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Original article

CGDSNPdb: a database resource for error-checked and imputed mouse SNPs

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The Center for Genome Dynamics Single Nucleotide Polymorphism Database (CGDSNPdb) is an open-source value-added database with more than nine million mouse single nucleotide polymorphisms (SNPs), drawn from multiple sources, with genotypes assigned to multiple inbred strains of laboratory mice. All SNPs are checked for accuracy and annotated for properties specific to the SNP as well as those implied by changes to overlapping protein-coding genes. CGDSNPdb serves as the primary interface to two unique data sets, the 'imputed genotype resource' in which a Hidden Markov Model was used to assess local haplotypes and the most probable base assignment at several million genomic loci in tens of strains of mice, and the Affymetrix Mouse Diversity Genotyping Array, a high density microarray with over 600 000 SNPs and over 900 000 invariant genomic probes. CGDSNPdb is accessible online through either a web-based query tool or a MySQL public login.

Database URL: http://cgd.jax.org/cgdsnpdb/

Introduction

Single nucleotide polymorphisms (SNPs) are variable single base positions within a genome that represent the simplest and possibly most common type of genetic variation. Accordingly, SNPs have emerged as a powerful tool for tracking heredity and genetic variation, and have become especially popular for phenotype genome-wide association studies (1, 2). The critical role of the laboratory mouse has led to several efforts aimed at large-scale collection and analysis of mouse SNPs (3–7).

The Center for Genome Dynamics Single Nucleotide Polymorphism database (CGDSNPdb) was designed to bring together multiple sources of mouse SNP data, while checking them for accuracy and consistency among sources. CGDSNPdb is distinguished by the inclusion of two unique data sets:

- The Imputed SNP Genotype Resource (IGR) (8) generated by a Hidden Markov Model (HMM) that assigns probable genotype and associated confidence levels for over 8 million SNPs in 74 strains of mice.
- Data collected from over 140 strains of laboratory mice (filtered to 72 inbred strains in the current release, version 1.3) with the Mouse Diversity Genotyping Array [MusDiv; (9)], a high density microarray with probes that target 623 124 SNPs and over 900 000 invariant genomic regions targeting features such as exons and copy number variations. MusDiv SNP data will also be submitted to dbSNP following publication of an analysis manuscript (in preparation).

The CGDSNPdb search engine facilitates a number of different queries, including search by chromosome region(s), nearby gene annotations, or SNP identifiers. Results can be returned as dynamic html or in flat-text comma-separated-value (CSV) format.

Annotations in CGDSNPdb include characteristics of the SNP (e.g. presence in CpG dinucleotide, major/minor allele frequencies), along with functional characteristics of protein-coding genes affected by the SNP (e.g. changes in amino-acid physical and chemical characteristics, changes in codon usage, and overlapping or closest

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neighboring genes). All annotations were generated using an automated analysis pipeline with subsequent quality controls, described below.

CGDSNPdb was constructed primarily as a resource to support the imputation and mouse diversity array projects, however, it is being made available as a somewhat reduced size, but high confidence, collection of mouse SNPs. Database updates will be driven by the availability of new or updated genome assemblies, updated releases of major external SNP data sets, new SNP data sources, and maintenance. Future growth of the database will be targeted primarily at large-scale projects such as the 'mouse genomes project' (http://www.sanger.ac.uk/resources/mouse/genomes/) as well as data sets that can increase the represented strain diversity. Minor releases of CGDSNPdb may also be generated for improved data visualization or underlying quality control procedures. This manuscript provides a high-level overview of the main components of CGDSNPdb, as of version 1.3 (January 2010).

Implementation details

The database

CGDSNPdb was implemented using the open source MySQL relational database management system. The database consists of the core tables, containing all pertinent data for the SNP, including all data from the source download, and gene related tables that facilitate associations between SNPs and neighboring genes. Database schemas are available as Supplementary Figures S1 and S2. Original SNP files from sources and genome assembly files for flanking sequence data are retained and stored separately from the database.

Automated load programs, written in Perl and C++ with SQL queries, integrate data sets from various external sources into the database. These data files have, in general, been obtained directly from the generating source rather than another accumulative resource, such as dbSNP at NCBI (10) or the Mouse Phenome Database (MPD) (11). The load process (Supplementary Figure S3) includes a number of quality control checks that identify problems or ambiguities with SNPs and correct them, if possible. Quality control checks include comparison of the provided SNP call in C57BL/6J with the same position within the reference genome, genomic comparison of the provided flanking sequences (typically 50-nt up and downstream, with a requirement of at least 60% sequence identity in each direction), identification and resolution of duplicate entries (as defined by chromosome and position), and comparison of genotype calls (strain and genotype) among the different data sources. Genomic coordinates provided by the SNP source were assumed to be correct, and only challenged and further tested if the checks of the SNP or flanking sequence failed. No minimum length requirements were placed on the length of the flanking sequences, but SNP correction was only possible if flanking sequences were provided with the source data.

MusDiv (9) SNPs were subjected to additional quality tests intended to assist in the interpretation of the microarray hybridization patterns. The MusDiv SNPs hybridization probes are primarily 25-mers, with the SNP typically centered within the probe. Probes included both forward and reverse sense probes for both the reference C57BL6/J base and the known variant. Alignment of the flanking and probe sequences to the reference C57BL6/J genome was made using PASS (12), as it provided the best tradeoff of speed and alignment sensitivity (data not shown), especially for the analysis of near matches necessary for the mouse diversity array probes. PASS was used to align all four variants classes of probes, identifying all genomic matches with 0, 1 or 2 mismatches to the probe.

SNPs with conflicting genotype calls from different sources were loaded into the database, but flagged to indicate their conflict status. SNPs with conflicting duplicate calls from the same source were removed. SNPs with other unrecoverable errors (e.g. flanking sequences that do not align to the genome) were also flagged and removed as SNP entries in the database. SNPs in disagreement with the genome were identified and deleted prior to comparison for source-to-source conflicts, removing one systematic class of conflict. All details of the SNP processing and filtering are stored in log files that are subsequently loaded into the database, making deleted SNPs searchable, including the evidence that supports exclusion. Complete results of the CGDSNPdb data load processing and data quality check are available online at the CGDSNPdb website (http://cgd.jax.org/CGDSNPdbdb/utils/snp_data_report.php).

SNP-to-gene associations were generated through exhaustive testing of all SNPs in each data source with all transcripts currently in the ENSEMBL mouse (version 56_37i) transcript collection (13). SNPs that overlap genes with alternatively processed (splicing, transcription initiation, or polyadenylation) transcripts were compared against all isoforms in the collection. The comparison with all isoforms resulted in some SNPs with multiple classes of genomic location, since alternative processing can change whether or not the specific SNP is included in the final transcript (e.g. Figure 3).

CGDSNPdb currently (version 1.3) holds 9 686 537 distinct SNPs (Table 1), drawn from eight sources, including the previously mentioned IGR (8) and MusDiv (9) sets. Data were also drawn from large SNP collections generated by NIEHS (4), the Broad Institute (14), Genomics Institute of the Novartis Research Foundation (6), and Celera (15). Finally, two manually curated sets were included, specifically to support the needs of the IGR and MusDiv analysis.

Table 1. A summary of the total SNP data included in CGDSNPdb, version 1.3

Classification	Count
Total	9 686 537
Transition	6 607 155
Transversion	3 079 382
Intergenic	5 617 609
Genic	4 068 928
Intronic	3 850 229
Exonic	247 920
UTR	112 067
CDS	126 078
CDS:nonsynonymous	85 090
CDS:synonymous	43 698
Noncoding gene exon	17 032
Noncoding gene intron	5910

Table 2. A summary of the data for the sources of SNP data in CGDSNPdb, version 1.3

Source	Available	Loaded	Strains	Genomic mismatch	Duplicate
Imputed	7 868 024	7 867 856	74	0	0
MusDiv	584 920	548 363	72	612	0
NIEHS	8 238 764	8 230 026	16	1830	22 620
GNF	156 513	155 677	76	611	243
Broad	138 602	138 594	48	233	9
Celera	2 122 060	2 122 059	5	0	0
Paigen	24 608	24 608	50	0	0
Wild Derived	667	667	37	0	0

These included a set of SNPs targeted for studies of atherosclerosis and related diseases, drawn from a variety of sources (B. Paigen, personal comm.), and a collection of SNPs identified specifically for differentiating between wild-derived inbred and standard laboratory inbred strains, drawn from existing sources (10,11,16–18). A summary of the characteristics of all of our data sets is available here (Tables 2 and 3) and more detailed data are available at the CGDSNPdb web site.

Web interface

The web interface to CGDSNPdb was built using open source Linux-Apache-MySQL-PHP (LAMP) tools. The database is searchable through an online interface (Figure 1) that can accept as input genome coordinates, SNP accession ids, ENSEMBL gene predictions and Mouse Genome Informatics (MGI) gene symbols (11) and Entrez gene IDs (19).

Table 3. Conflicts in SNP genotypes between different data sources

	NIEHS	MusDiv	Broad	GNF	Celera	Paigen	Wild derived
Imputed	17 339	393 768	2793	5065	19 914	5374	19
NIEHS		37 916	5526	10 141	18 118	1048	0
MusDiv			21 222	46 475	1343	2404	0
Broad				354	886	247	0
GNF					3292	6138	1
Celera						728	1
Paigen							0

Each cell counts for the number of SNPs where the indicated sources disagree on at least one strain genotype.

Searches can be restricted to source of SNP data, and specific strains of interest. As noted above, choices of data source include the IGR and MusDiv sets (Figure 1). Pop-ups are used for help tips such as the acceptable formats for a search term and the data sources that include SNPs for any particular strain of mouse.

All searches return a common result page (Figure 2), which includes a summary of the parameters of the search followed by a table representation of the SNP genotypes and annotations. Currently displayed annotations include SNP accession ids (from all sources that match a SNP), mutation type (transition or transversion), MGI gene symbol (11), ENSEMBL transcript (13), CpG site status, minor allele frequency and frequency of failed SNP assay. The returned table is a dynamic object that allows the user to re-order or remove individual columns. The summary page also contains links that allow further restriction of the displayed SNPs by class of location within the gene annotations on the genome, including intergenic, exonic, intronic, etc. All displayed data from external sources such as MGI or ENSEMBL, as well as genomic location within the CGD genome browser, are active links that enable direct access to the appropriate external websites. As of version 1.3, ENSEMBL annotations are for mouse release 56_37i, and MGI annotations were downloaded as of 12 January 2010.

In the HTML representation, the result table contains both text and background color representations of the genotype data for the selected strains (Figure 2). When the imputed SNP genotypes are included in the analysis, the background color intensity is varied to show the 'confidence level' associated with the imputed call, with darkest colors representing high confidence and lightest colors representing low confidence (Figure 2). Experimentally assessed and imputed genotypes are shown as upper and lower case letters, respectively. Genotypes with a conflict

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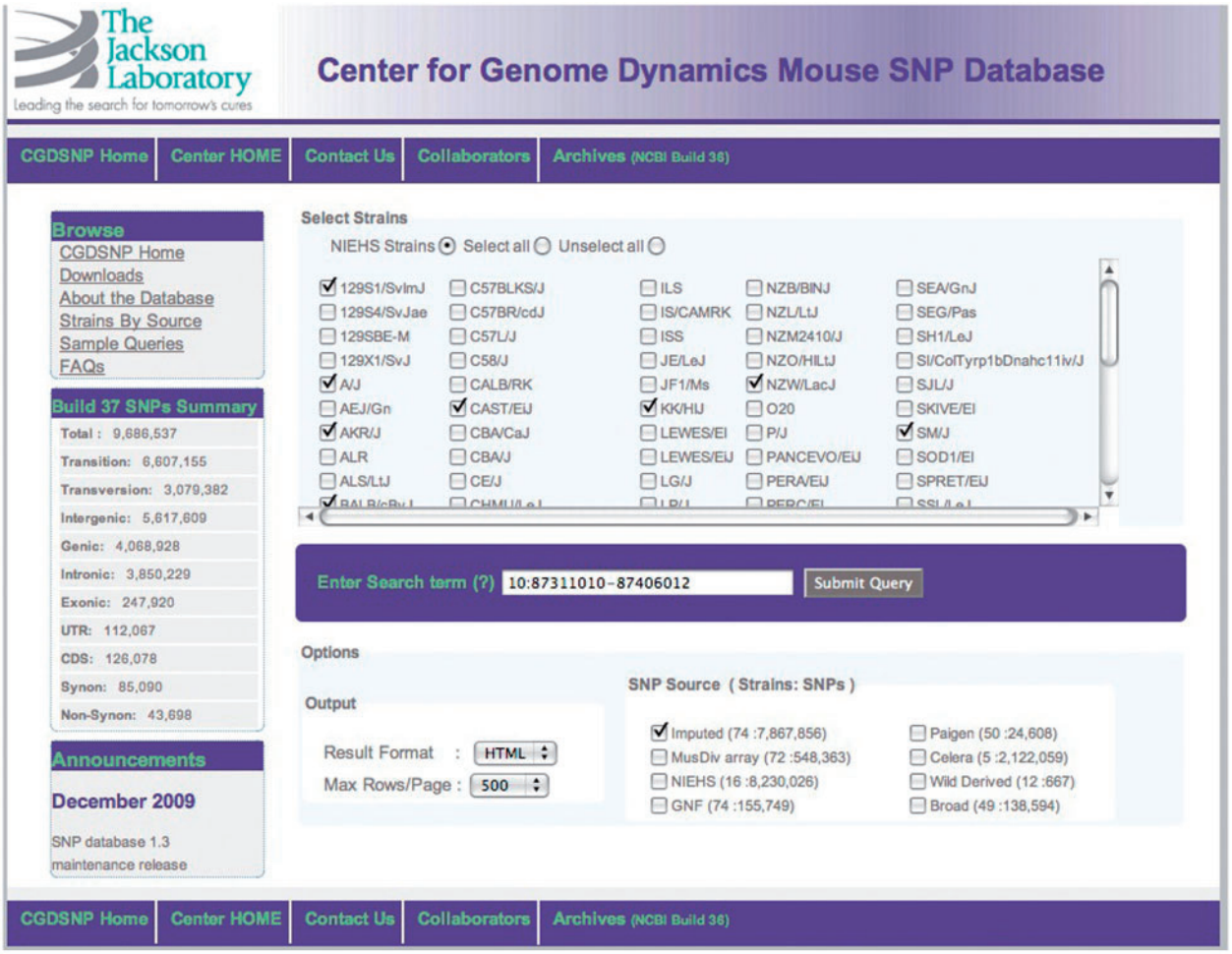


Figure 1. A screen capture of the CGDSNP interface search input form.

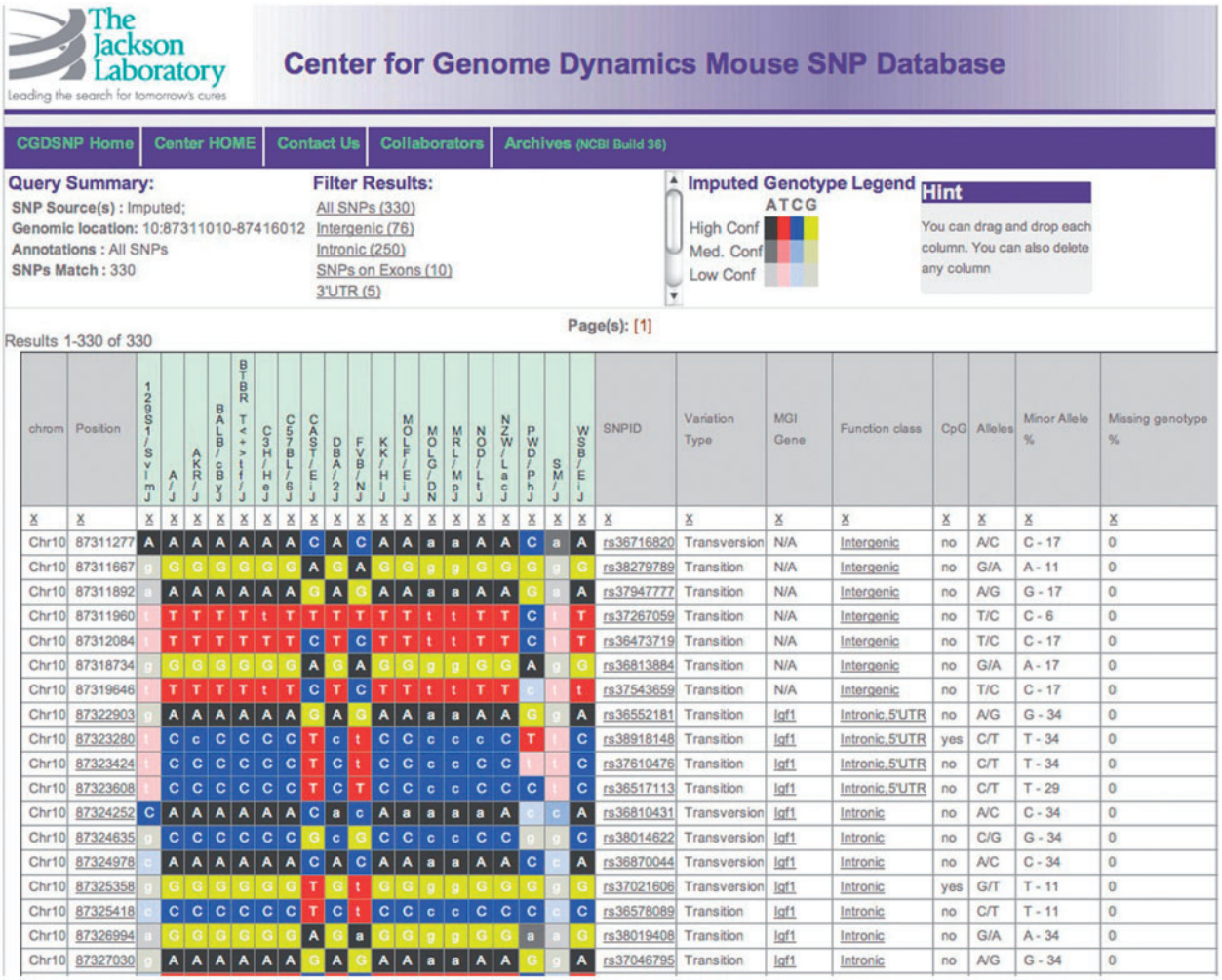
between data sources are shown as question marks if both sources are included in the query.

Every SNP is also further described in a SNP detail page (Figure 3). The SNP detail page takes on different forms depending upon the genomic nature of the SNP location. All SNP detail pages display a standard set of information that includes: data source and source-specific identification, genomic location (chromosome and base position), major and minor alleles, a summary of the results of quality control analysis, and the class of SNP location. Further data display on the SNP detail page is dependent on whether or not the specific SNP is located within the genomic boundaries of any annotated transcripts (Figure 3).

The detail page for SNPs within the genomic bounds of a transcript, whether within coding sequence (CDS), untranslated region (UTR), or introns includes details of the gene and transcript(s) that were matched, including reference genomic coordinates, with links to external databases. CDS SNP descriptions also include the rank of the exon within the transcript structure, position within amino-acid codon, and functional characteristics of the variants, including both codons and their usage frequencies in mouse, both amino acids and their associated BLOSUM62 substitution score (20), side chain chemical characteristics, hydropathy indexes, and the molecular structure of each amino acid (Figure 3). The detail page for Intergenic SNPs (Supplementary Figure 4) includes information on the immediate neighboring genes, including relative orientation and distance to the SNP.

Access

The CGDSNPdb webserver interface URL is http://cgd.jax.org/cgdsnpdb/. CGDSNPdb can also be accessed directly with read-only privileges at host cgd.jax.org. Login instructions are available on the webserver interface. Raw data files, including those for the MusDiv and IGR can be found at http://cgd.jax.org/datasets/popgen.shtml.



chrom	Position	129S1/SvImJ	A/J	AKR/J	BALB/cByJ	BTBR T<+>tf/J	C3H/HeJ	C57BL/6J	CAST/EiJ	DBA/2J	FVB/NJ	KK/HiJ	MOLF/EiJ	MOLG/DnJ	MRL/MpJ	NOD/LtJ	NZW/LacJ	PWD/PhJ	SM/J	WSB/EiJ	SNPID	Variation Type	MGI Gene	Function class	CpG	Alleles	Minor Allele %	Missing genotype %
Chr10	87311277	A	A	A	A	A	A	A	C	A	C	A	A	a	a	A	A	C	a	A	rs36716820	Transversion	N/A	Intergenic	no	A/C	C - 17	0
Chr10	87311667	g	G	G	G	G	G	G	A	G	A	G	G	g	g	G	G	G	g	G	rs38279789	Transition	N/A	Intergenic	no	G/A	A - 11	0
Chr10	87311892	a	A	A	A	A	A	A	G	A	G	A	A	a	a	A	A	G	a	A	rs37947777	Transition	N/A	Intergenic	no	A/G	G - 17	0
Chr10	87311960	t	T	T	T	T	t	T	T	T	T	T	T	t	t	T	T	C	t	T	rs37267059	Transition	N/A	Intergenic	no	T/C	C - 6	0
Chr10	87312084	t	T	T	T	T	T	T	C	T	C	T	T	t	t	T	T	C	t	T	rs36473719	Transition	N/A	Intergenic	no	T/C	C - 17	0
Chr10	87318734	g	G	G	G	G	G	G	A	G	A	G	G	g	g	G	G	A	g	G	rs36813884	Transition	N/A	Intergenic	no	G/A	A - 17	0
Chr10	87319646	t	T	T	T	T	t	T	C	T	C	T	T	t	t	T	T	c	t	t	rs37543659	Transition	N/A	Intergenic	no	T/C	C - 17	0
Chr10	87322903	g	A	A	A	A	A	A	G	A	G	A	A	a	a	A	A	G	g	A	rs36552181	Transition	Igf1	Intronic,5'UTR	no	A/G	G - 34	0
Chr10	87323280	t	C	c	C	C	C	C	T	c	t	C	C	c	c	c	C	T	t	C	rs38918148	Transition	Igf1	Intronic,5'UTR	yes	C/T	T - 34	0
Chr10	87323424	t	C	C	C	C	C	C	T	C	t	C	C	c	c	C	C	t	t	C	rs37610476	Transition	Igf1	Intronic,5'UTR	no	C/T	T - 34	0
Chr10	87323608	t	C	C	C	C	C	C	T	C	T	C	C	c	c	C	C	C	t	C	rs36517113	Transition	Igf1	Intronic,5'UTR	no	C/T	T - 29	0
Chr10	87324252	C	A	A	A	A	A	A	C	a	c	A	a	a	a	a	A	c	c	A	rs36810431	Transversion	Igf1	Intronic	no	A/C	C - 34	0
Chr10	87324635	g	C	C	C	C	C	C	G	c	G	C	C	c	c	C	C	g	g	C	rs38014622	Transversion	Igf1	Intronic	no	C/G	G - 34	0
Chr10	87324978	c	A	A	A	A	A	A	C	A	C	A	A	a	a	A	A	C	c	A	rs36870044	Transversion	Igf1	Intronic	no	A/C	C - 34	0
Chr10	87325358	g	G	G	G	G	G	G	T	G	t	G	G	g	g	G	G	G	g	G	rs37021606	Transversion	Igf1	Intronic	yes	G/T	T - 11	0
Chr10	87325418	c	C	C	C	C	C	C	T	C	t	C	C	c	c	C	C	C	c	C	rs36578089	Transition	Igf1	Intronic	no	C/T	T - 11	0
Chr10	87326994	a	G	G	G	G	G	G	A	G	a	G	G	g	g	G	G	a	a	G	rs38019408	Transition	Igf1	Intronic	no	G/A	A - 34	0
Chr10	87327030	g	A	A	A	A	A	A	G	A	G	A	A	a	a	A	A	G	g	A	rs37046795	Transition	Igf1	Intronic	no	A/G	G - 34	0

Figure 2. A screen capture of the standard summary page for SNP search, specifically showing results for the tumor suppressor *P53*, using the imputed data resource. Background colors in the genotype table represent the specific nucleotide and the imputed confidence level, with darkest colors representing the highest confidence.

Additional features

Additional features available at the online site include user help pages, a Frequently Asked Questions (FAQ) page, and database report pages. The database report page provides details generated automatically during the process of preparing and inserting the external data sources into the database, including numbers of SNPs processed, number inserted successfully, and numbers with each of the types of problems and/or inconsistencies. Users can ultimately drill down to obtain lists of SNPs that fall into each specific category.

Future Plans and Upgrades

Future plans include increased integration of additional annotation data that assess the functional implications of the variation, including but not limited to intersection of SNP locations with known or predicted functional elements such as splice sites, promoter elements, and polyadenylation sites or overlap with regions of evolutionary conservation, such as provided by the PhastCons scores (21). The extended ambiguity analysis implemented for the MusDiv (9) SNPs and associated array probes will be extended to all SNPs in the database for which flanking sequence is available. Finally, search capabilities will be improved with further integration of gene identification data, such as Unigene (22) IDs. Any additional upgrades will be focused on requests generated by users of the database.

Citing CGDSNPdb

The CGDSNPdb should be referenced using this publication. In addition, since CGDSNPdb is planned to include regular updates, references to use of the database should include the current database version and month and year of access.

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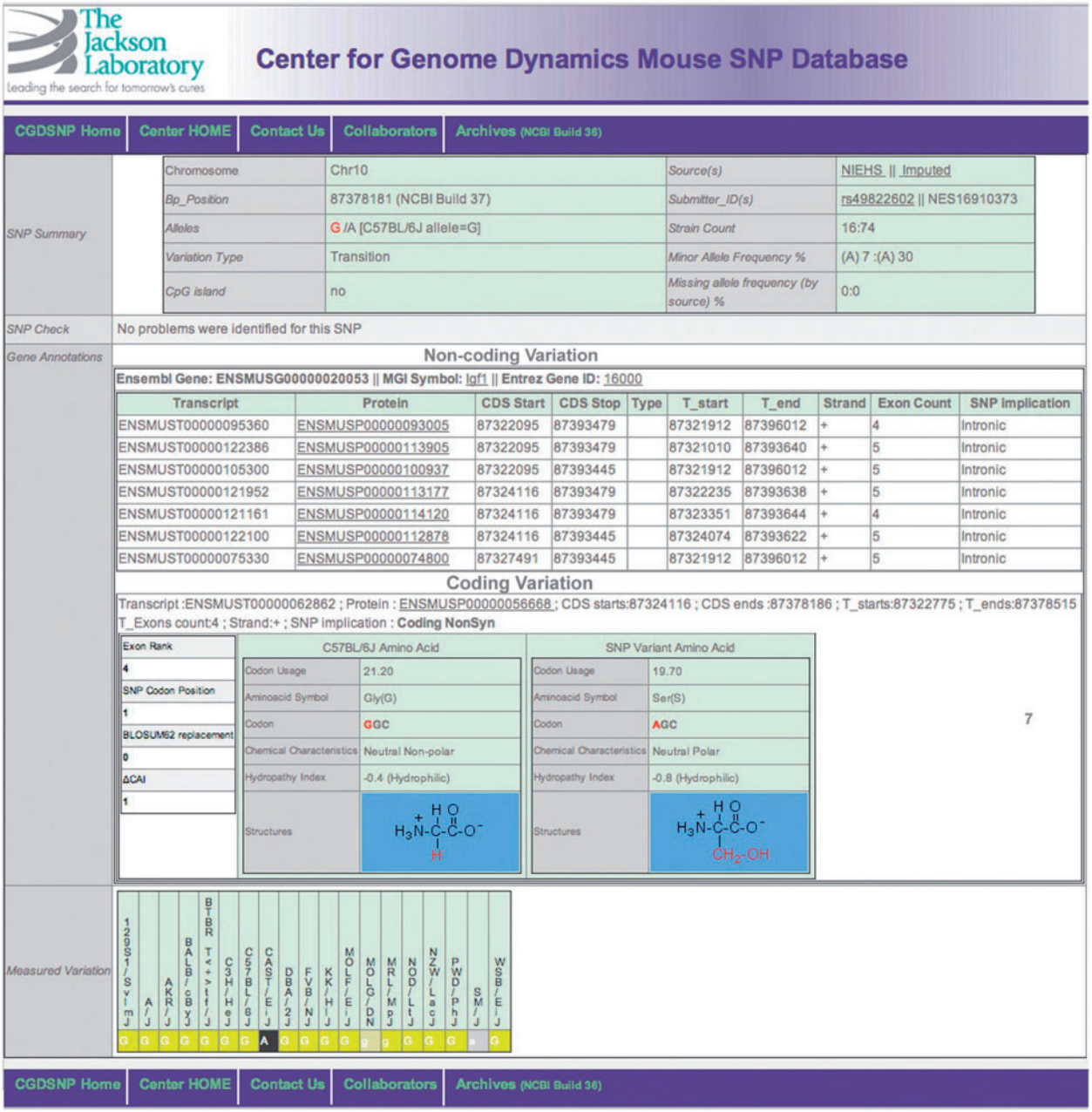

Figure 3. A screen capture for the SNP detail page, showing SNPs within the bounds of a transcript, whether in an UTR, intron or CDS. If multiple transcripts have been annotated for a given gene, the results are grouped by whether the change is within the coding sequence. A detail page for intergenic SNPs is available as Supplementary Figure S4.

Supplementary Data

Supplementary Data are available at *Database* Online.

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References

1. Hirschhorn,J.N. and Daly,M.J. (2005) Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.*, **6**, 95–108.
2. Ioannidis,J.P., Thomas,G. and Daly,M.J. (2009) Validating, augmenting and refining genome-wide association signals. *Nat. Rev. Genet.*, **10**, 318–329.
3. Abe,K., Noguchi,H., Tagawa,K. *et al*. (2004) Contribution of Asian mouse subspecies Mus musculus molossinus to genomic constitution of strain C57BL/6J, as defined by BAC-end sequence-SNP analysis. *Genome Res.*, **14**, 2439–2447.
4. Frazer,K.A., Eskin,E., Kang,H.M. *et al*. (2007) A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature*, **448**, 1050–1053.
5. Petkov,P.M., Cassell,M.A., Sargent,E.E. *et al*. (2004) Development of a SNP genotyping panel for genetic monitoring of the laboratory mouse. *Genomics*, **83**, 902–911.
6. Pletcher,M.T., McClurg,P., Batalov,S. *et al*. (2004) Use of a dense single nucleotide polymorphism map for in silico mapping in the mouse. *PLoS Biol.*, **2**, e393.
7. Shifman,S., Bell,J.T., Copley,R.R. *et al*. (2006) A high-resolution single nucleotide polymorphism genetic map of the mouse genome. *PLoS Biol.*, **4**, e395.
8. Szatkiewicz,J.P., Beane,G.L., Ding,Y. *et al*. (2008) An imputed genotype resource for the laboratory mouse. *Mamm. Genome*, **19**, 199–208.
9. Yang,H., Ding,Y., Hutchins,L.N. *et al*. (2009) A customized and versatile high-density genotyping array for the mouse. *Nat. Methods*, **6**, 663–666.
10. Sherry,S.T., Ward,M.H., Kholodov,M. *et al*. (2001) dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.*, **29**, 308–311.
11. Grubb,S.C., Maddatu,T.P., Bult,C.J. *et al*. (2009) Mouse phenome database. *Nucleic Acids Res.*, **37**, D720–D730.
12. Campagna,D., Albiero,A., Bilardi,A. *et al*. (2009) PASS: a program to align short sequences. *Bioinformatics*, **25**, 967–968.
13. Hubbard,T.J., Aken,B.L., Ayling,S. *et al*. (2009) Ensembl 2009. *Nucleic Acids Res.*, **37**, D690–D697.
14. Wade,C.M. and Daly,M.J. (2005) Genetic variation in laboratory mice. *Nat. Genet.*, **37**, 1175–1180.
15. Mural,R.J., Adams,M.D., Myers,E.W. *et al*. (2002) A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. *Science*, **296**, 1661–1671.
16. Ideraabdullah,F.Y., de la Casa-Esperon,E., Bell,T.A. *et al*. (2004) Genetic and haplotype diversity among wild-derived mouse inbred strains. *Genome Res.*, **14**, 1880–1887.
17. Salcedo,T., Geraldes,A. and Nachman,M.W. (2007) Nucleotide variation in wild and inbred mice. *Genetics*, **177**, 2277–2291.
18. Yang,H., Bell,T.A., Churchill,G.A. *et al*. (2007) On the subspecific origin of the laboratory mouse. *Nat Genet.*, **39**, 1100–1107.
19. Maglott,D., Ostell,J., Pruitt,K.D. *et al*. (2007) Entrez Gene: gene-centered information at NCBI. *Nucleic Acids Res.*, **35**, D26–D31.
20. Henikoff,S. and Henikoff,J.G. (1992) Amino acid substitution matrices from protein blocks. *Proc. Natl Acad. Sci. USA*, **89**, 10915–10919.
21. Siepel,A., Bejerano,G., Pedersen,J.S. *et al*. (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.*, **15**, 1034–1050.
22. Wheeler,D.L., Barrett,T., Benson,D.A. *et al*. (2008) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.*, **36**, D13–D21.