Completion of genomic rat variants analysis of the Hybrid Rat Diversity Panel

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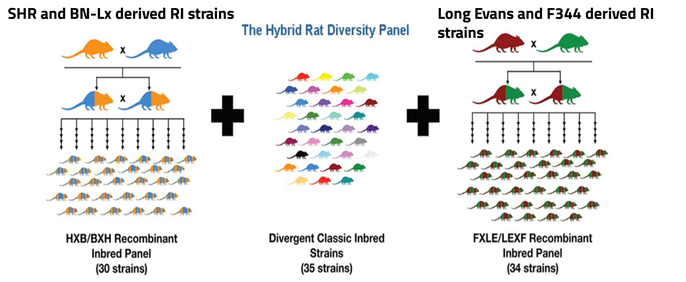
Abstract

The Hybrid Rat Diversity Panel (HRDP) is a group of 96 rat inbred strains selected to study mechanisms of complex traits similar in their pathology to common human diseases. We have analyzed the HRDP whole genomic sequencing data (Illumina short reads) using the high-quality rat reference mRatBN7.2 and variant discovery GATK4 Best Practices recommendations (Broad Institute 2019). The average sequence coverage ranges from 15x to 69x per rat strain sample. We have found that more than 21 mln of germline variants, ~8 mln of short indels and ~13 mln of SNVs, characterize the rat cohort. In addition, we observed a remarkable drop in the number of indels discovered with the new genome reference compared with the old rat assembly from 2014, Rnor6. Our results provide high confidence variants that represent the sequence cohort heterogeneity but also variants that require additional quality testing. Low coverage variants, a fraction of private variants of Brown Norway reference rat strain and genomic regions with accumulation of heterozygous variants will require further analysis. Currently we are incorporating the data into Rat Genome Database (https://rgd.mcw.edu) to provide functional annotations to the rat community, as well as assist in prioritization of potential disease-causing mutations. Thus, researchers can access information about strain specific variants on gene report pages, in Variant Visualizer tool and in genomic browser. The data in vcf format are available for retrieval in the 'Download' section of the RGD site.

What is the Hybrid Rat Diversity Panel ?

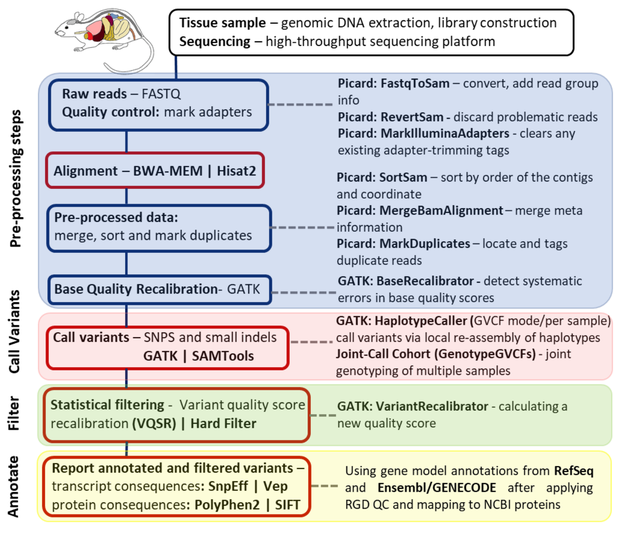


- blood pressure regulation
- glucose and fatty acid metabolism
- taste aversion
- herpes simplex encephalitis
- metabolic syndrome
- alcohol consumption
- cardiovascular diseases
- renal diseases
- obesity
- diabetes
- pulmonary diseases
- behavioral variation
- anxiety
- autoimmunity
- seizures
- epilepsy
- male reproductive disorders
- lymphoma and leukemia

Hybrid Rat Diversity Panel was selected to:

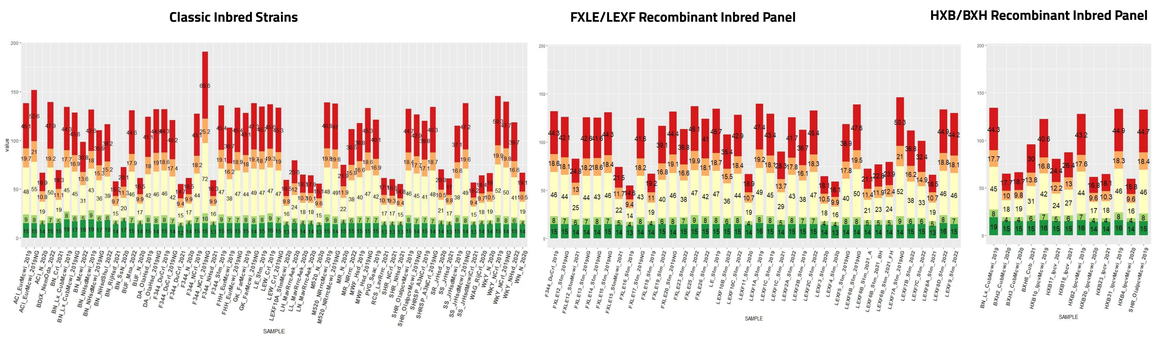
1. Provide stable genetic and phenotypic strains to allow researchers to conduct reproducible experiments
2. Maximize the genetic diversity among strains and to maximize power to detect specific genetic loci associated with a complex trait (QTL mapping resolution)
3. Extend the whole genome sequencing to all HRDP inbred rat strains with susceptibility to different complex diseases
4. Facilitate the translation of disease-related genetics and genomics research to pre-clinical and clinical studies

Analysis workflow



1. Pre-processing steps involve marking adapter sequences, alignment to the new rat genome reference **mRatBN7** and marking duplicates.
2. Base Quality Scores Recalibration corrects biases introduced by sequencing platforms and assigns scores empirically determined from the read data using validated variants.
3. Variant calling is accomplished by running the GATK HaplotypeCaller that simultaneously detects SNVs and indels via local de-novo assembly of haplotypes (method to increase accuracy of the variant call comparing with position-based algorithm).
4. In the filtering process we remove less reliable variant calls: variants with low coverage, low quality, strand biased, located in SNV clusters, and supported by low-confidence read alignment.

Sequencing Coverage and Variants Discovery



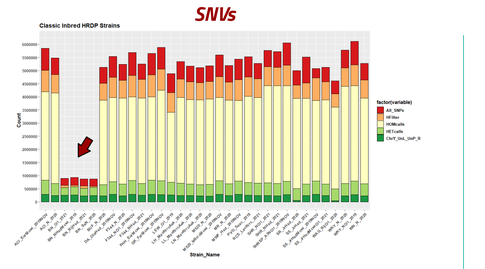
The mean coverage in bases of the genome territory, after all filters are applied.
The standard deviation of coverage of the genome after all filters are applied.
The median coverage in bases of the genome territory, after all filters are applied.
The theoretical HET SNP sensitivity.
The Phred Scaled Q Score of the theoretical HET SNP sensitivity.

Mean Coverage	**14.6 – 68.6**
% of Reads Aligned	**99.1 – 99.6**
% of Reads improperly aligned in pairs	**0.02 – 0.043**
% of Chimeric reads	**0.003 – 0.007**

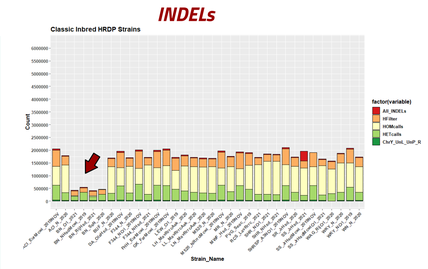
mRatBN7

Mapping analysis shows the coverage and performance of the whole genome sequencing. ***Mean base coverage*** *ranges from* ***15x to 69x*** *and the estimated* ***sensitivity to detect heterozygous sites*** *(as a function of coverage and base quality distribution ranges) drops quickly when mean coverage is below* ***20X.***

SNVs

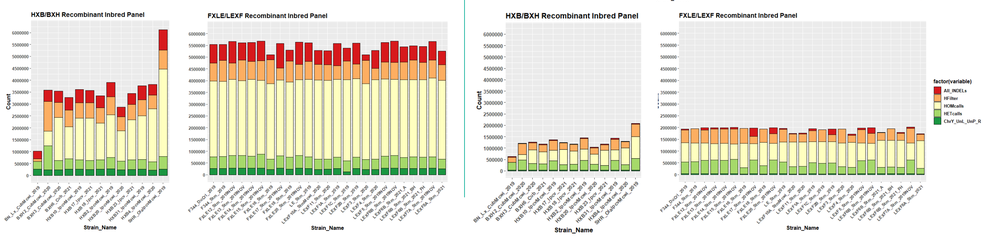


INDELs

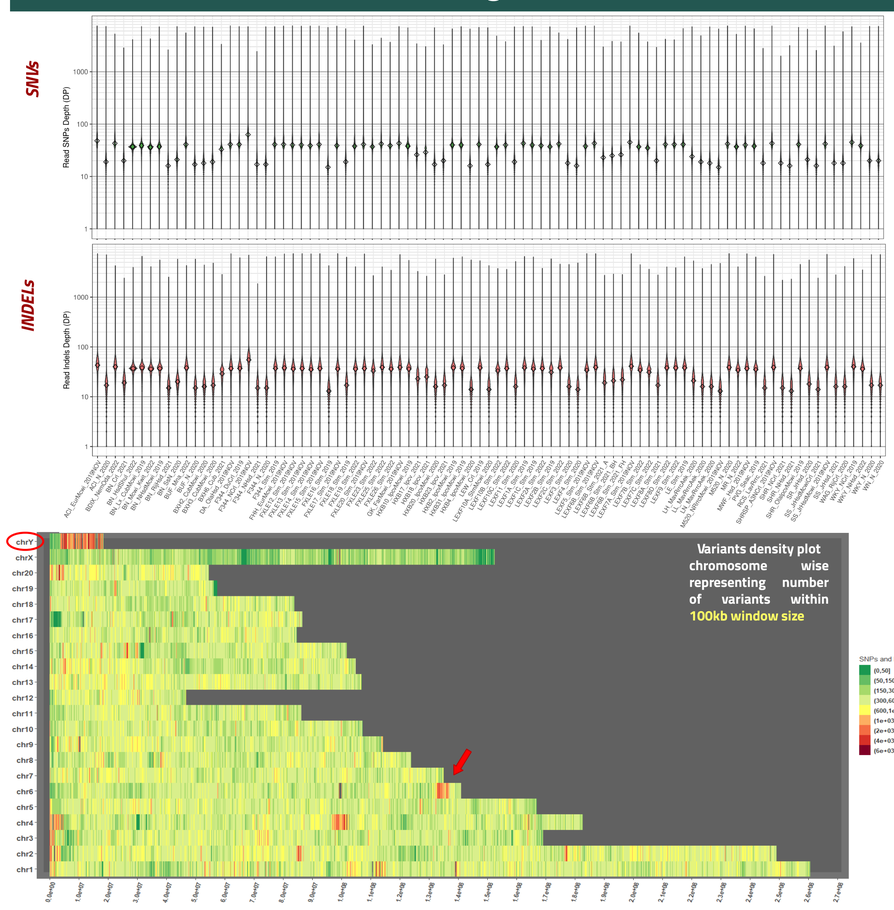


Number of SNVs and indels subsets per sample *- total number of SNVs per sample was up to* ***6mln*** *and around* ***2mln*** *of indels,* ***except Brown Norway*** *- classic and recombinant strains, that have* ***~5-6x lower number of variants.***

Classic Inbred Strains / Recombinant Inbred Panel



High Confidence Variants



Hard Filter - 6 parameters PASS

1. *QualByDepth (QD)*
2. *FisherStrand (FS)*
3. *StrandOddsRatio (SOR)*
4. *RMSMappingQuality (MQ)*
5. *MappingQualityRankSumTest (MQRankSum)*
6. *ReadPosRankSumTest (ReadPosRankSum)*

All SNVs	17,550,340
HF SNVs	13,205,288
All INDELs	9,428,709
HF INDELs	8,373,427

High confidence variants *- Number of variants decrease after applying hard filtering parameters and only variants supported by more than* ***10 reads*** *were selected as high confidence set.*

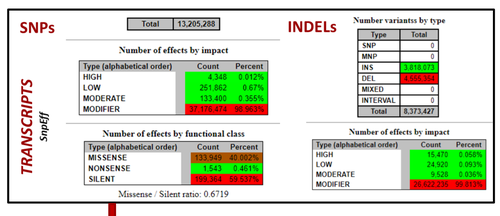
Variants density plot *shows genome location with high number of* ***variants accumulation*** *that require further analysis.*

HRDP variants in RGD

	Atanur et al. 2013	*Hermsen et al. 2015*	*RGD 2018*	*HRDP 2022*		
Number of analyzed rat strains	27	40	25	47		89
Number of identified high confidence SNVs	9,665,340	9,183,702	8,953,897	11,585,641	12,354,591	13,205,288
Reference Genome	RGSC 3.4 - chromosomes	RGSC 5.0 - whole	RGSC 6.0 - whole	RGSC 6.0 - whole	mRatBN7v2- whole	
Alignment Software	BWA-0.5.8c	BWA mem –M 0.7.5a	BWA mem 0.7.15	BWA mem 0.7.17		
Genomic variants call	GATK v. 1.0.6001	GATK HaplotypeCaller v2.8-1	GATK HaplotypeCaller v3.6	GATK HaplotypeCaller v.4.1.3.0		
Variant quality recalibration (VQSR) - true training set	Top 30% of high quality SNVs	Not defined	273,568 selected SNVs	In preparation		
dbSNPs	dbSNP125 - 41,658 (1,291) (35,186)	dbSNP138 - 5,076,239 (5,043,831)	dbSNP149 - 5,075,461 (5,042,280) 4,721,043	dbSNP149 - 5,075,461 (5,042,280) 4,721,043		dbSNP_EVAv3 9653928
Tranche sensitivity threshold	99.0	99.5	95.0	In preparation		

Comparison of previously reported analysis of ***single nucleotide variants*** ***identified in different rat populations.***

Variants Functional Consequences



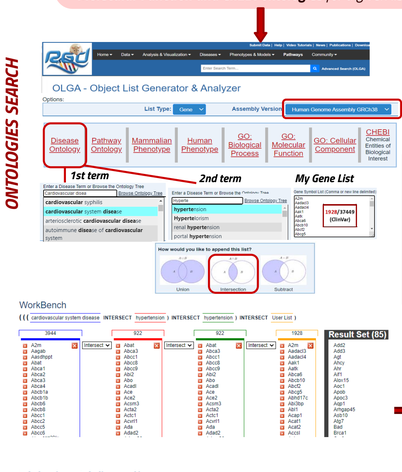
Gene type	ALL Genes	Cardiovascular system disease	Heart Disease	Hypertension	Disease of metabolism	Hypercholesterolemia	Nervous system disease
Impacted Genes with NM_Transcript	12,416	2713/3944	1605/2809	916/922	5571/5845	35/116	8672/12450
Impacted Genes with NR_Transcript	451	72/3944	61/2809	7/922	103/5845	0/116	160/12450
Functional consequences							
All Genes with DMG effect	2924	417	286	90	673	16	1419
Shared with Human Genes with pathogenic variants	1928/37449 (ClinVar)	395	270	85	639	16	1295

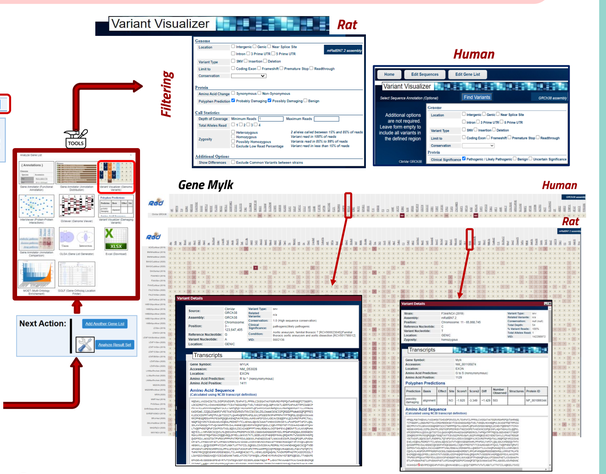
PROTEINS PolyPhen2

Gene counts with damaging variants by ***Disease Ontology*** ***terms***

OLGA Cross Analysis: *tool allows to select terms from* ***Disease, Pathway, Mammalian Phenotype, Biological Process, Cellular Component, Molecular Function and ChEBI ontologies*** *for a given list of genes and further analyze it with other RGD tools.*

ONTOLOGIES SEARCH





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Summary

- ***Whole genome sequencing of rat diversity panel provides high confidence variants that represent the sequence cohort heterogeneity but also variants that require additional quality testing.***
- ***Genetic variation analysis in rat strains selected for HRDP helps to generate high resolution association mapping and complete systems genetics on complex traits***
- ***We are building the HRDP Portal within RGD that will provide data mining and visualization functions for genomic and phenotypic data***