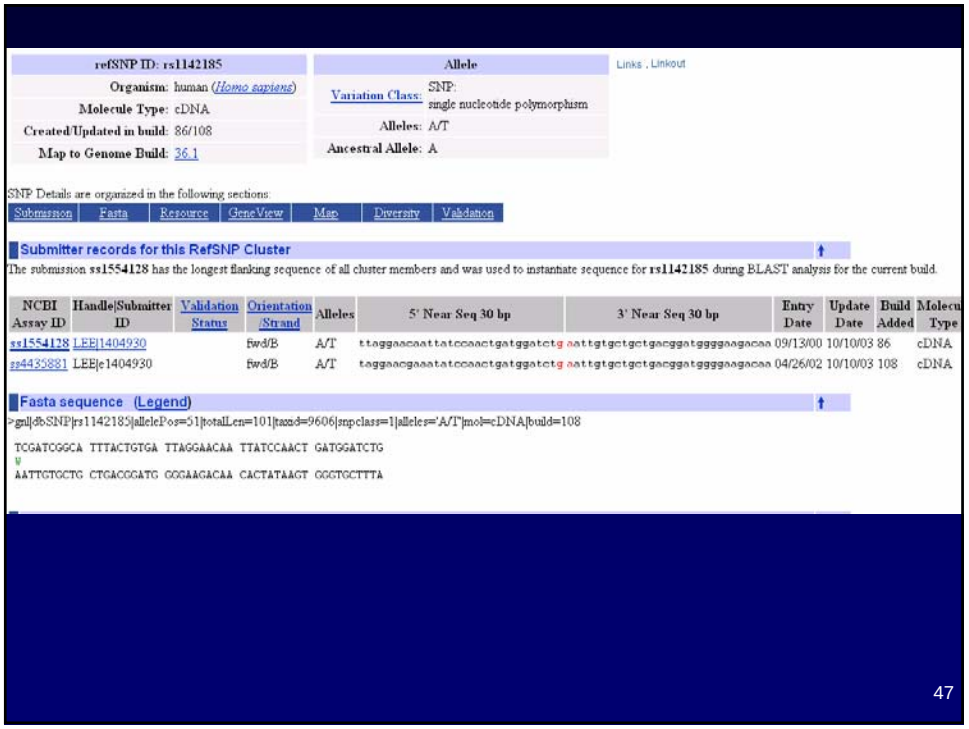


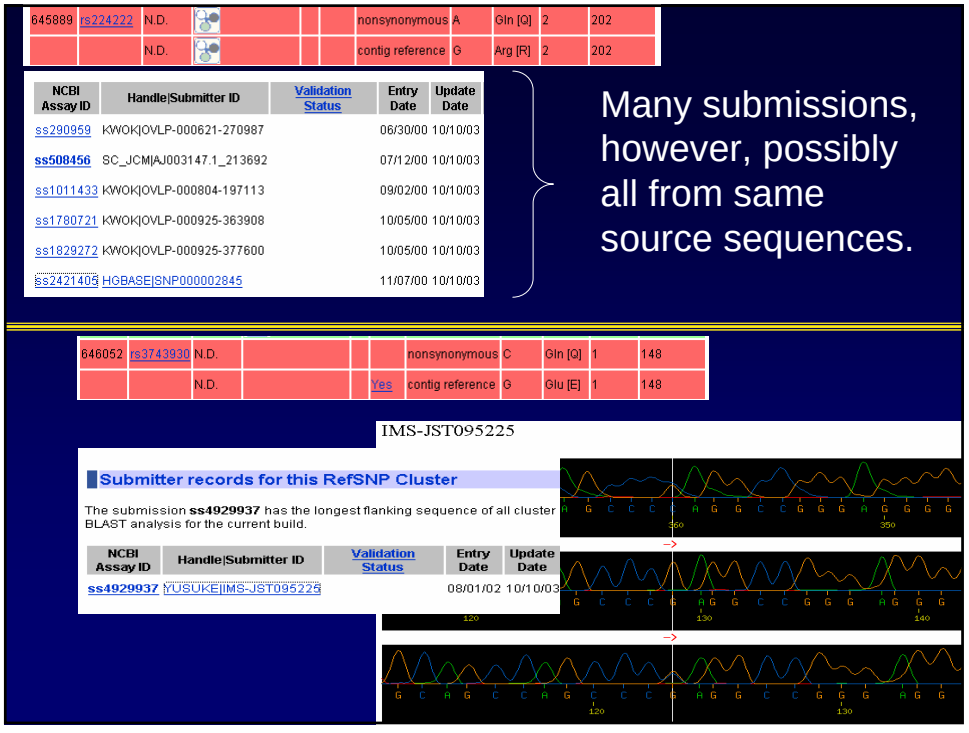
Variations in ENST00000234701

ID	Type	Chr: bp	Alleles	Ambiguity	AA change	AA co-ordinate	Class	Source	Validation
rs2145412	NON_SYNONYMOUS_CODING	1: 86711718	C/T	Y	L/F	65 (1)	snp	HGVbase, dbSNP, TSC	cluster, freq, submitter, doublehit
rs5742891	SPLICE_SITE, INTRONIC	1: 86712151	T/A	W	-	-	snp	dbSNP	-
rs1005569	SPLICE_SITE, INTRONIC	1: 86712151	T/A	W	-	-	snp	HGVbase, dbSNP, TSC	-
rs2753386	NON_SYNONYMOUS_CODING	1: 86714722	G/A	R	R/K	152 (2)	snp	HGVbase, dbSNP	-
rs1321694	SYNONYMOUS_CODING	1: 86720563	T/A	W	V	215 (3)	snp	HGVbase, dbSNP, TSC, Affy GeneChip 500K Mapping Array	cluster, freq, submitter, doublehit
rs4630108	SYNONYMOUS_CODING	1: 86724802	T/C	Y	G	320 (3)	snp	HGVbase, dbSNP	-
rs2734705	NON_SYNONYMOUS_CODING	1: 86724912	A/G	R	N/S	357 (2)	snp	HGVbase, dbSNP, Affy GeneChip 500K Mapping Array	freq, doublehit
rs1142185	NON_SYNONYMOUS_CODING	1: 86727301	A/T	W	E/V	406 (2)	snp	HGVbase, dbSNP	-
rs4647852	NON_SYNONYMOUS_CODING	1: 86727361	A/G	R	K/R	426 (2)	snp	HGVbase, dbSNP	freq
rs1064880	NON_SYNONYMOUS_CODING	1: 86727365	A/T	W	Q/H	427 (3)	snp	HGVbase, dbSNP	-
rs1064881	NON_SYNONYMOUS_CODING	1: 86727375	A/C	M	I/L	431 (1)	snp	HGVbase, dbSNP	-
rs4647854	SYNONYMOUS_CODING	1: [illegible]	C/T	Y	V	435 (3)	snp	HGVbase, dbSNP	freq

46


refSNP ID: rs1142185
Organism: human (Homo sapiens)
Molecule Type: cDNA
Created/Updated in build: 86/108
Map to Genome Build: 36.1
Allele
Variation Class: SNP: single nucleotide polymorphism
Alleles: A/T
Ancestral Allele: A
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 ss1554128 has the longest flanking sequence of all cluster members and was used to instantiate sequence for rs1142185 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 | Molecu Type
ss1554128 LEE|1404930 fwd/B A/T ttaggaacaattatccaactgatggatctg aattgtgctgctgacggatggggaagacaa 09/13/00 10/10/03 86 cDNA
ss4439881 LEE|c1404930 fwd/B A/T taggaacgaaatatccaactgatggatctg aattgtgctgctgacggatggggaagacaa 04/26/02 10/10/03 108 cDNA
Fasta sequence (Legend)
>gnl|dbSNP|rs1142185|allelePos=51|totalLen=101|taxid=9606|snpclass=1|alleles='A/T'|mol=cDNA|build=108
TCGATCGGCA TTTACTGTGA TTAGGAACAA TTATCCAACT GATGGATCTG
AATTGTGCTG CTGACCGATG GCGAAGACAA CACTATAAGT GGGTGCTTTA

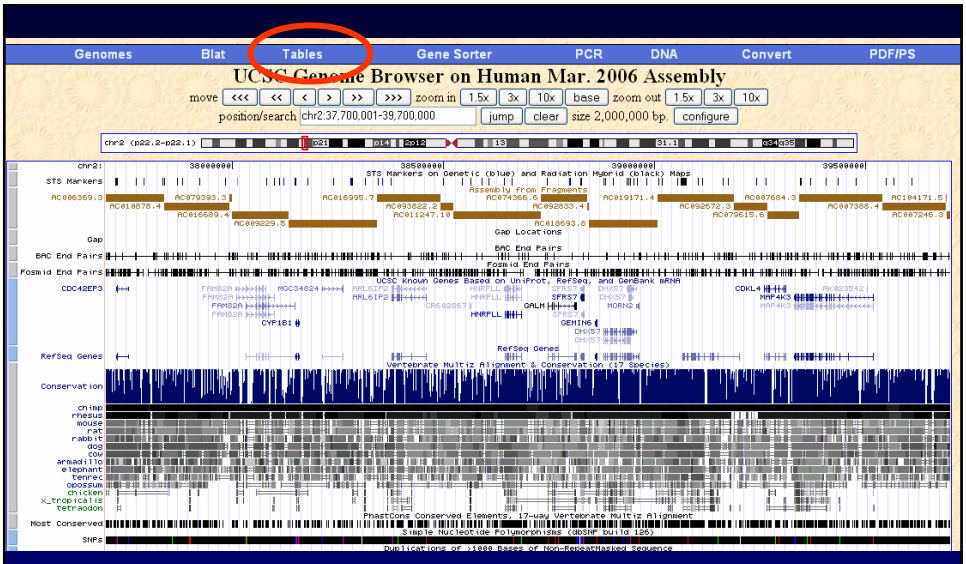
47


645889 rs224222 N.D. nonsynonymous A Gln [Q] 2 202
N.D. contig reference G Arg [R] 2 202
NCBI Assay ID | Handle|Submitter ID | Validation Status | Entry Date | Update Date
ss290959 KWOK|OVLP-000621-270987 06/30/00 10/10/03
ss508456 SC_JCM|AJ003147.1_213692 07/12/00 10/10/03
ss1011433 KWOK|OVLP-000804-197113 09/02/00 10/10/03
ss1780721 KWOK|OVLP-000925-363908 10/05/00 10/10/03
ss1829272 KWOK|OVLP-000925-377600 10/05/00 10/10/03
ss2421405 HGBASE|SNP000002845 11/07/00 10/10/03
Many submissions, however, possibly all from same source sequences.
646052 rs3743930 N.D. nonsynonymous C Gln [Q] 1 148
N.D. Yes contig reference G Glu [E] 1 148
IMS-JST095225
Submitter records for this RefSNP Cluster
The submission ss4929937 has the longest flanking sequence of all cluster BLAST analysis for the current build.
NCBI Assay ID | Handle|Submitter ID | Validation Status | Entry Date | Update Date
ss4929937 YUSUKE|IMS-JST095225 08/01/02 10/10/03

How to find SNPs in a region of interest

- Gene based example
- A 2 Mbp region
- From a list of candidate genes

49



http://genome.ucsc.edu

50

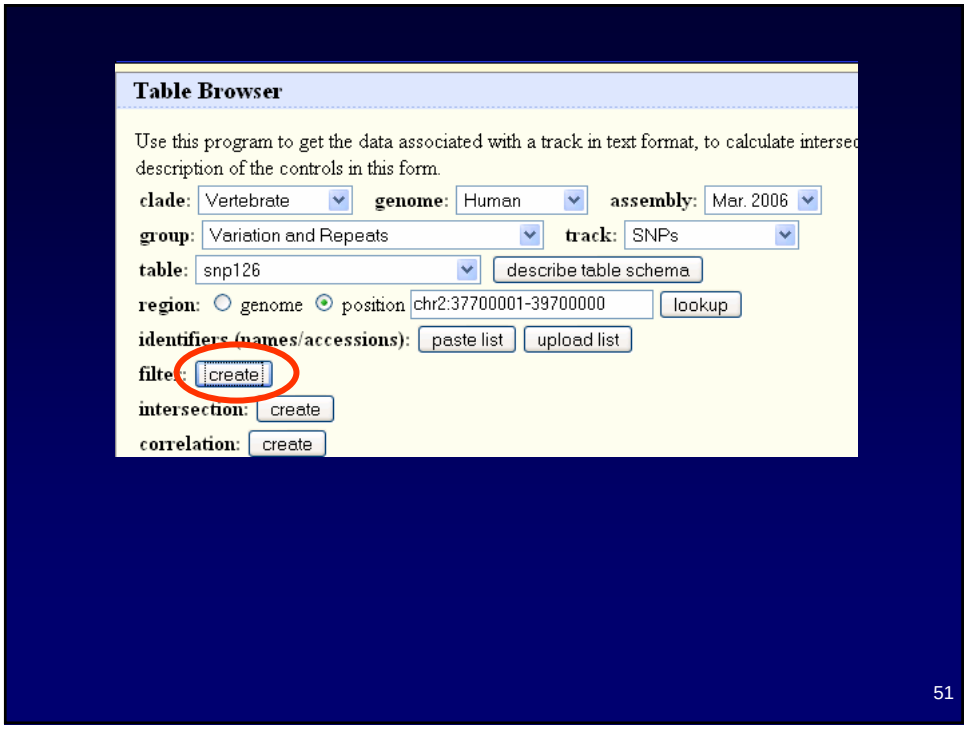
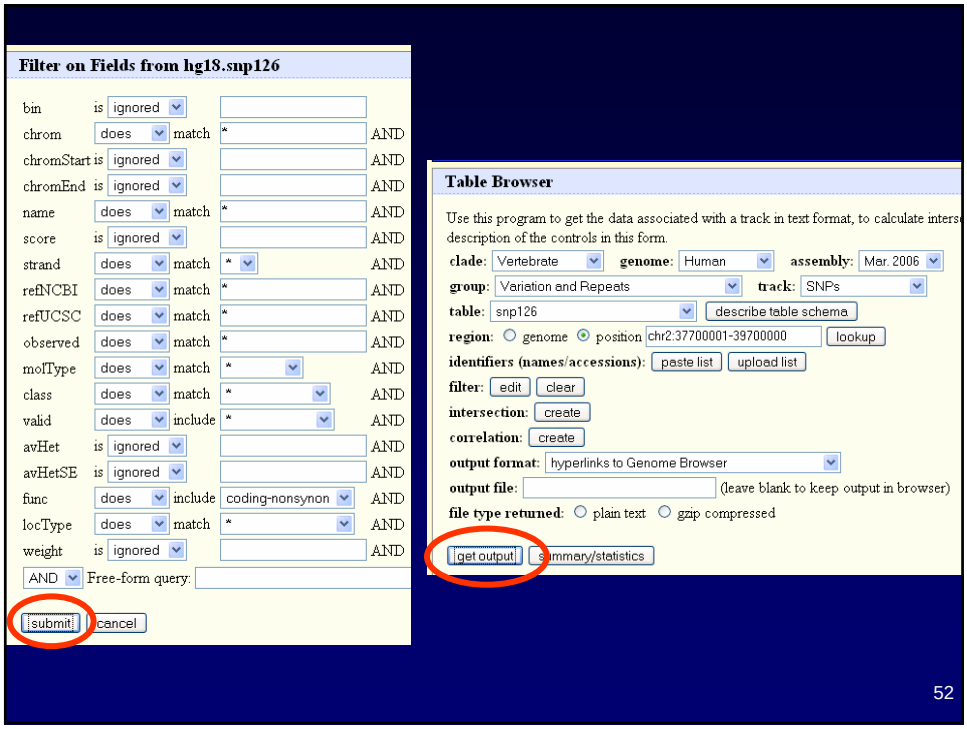
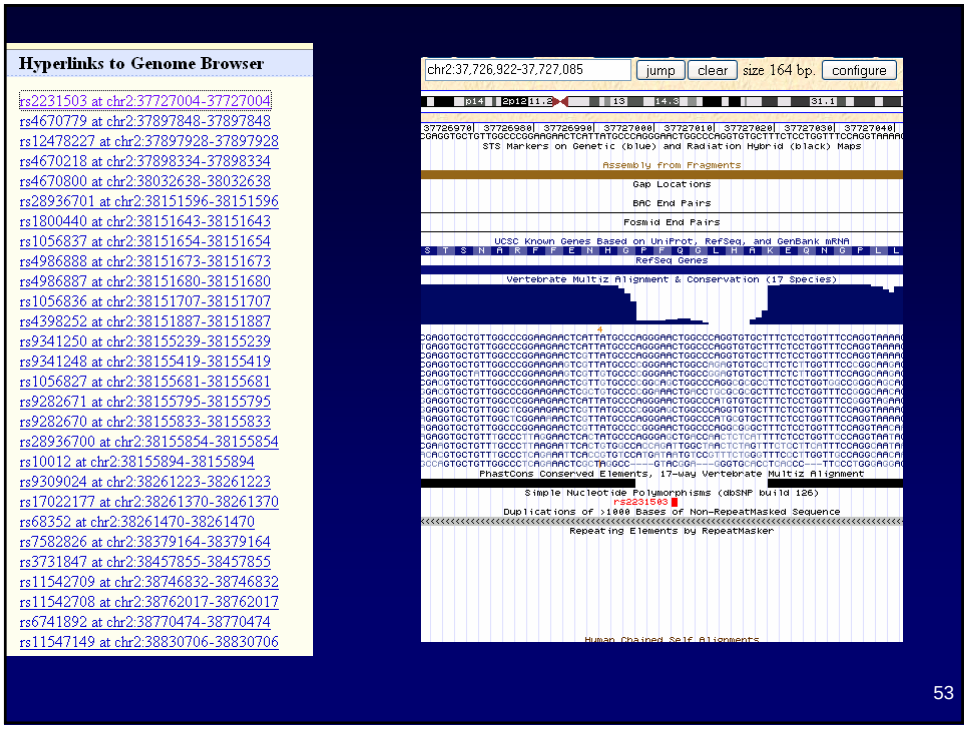


Table Browser
Use this program to get the data associated with a track in text format, to calculate intersec
description of the controls in this form.
clade: Vertebrate
genome: Human
assembly: Mar. 2006
group: Variation and Repeats
track: SNPs
table: snp126
describe table schema
region: genome position chr2:37700001-39700000
lookup
identifiers (names/accessions):
paste list
upload list
filter: create
intersection: create
correlation: create
51


Filter on Fields from hg18.snp126
bin is ignored
chrom does match * AND
chromStart is ignored AND
chromEnd is ignored AND
name does match * AND
score is ignored AND
strand does match * AND
refNCBI does match * AND
refUCSC does match * AND
observed does match * AND
molType does match * AND
class does match * AND
valid does include * AND
avHet is ignored AND
avHetSE is ignored AND
func does include coding-nonsynon AND
locType does match * AND
weight is ignored AND
AND Free-form query:
submit cancel
Table Browser
Use this program to get the data associated with a track in text format, to calculate inters
description of the controls in this form.
clade: Vertebrate
genome: Human
assembly: Mar. 2006
group: Variation and Repeats
track: SNPs
table: snp126
describe table schema
region: genome position chr2:37700001-39700000
lookup
identifiers (names/accessions):
paste list
upload list
filter: edit clear
intersection: create
correlation: create
output format: hyperlinks to Genome Browser
output file: (leave blank to keep output in browser)
file type returned: plain text gzip compressed
get output
summary/statistics
52



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How to find SNPs in a region of interest

- Gene based example
- A 2 Mbp region
- From a list of candidate genes

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Selecting SNPs from a list of candidate genes

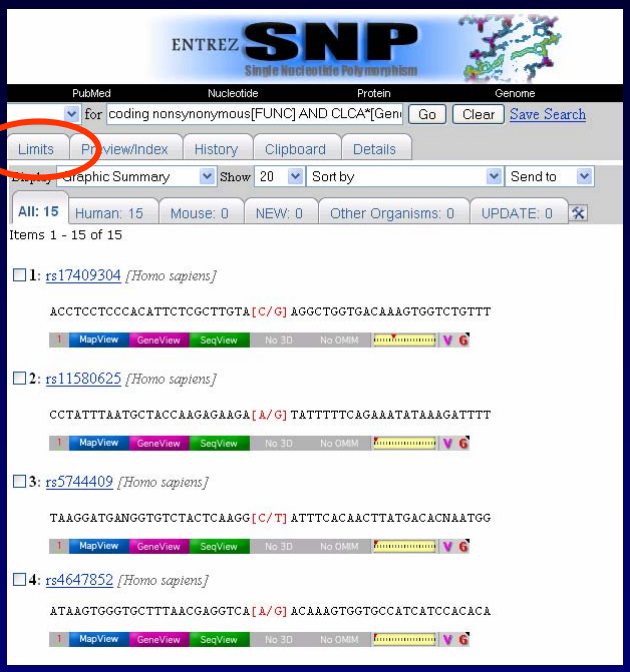
- Use the Entrez SNP query:

  coding nonsynonymous[FUNC] AND CLCA*[Gene name] AND human[orgn]

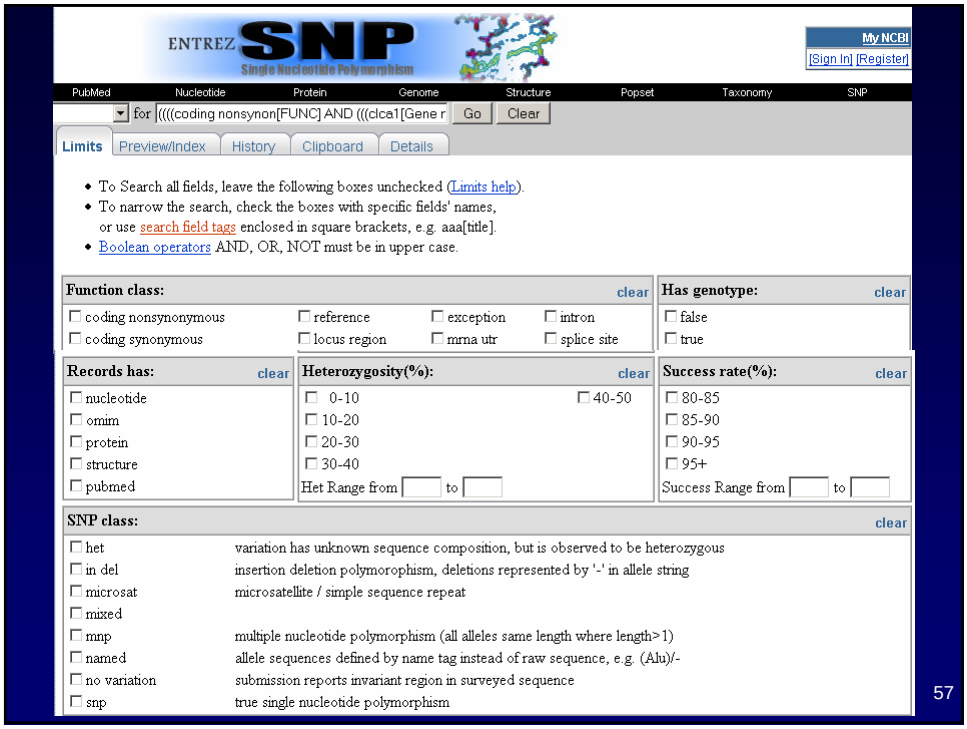
- Download dbSNP database and cross reference with candidate gene list coordinates

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp

55



56



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Overview of Topics

- Genome variation origins
- Types of polymorphisms
- SNP discovery methods
- Access to genetic variation data
- How to find SNPs in a region of interest
- Haplotype Map project

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Haplotype Map project

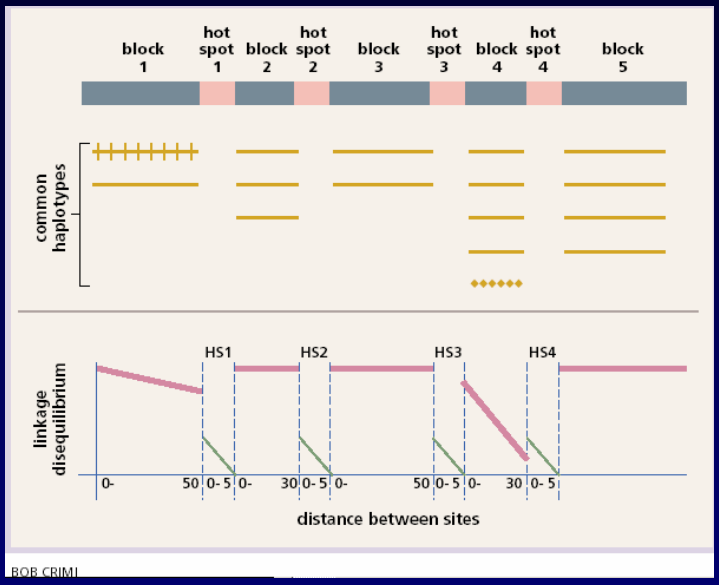
- What is a Haplotype?
- What is Linkage Disequilibrium (LD)?
- What is the Haplotype Map Project?

59

What is a Haplotype?

- A set of closely linked genetic markers present on one chromosome which tend to be inherited together (not easily separable by recombination).
- Recombination occurs between homologous chromosomes when cells divide.
- It is believed that recombination is not equally likely across the genome, but that it is punctuated by hot-spots.

60



From: Goldstein DB. Islands of linkage disequilibrium. Nat Genet. 2001 Oct;29(2):109-11.

61

What is Linkage Disequilibrium?

- When the observed frequencies of genetic markers in a population does not agree with haplotype frequencies predicted by multiplying together the frequency of individual genetic markers in each haplotype.

139	0.352
140	0.5
141	0.499
142	0.5
143	0.499
144	0.453
145	0.499
146	0.497

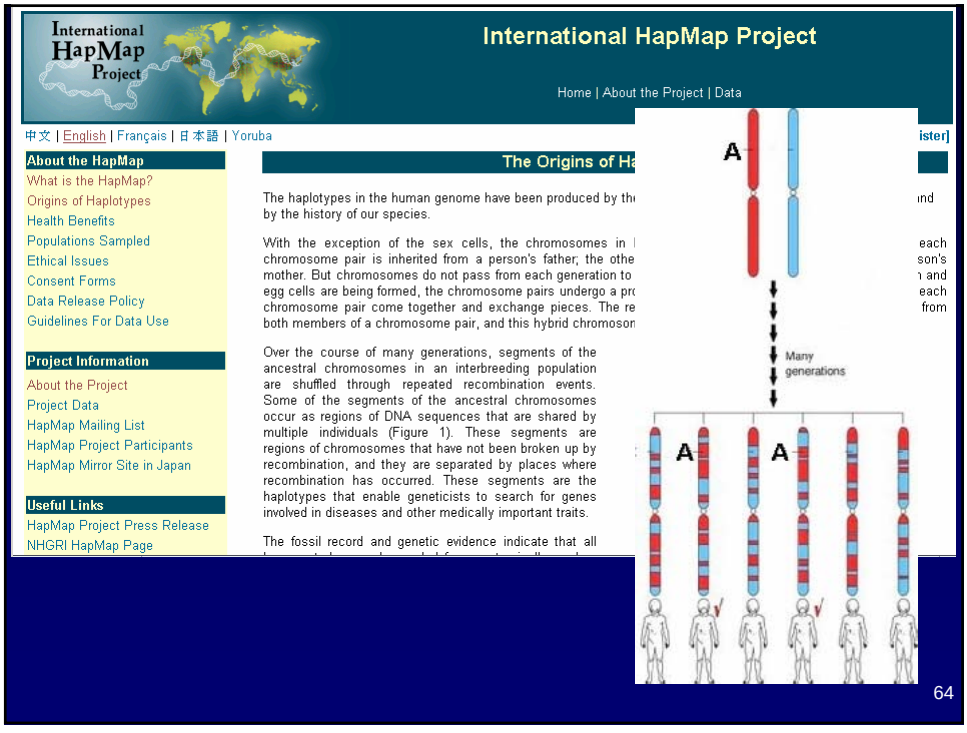
139 140 141 142 143 144 145 146

CAACTCAT .217	0.352*0.5^7=0.00275
TGGTCTGC .365	0.648*0.5^7=0.00534
TGGTCCGC .127	0.648*0.5^7=0.00534
TAACTCAT .266	0.648*0.5^7=0.00534

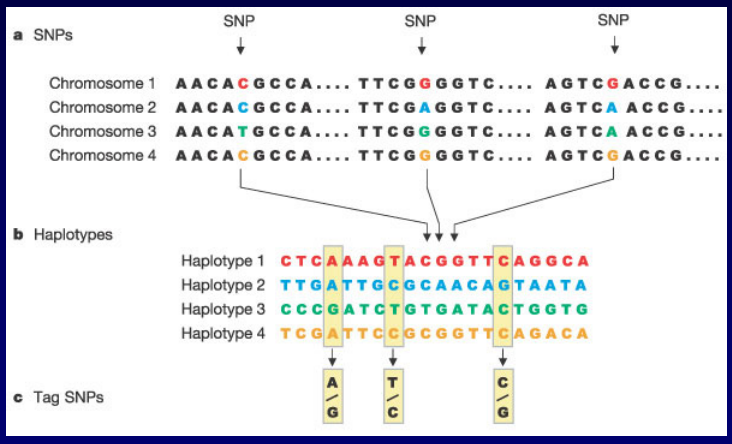
0.975

62


International
HapMap
Project
www.hapmap.org


International HapMap Project
Home | About the Project | Data
中文 | English | Français | 日本語 | Yoruba
About the HapMap
What is the HapMap?
Origins of Haplotypes
Health Benefits
Populations Sampled
Ethical Issues
Consent Forms
Data Release Policy
Guidelines For Data Use
Project Information
About the Project
Project Data
HapMap Mailing List
HapMap Project Participants
HapMap Mirror Site in Japan
Useful Links
HapMap Project Press Release
NHGRI HapMap Page
The Origins of Ha
The haplotypes in the human genome have been produced by th
by the history of our species.
With the exception of the sex cells, the chromosomes in
chromosome pair is inherited from a person's father; the othe
mother. But chromosomes do not pass from each generation to
egg cells are being formed, the chromosome pairs undergo a pr
chromosome pair come together and exchange pieces. The re
both members of a chromosome pair, and this hybrid chromoson
Over the course of many generations, segments of the
ancestral chromosomes in an interbreeding population
are shuffled through repeated recombination events.
Some of the segments of the ancestral chromosomes
occur as regions of DNA sequences that are shared by
multiple individuals (Figure 1). These segments are
regions of chromosomes that have not been broken up by
recombination, and they are separated by places where
recombination has occurred. These segments are the
haplotypes that enable geneticists to search for genes
involved in diseases and other medically important traits.
The fossil record and genetic evidence indicate that all
A
Many
generations
A
A
64

Identification of Haplotypes Through Genotyping



International HapMap Project

- **Goal: to develop a haplotype map covering 80 - 90% of the genome**
- **The map should be usable in all populations**
- **Three year project started October 2002 and completed in October 2005 (Phase I)**
- **International collaboration, involving Canada, China, Nigeria, Japan, the United Kingdom, and the United States**
- **All data publicly accessible at www.hapmap.org**

International HapMap Project: Sample Collection

- **Similarity in haplotypes worldwide limits the need to collect samples from many populations**
- **No clinical information collected, samples anonymous**
- **Individual consent and extensive community consultation**
- **270 samples collected and genotyped**
  - **Africa (Yoruba in Ibadan, Nigeria)**
  - **Asia (Japanese in Tokyo, Han Chinese in Beijing)**
  - **Europe (CEPH family samples, Utah)**
- **Samples are available as DNA or cell lines from Coriell**
- **Additional populations being studied in a pilot phase**

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International HapMap Project: Experimental Strategy

- **Participating centers have divided up the genome, according to capacity of each center**
- **Different centers use different platforms: Illumina, Third Wave, Sequenom, TaqMan, ParAllele**
- **Data Coordination Center provides lists of SNPs, and receives genotypes**
- **Phase I HapMap – Obtain genotypes from a working SNP every 5 kb across the genome**
- **Phase II – Fill in gaps in linkage disequilibrium map: completed by Perlegen**

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HapMap Milestones

- Fall 2004 – Phase I map of 600,000 SNPs in European samples
- Early 2005 – Phase I map in Asian and African samples
- Fall 2005 – Perlegen contributes another 3M SNPs to the map
- Fall 2005 – Final HapMap, including gap filling
- "HapTag" SNPs able to represent 80-90% of common variation with
  - 200,000 SNPs for European or Asian samples
  - 400,000 SNPs for African samples

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HapMap Gbrowse

http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/

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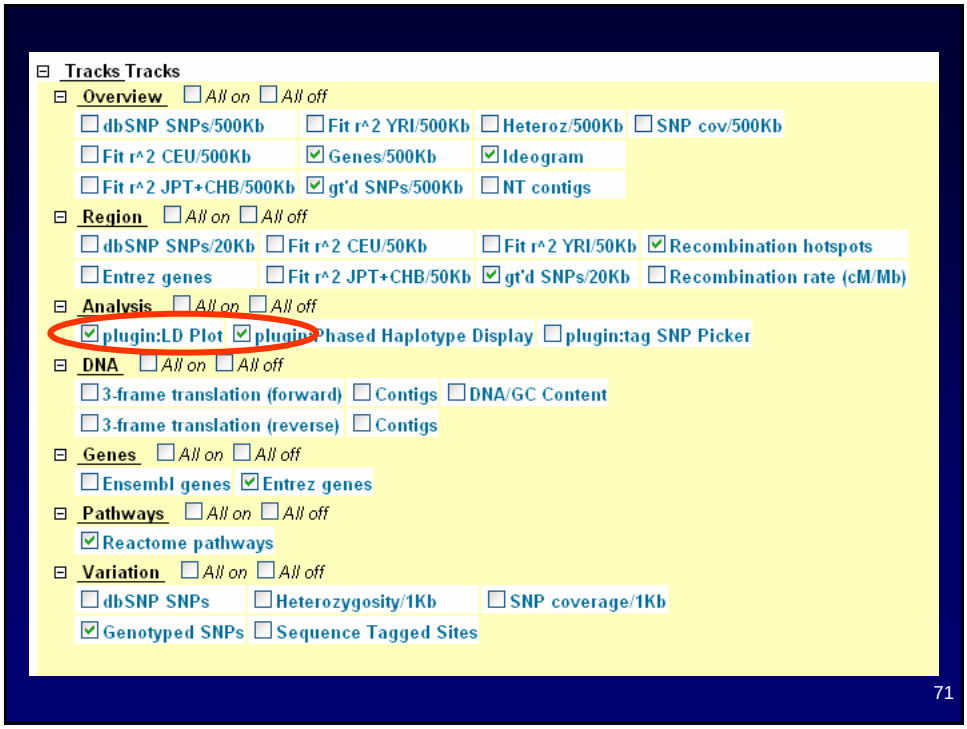
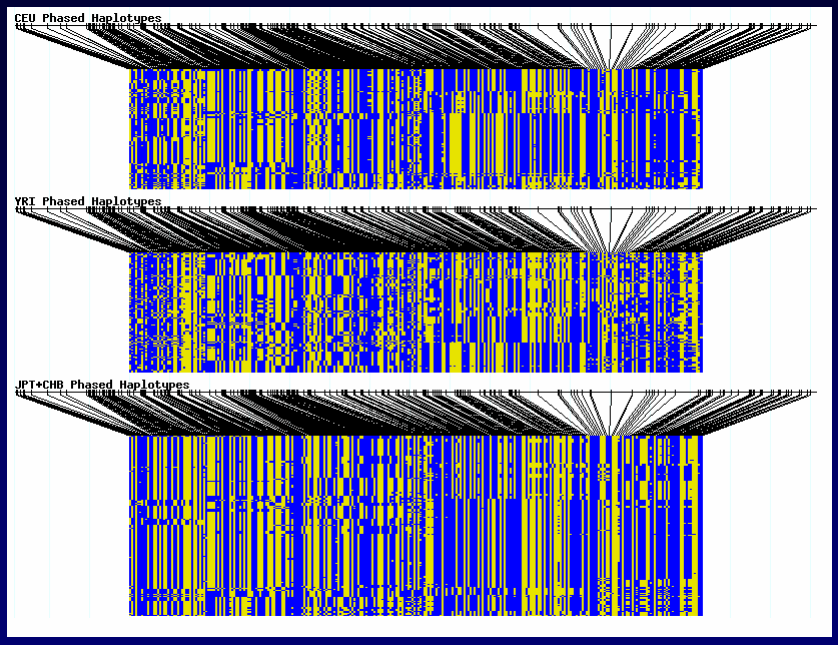

Tracks Tracks
Overview All on All off
dbSNP SNPs/500Kb
Fit r^2 YRI/500Kb
Heteroz/500Kb
SNP cov/500Kb
Fit r^2 CEU/500Kb
Genes/500Kb
Ideogram
Fit r^2 JPT+CHB/500Kb
gt'd SNPs/500Kb
NT contigs
Region All on All off
dbSNP SNPs/20Kb
Fit r^2 CEU/50Kb
Fit r^2 YRI/50Kb
Recombination hotspots
Entrez genes
Fit r^2 JPT+CHB/50Kb
gt'd SNPs/20Kb
Recombination rate (cM/Mb)
Analysis All on All off
plugin:LD Plot
plugin:Phased Haplotype Display
plugin:tag SNP Picker
DNA All on All off
3-frame translation (forward)
Contigs
DNA/GC Content
3-frame translation (reverse)
Contigs
Genes All on All off
Ensembl genes
Entrez genes
Pathways All on All off
Reactome pathways
Variation All on All off
dbSNP SNPs
Heterozygosity/1Kb
SNP coverage/1Kb
Genotyped SNPs
Sequence Tagged Sites

71


Region of chr13
30M
31M
gt'd SNPs/20Kb
200
0
Recombination hotspots
30600k
30700k
Genotyped SNPs
Entrez genes
NM_006644
HSPH1: heat shock 105kD
NM_194318
B3GTL: beta 3-glycosyltransferase-like
CEU:lod

72



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SLC24A5, a Putative Cation Exchanger, Affects Pigmentation in Zebrafish and Humans

Rebecca L. Lamason,[1]* Manzoor-Ali P.K. Mohideen,[1]† Jason R. Mest,[1] Andrew C. Wong,[1]‡ Heather L. Norton,[6] Michele C. Aros,[1] Michael J. Jurynec,[8] Xianyun Mao,[6] Vanessa R. Humphreville,[1]§ Jasper E. Humbert,[2,9] Soniya Sinha,[2] Jessica L. Moore,[3]‖ Pudur Jagadeeswaran,[10] Wei Zhao,[9] Gang Ning,[7] Izabela Makalowska,[7] Paul M. McKeigue,[11] David O'Donnell,[11] Rick Kittles,[12] Esteban J. Parra,[13] Nancy J. Mangini,[14] David J. Grunwald,[8] Mark D. Shriver,[6] Victor A. Canfield,[4] Keith C. Cheng[1,4,5]¶

Science 16 December 2005:
Vol. 310. no. 5755, pp. 1782 - 1786

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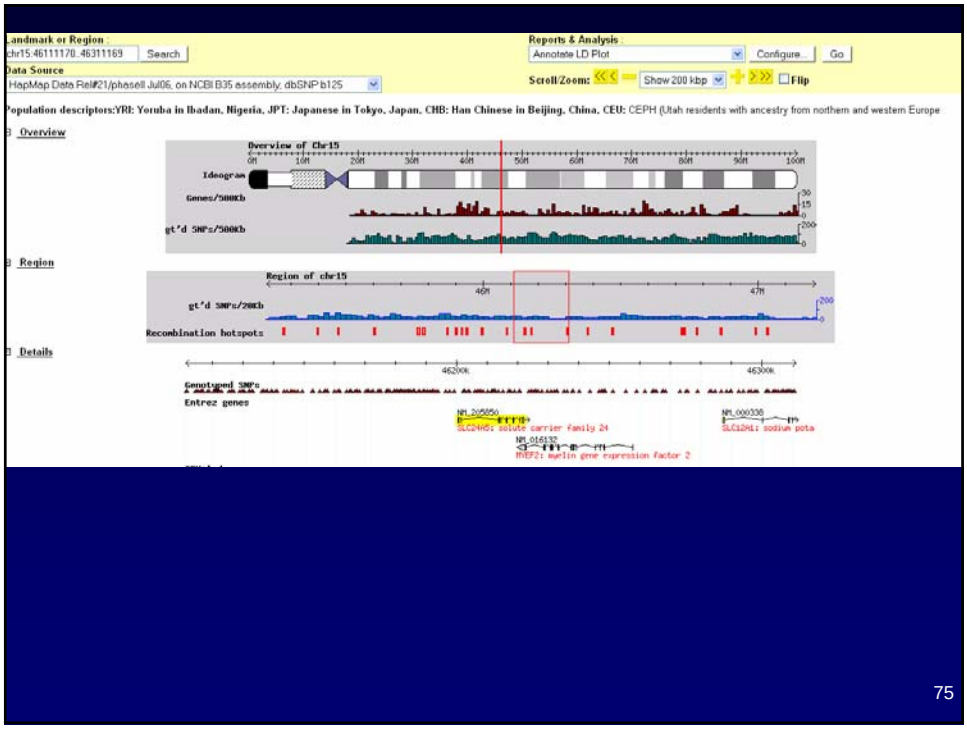
Landmark or Region :
chr15:46111170..46311169
Search
Reports & Analysis :
Annotate LD Plot
Configure...
Go
Data Source
HapMap Data Rel#21/phaseII Jul06, on NCBI B35 assembly, dbSNP b125
Scroll/Zoom:
Show 200 kbp
Flip
Population descriptors:YRI: Yoruba in Ibadan, Nigeria, JPT: Japanese in Tokyo, Japan, CHB: Han Chinese in Beijing, China, CEU: CEPH (Utah residents with ancestry from northern and western Europe
Overview
Overview of Chr15
Ideogram
Genes/500Kb
gt'd SNPs/500Kb
Region
Region of chr15
gt'd SNPs/20Kb
Recombination hotspots
Details
Genotyped SNPs
Entrez genes
NM_205850
SLC24A5: solute carrier family 24
NM_000338
SLC12A1: sodium pota
NM_016132
MYEF2: myelin gene expression factor 2

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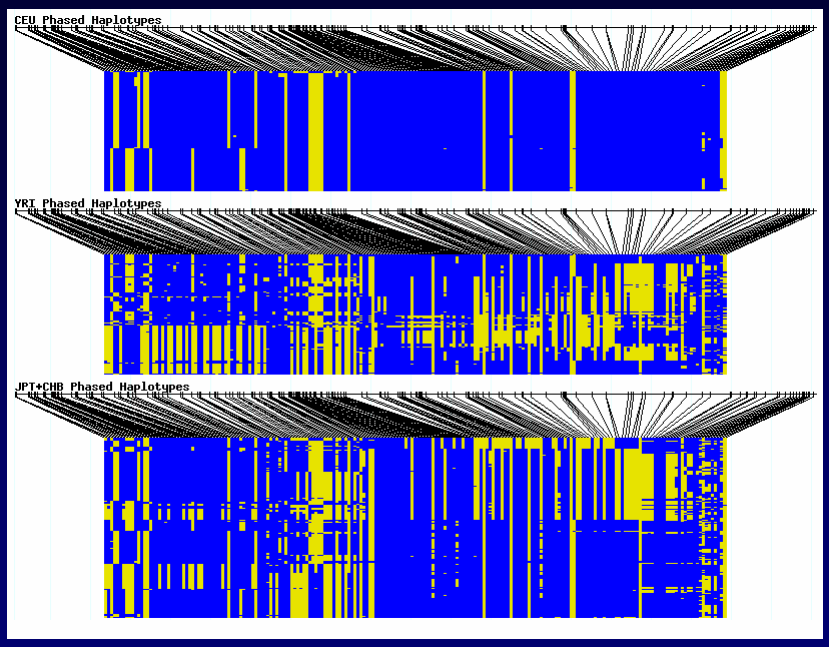
Region of chr15
gt'd SNPs/20Kb
Recombination hotspots
Genotyped SNPs
Entrez genes
NM_205850
SLC24A5: solute carrier family 24
NM_000338
SLC12A1: sodium pota
NM_016132
MYEF2: myelin gene expression factor 2
CEU:lod

76



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Tracks Tracks

Overview All on All off

dbSNP SNPs/500Kb | Fit r^2 YRI/500Kb | Heteroz/500Kb | SNP cov/500Kb

Fit r^2 CEU/500Kb | Genes/500Kb | Ideogram

Fit r^2 JPT+CHB/500Kb | gt'd SNPs/500Kb | NT contigs

Region All on All off

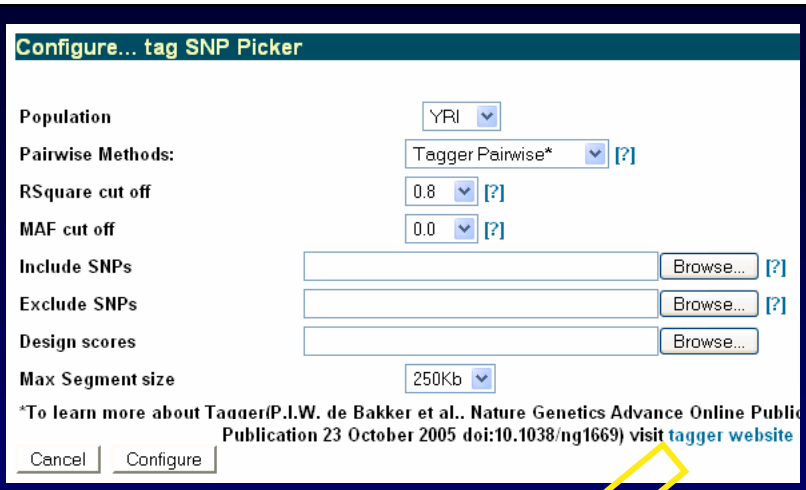
dbSNP SNPs/20Kb | Fit r^2 CEU/50Kb | Fit r^2 YRI/50Kb | Recombination hotspots

Entrez genes | Fit r^2 JPT+CHB/50Kb | gt'd SNPs/20Kb | Recombination rate (cM/Mb)

Analysis All on All off

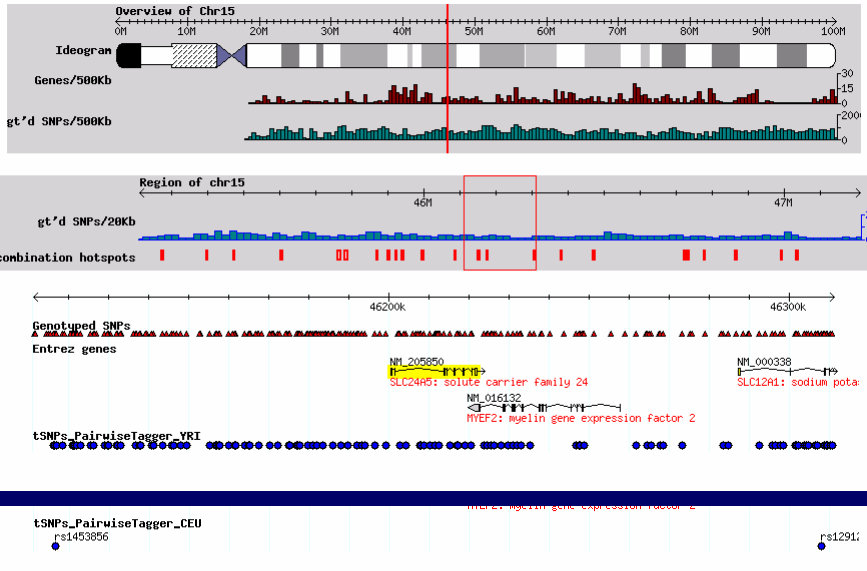
plugin:LD Plot | plugin:Phased Haplotype Display | plugin:tag SNP Picker

78

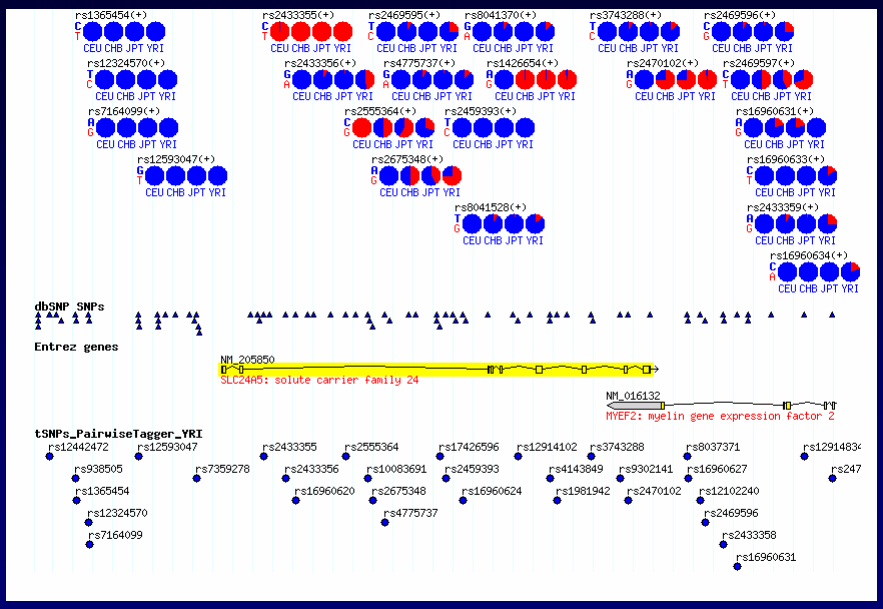


http://www.broad.mit.edu/mpg/tagger/

79



80



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Overview of Topics

- Genome variation origins
- Types of polymorphisms
- SNP discovery methods
- Access to genetic variation data
- How to find SNPs in a region of interest
- Haplotype Map project
- Medical Sequencing
- SNPs for Other Species
- New Sequencing Technologies

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A Brief Tour of a Medical Sequencing Pipeline

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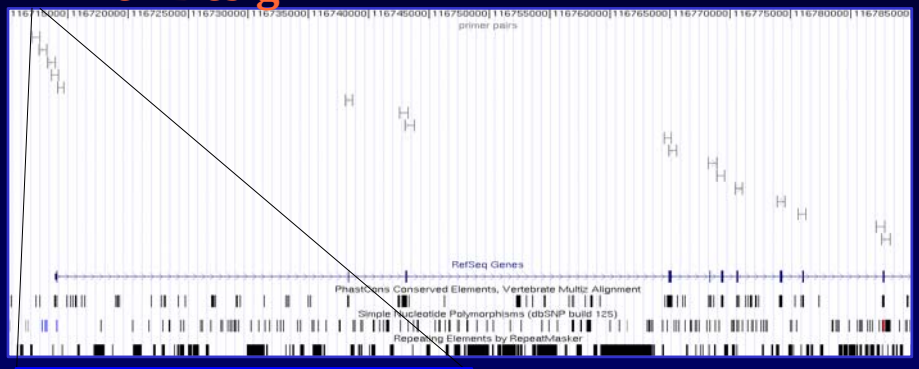
Primer Design



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.

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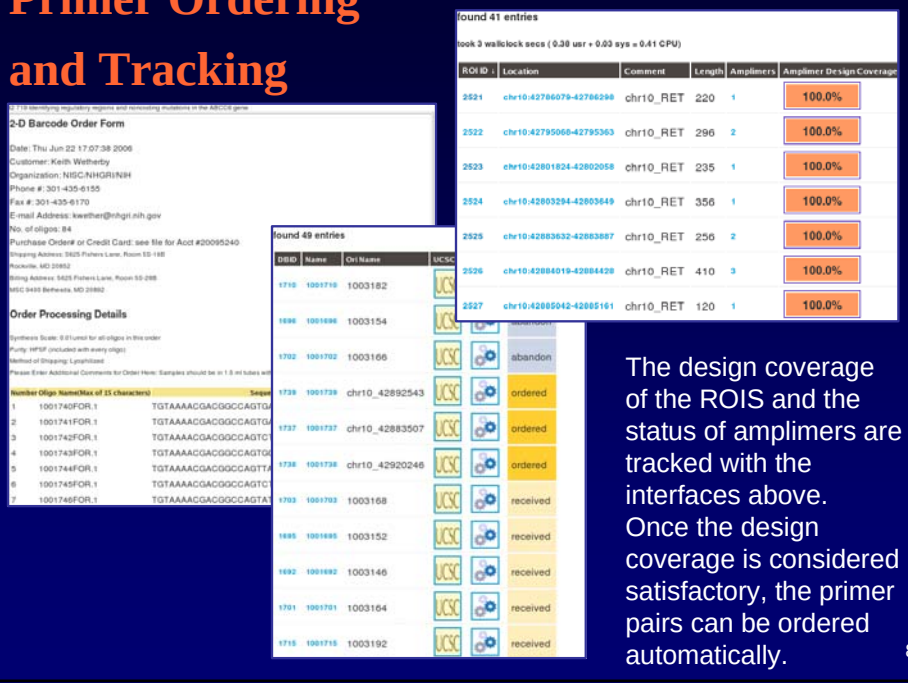
Primer Ordering and Tracking



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.

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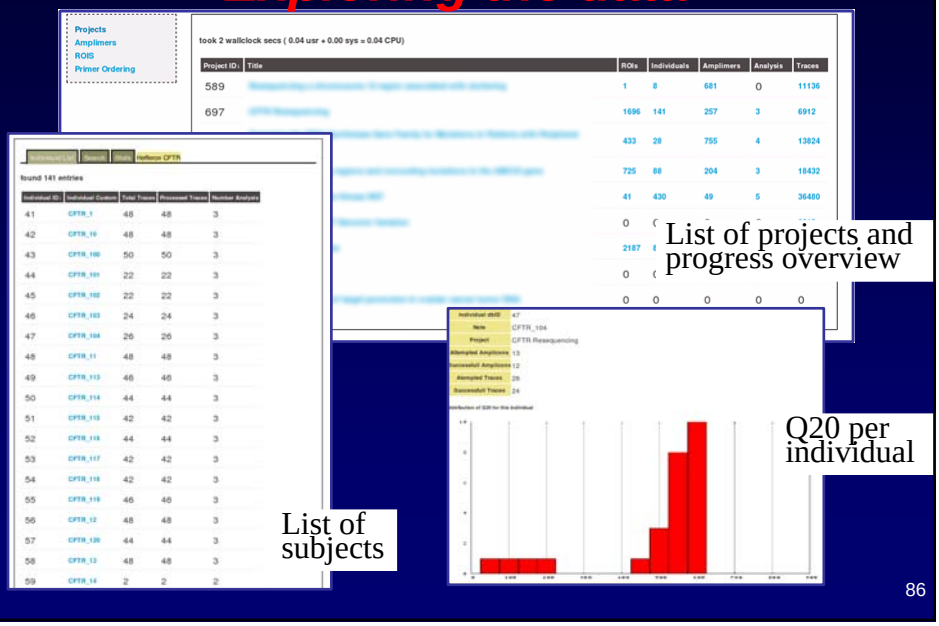
Exploring the data



List of projects and progress overview

Q20 per individual

List of subjects

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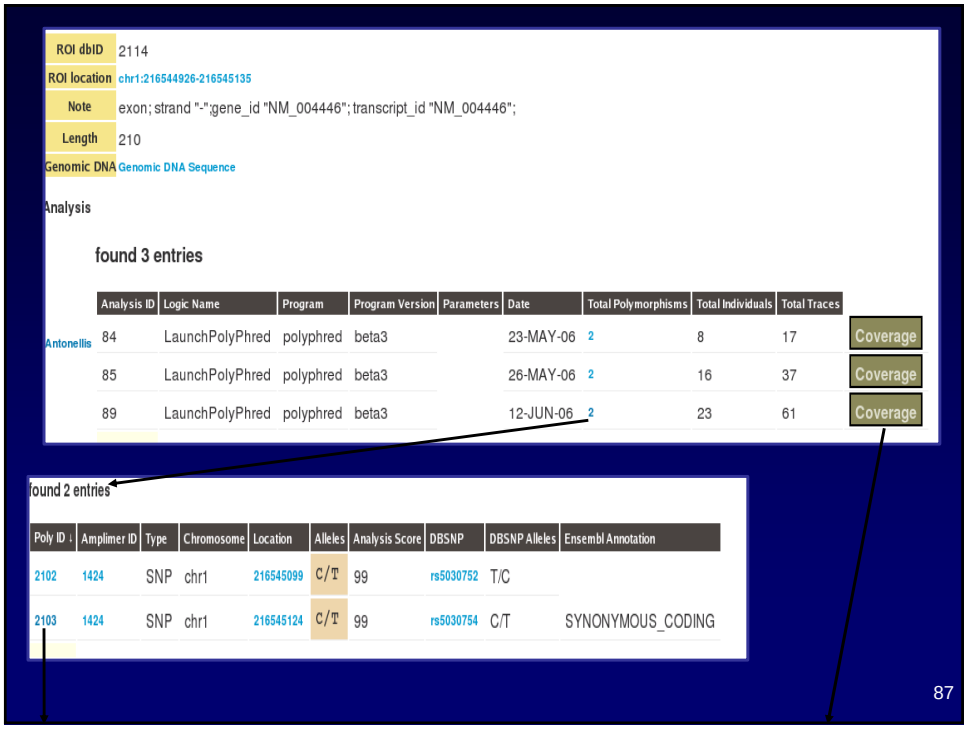
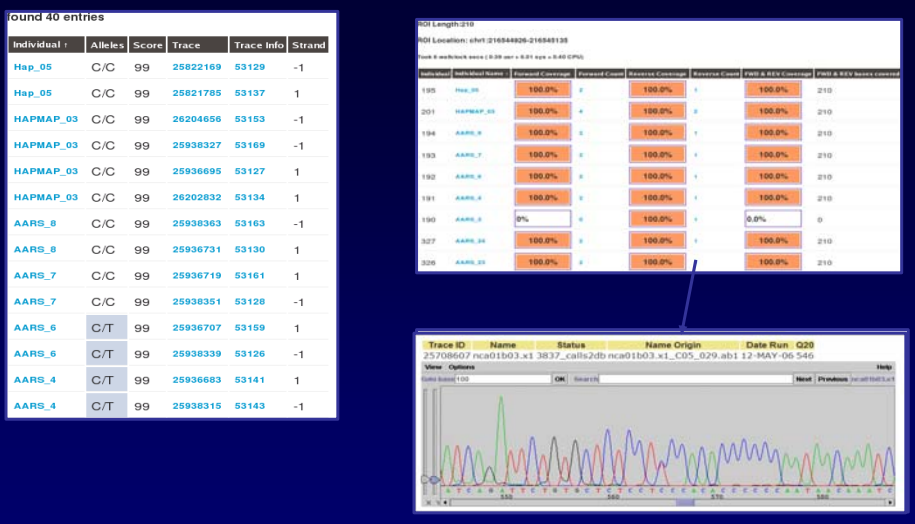
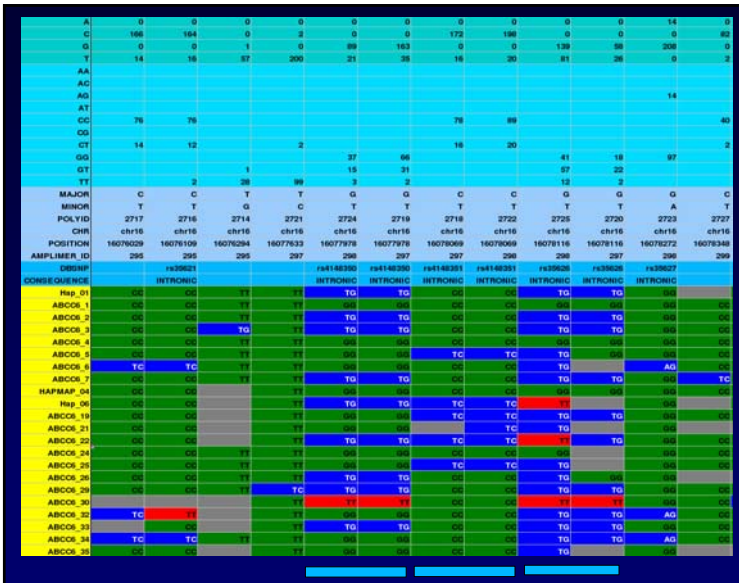


87



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.

88



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.

89

Some of the challenges of variation detection

INDEL

"Dye blob"

Detection saturation

90

SNPs for Other Species

- Mouse
  - The reference strain sequenced, C57BL/6J, was inbred for sufficient generations to result in a homozygous genome, however, 15 mouse strains have been sequenced and the variations are available from dbSNP (http://www.nih.gov/news/pr/oct2006/niehs-25.htm)
  - This is a great resource for mouse genetics. For example, crossing two different mouse strains where one mouse has given disease causing mutation.
- Dog
  - The reference dog genome sequence comes from a fairly inbred individual (a boxer named Tasha). This individual is 60% homozygous with the heterozygous regions showing 1SNP per 900 bases, giving 770k SNPs.
  - Celera sequenced a poodle, Shadow, and comparing this genome to Tasha's sequence give 1.46M SNPs
  - The public sequencing effort also generated whole genome shotgun sequence from 9 other dogs breeds as well as 4 wolves and a coyote

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SNPs for Other Species

- Chimpanzee
  - The reference sequence in based on Clint along with light WGS of four other West African and three central African chimpanzees giving a total of 1.66M SNPs.
  - Chimpanzee sequence can also be used together with human SNPs to determine the ancestral allele state, as noted in many of the dbSNP records.
- Cat
  - The reference cat sequence, like dog, comes from an inbred individual (an Abyssinian named Cinnamon) which is also about 60% homozygous, with the heterozygous regions showing 1 SNP per 600 bases.

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Cat SNP Analysis

- Cinnamon is of the Abyssinian breed, and its genome is diploid
- Thus, when two sequence traces overlap, there is a 50% chance that these two traces came from different chromosomes
- If Cinnamon were an out-bred cat, then traces that arise from different chromosomes should exhibit sequence polymorphisms
- However, due to inbreeding, the locus of these two chromosomes may have been derived from an ancestor's chromosome only a few generations back, thus exhibiting no polymorphisms

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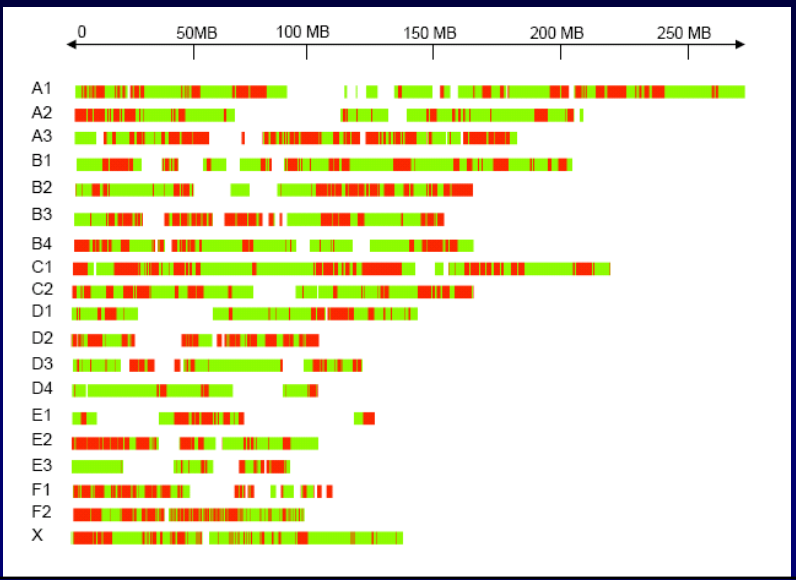
Heterozygosity Profile of Cinnamon



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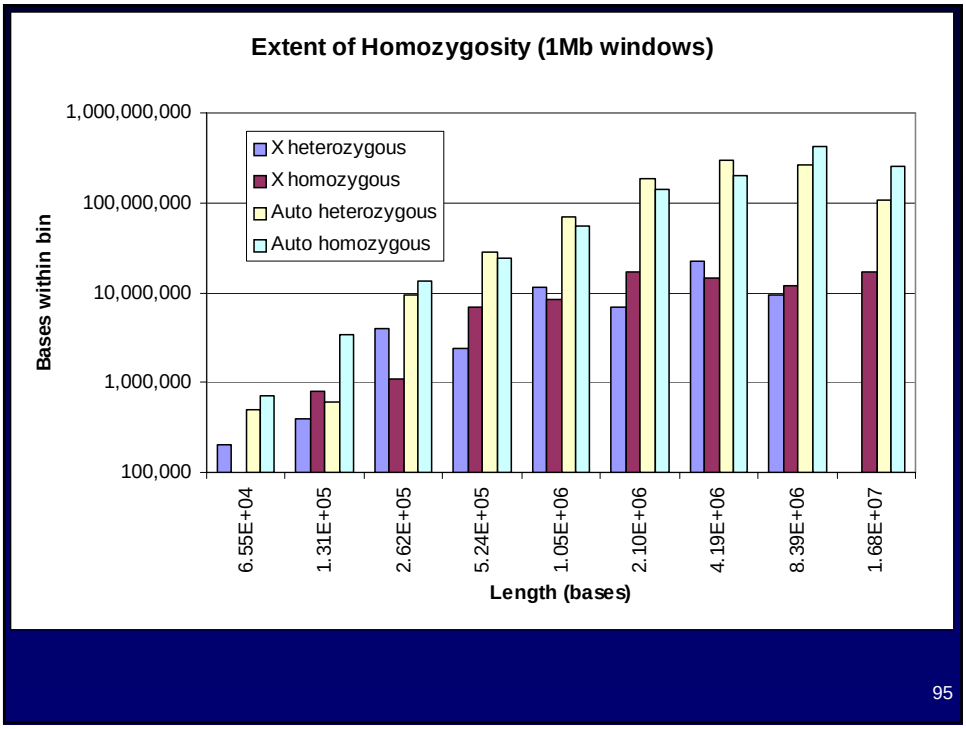

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Cinnamon's Polymorphism Statistics

- 57% of Cinnamon's autosomes are homozygous
- Within the heterozygous segments of this individual, we discovered over 325,000 SNPs and over 37,000 deletion/insertion polymorphisms
- The heterozygosity level of heterozygous regions is 0.17%, or about 70% higher than human heterozygosity levels
- Comparing Cinnamon to another cat (Gus), a brown classic tabby (RPCI-86), yields a heterozygosity level of about 0.2%, or about twice the level of humans.

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Linkage Disequilibrium Across Cat Breeds

- Selected SNPs detected from Cinnamon's genome within heterozygous regions on 10 different chromosomes.
- 35 SNPs were selected per chromosome, with the first 8 SNPs within a 15kb window and rest selected every approximately every 15kb away from the previous SNP.
- These SNPs were genotyped across 97 cats from 24 breeds, 7 outbred "alley" cats and 12 wild species.
- Linkage disequilibrium (LD) was calculated for those individuals that were homozygous within the first 15kb window, and the length of LD was derived from the extent of the homozygous interval.

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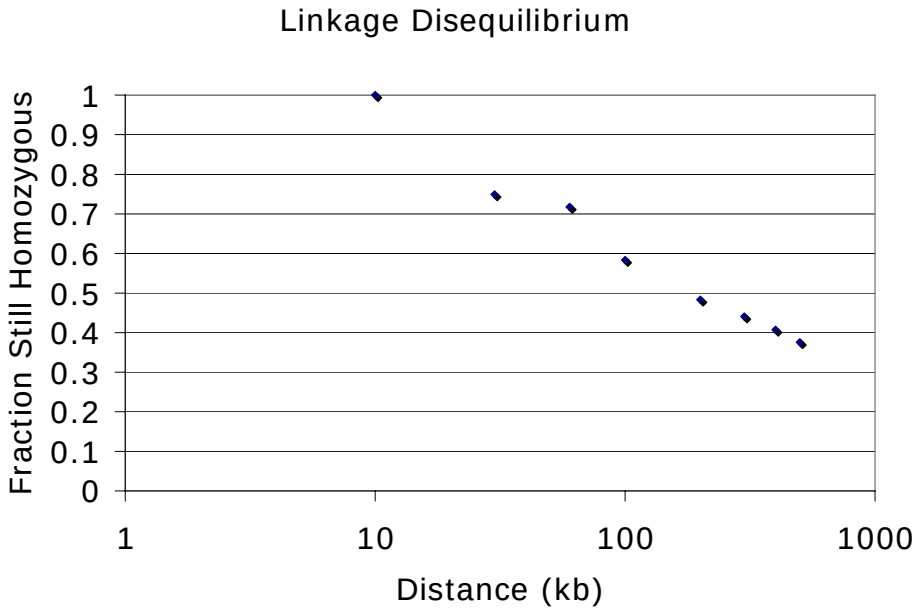

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Summary of Cat LD Results

- ~60% of 10 kb regions are homozygous within an individual. This is very similar to dogs.
- Conditional on being homozygous within the 10 kb region, 50% of cases are still homozygous at 150 kb. The extent of linkage disequilibrium is roughly a third that in dogs.
- The number of markers needed for genome-wide association: current estimate about 45k markers.

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New Sequencing Technologies

- 454 Life Sciences
  - 100-200 base reads
  - 20-40Mb per run
  - 2 runs per day
- Solexa
  - 25-40 base reads
  - 8*125Mb per run
  - 2 runs per week
- ABI SOLiD
  - Similar to Solexa
  - Run performance like Solexa

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SNP Detection with New Sequencing Technologies

- Need to greatly over-sample each base to insure high quality SNP detection, about 30 fold redundancy
- To sequence an entire individual's genome requires 3Gb*30/1Gb/run or about 90 runs on a Solexa machine (45 weeks)
- Targeted sequencing requires additional preparation, e.g. long range (10kb) PCR
  - Introduces variable product amplification levels requiring greater average sequencing redundancy to ensure a minimum redundancy of 30 fold
  - Allelic PCR dropout resulting in missed genetic diversity
  - Approach has been successfully applied to a 140kb genomic interval

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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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WEB pages

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http://www.ncbi.nlm.nih.gov/SNP/index.html : dbSNP home page
http://www.ensembl.org : Ensembl home page
http://www.ucl.ac.uk/~ucbhdjm/courses/b242/2+Gene/2+Gene.html
http://www.hapmap.org/: Haplotype Map Project home page
http://www.hapmap.org/cgi-perl/gbrowse/gbrowse/hapmap
http://www.broad.mit.edu/personal/jcbarret/haploview/
http://genome.perlegen.com/browser/index.html: Perlegen's HapMap

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