Analysis of single nucleotide polymorphisms in human chromosomes 21 and 22

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Abstract

Single nucleotide polymorphisms (SNPs) are useful for genome-wide mapping and study of disease genes. Previous studies have focused on specific genes or SNPs pooled from a variety of different sources. Here, we present a systematic approach to the analysis of SNPs in relation to various features on a genome-wide scale. We have performed a comprehensive analysis of 39,408 SNPs on human chromosomes 21 and 22 from The SNP Consortium (TSC) database, where SNPs are obtained by random sequencing using consistent and uniform methods. Our study indicates that the occurrence of SNPs is lowest in exons and higher in repeats, introns and pseudogenes. Moreover, in comparing genes and pseudogenes, we find that the SNP density is higher in pseudogenes and the ratio of nonsynonymous to synonymous changes is much higher as well. These observations may be explained by the increased rate of SNP accumulation in pseudogenes, which presumably are not under selective pressure. We have also performed secondary structure prediction on all coding regions and found that there is no preferential distribution of SNPs in α-helices, β-sheets or coils. This could imply that protein structures, in general, can tolerate a wide degree of substitutions. Tables relating to our results are available from http://genecensus.org/pseudogene.

Keywords: Single nucleotide polymorphisms, SNP, pseudogenes, synonymous, nonsynonymous, secondary structure.

Introduction

Single nucleotide polymorphisms are single base variations between genomes within a species. SNPs are useful markers for diseases in haplotype-based association studies and in linkage disequilibrium analysis [1-3]. It is also believed that these small genomic-level differences may be used to explain the differential drug-response behavior of individuals towards a drug and can be used to tailor drugs based on an individual's genetic makeup [3].

The sequence variations in many important human genes have been extensively studied. In these studies, it was found that approximately half of the coding SNPs resulted in an amino acid change in the protein sequence [4-6]. Ng and Henikoff have predicted the effect of SNPs on protein function based on sequence homology methods [7]. Several groups have predicted the effect of SNPs on the structure of proteins in order to rationalize the effect of SNPs on protein function [8-11]. Wang and Moult showed that SNPs resulting in deleterious amino acid changes predominantly affect the stability of the protein. Sunyaev and coworkers estimate that about 20% of common non-synonymous SNPs will have deleterious effects on protein structure based on the location of SNPs mapped onto 3D-structures and comparative homology analyses [10]. Chasman and Adams estimated that 26-32 % of nonsynonymous SNPs have effects on function [8]. With the release of the human genome sequence, broad overviews of its SNP landscape have been published [12,13]. The analyses by both the Celera and TSC groups on different SNP datasets indicate that the distribution of SNPs is not uniform throughout the genome.

The publication of the human genome sequence [13,14] and a plethora of other genomic sequences has made it possible to perform large-scale surveys of different features relating to the whole genome [15-18]. Many different databases containing data on SNPs are publicly available now [19,20]. A huge repository of coding SNPs obtained by data mining of expressed sequence tags database using statistical methods is also available [21,22]. Our study pertains to a large-scale survey of SNPs in TSC database [12,23]. We chose the TSC data set because the SNPs have been obtained by randomly sequencing human genomic DNA of 24 unrelated individuals and thus represents an unbiased random sampling of the SNPs in the human genome. In addition, the TSC data is homogenous as the majority of SNPs are obtained by the application of uniform automated methods. It must be noted that the TSC data set consists of mostly high frequency SNPs i.e. SNPs which occur at frequencies > 10% of the populations surveyed. It is estimated that 77% of SNPs have a minor allele frequency of more than 20% in at least one population surveyed [12].

We have performed a detailed analysis of SNPs in TSC database in human chromosomes 21 and 22. Chromosomes 21 and 22 were chosen for this analysis because of the completeness and the high quality of sequence and assembly available when this work was begun [24,25].

Methods

Release 10 of the TSC data was used for this analysis. The data was downloaded from snp.cshl.org. Assembled sequences of chromosome 21 (Chr21) and chromosome 22 (Chr22) were obtained from NCBI and The Sanger Center respectively. We have explicitly listed the exact files used because data and databases are constantly updated and we used these files throughout our analyses to make sure that all results obtained were consistent with each other. For Chr21, the file, hs_chr21all.fna, dated May 17,2000, was retrieved from ftp://ncbi.nlm.nih.gov/genbank/genomes/H_sapiens and the May 19, 2000 release of Chr22 sequence was retrieved from ftp://ftp.sanger.ac.uk/pub/human/chr22/sequences/Chr_22/complete_sequence/Chr_22_19-05-2000.fa. The exact sequences that were used can also be found at http://bioinfo.mbb.yale.edu/genome/snps**.**

The sequences corresponding to SNPs in Chr21 and 22 were extracted using the tables that contained SNPs mapped on to them using the "Golden Path" assembly (http://genome.ucsc.edu) in the TSC database. We remapped them onto the above-mentioned versions of Chr21 and 22 using BLASTN [26] to maintain consistency with all our data analyses.

Exonic regions were identified from homology matches to all the SWISSPROT proteins. We used this procedure because the number of genes and their locations and gene annotations vary depending on the gene prediction program or method of analysis used. In addition, many pseudogenes have been erroneously annotated as genes. We used the entire SWISSPROT because a complete set of human proteins is not yet available. Significant matches to exonic regions of non-human proteins indicate a high likelihood of a corresponding human-complement. In addition, homology to non-human proteins is relevant to our analysis as they may represent either laterally transferred pseudogenes or extinct pseudogenes.

Homology matches to proteins were obtained by performing a six-frame translational BLAST search of the genomic sequences of Chr21 and 22 against the SWISS-PROT database [27]. All the sequence coordinates were translated to absolute coordinates with respect to the chromosomal assembly.

For finding the homology matches to the SWISS-PROT database, all matches that corresponded to repeat regions were eliminated: these consist of the known human repeat sequences, retroviral elements and low complexity regions identified by the RepeatMasker2 program [28]. Although some coding regions may contain repeats, we had to remove such matches because the genome is riddled with numerous repeats such as Alu elements. Often, a lot of good matches are obtained to a wide variety of different sequences primarily because of presence of low complexity and repeat sequences. The only automated way to remove such spurious matches in such large-scale analyses is by eliminating such matches. Thus, this set of matches represents a conservative lower estimate of exonic regions. This set was further reduced to get a nonredundant set. First, only matches with e-values $< 10^{-4}$ were considered as significant matches. These matches were then sorted in decreasing order of significance. This set was reduced for mutual overlap by deleting matches that overlap substantially with a picked match (if two matches overlapped by more than 30 nucleotides, they were appropriately merged or removed if one was a subset of the other). Pseudogenic matches were removed from this set as described below and the remaining matches constituted the exonic matches to SWISS-PROT proteins.

Pseudogenes were obtained from the above set of matches by identifying matches that contained premature stop codons. A non-redundant set of pseudogene matches was obtained after discarding potential pseudogene sequences that overlapped artefactually with known genes [29,30].

Amino acids changes were classified as conservative and non-conservative based on Gonnet Pam250 matrix [31]. All amino acids changes within groups with a score > 0.5

were considered to be conservative. For our analysis, we considered amino acid changes within the following groups to be conservative: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, and FYW.

Secondary structure prediction of the SNP sequences pertaining to matches to exons and pseudogenes were performed using the GORIV program [32]. The accuracy of secondary structure predictions vary depending on the method used [33,34]. Multiple sequence alignments at best have an accuracy of about 72% [33]. Being a single sequence prediction method, GORIV is less accurate than methods based on multiple sequence alignments. The version IV of GOR has a mean accuracy of 64.4% for a three state prediction (Q3) (http://genamics.com/expression/strucpred.htm). However, GORIV is a useful prediction program for large-scale analyses where multiple sequence alignments are often not possible or very time-consuming. Exon predictions were obtained from GenomeScan [35]. This data was used to extract predicted intron coordinates based on the predicted exon coordinates.

SNPs were modeled to fit a Poisson distribution using the following equation:

$$P(y)=(m^{y}e^{-m}) /y!$$

Where m= mean value of y and y = number of SNPs observed

Optimized runs of the translational BLAST search of chromosome 22 against SWISSPROT took about 8 days of CPU time (on a 600MHz processor). The repeatmasking of chromosomes 21 and 22 took about 10 days each on a 600 MHz processor. Chromosomes 21 and 22 are the smallest chromosomes in the human genome. When this work was begun, the human genome sequence data was in a continuous state of flux. Clearly, the remapping of all the features on to a dynamically changing human genome sequence data would be computationally very intensive.

Results

Distribution of SNPs in the various features of Chr21 and 22

Our analysis basically incorporates three steps: A. Mapping of the different genomic features on to the genomic DNA sequences of chromosomes 21 and 22. B. Mapping of SNPs onto the two chromosomes. C. Mapping SNPs on to repeats, exons, pseudogenic regions and introns by combining the results obtained from steps A and B. This is illustrated in Figure 1. A prerequisite for this kind of systematic analysis is the availability of high quality sequence information, both in terms of completeness and assembly of the chromosomes because all the genomic features are annotated with reference to a stable coordinate system of the chromosomal sequence. Step A is computationally time-intensive. Step B is a straightforward BLAST search and therefore, this approach enabled us to map the SNPs on to chromosomes quickly as the TSC database changed fairly rapidly. The mapping of SNPs directly on to the various features (for example, mapping on to exons by a translational BLAST search against SWISSPROT proteins) would take longer computational times and would have to be performed with every new release of the TSC database.

Most studies show that SNPs are found at the rate of 1-2 per kb of the human genome [36]. In the TSC data set, an average of one SNP per 2kb is observed for both Chr21 and 22, with Chr22 having a slightly higher SNP density. An overview of the SNP distribution is shown in Table 1. While nucleotide diversity and heterozygosity measures are normally used to measure polymorphism density [4-6], it was not possible to perform such an analysis from the TSC data because the sequence reads are not available. Instead, we define SNP density as the total number of SNPs normalized to the total number of bases in any given genomic feature. This kind of analysis is useful for looking at the TSC data, as our aim was to glean information from randomly sampled SNPs obtained by consistent and uniform methods.

As detailed in the methods section, exons and pseudogenes where identified by homology matches to proteins in SwissProt, repeats were identified using RepeatMasker and intron coordinates were derived from GenomeScan predictions. This is clearly depicted in the flow chart labeled as Figure 2.

The occurrence of SNPs is about the same in repeats and introns and higher than in exons. The SNP density in exons is significantly lower than that of the entire chromosome. In contrast, the SNP density is higher in pseudogenes than in exons. In chromosome 22, the SNP density in pseudogenes is even higher than the chromosome at large. This is surprising, as we would have expected the SNP density in pseudogenes to be similar to other intergenic regions. With the exception of SNPs in pseudogenes, the distribution of SNPs amongst the other regions of the chromosome is remarkably similar in both Chr21 and 22, as seen in Table 1. A detailed analysis of exonic or coding SNPs (cSNPs) and the SNPs in pseudogenes is given below.

Exonic SNPs (cSNPs)

About 0.35% and 0.66% of the SNPs in Chr21 and 22 respectively are found in exons determined by homology matches to proteins in SWISS-PROT (Table 1). The occurrence of SNPs is lowest in exons compared to pseudogenes, repeats and introns. The occurrence of SNPs can be modeled by a Poisson distribution because the number of SNPs per kilobase of sequence is relatively small. Based on the average chromosomal SNP density, we see that the number of SNPs in exons is significantly lower than would be expected based on a random distribution of SNPs in the genome as seen from the P-values. The P value for the occurrence of 67 or fewer SNPs in exons for Chr21 is 2.0e-5 and that of finding 136 or fewer SNPs in exons in Chr22 is 5.3e-5. This is clearly illustrated in Figure 3.

Previous reports on SNPs indicate that the number of SNPs that result in a change of amino acid (nonsynonymous) are lower or about the same as substitutions which result in silent changes (synonymous) [4,6]. The ratio of nonsynonymous to synonymous changes due to SNPs varies between 0.3-1.0 [13] depending on the data set used. In our analysis of SNPs in exons, we see that there are more number of nonsynonymous changes than synonymous changes in chromosomes 21 and 22 (Table 2). Of the 38 nonsynonymous SNPs in Chr21, 20 changes are conservative changes. Of the 85 nonsynonymous SNPs, 39 changes are conservative amino acid substitutions. For both Chr21 and 22, the ratio of nonsynonymous to synonymous changes are less than one when corrected for the frequency of synonymous and nonsynonymous sites. This corrected ratio is slightly higher in Chr22 than in Chr21 (Table 2). The fact that SNP density is higher for synonymous sites underscores the fact that natural selection pressure in genes operate presumably to maintain their structural/functional integrity.

Four nonsynonymous SNPs in Chr21 and two nonsynonymous SNPs in Chr22 result in termination codons. Of the total six nonsynonymous SNPs that result in Stop codons, three of them contain glutamine as the other variant. None of these codons are close to the 3'- end of the gene and therefore the premature truncation of proteins due to such SNPs could potentially affect their structure and/or function.

Correlation with predicted secondary structure

Secondary structure predictions of the exon sequences containing the SNPs show that SNPs are found in all the secondary structural elements: helical, beta-sheet and coil regions. While the absolute number of SNPs in coils is generally more than the number of SNPs in helices and sheets put together, the SNP densities of the exonic SNPs (normalized to the total number of residues in the corresponding secondary structural class) are not significantly different from each other (Table 3). In general, coil regions tend to be more variable in protein structures and we may have expected to see more SNPs in coils. We do not see a preponderance of SNPs in coils. This may indicate that protein structures may have evolved to accommodate amino acid changes [37].

Proline and glycine residues are generally not favored residues in helices and sheets. Therefore, SNPs that result in amino acid substitutions to proline or glycine could affect the structure of the protein. SNPs pertaining to prolines and glycines are discussed below:

- In Chr21, there are four nonsynonymous SNPs that result in an amino acid variation involving proline residues. In all four cases, leucine is a second variant. Of the four, three occur in coil regions and presumably do not affect the structure of the protein. However, the other Pro/Leu variation is in an extended sheet region and could be deleterious to the protein fold.

- Of the three nonsynonymous SNPs involving glycines in Chr21, two occur in coils and presumably do not affect the structure of the protein. The third SNP involving a glycine occupies a β-sheet and may affect the structure of the protein.
- In Chr22, five SNPs lead to variations involving proline residues. Of the five, three of them occur in helices and the other two in coils. The three SNPs in helices could affect the structure of the protein, as proline is known to disrupt helices.
- Of the twelve nonsynomous SNPs that code for glycine in Chr22, there are four potentially disruptive variants: two in helices and one in a beta strand.

While prolines and glycines in coils may not affect the structure of the protein, it is quite possible that such changes may affect the function of the protein. In particular, prolines and glycines are known to be conserved critical residues important for the function of some proteins [38-40].

Pseudogenes

We observed that the SNP density is higher in pseudogenes than in exons in both chromosomes 21 and 22 (Table 1). The exonic matches and pseudogenes are derived from homology matches to SWISS-PROT proteins. Therefore, we modeled the distribution of SNPs in pseudogenes as a Poisson assuming that SNP density in exons reflects the probability of SNP occurrence. We see that the number of SNPs in pseudogenes in chromosome 22 is far greater than that expected from the SNP density in exons. The P value for the occurrence of 94 or more SNPs in pseudogenes for Chr22 is 4.7e-5. However, in chromosome 21, the increased SNP density of pseudogenes over exons is not statistically significant ($P > 0.1$). The occurrence of more SNPs in pseudogenes than exons in chromosome 22 may be due to higher substitution rates seen in pseudogenes [41] presumably due to the lack of natural selection pressure, thus allowing organisms to accumulate SNPs in non-functional pseudogenes.

The ratio of nonsynonymous to synonymous amino acid changes are 2.80 and 2.76 for Chr 21 and 22 respectively, both higher than the corresponding numbers for exons (Table 2). Also, the ratio of nonsynonymous to synonymous changes when corrected for frequency of synonymous and nonsynonymous sites is about one for both Chr21 and Chr22. In both cases, this corrected ratio is much higher than the corresponding ratio seen in exons. This could mean that nonsynonymous SNPs are more prevalent in pseudogenes because they may not have any deleterious functional consequences.

It is of interest to see if pseudogenes have a preponderance of SNPs that result in an amino acid change to a termination codon. Interestingly, there are no SNPs that result in such a change in Chr21. Of the 94 SNPs in pseudogenes in Chr22, seven result in premature truncation of proteins due to a codon change to a Stop codon.

Discussion

We have performed a systematic analysis of SNPs in human chromosomes 21 and 22 from TSC database. The SNP density in repeats and introns are about the same and much higher than in exons. The density of SNPs in exons is significantly lower than the average chromosomal SNP density. This is not surprising because genes are under selective pressure to maintain their biological functions. Pseudogenes have a higher SNP density than exons. It is possible that SNPs have a greater propensity to accumulate in pseudogenes, as they are not under any selective pressure to maintain functional integrity. The higher SNP density in pseudogenes relative to repeats and introns for chromosome 22 suggests that repeat regions and other noncoding DNA regions have some selection against sequence changes. It has been shown that a large fraction of conserved elements in Chromosome 21 comprise noncoding DNA [42]. Several noncoding DNA regions have regulatory roles and presumably have other hitherto unknown important functions that may explain the lower rate of polymorphism in other intergenic regions relative to pseudogenes [43-45].

SNPs are found in all secondary structural elements of the proteins: α-helices, β-sheet and coil regions and do not seem to preferentially populate any particular secondary structure. This implies that proteins have evolved to maintain their structural integrity and are able to accommodate a variety of substitutions. This kind of structural plasticity has been experimentally shown in several lysozyme mutants as well as other examples [37,46-49]. Perhaps, proteins maintain or adapt their three-dimensional structures to fulfill their biological roles. While there would certainly be some SNPs that would have deleterious structural consequences, it is possible that a majority of them are fairly benign to protein structure [8,10].

The number of SNPs in exons and pseudogenes are fairly small in this study. Nevertheless, the difference in the SNP rates in exons and pseudogenes is clearly significant. This approach is being extended to the study of all the SNPs in TSC database with the primary objective of understanding the impact of these SNPs on protein structure and function and the prevalence of SNPs in pseudogenes. The rate of SNP accumulation in pseudogenes could potentially be used to estimate the age of genes and could provide insights into evolution and divergence of genes.

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Figure Legends

Figure 1: Mapping of SNPs on to the various genomic features is done by mapping all features on to a fixed chromosome coordinate and merging the data.

Figure 2: This flow chart shows the various databases and methods used to map the various genomic features on to a reference chromosome sequence. The SNPs, exons and pseudogenes have been mapped on to the chromosome using BLAST. The various resources and inputs for these runs are denoted by ovals. Processes are denoted by rectangles. Coordinates for repeats were obtained using RepeatMasker2. Gene predictions from GenomeScan on the reference chromosomal sequence were used to extract the coordinates of introns. The big rectangle comprising the smaller boxes indicate the mapping of all the features on to a reference chromosome sequence. The circle indicates the final unified chromosome with all the different features mapped on to it. More details are given in the Methods section.

Figure 3: The number of SNPs in exons compared to that expected based on the SNP density in exons is shown here. Gray bars indicate the observed number of SNPs in exons. White bars indicate the expected number of SNPs in exons based on the average chromosomal SNP density. The brackets indicate the 95% confidence interval about this expectation.

Table 1

Chromosome	Average SNP density	SNP density in repeats	SNP density in exons[*]	SNP density in pseudogenes[*]	SNP density in introns[#]
21	0.56/kb (18977)	0.55/kb (8406)	0.35/kb (67)	0.40/kb (38)	0.52/kb (6371)
22	0.61/kb (20431)	0.53/kb (8365)	0.45/kb (136)	0.70/kb (94)	0.53/kb (8934)

The numbers in parentheses indicate the number of SNPs.

The SNP density is the number of SNPs per kb of sequence.

* : Exons and pseudogenes obtained based on homology matches to SWISSPROT.

# : Coordinates for introns obtained from predicted gene assignments by GenomeScan

Table 2

Chromosome	Category	Synonymous	Nonsynonymous	Ratio (NSyn/Syn)	Corrected ratio *(Nsyn/Syn)
21	**Exons**	29 *(0.59/kb)	38 *(0.27/kb)	1.31	0.46
	Pseudogenes	10 *(0.40/kb)	28 *(0.39/kb)	2.80	0.98
22	**Exons**	51 *(0.74/kb)	85 *(0.38/kb)	1.66	0.51
	Pseudogenes	25 *(0.72/kb)	69 *(0.69/kb)	2.76	0.96

Nsyn : Nonsynonymous changes Syn : Synonymous changes

The numbers in parentheses indicate the SNP density, the number of SNPs per kb of sequence.

*: The number of synonymous sites was calculated as the sum of fourfold degenerate sites and half the number of twofold degenerate sites. The number of nonsynonymous sites was calculated as the sum of nondegenerate sites and half the number of twofold degenerate sites [4]

Table 3

Chromosome	Category	SNPs in helices	SNPs in β-strands	SNPs in coils
21	**Exons**	10 (0.63)	17 (1.26)	40 (1.16)
	Pseudogenes	5 (0.95)	11 (1.68)	22 (1.09)
22	**Exons**	38 (1.43)	27 (1.30)	71 (1.31)
	Pseudogenes	12 (1.40)	23 (2.37)	59 (2.21)

The number in parentheses indicates the SNP density normalized to the number of amino acid residues in the corresponding secondary structural class i.e. helices, strands and coils. The SNP density is reported as the number of SNPs per 1000 amino acid residues in the corresponding secondary structural class.

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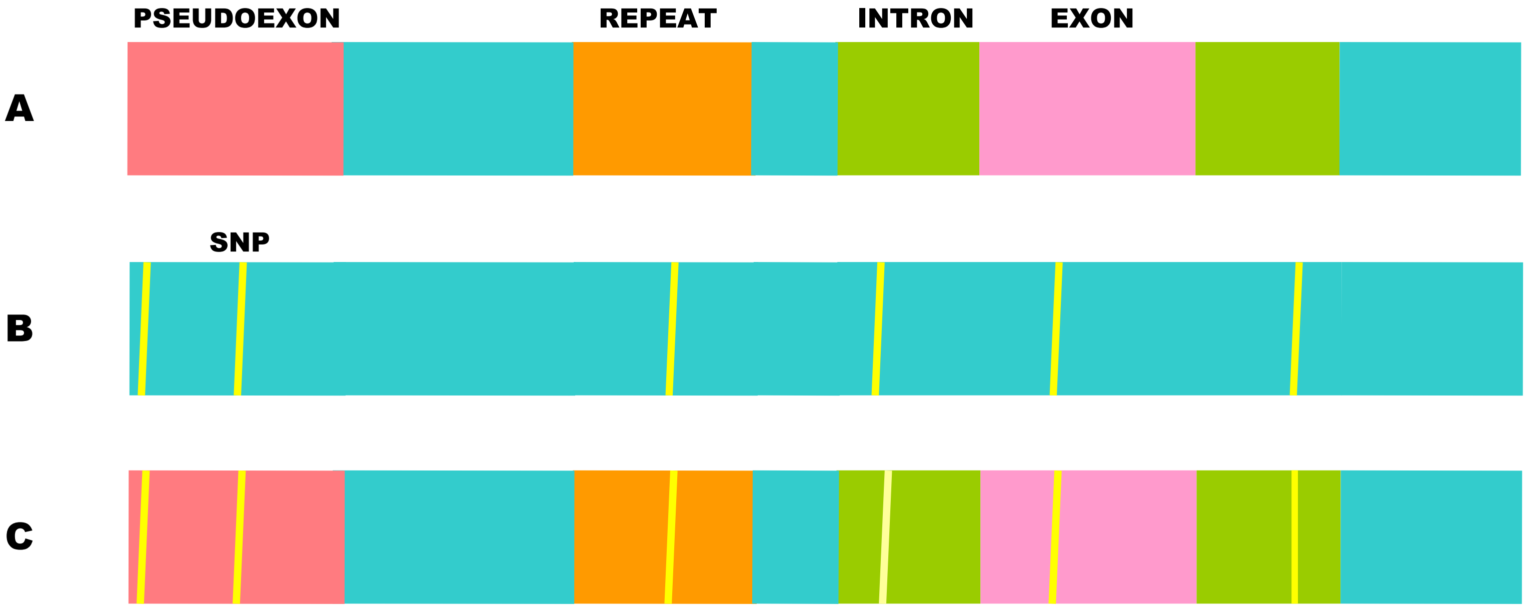
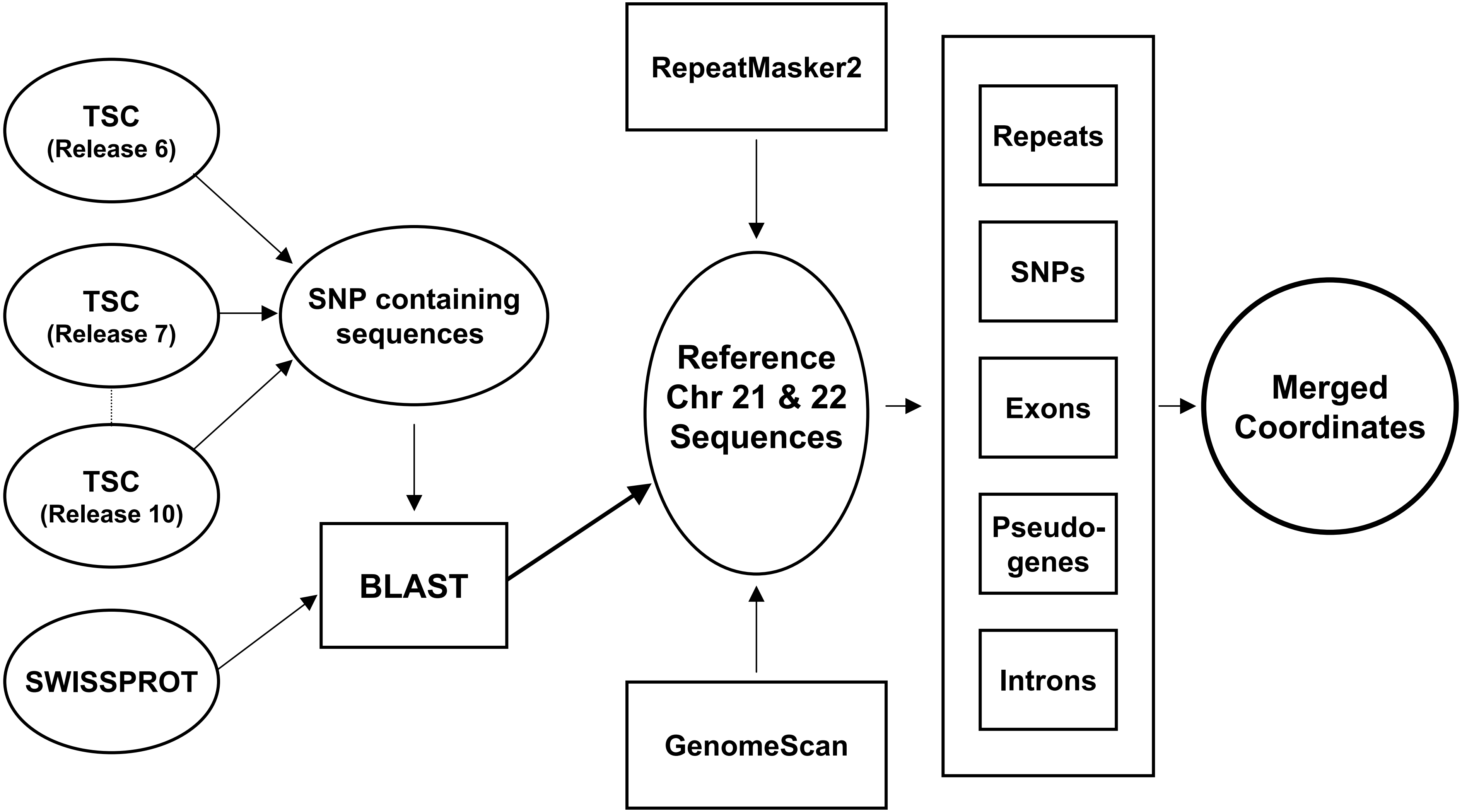
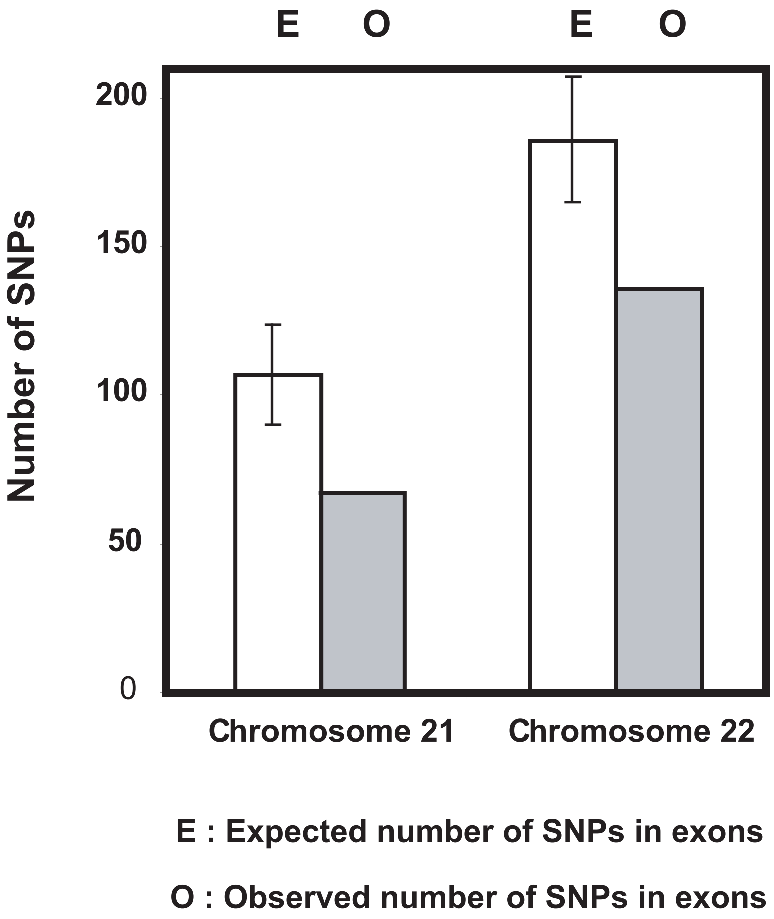


A. Mapping repeats, exons, pseudoexons and introns on to a fixed coordinate framework of the chromosome sequence

B. Mapping SNPs on to the chromosome

C. Mapping SNPs on to the various features using A and B.


TSC (Release 6)
TSC (Release 7)
TSC (Release 10)
SWISSPROT
SNP containing sequences
BLAST
RepeatMasker2
Reference Chr 21 & 22 Sequences
GenomeScan
Repeats
SNPs
Exons
Pseudo-genes
Introns
Merged Coordinates



E
O
E
O
Number of SNPs
200
150
100
50
0
Chromosome 21
Chromosome 22
E : Expected number of SNPs in exons
O : Observed number of SNPs in exons