On Monday, you explored your DNA data. Today, we will take it further and find out what you need to know about your SNPs and their functionality.

Our first example will be *PON1* gene rs662
https://www.ncbi.nlm.nih.gov/pubmed/
Free radicals

PON1 rs662 CT/CC THEN tomato >5 g/kg or lycopene >0.5 mg/kg

rs662 is also known as Q192R

Br J Nutr. 2005 Mar;93(3):291-7.
Paraoxonase 1 Q192R (PON1-192) polymorphism is associated with reduced lipid peroxidation in healthy young men on a low-carotenoid diet supplemented with tomato juice.

Bub A1, Barth SW, Watzl B, Briviba K, Rechkemmer G.

Abstract
The HDL-bound enzyme paraoxonase (PON) protects LDL from oxidation and may therefore attenuate the development of atherosclerosis. We examined the effect of tomato and carrot juice consumption on PON1 activity and lipid peroxidation in healthy young volunteers with different PON1-192 genotypes (Q/R substitution at position 192). In this randomized cross-over study twenty-two healthy, non-smoking men on a low-carotenoid diet received 330 ml/d tomato juice (37.0 mg lycopene, 1.6 mg beta-carotene) or carrot juice (27.1 mg beta-carotene, 13.1 mg alpha-carotene) for 2 weeks. Intervention periods were preceded by 2-week low-carotenoid intake. We determined the PON1-192 genotype by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) and measured ex vivo LDL oxidation (lag time), plasma malondialdehyde and PON1 activity at the beginning and end of each intervention period. At baseline, lag time was higher (P<0.05) in QQ (111 (sd 9) min) than in QR/RR subjects (101 (sd 8) min). Neither tomato nor carrot juice consumption had significant effects on PON1 activity. However, tomato juice consumption reduced (P<0.05) plasma malondialdehyde in QR/RR (Delta: -0.073 (sd 0.11) micromol/l) as compared to QQ subjects (Delta:+0.047 (sd 0.13) micromol/l). Carrot juice had no significant effect on malondialdehyde irrespective of the PON1-192 genotype. Male volunteers with the QR/RR genotype showed an increased lipid peroxidation at baseline. Although tomato and carrot juice fail to affect PON1 activity, tomato juice intake reduced lipid peroxidation in healthy volunteers carrying the R-allele of the PON1-192 genotype and could thus contribute to CVD risk reduction in these individuals.

Exp Biol Med (Maywood). 2016 Mar 27. pii: 1535370216641786. [Epub ahead of print]
The common variant Q192R at the paraoxonase 1 (PON1) gene and its activity are responsible for a portion of the altered antioxidant status in type 2 diabetes.

Zargari M1, Sharafeddin F1, Mahrooz A2, Alizadeh A3, Masoumi P1.

Abstract
In this study, we investigated the effects of paraoxonase 1 (PON1) activities and the variant PON1-Q192R on the ferric reducing ability of plasma (FRAP) and total thiol. In addition, we examined the distribution of genotypes of this variant and the relationship of the genotypes with age in patients with type 2 diabetes (T2D). A total of 115 patients with T2D were enrolled in this study. Paraoxonase activity (PON-para) and arylesterase activity (PON-aryl) were determined using spectrophotometric assays. The distribution of the Q192R genotypes was determined by the double substrate method. The antioxidant status was evaluated by determining FRAP and total thiol. The frequencies of Q and R allozyme were 0.78 and 0.22, respectively. The multivariate analysis identified a significant association between the variables PON1-Q192R (Wilks' $\lambda = 0.85, P = 0.002$) and PON-aryl (Wilks' $\lambda = 0.896, P = 0.017$), with FRAP and total thiol. The significant difference observed for PON1-Q192R and PON-aryl is primarily due to the changes in FRAP levels ($\eta 2 = 0.127, P = 0.002$ for PON1-Q192R; $\eta 2 = 0.083, P = 0.011$ for PON-aryl). The interaction PON1-Q192R-PON-aryl increased the effect sizes from 8 to 19% for FRAP. Only in R-carrying genotypes, there were significant correlations between both PON-para/HDL ($r = -0.574, P < 0.001$) and PON-aryl/HDL ($r = -0.577, P < 0.001$) with age. Our data suggest that the variant PON1-Q192R and PON1 activity, particularly PON-aryl, influenced the antioxidant status in T2D. The interaction of this variant and PON1 activity increased the effect size on the antioxidant capacity. Moreover, the presence of the R allozyme may potentiate the effects of age on susceptibility to cardiovascular diseases in T2D.

Endokrynol Pol. 2016 Feb 17. doi: 10.5603/EP.a2016.0027. [Epub ahead of print]

Paraoxonase 1 polymorphisms (L55M and Q192R) as a genetic marker of diabetic nephropathy in youth with type 1 diabetes.

Fekih O1, Triki S, Rejeb J, Neffati F, Douki W, Ommezzine A, Chouchane S, Guediche MN, Bouslama A, Najjar MF.

Abstract

INTRODUCTION:

Paraoxonase 1 (PON1) polymorphisms have been largely involved in diabetes complications. The aim of the study is to evaluate effects of PON1 polymorphisms (L55M and Q192R) on Diabetic nephropathy (DN).

MATERIAL AND METHODS:

The study involved 116 children and adolescents with Type 1 diabetes (T1D) and 91 healthy subjects. Albumin excretion rate (AER) was determined by immunoturbidimetry. PON1 activity was measured by a spectrophotometric method and genotyping of PON1 gene was assessed by multiplex PCR followed by RFLP.

RESULTS:

PON1 activity was inversely correlated to AER (r = - 0.245, p = 0.008). A significant decrease (p = 0.037) in PON1 activity has been shown between patients with nephropathy and those without (162 (57 - 618) Vs 316 (37 - 788) IU/L, respectively). The distribution of AER was, for L55M polymorphism MM > LM > LL (p = 0.002) and for Q192R polymorphism QQ > QR > RR (p < 0.001). The opposite distribution was noted for PON 1 activity (p < 0.001). LMQQ and MMQQ haplotypes seem to increase AER (p = 0.004, p = 0.003, respectively) and to reduce PON1 activity (p = 0.011, p = 0.052, respectively) in youth with T1D. However LLRR haplotype seems to have the opposite effect.

CONCLUSION:

This study demonstrated that PON1 polymorphisms L55M and Q192R seem to be a genetic marker involved in the development of DN in T1D.

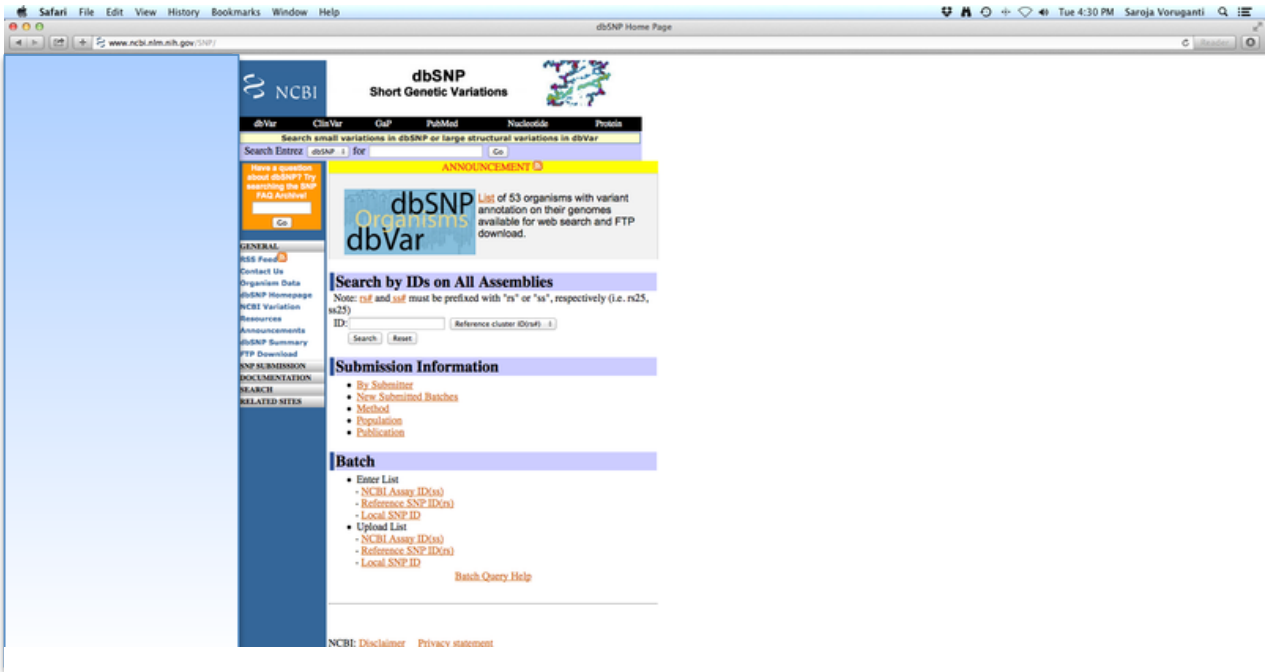
Workshop Task

dbSNP – NCBI database of genetic variation

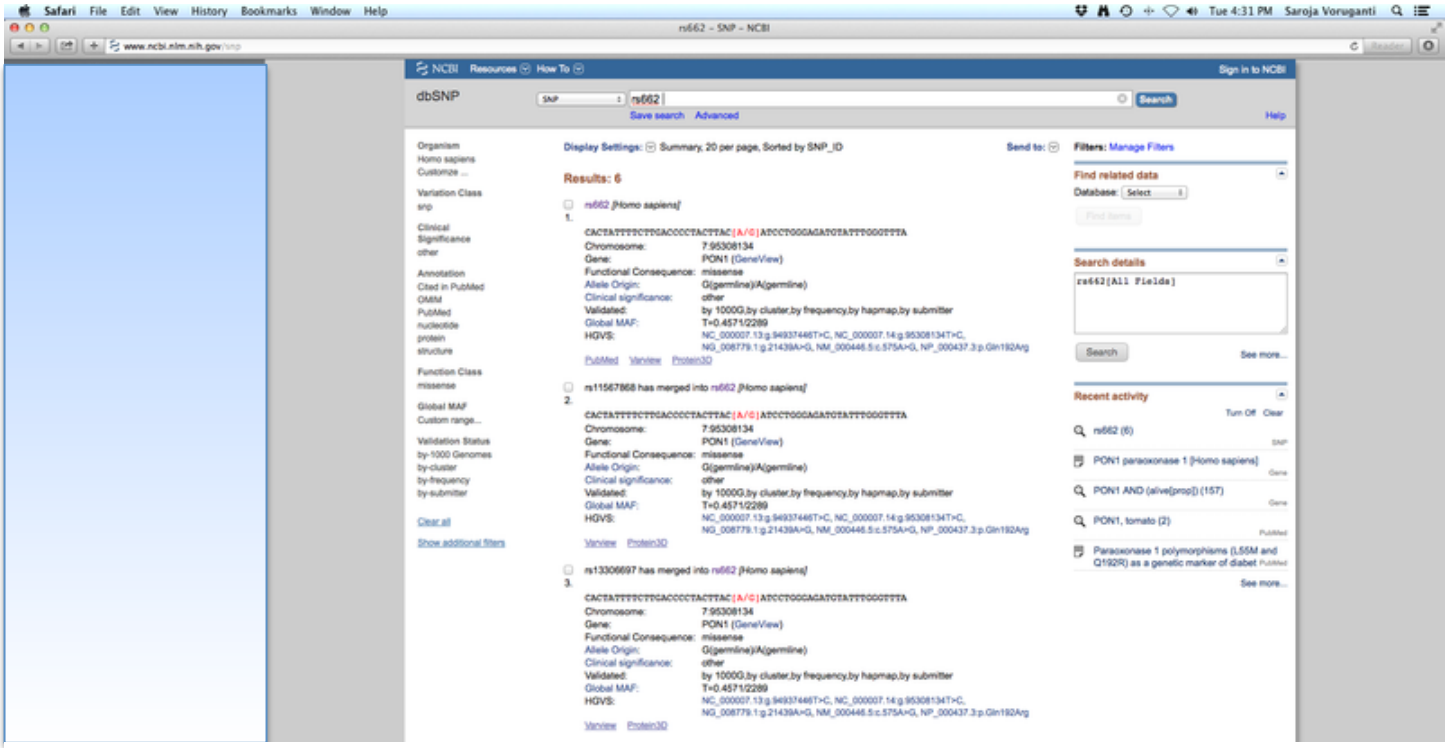
1. dbSNP page

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

http://www.ncbi.nlm.nih.gov/books/NBK44455/

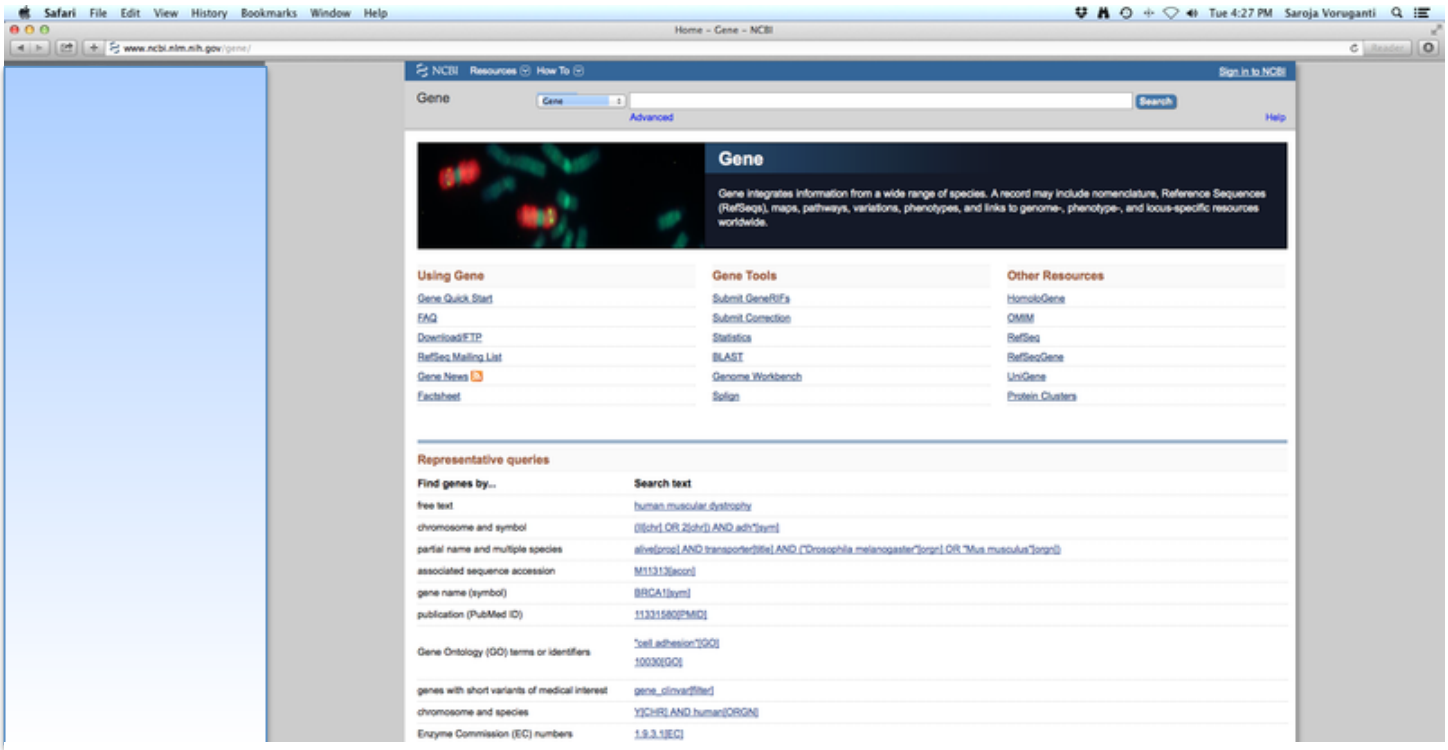


2. Enter rs662 into the search panel

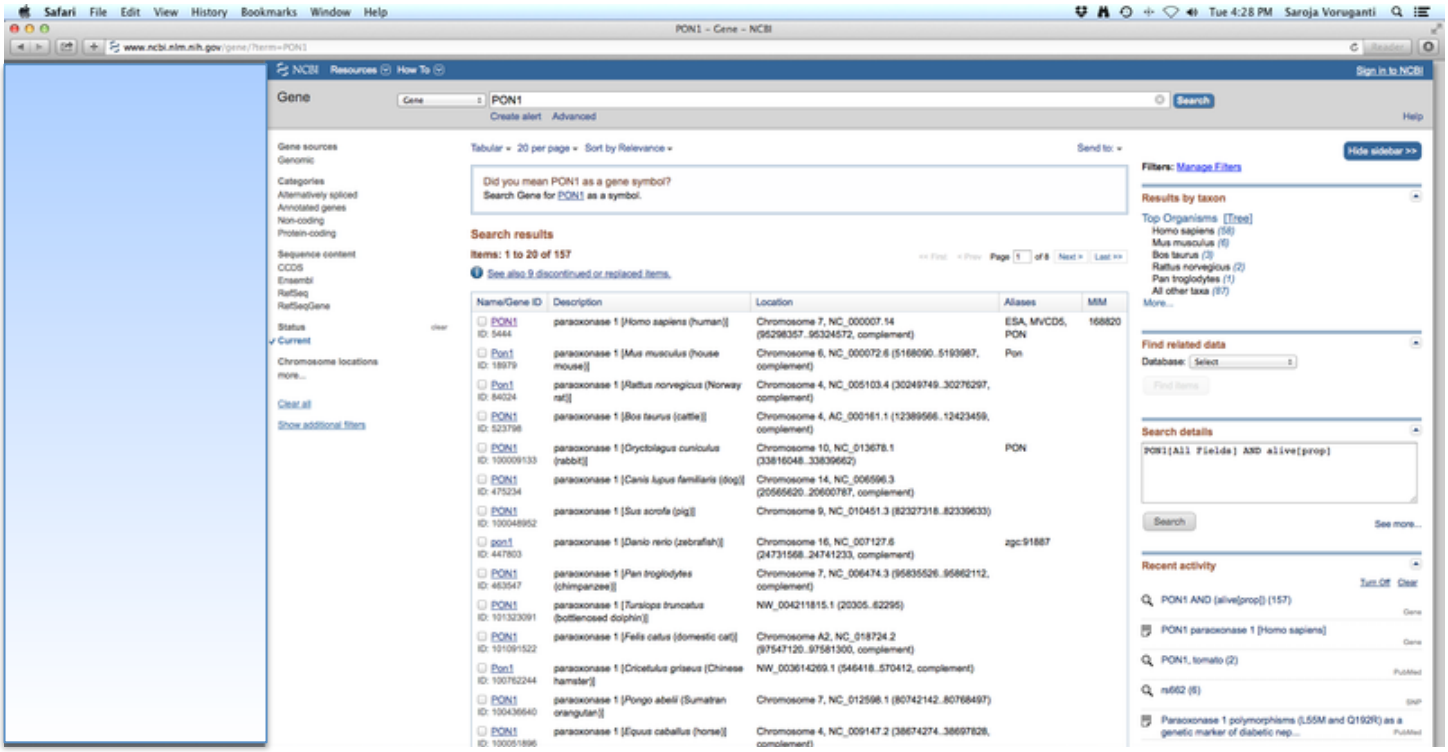


3. Click on rs662
4. Information about rs662 provided.

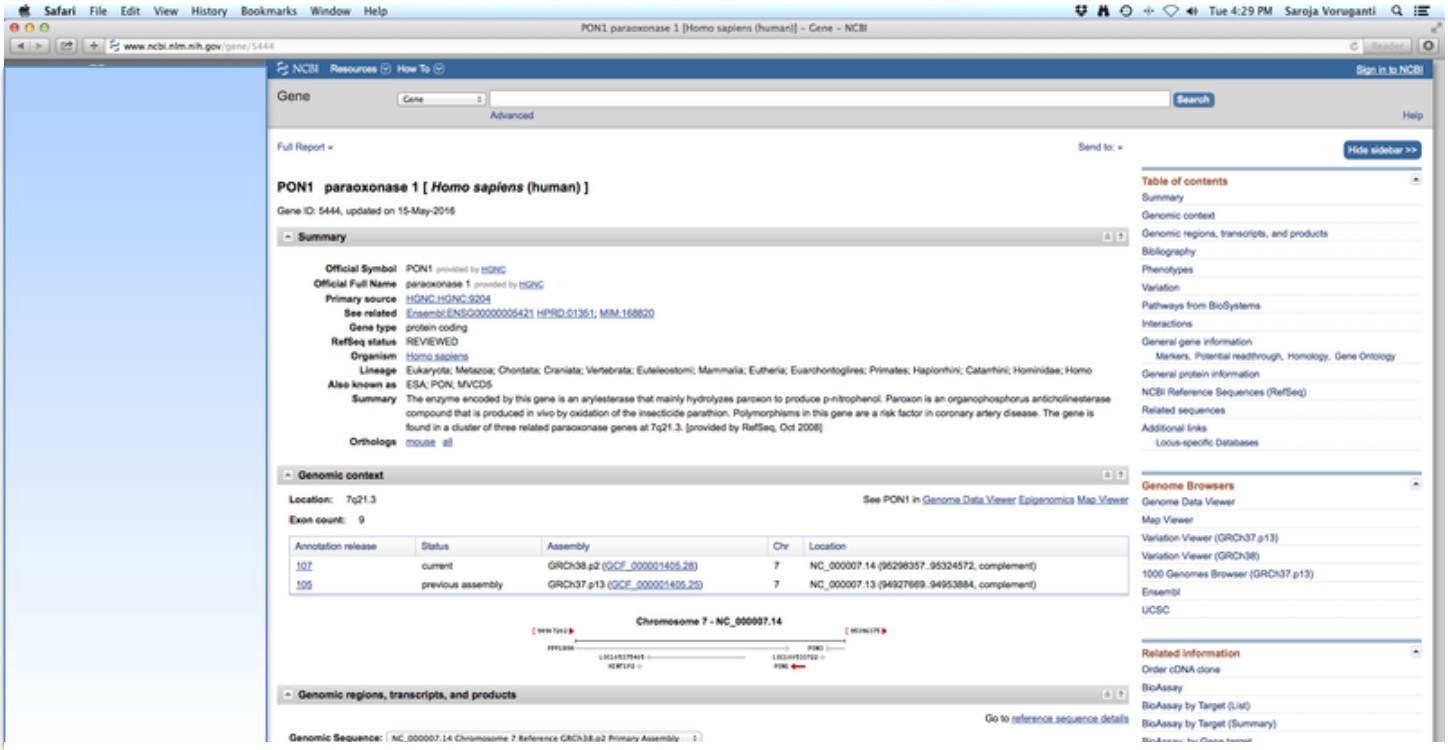
5.For information on the gene *PON1*
http://www.ncbi.nlm.nih.gov/gene/ - A portal to *gene*-specific content based on *NCBI's* RefSeq project, information from model organism databases, and links to other resources.



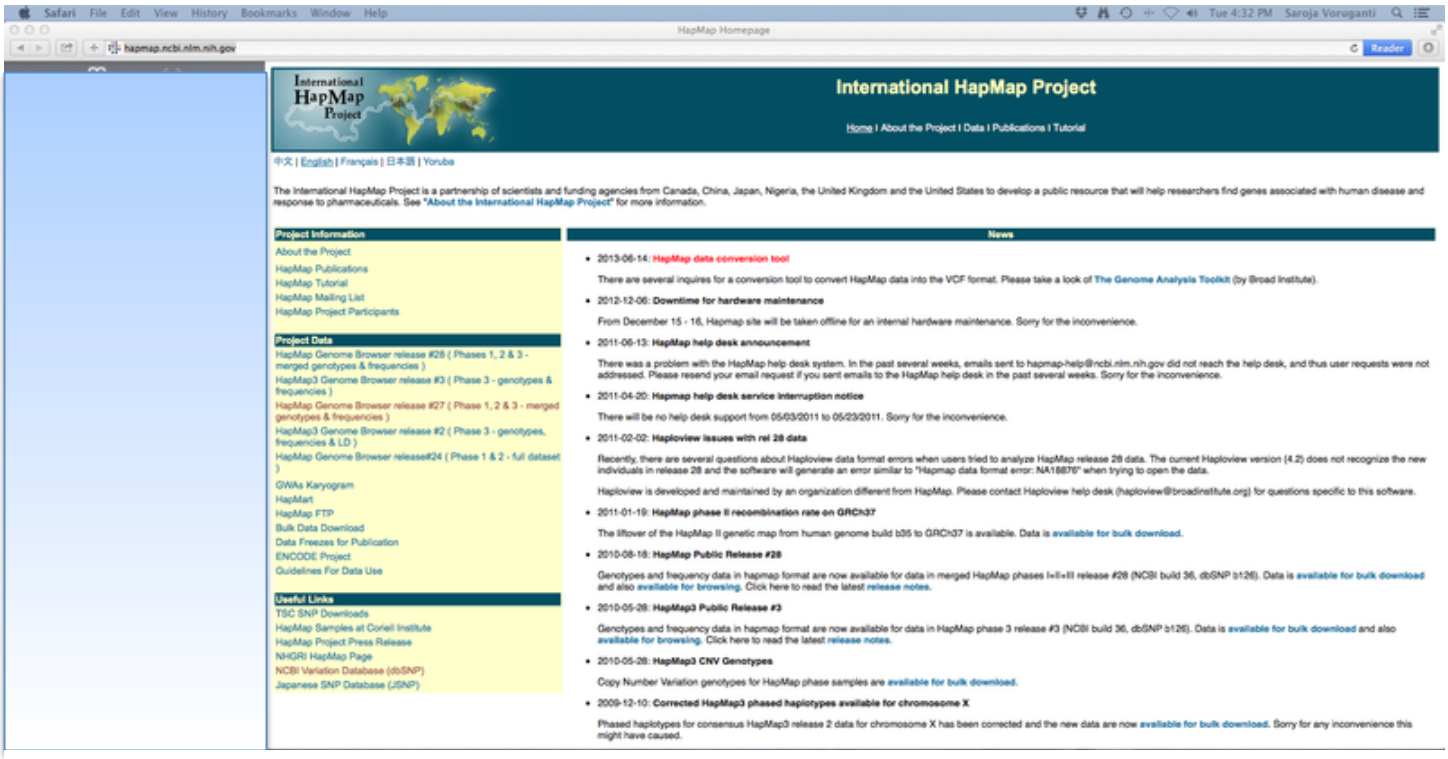
6. Enter the word PON1 in search



7. Click on Paraoxanase 1 (Homo Sapiens (human))



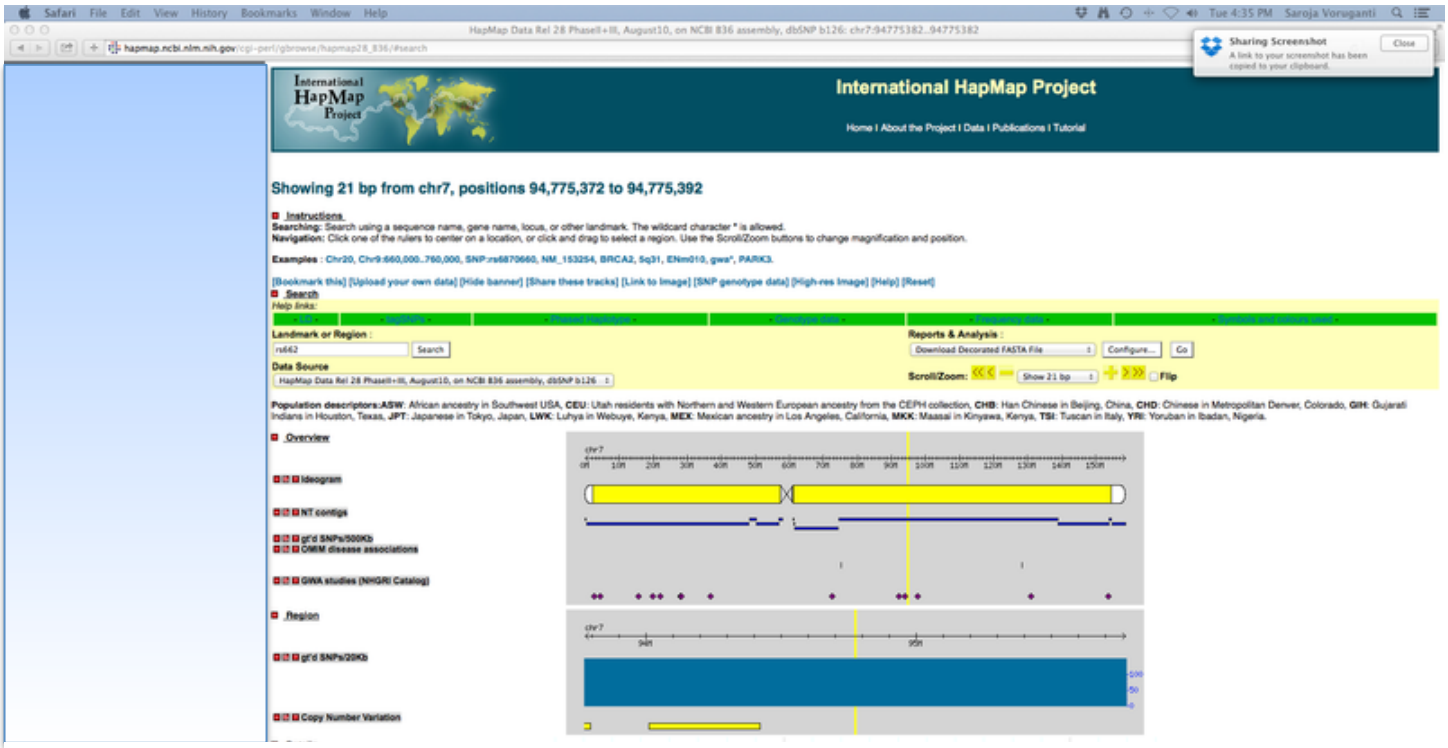
8. HapMap http://hapmap.ncbi.nlm.nih.gov - The HapMap is a catalog of common genetic variants that occur in human beings. It describes what these variants are, where they occur in our DNA, and how they are distributed among people within populations and among populations in different parts of the world.



9. Click on “HapMap Genome Browser release #28 (Phases 1,2 &3-merged genotypes & frequencies)

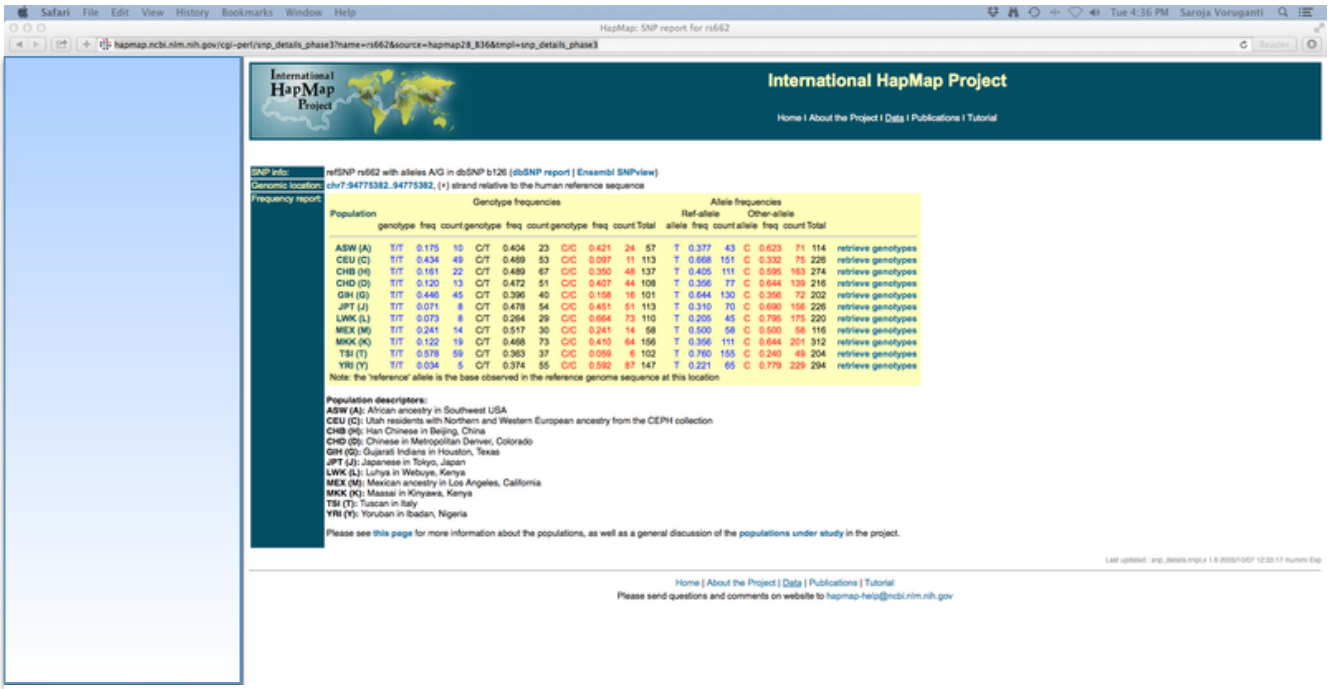
http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/

10. enter rs662 in the search panel

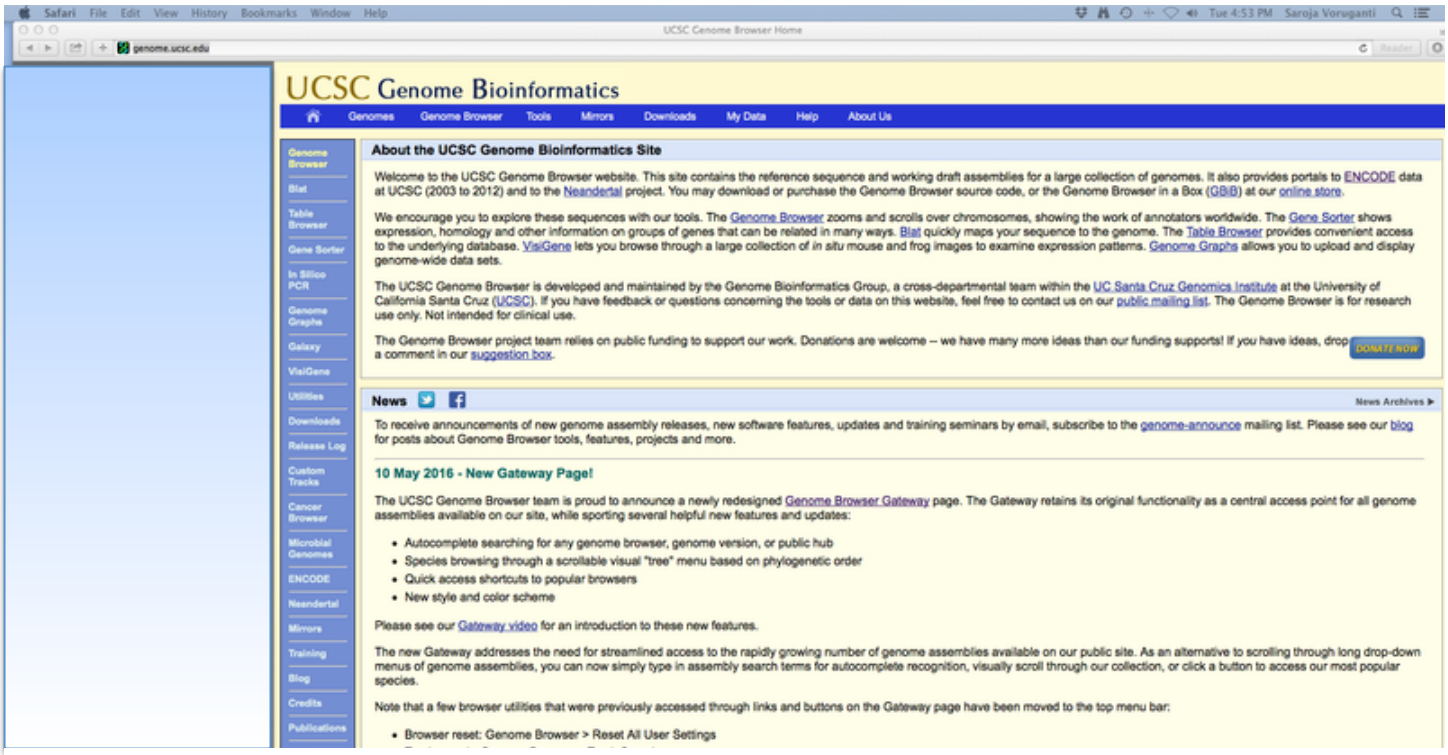


11. click on rs662 for allele and genotype frequencies

http://hapmap.ncbi.nlm.nih.gov/cgi-perl/snp_details_phase3?name=rs662&source=hapmap28_B36&tmpl=snp_details_phase3



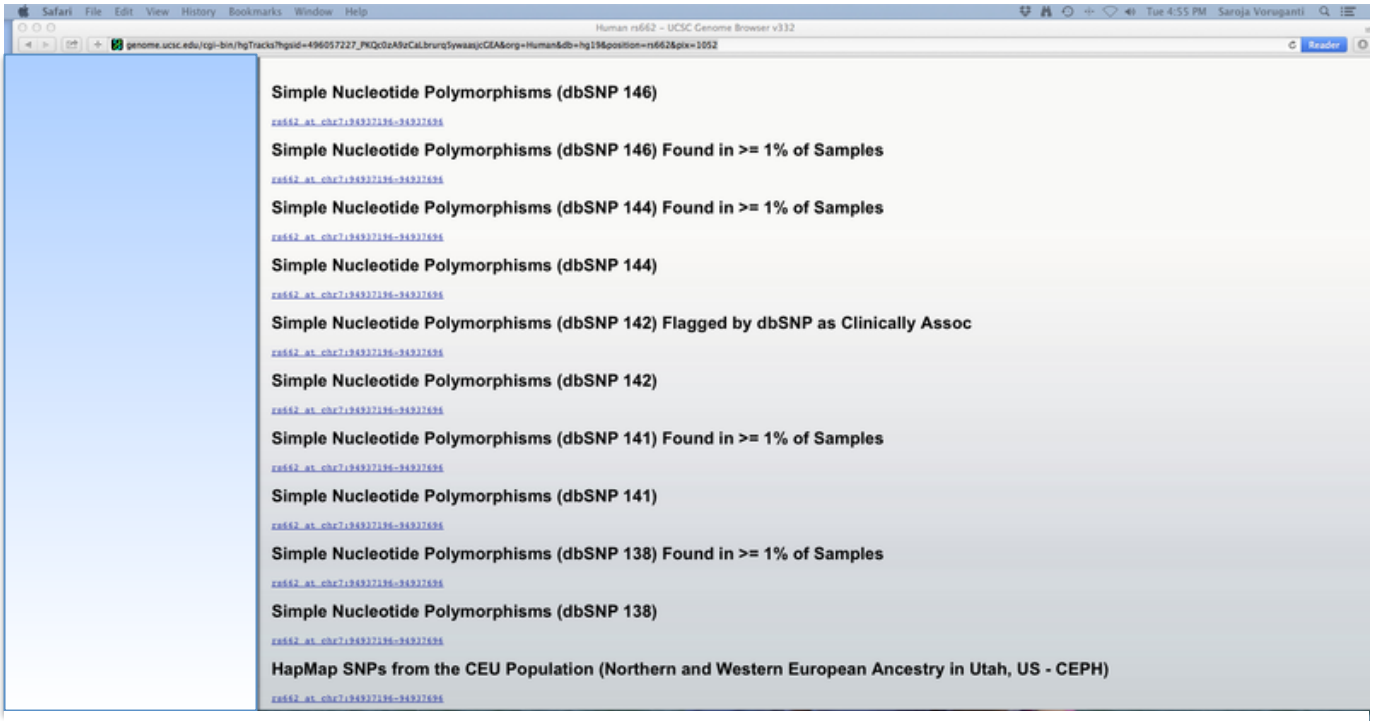
12. Another website to provide more information related to the SNP and the gene
UCSC Genome Browser - The UCSC Genome Browser is an interactive website offering access to genome sequence data from a variety of vertebrate and invertebrate species and major model organisms, integrated with a large collection of aligned annotations
http://genome.ucsc.edu



13. Click on Genome Browser on top left side

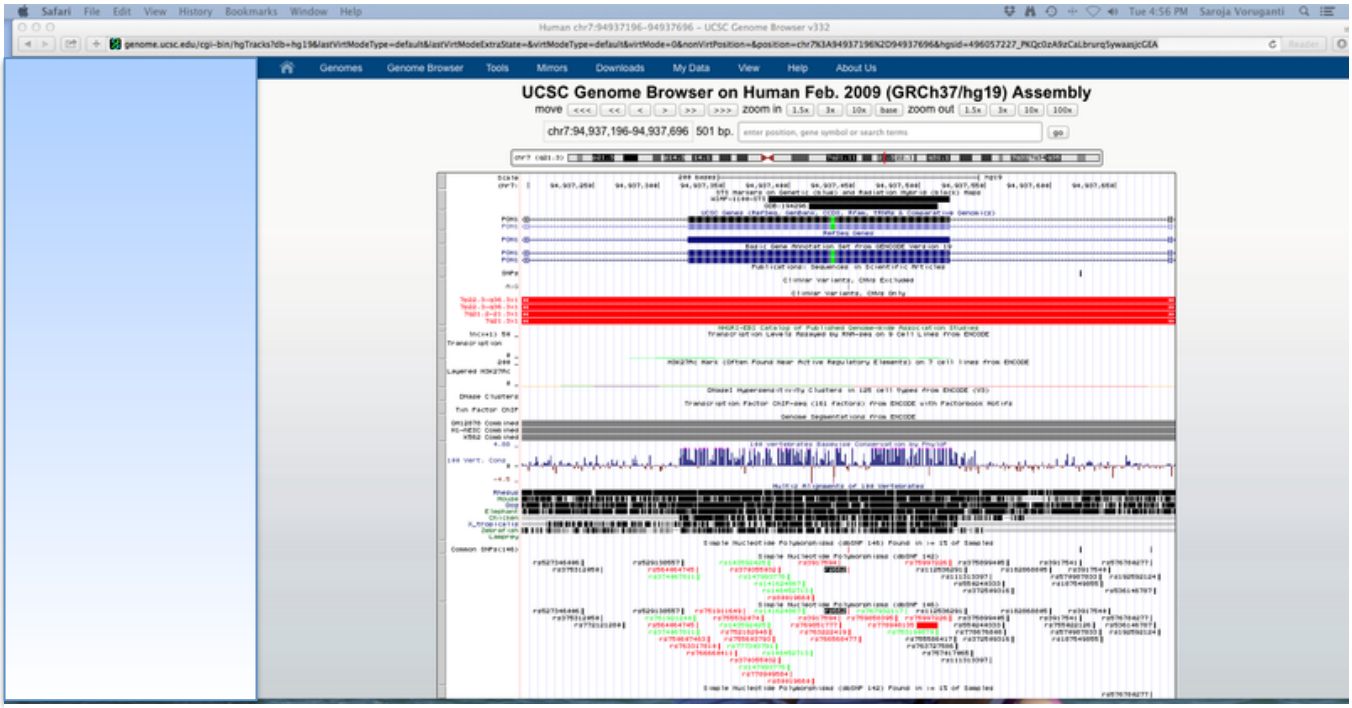
http://genome.ucsc.edu/cgi-bin/hgGateway

http://genome.ucsc.edu/cgi-bin/hgTracks?hgsid=496057227_PKQc0zA9zCaLbrurq5ywaasjcGEA&org=Human&db=hg19&position=rs662&pix=1052

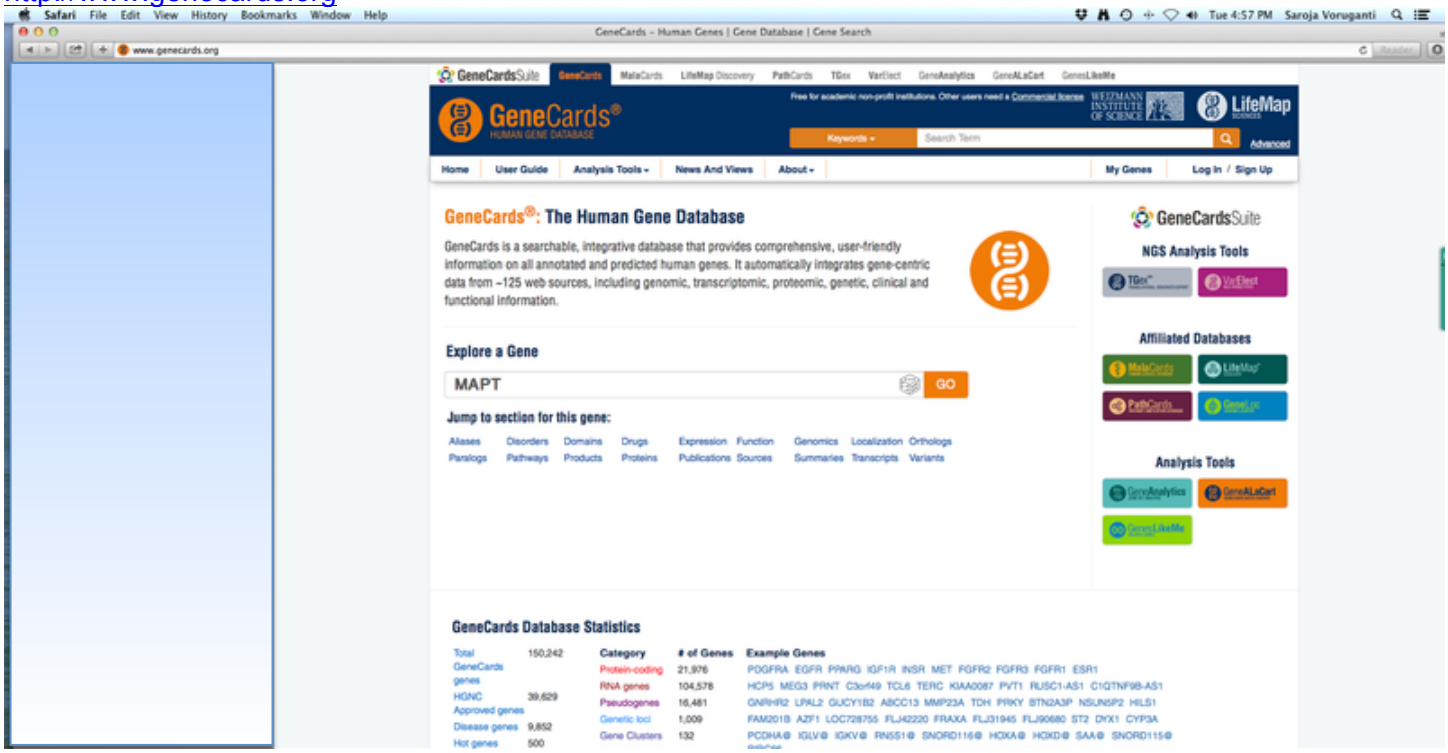


Click on the first rs662

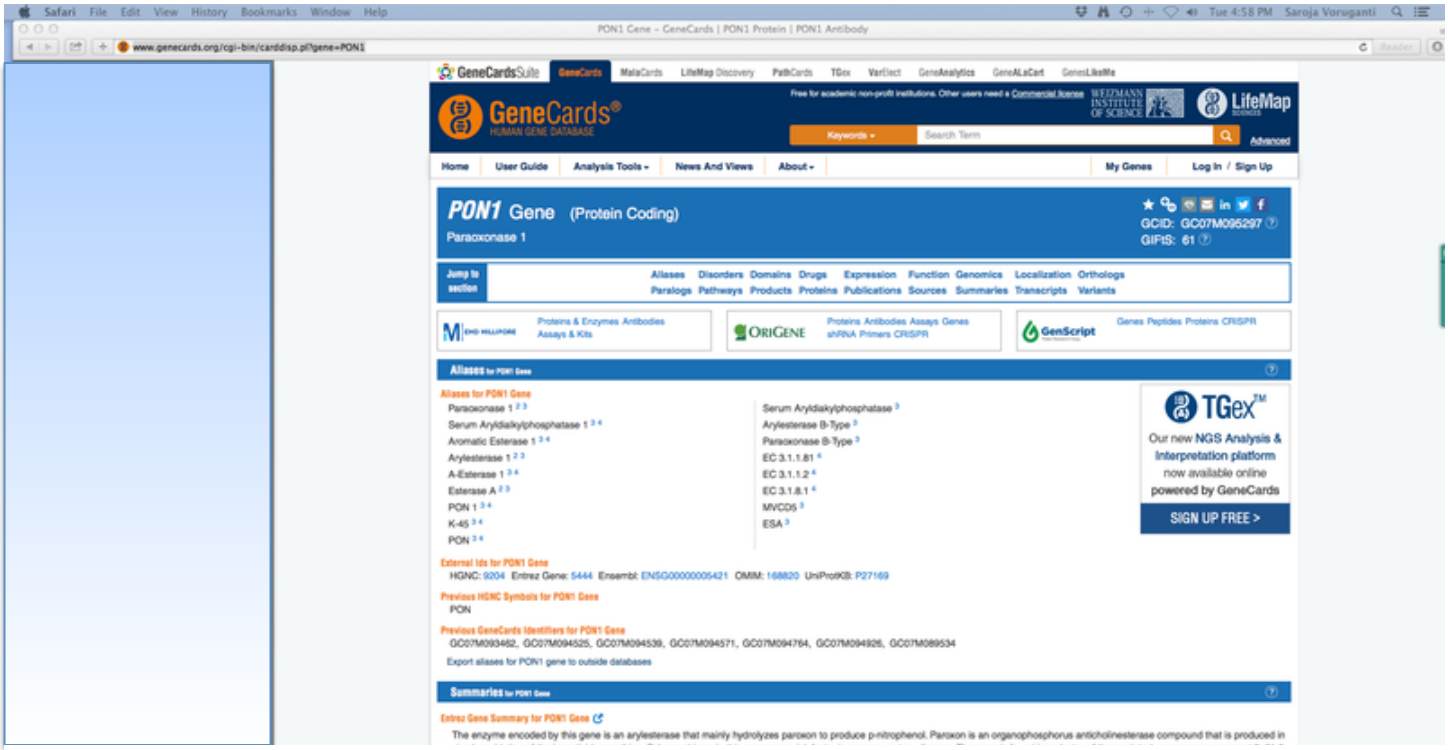
http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr7%3A94937196%2D94937696&hgsid=496057227_PKQc0zA9zCaLbrurq5ywaasjcGEA

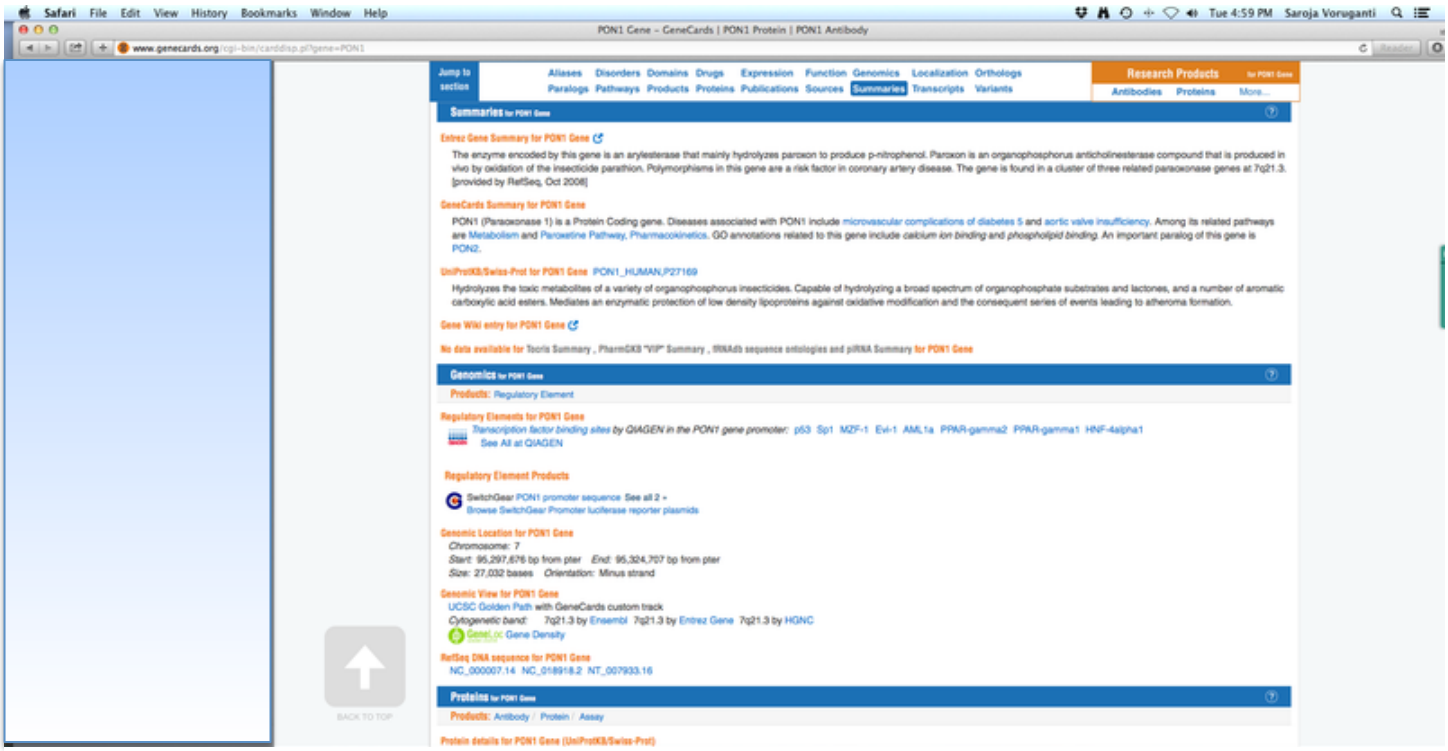


Another useful website is Gene Cards- *GeneCards* is a searchable, integrated, database of human genes that provides concise genomic related information, on all known and predicted human genes.
http://www.genecards.org



Enter PON1
http://www.genecards.org/cgi-bin/carddisp.pl?gene=PON1

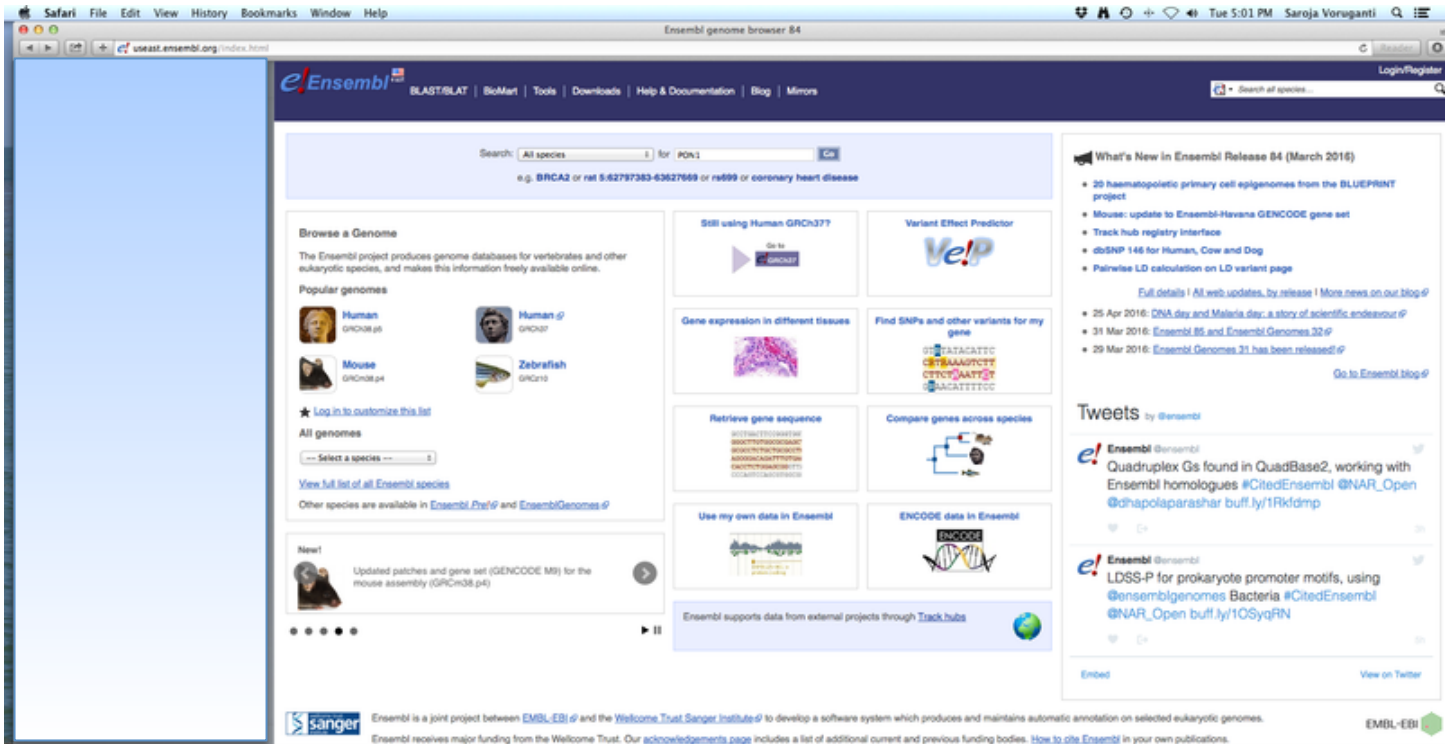


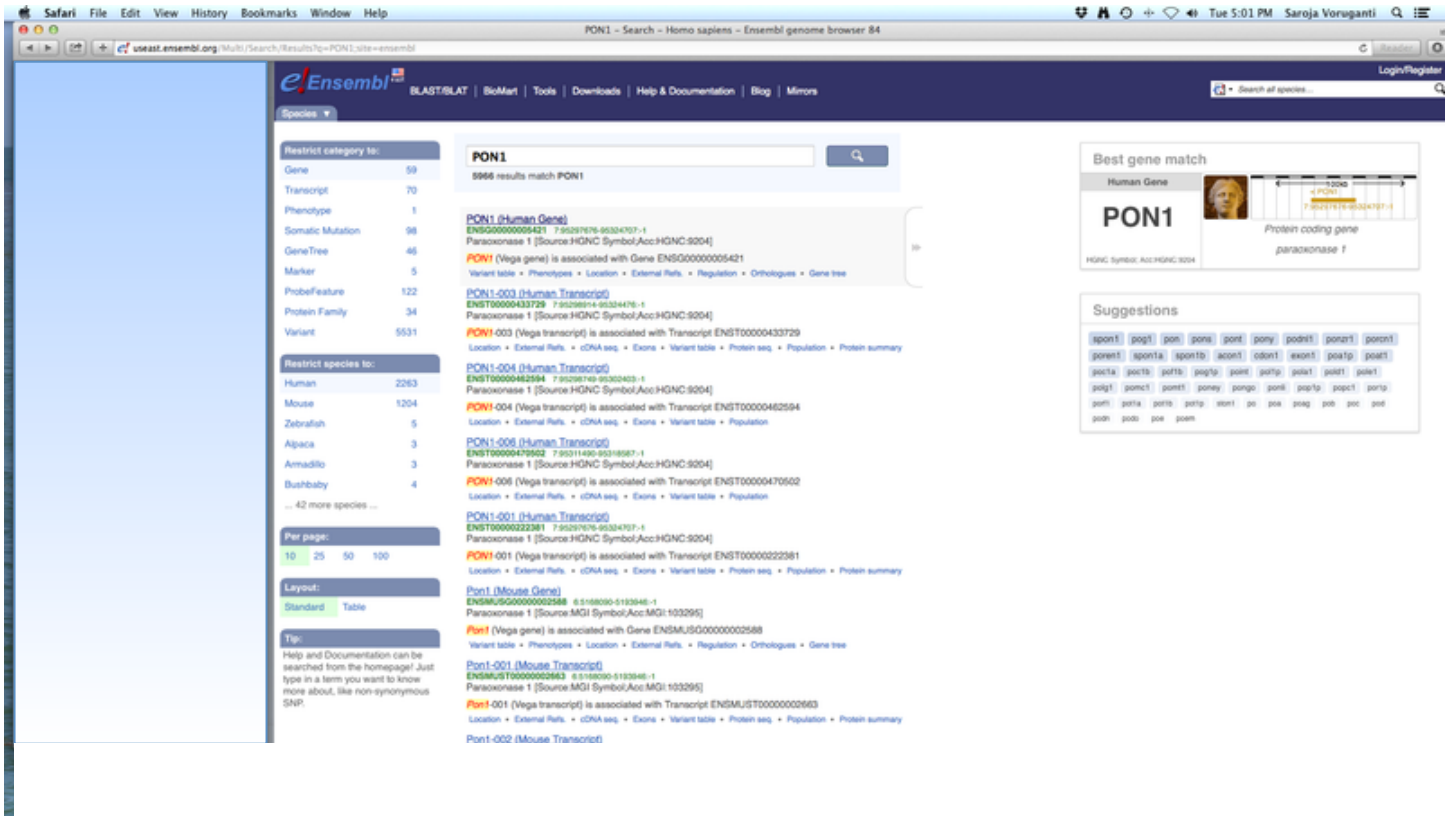


One more “Ensembl” http://useast.ensembl.org/index.html - Ensembl's aim is to provide a centralized resource for geneticists, molecular biologists and other researchers studying the genomes of our own species and other

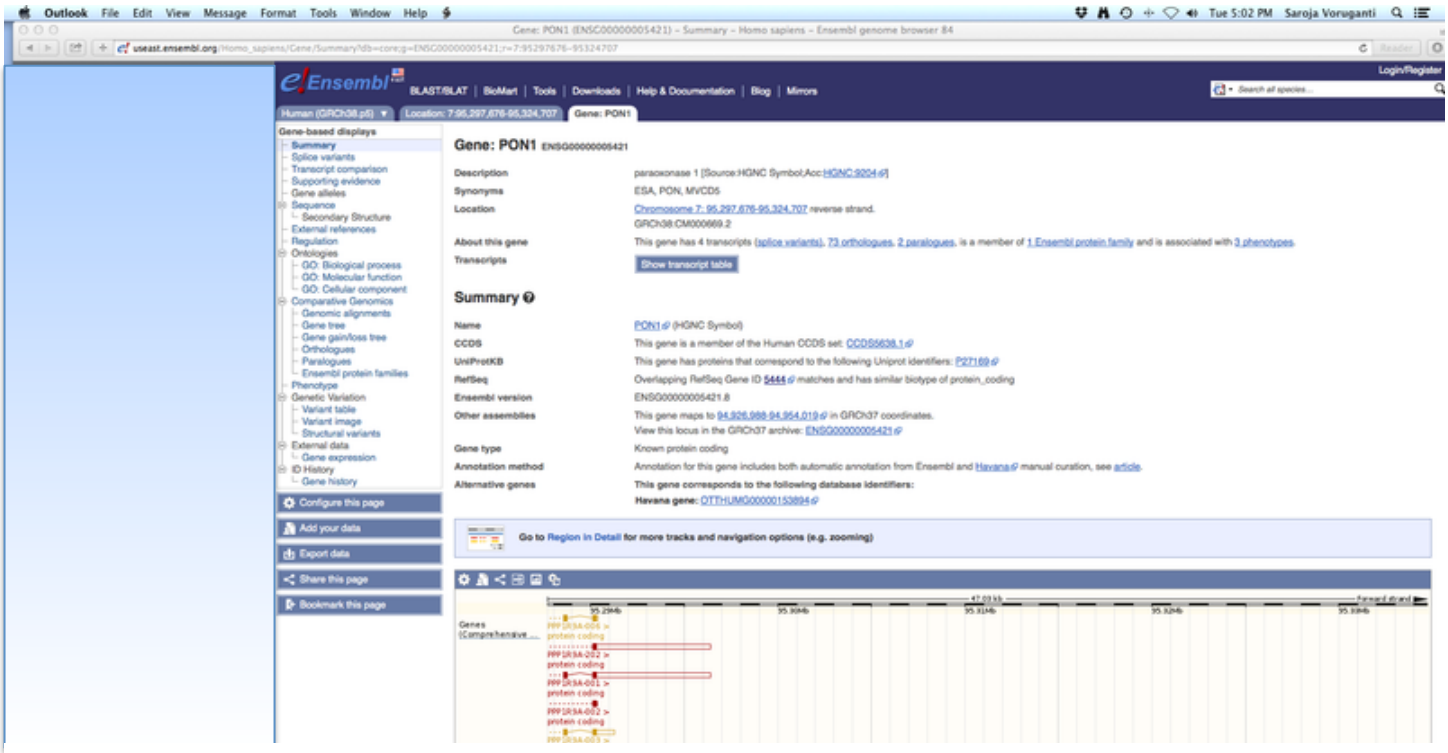
vertebrates and model organisms. Ensembl is one of several well known genome browsers for the retrieval of genomic information.

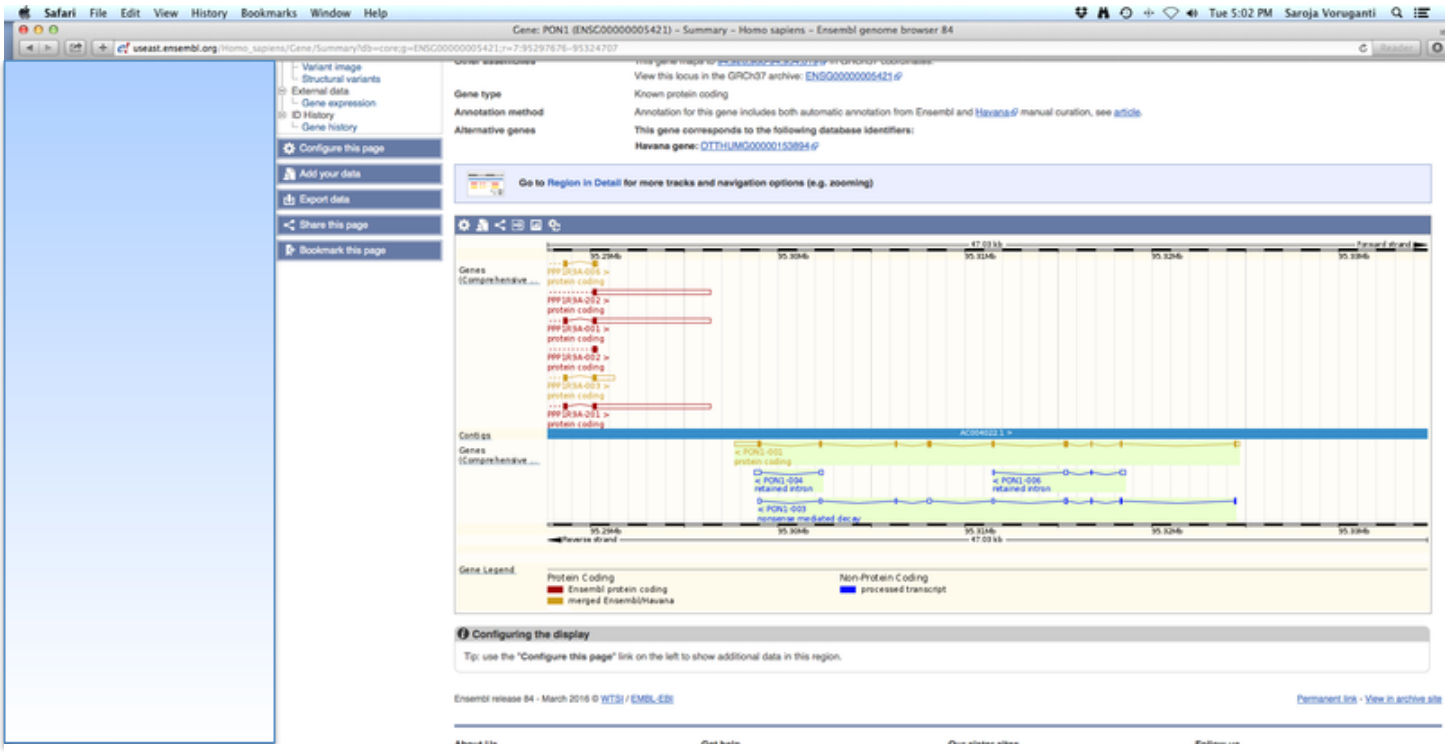
Enter PON1 in the search panel





Click on *PON1* Human gene

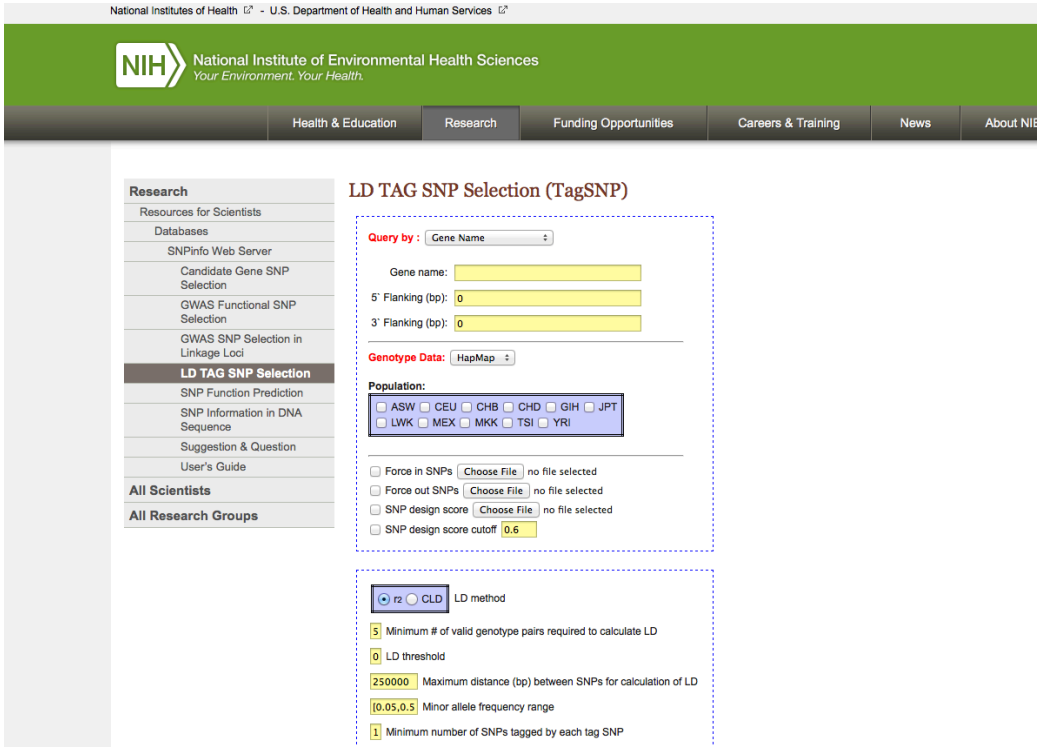


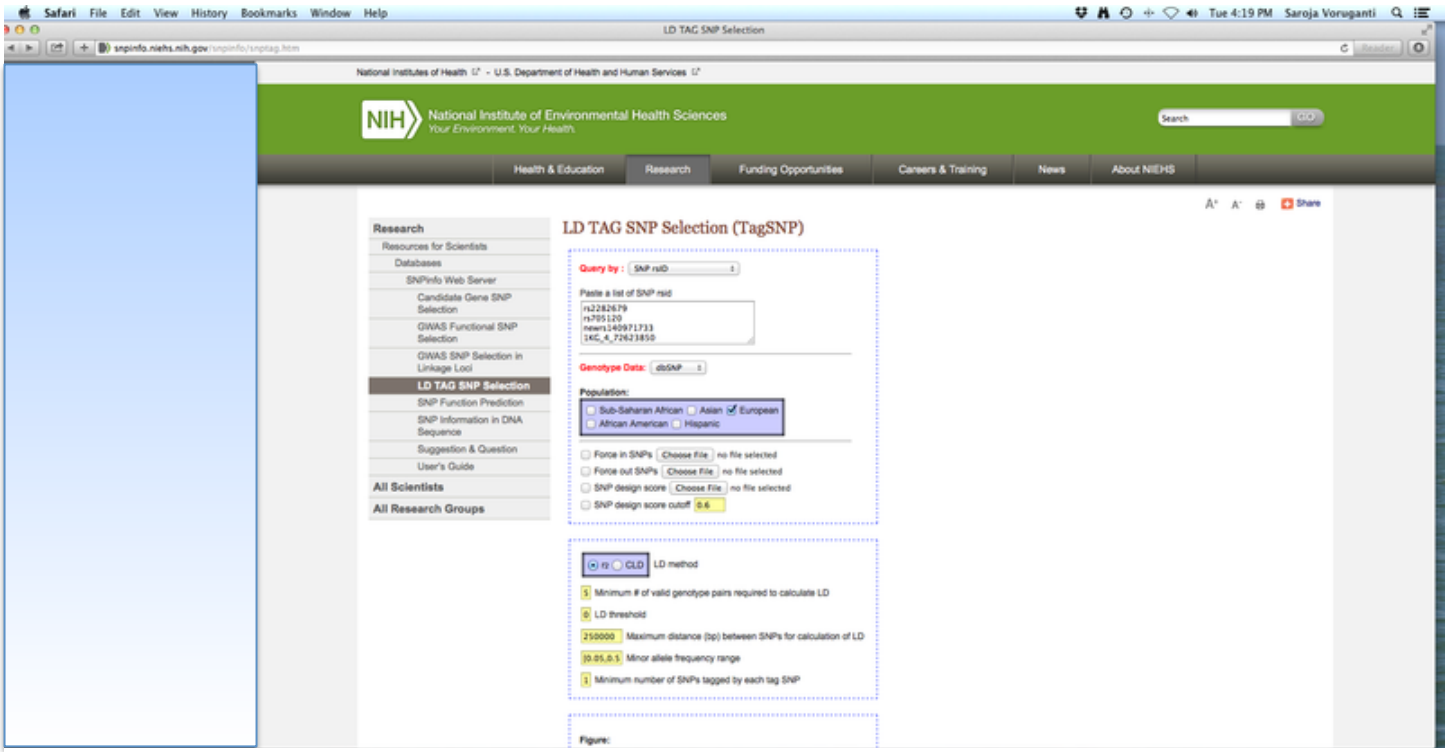


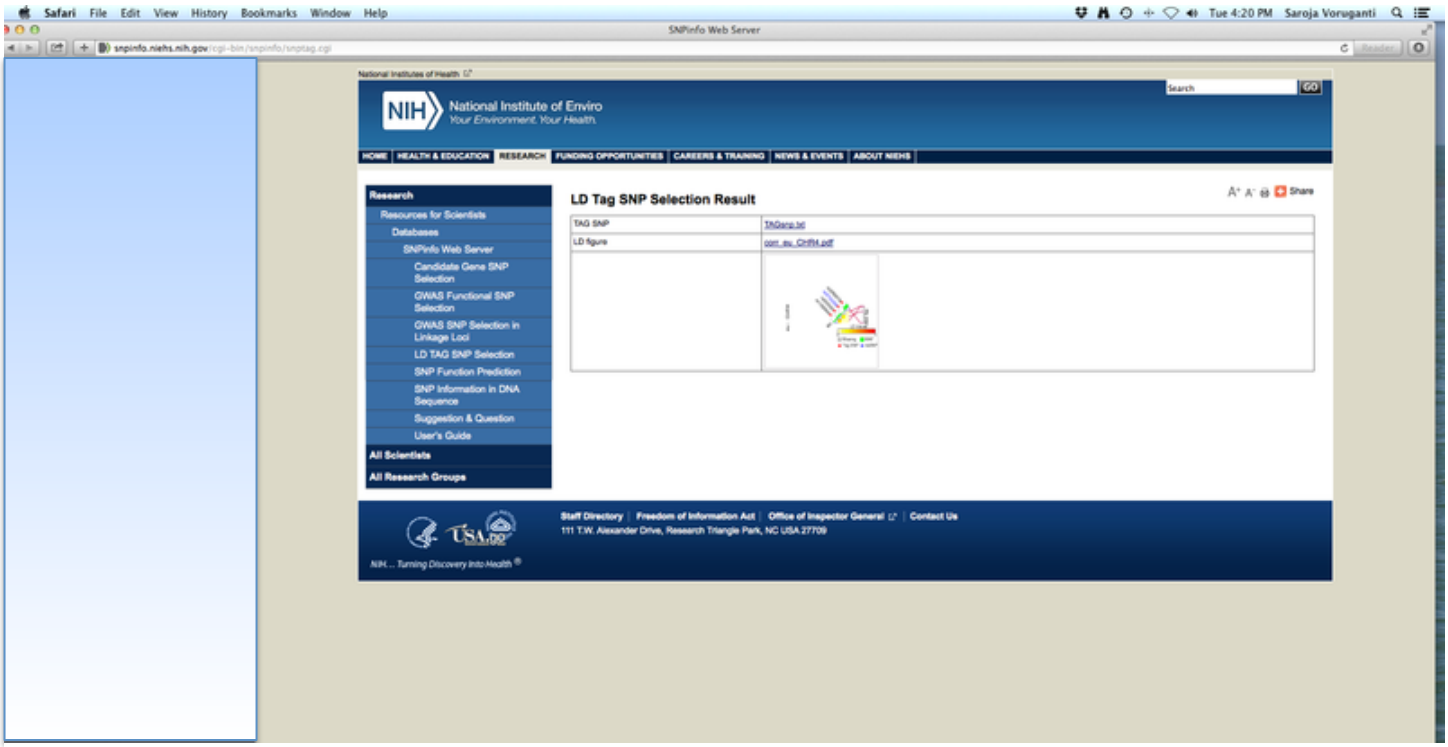
For information on LD and Tag SNPs
https://snpinfo.niehs.nih.gov/snpinfo/snptag.htm - SNPinfo is designed to comprehensively utilize computational (predicted functional SNPs that have differential affect between reference allele and alternative allele), experimental and epidemiological information together with recent genome wide association study

(GWAS) results and linkage disequilibrium (LD) information to prioritize SNPs for further genetic mapping studies

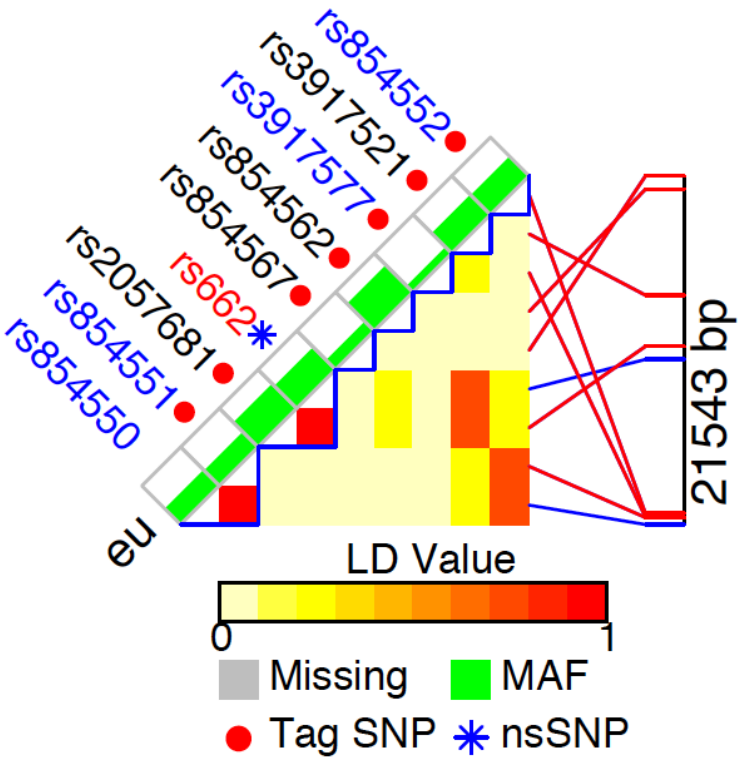
Select SNPrsID if you are entering SNP ID. I selected SNP rsID
Select either dbSNP or HapMap. I selected dbSNP

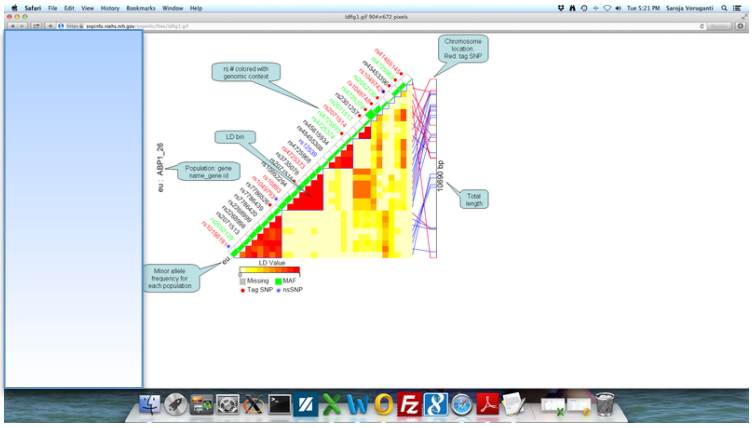






Click on the pdf link and you will get the LD plot for *PON1* SNPs



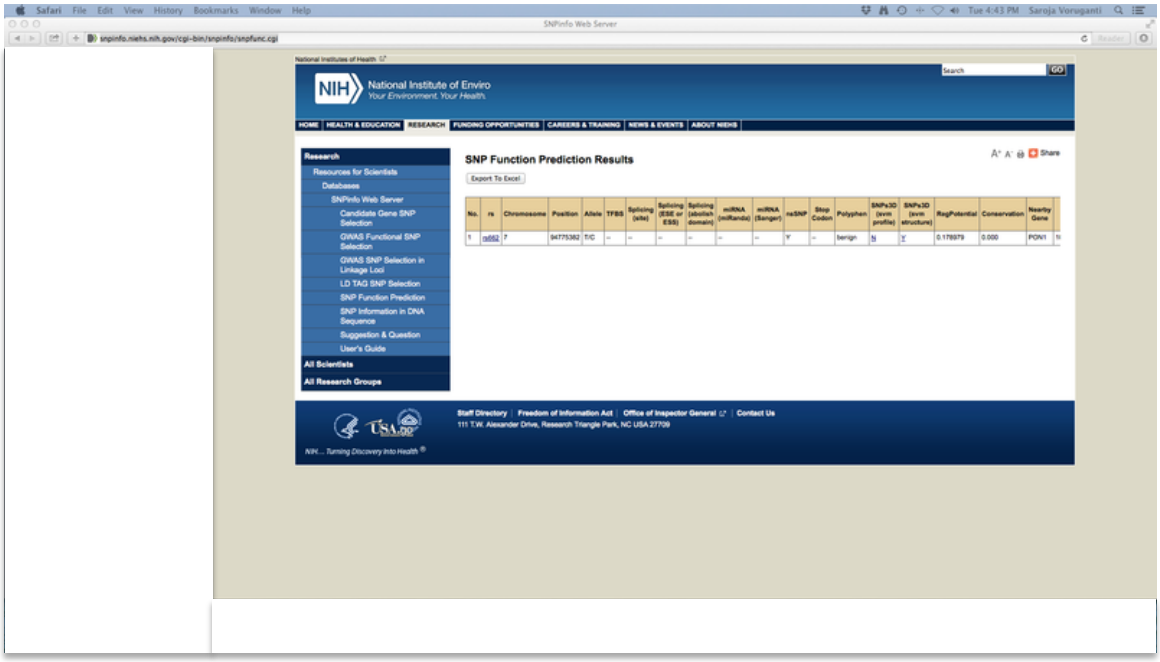


10. For functional significance of rs662 click on SNP function prediction

https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm

enter rs662 in “paste a list of SNP rsid panel” and select a population

https://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi



FuncPred: Functional SNP Prediction

SNPs may have functional effects including: transcriptional regulation by affecting transcription factor binding sites (TFBS) activity; premature termination of amino-acid sequence (stop codons); changing of splicing pattern or efficiency by disrupting splice site, exonic splicing enhancers (ESE) or silencers (ESS); alteration of protein structures or properties by changing single amino acids or changing the frame of the protein-coding region; regulation of protein translation by affecting microRNA (miRNA) binding sites activity. Many software tools or web servers can be used to predict TFBSs, ESE site, ESS site or miRNA binding sites. However, although SNPs may be located in such binding sites, the alternative alleles of a SNP may not necesarily have different activities. We designed several pipelines to predict SNPs that may affect biological function with alternative alleles.

Include SNPs with LD> ... in population

Display SNPs that are in high LD (in population selected) with SNPs in the query SNP list.

nsSNP

SNPs in protein-coding regions that can cause amino acid change (non-synonymous coding SNPs, nsSNP).

Stop Codon

SNPs that may lead to premature termination of peptides (non-sense), which would disable the protein function.

Polyphen prediction Polyphen (http://genetics.bwh.harvard.edu/pph/) (Sunyaev, Ramensky et al. 2001) method predicted damaging nsSNPs.

SNPs3D prediction SNPs3D (http://www.snps3d.org/) (Yue, Melamud et al. 2006) method predicted damaging nsSNPs.

TFBS Prediction

If a non-coding SNP is located at a transcription factor-binding site (TFBS) of a gene, then it may affect the level, location, or timing of gene expression. We predicted such SNPs according to the procedure described in Xu and Taylor (submitted).

Refine TFBS Several studies (Elnitski et al 2003,2006; King et al, 2005) show that using both the predicted conserved TFBS together with the regulatory potential score(RP Score) (downloaded from UCSC genome bioinformatics web site(http://genome.ucsc.edu)) can result in more precise predictions, so we also provide this option on the web server.

Splicing regulation

SNPs that are located at 2 base pair of intron-exon junction region, exonic splicing enhancer (ESE), or exonic splicing silencer (ESS) may disrupt splicing activity and cause alternative splicing. We predict SNPs whose alternative alleles may affect splicing using the methods detailed in Xu and Taylor (submitted).

miRNA Binding Site Prediction

microRNAs (miRNA) are single-stranded RNA molecules of about 21-23 nucleotides in length, which can inhibit protein translation through binding to the end of a messenger RNA (mRNAs). We predicted SNPs that may affact miRNA binding site activity according to the methods described in Xu and Taylor (submitted).

Regulatory Potential Score

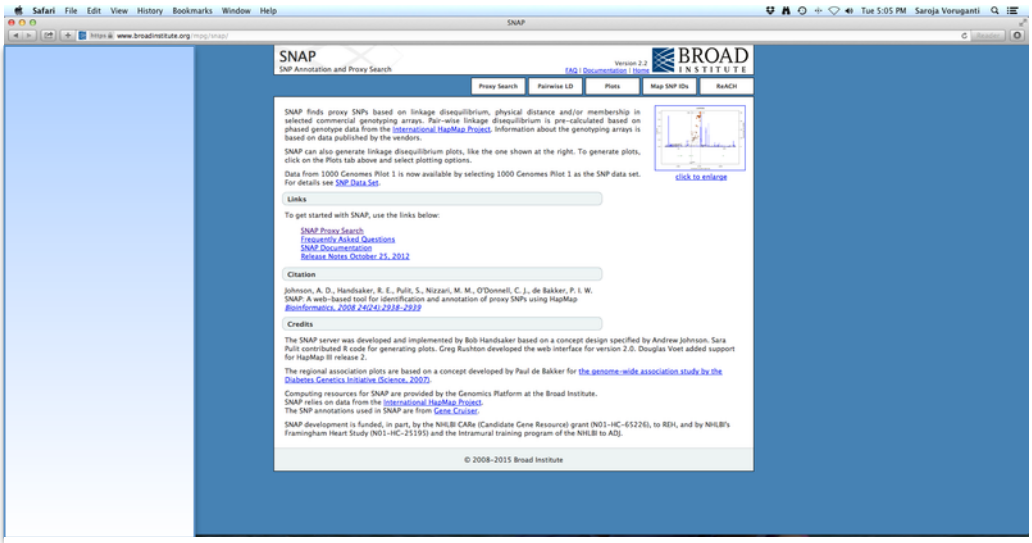
Regulatory potential score (ESPERR Regulatory Potential (7 Species)) downloaded from UCSC genome bioinformatics web site (http://genome.ucsc.edu/). Because SNPs in coding region are tend to have higher regulatory potential scores (see Figure, lines for all SNPs in human genome, non-coding SNPs and intron SNPs are overlapped), So we use this score only for SNPs that are outside of coding region in SNP selection.

Conservation Score

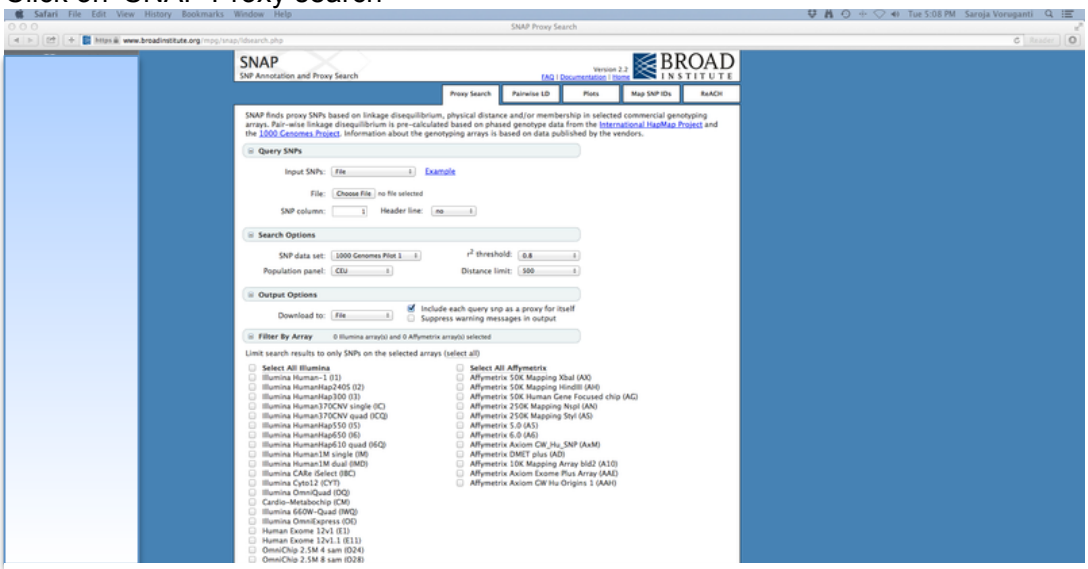
Vertebrate Multiz Alignment and Conservation score(17 Species) downloaded from UCSC genome bioinformatics web site (http://genome.ucsc.edu/). Because SNPs in coding region are tend to have higher conservation score (see Figure, lines for all SNPs in human genome, non-coding SNPs and intron snps are overlpped), so we use this score only for SNPs that are outside of the coding region of genes in SNP selection.

For more LD information

https://www.broadinstitute.org/mpg/snap/ - SNAP finds proxy SNPs based on linkage disequilibrium, physical distance and/or membership in selected commercial genotyping arrays. Pair-wise linkage disequilibrium is pre-calculated based on phased genotype data from the International HapMap Project. Information about the genotyping arrays is based on data published by the vendors. SNAP can also generate linkage disequilibrium plots, like the one shown at the right. To generate plots, click on the Plots tab above and select plotting options.



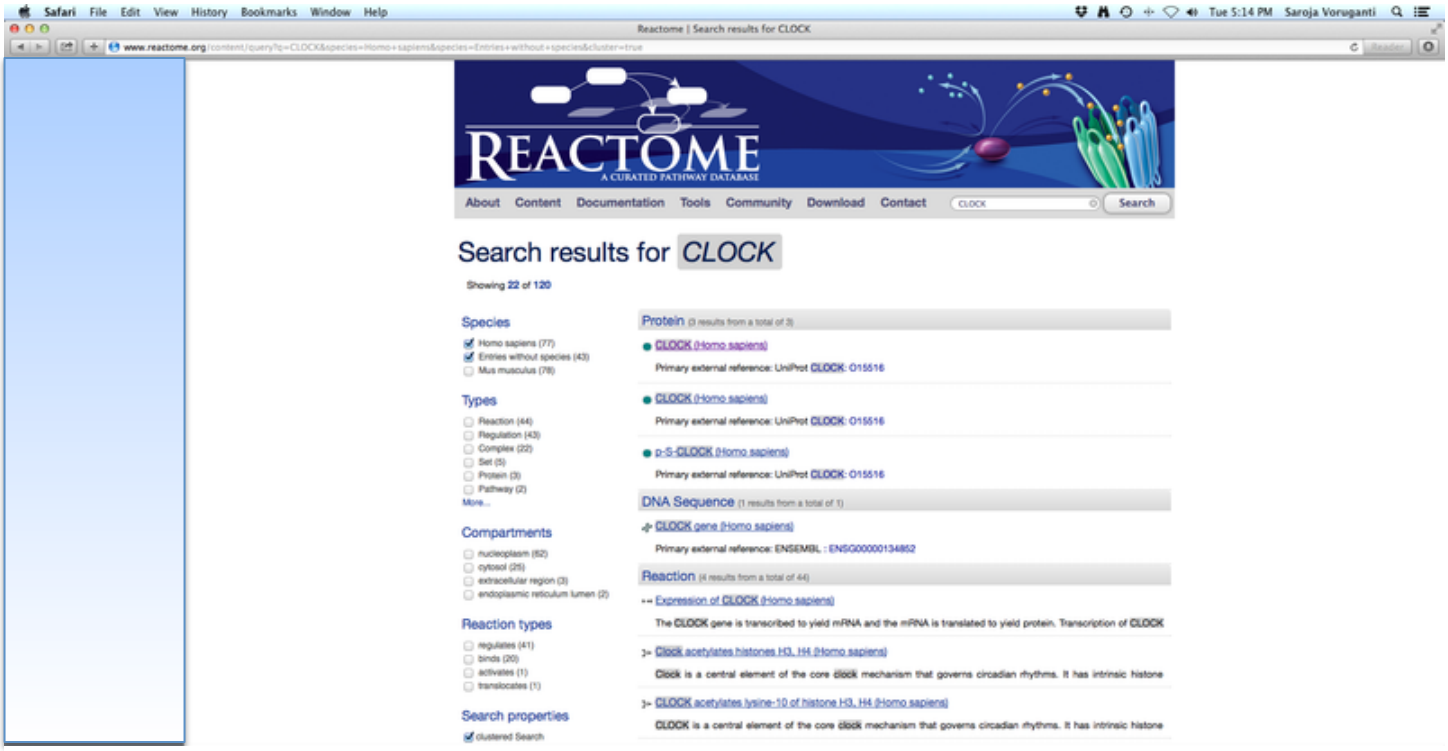
Click on ‘SNAP Proxy search’



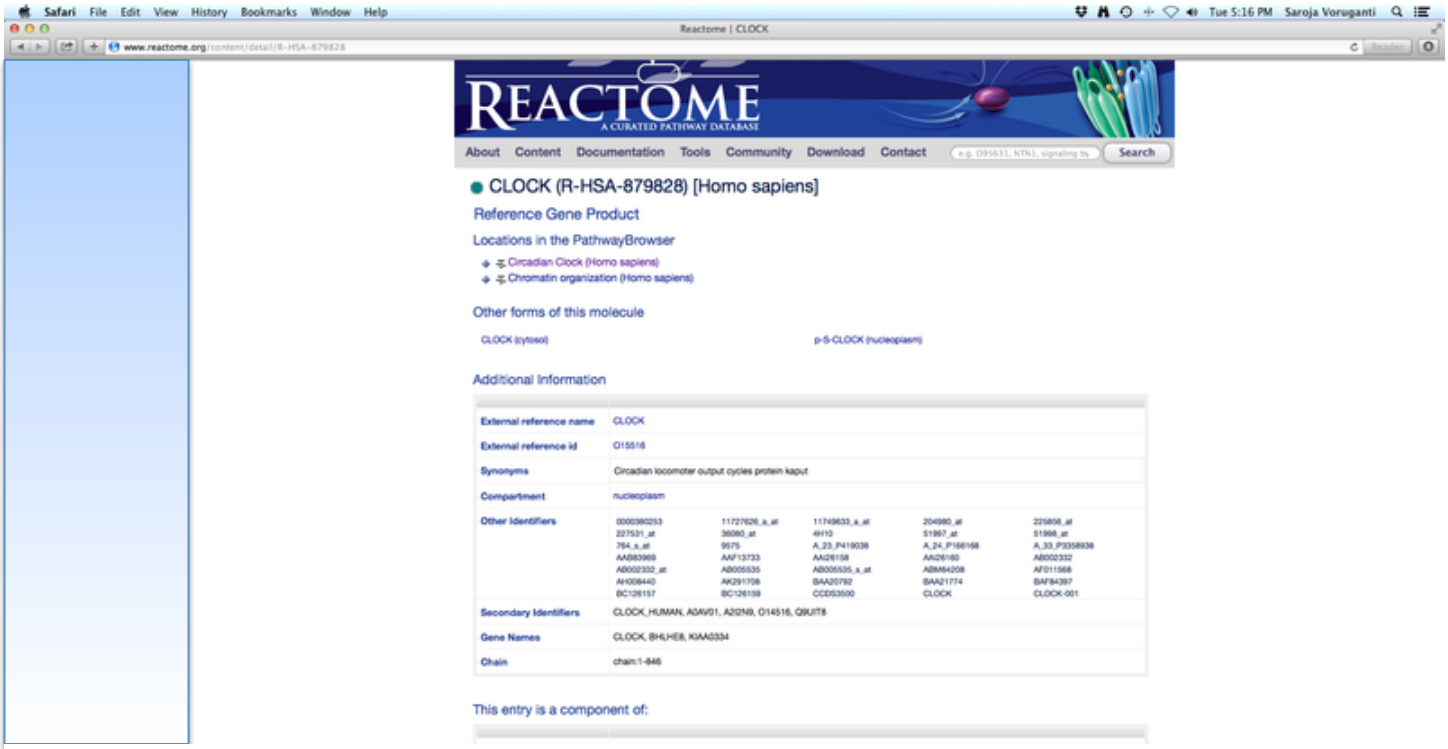
Select ‘Text Entry’ and enter rs662

Reactome
http://www.reactome.org - a free online curated pathway database

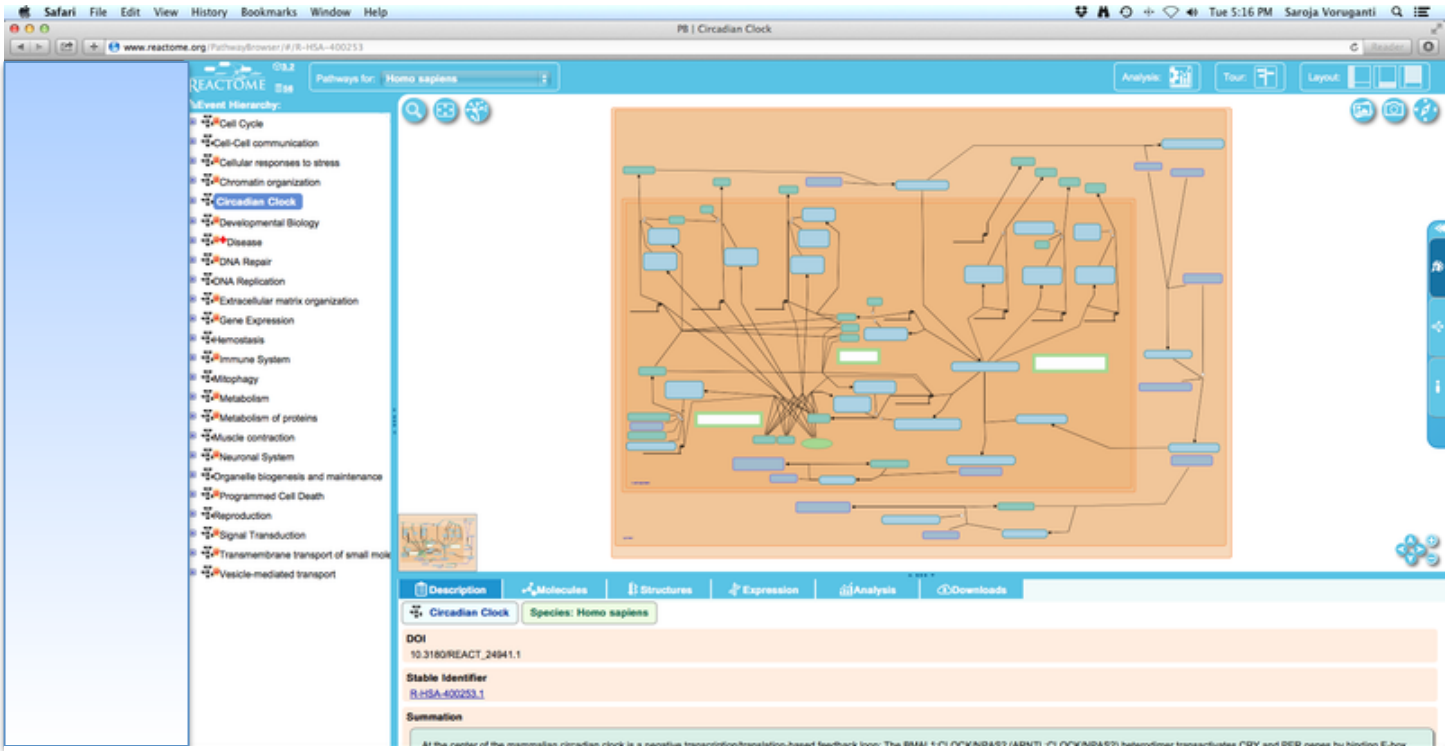
enter CLOCK in the search panel



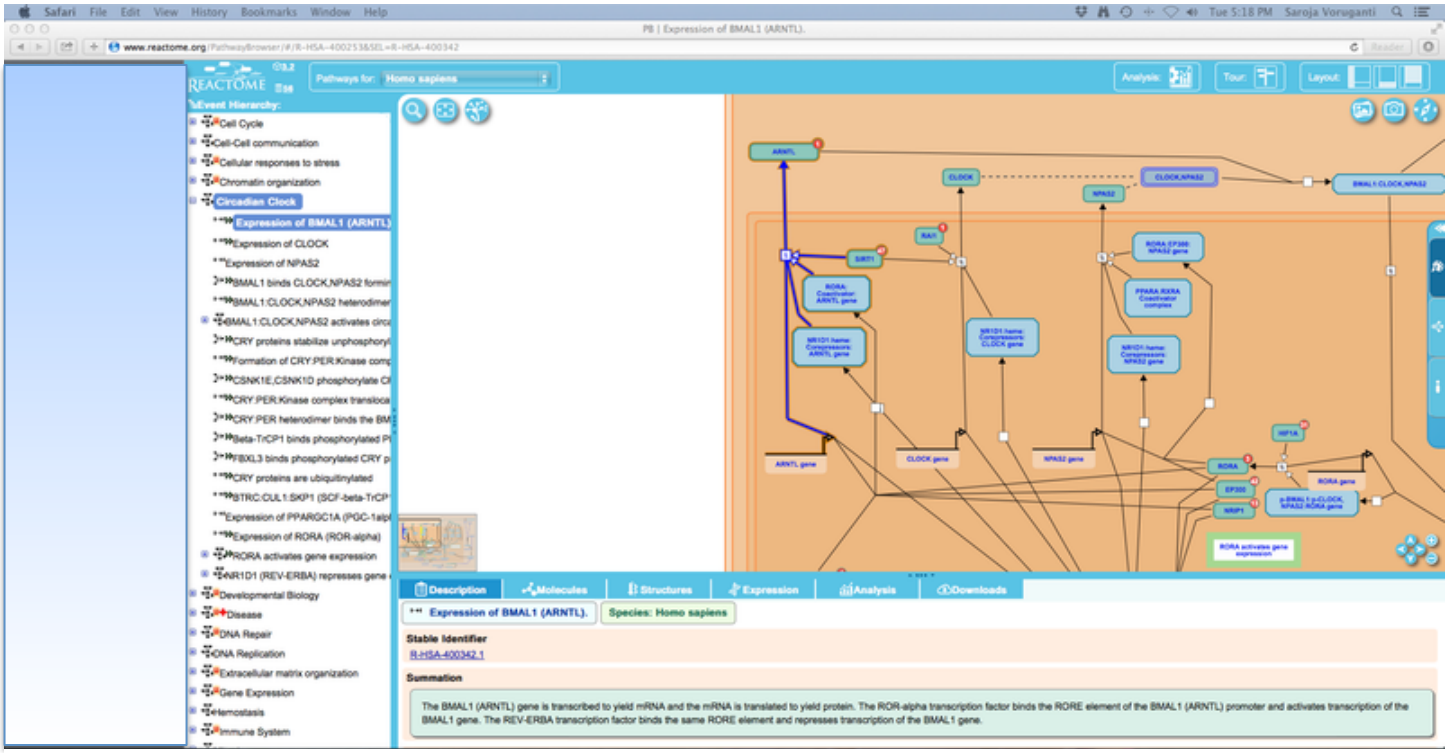
Click on CLOCK (Homo Sapiens)



Click on Circadian Clock (Homo Sapiens)



you can click on Circadian Clock on the left side and click on each subsection to uncover the pathways. First one is shown as an example



Maouche S and Schunkert H. Strategies beyond genome-wide association studies for atherosclerosis. Arterioscler Thromb Vasc Biol.2012;32:170-181

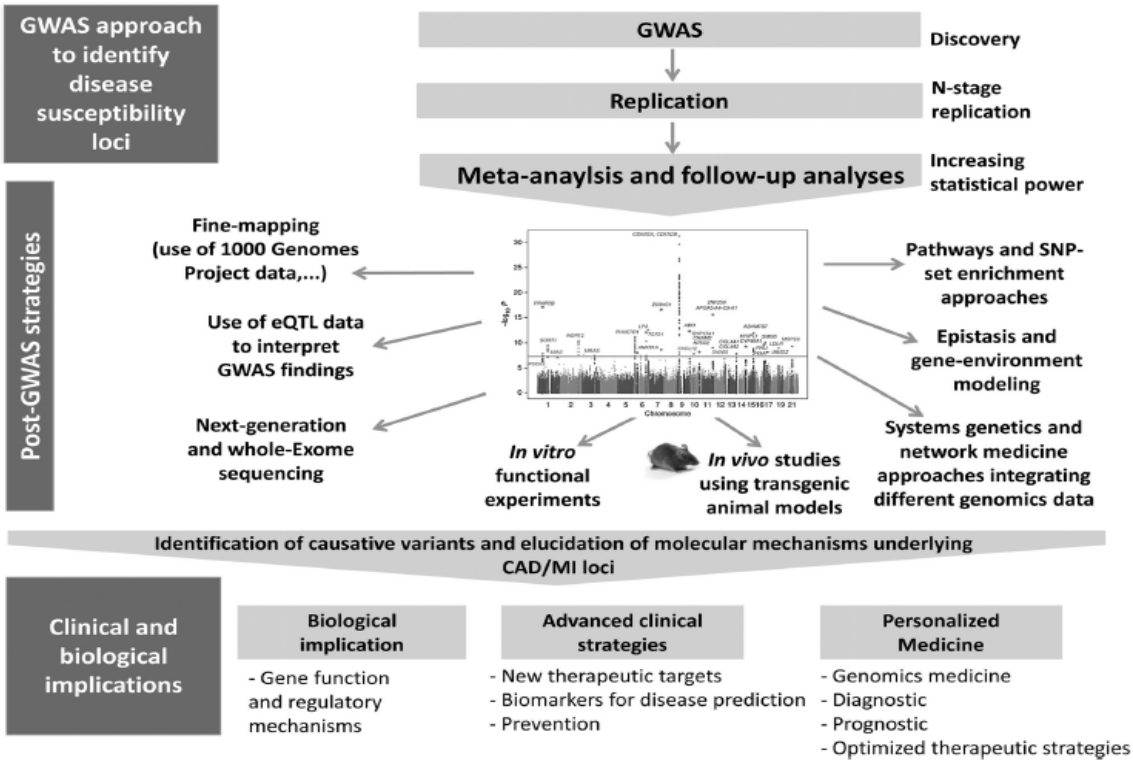


Figure: Post-genome-wide association study strategies and biological and clinical implications of GWAS findings.