Leveraging variant annotations for genotype-phenotype association testing

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SISG Module 17
Computational Pipeline for WGS Data

Overview of variant annotation session

Section I : Thursday + Friday (instructional part)

- What are variant annotations?
- Applications of annotations in rare variant association testing
- How to obtain annotations ?
- Approaches for aggregating and filtering variants for rare variant association testing
- Generating variant grouping files for conducting rare-variant aggregate test
- WGSAparsr

Section II: Friday (hands–on part)

- Parsing WGSA files using WGSAparsr
- Generate variant grouping files
- Association testing in aggregation units using variant grouping files

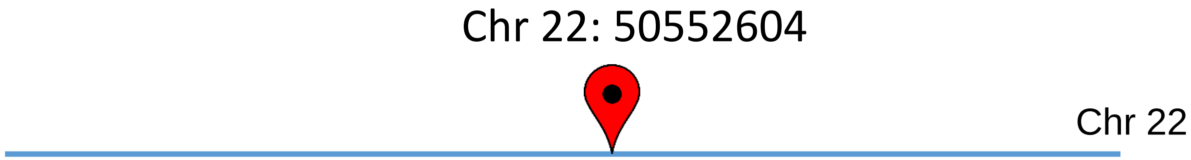
What is annotation?

- Annotation is a qualitative or quantitative information about the variant or its position
- Annotations can be
  - variant dependent (dependent on chrom, position, reference and alternate allele)
  - variant independent (dependent only on chromosome and position)

chromosome	position	Reference allele	Alternate allele	Gene	SIFT score	consequence	CADD score	Regulatory annotation
1	100	A	G	G1	5	missense	0.5	enhancer
1	100	A	T	G2	1	intronic	0.5	enhancer

Annotations

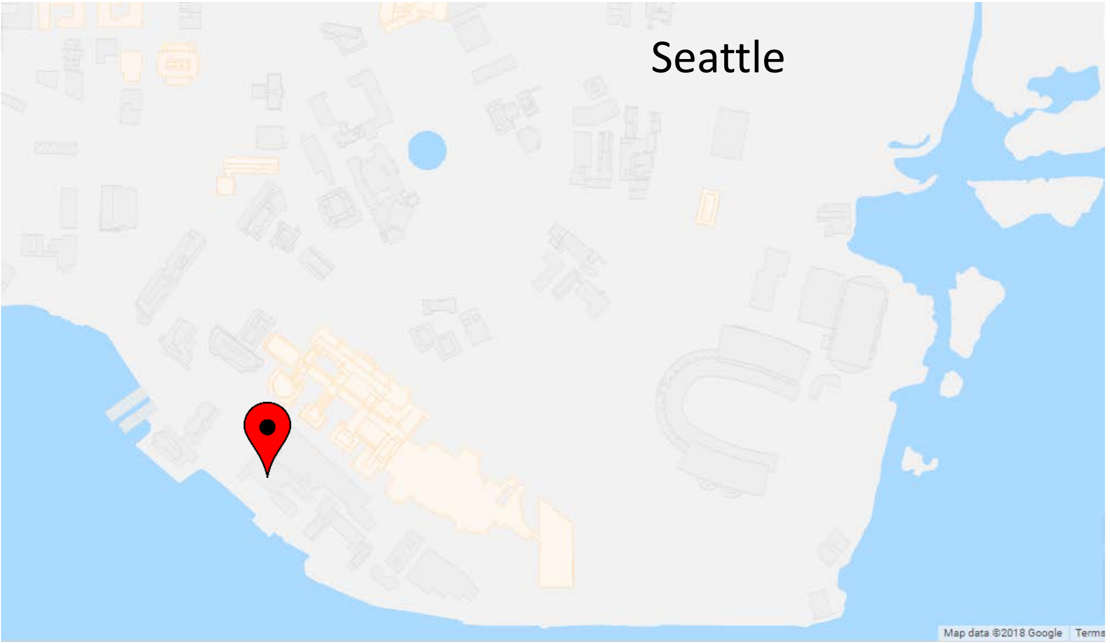
Annotating variants = generating map of the genome

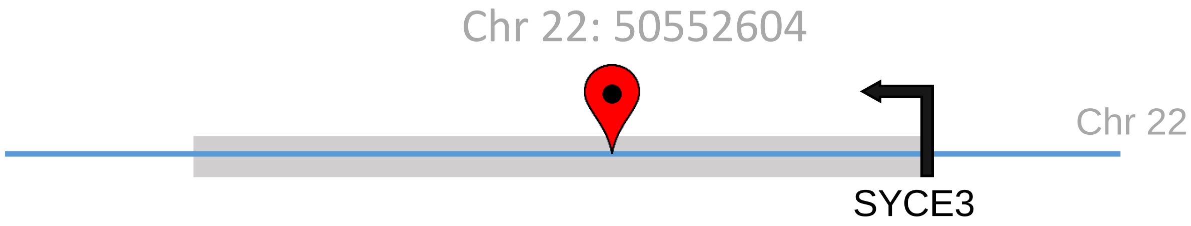


Name of the city provides some context about your location on Earth!

Chromosome and position provides some context about your location in the genome

Annotating variants = generating map of the genome

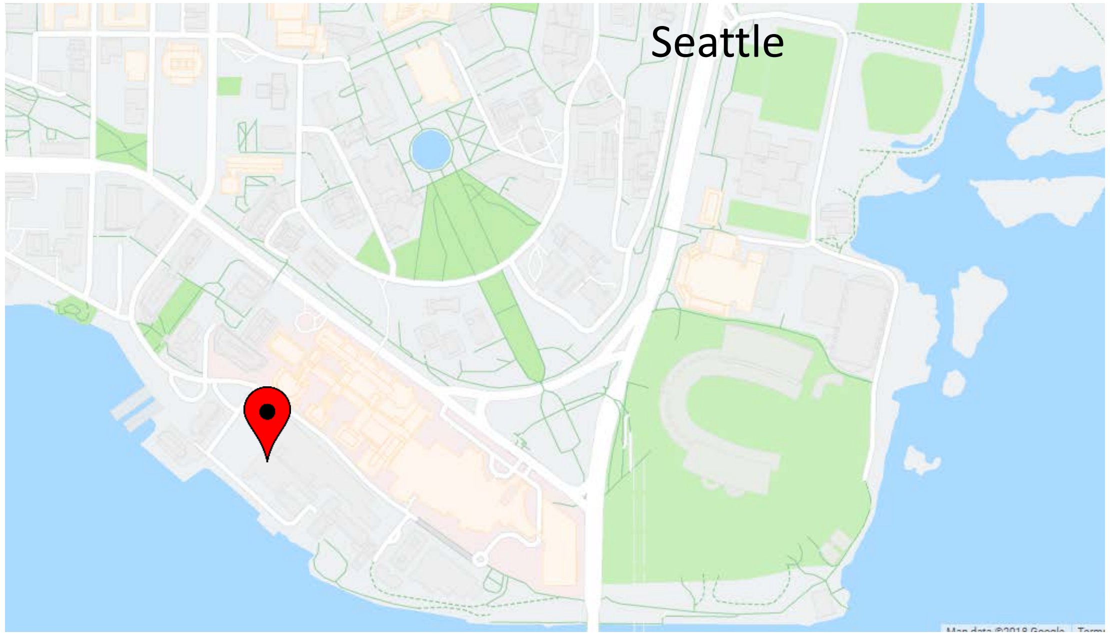




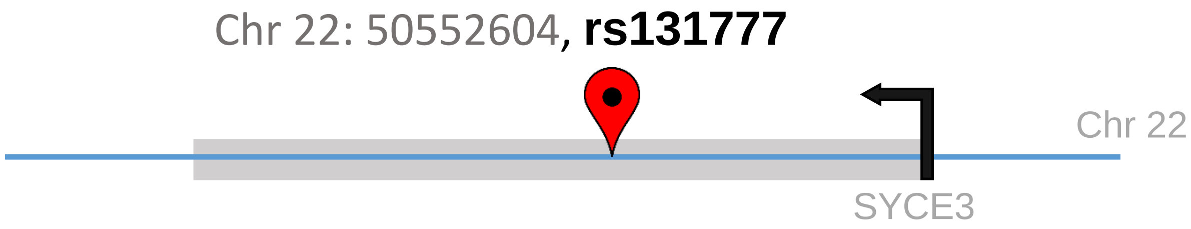
Building outlines overlaid indicate you are in a building

Gene name annotations identify that the variant overlaps with SYCE3 gene

Annotating variants = generating map of the genome



Roads overlaid show paths you can take to go from point A to B

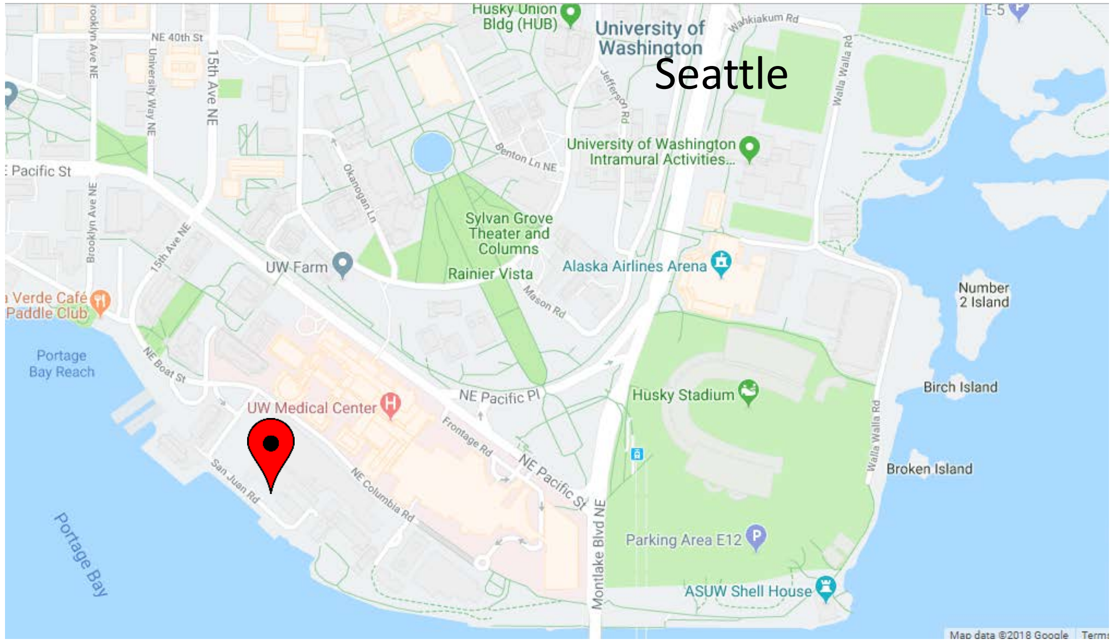

Chr 22: 50552604, **rs131777**

Chr 22

SYCE3

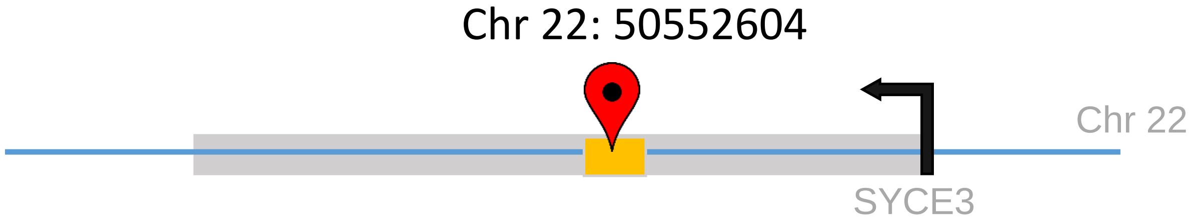
rs identifier and GWAS catalogue annotations help you identify that this variant is previously associated with red cell trait “Mean corpuscular volume”

Annotating variants = generating map of the genome



Road and building names overlaid

- You can take a walk to UW farm
- There is UW medical center nearby
- You can get lunch at Agua verde
- You can visit the Husky stadium

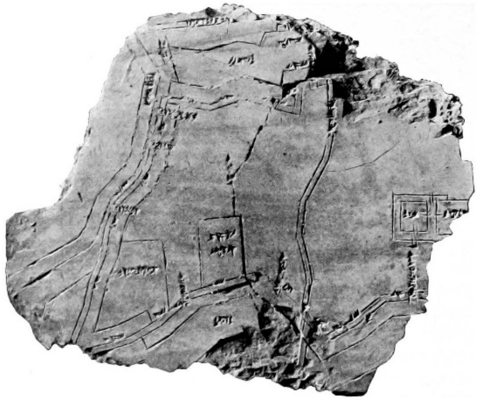

Chr 22: 50552604

Regulatory annotations help you identify that the variant overlaps with a regulatory element. The overlapping regulatory element is active in* :

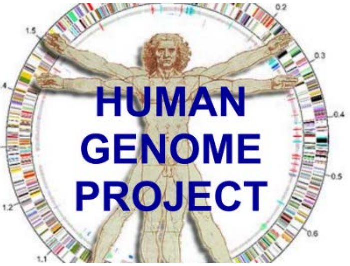
- Red cell cells
- Platelets
- Not in brain cells and bladder cells

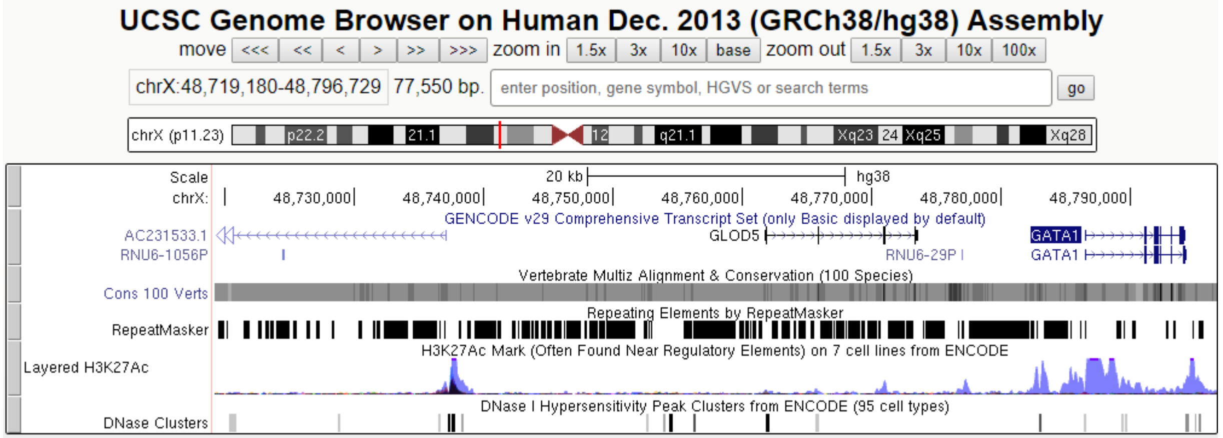
* hypothetical example

Maps evolve as we learn more!



https://www.google.com/earth/





UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

move
<<<
<<
<
>
>>
>>>
zoom in
1.5x
3x
10x
base
zoom out
1.5x
3x
10x
100x
go

chrX:48,719,180-48,796,729 77,550 bp.

enter position, gene symbol, HGVS or search terms

chrX (p11.23)
p22.2
21.1
12
q21.1
Xq23
24
Xq25
Xq28
Scale
20 kb
hg38
chrX:
48,730,000
48,740,000
48,750,000
48,760,000
48,770,000
48,780,000
48,790,000
GENCODE v29 Comprehensive Transcript Set (only Basic displayed by default)
AC231533.1
RNU6-1056P
GLOD5
RNU6-29P
GATA1
GATA1
Vertebrate Multiz Alignment & Conservation (100 Species)
Cons 100 Verts
Repeating Elements by RepeatMasker
RepeatMasker
H3K27Ac Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE
Layered H3K27Ac
DNase I Hypersensitivity Peak Clusters from ENCODE (95 cell types)
DNase Clusters

Applications of annotations in rare variant association testing

1. Boost power in rare-variant association testing
    - Define genomics regions to group variants over
    - Filter variants within grouped variant sets
    - Use annotation as weights
2. Interpret signals identified in GWAS
    - Predict causal variants
    - Develop hypotheses about biological mechanisms explaining variant-trait associations

Applications of annotations in rare variant association testing

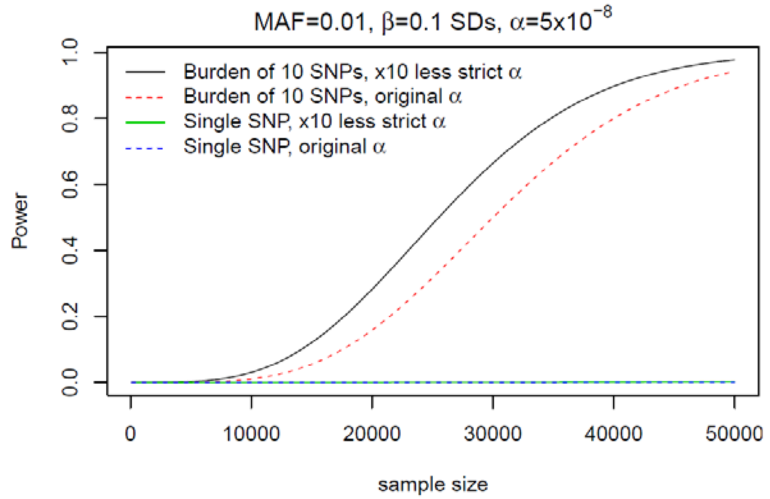
1. Boost power in rare-variant association testing
    - Group variants using annotations
    - Filter variants using annotations
    - Use annotation as weights
2. Interpret signals identified in GWAS
    - Predict causal variants
    - Develop hypotheses about biological mechanisms explaining variant-trait associations

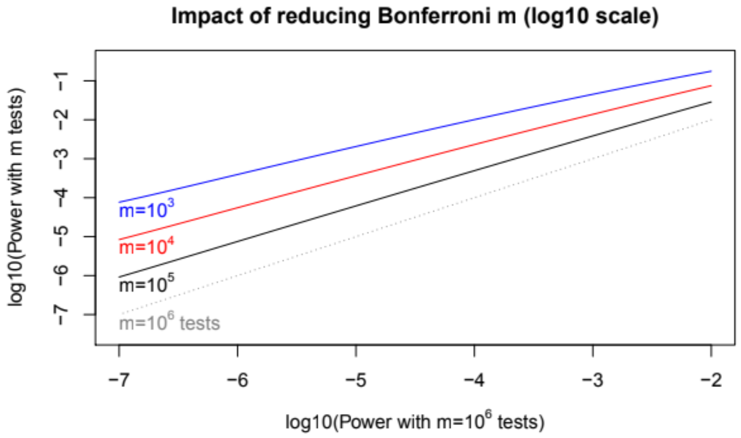
Aggregating variants boosts power

- Majority of variants from WGS studies are rare (MAF<0.05)

- Single variant analysis of rare variants lack power due to
  - an increased multiple testing burden
  - a decrease in statistical power owing to the rarity of individuals carrying these variant alleles

- To gain power, rare variants are grouped using annotations





For each ×10 reduction in m, get between ×2 and ×10 increase in power. Need several of these to move from 'hopeless' to 'hopeful'.

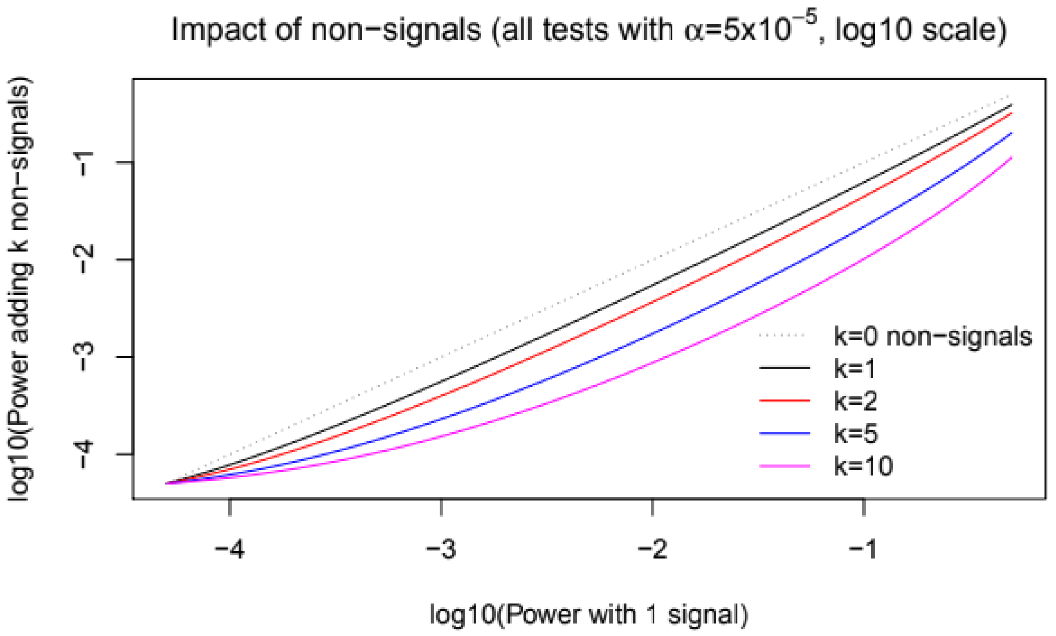
From Dr. Ken Rice

Applications of annotations in rare variant association testing

1. Boost power in rare-variant association testing
   - Define genomics regions to group variants over
   - Filter variants within grouped variant sets
   - Use annotation as weights
2. Interpret signals identified in GWAS
   - Predict causal variants
   - Develop hypotheses about biological mechanisms explaining variant-trait associations

Why filter variants within grouped variant sets?

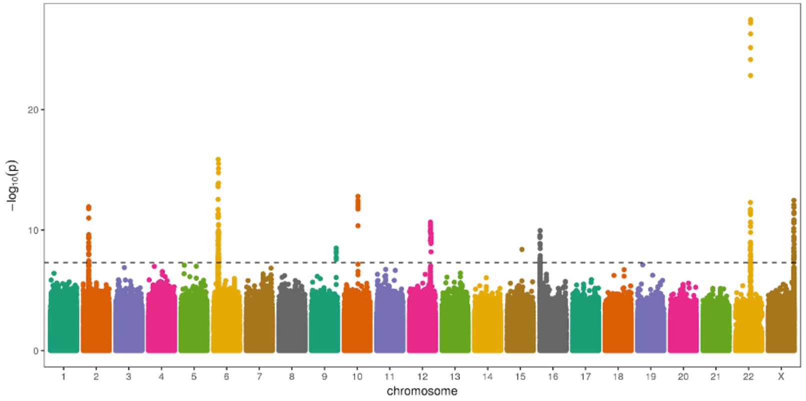
- Annotations are used to filter or weight variants in association tests
  - Down weight neutral/non-signal variants
  - Up-weight functional/deleterious signal variants
  - Good filtering strategy increases the signal to noise ratio & increases power to detect an association



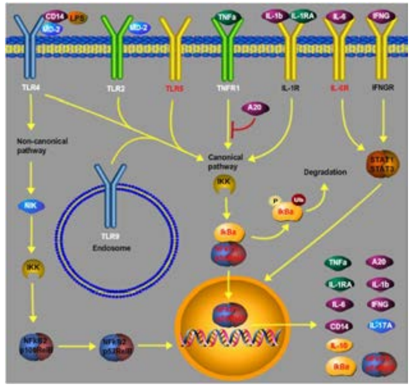
Reducing k by ×10 provides up to ×10 more power, but often less that that.

Applications of annotations in rare variant association testing

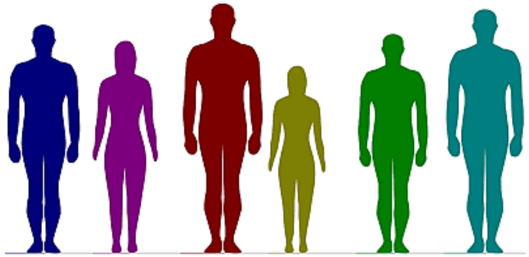
1. Boost power in rare-variant association testing
   - Define genomics regions to group variants over
   - Filter variants within grouped variant sets
   - Use annotation as weights
2. Interpret signals identified in GWAS
   - Predict causal variants
   - Develop hypotheses about biological mechanisms explaining variant-trait associations



Significant variants



Biological pathways



Explaining phenotypic variation

We will not go into details of this application but I am happy to chat more about this in-person

Section I : Outline

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What is the source of annotations?

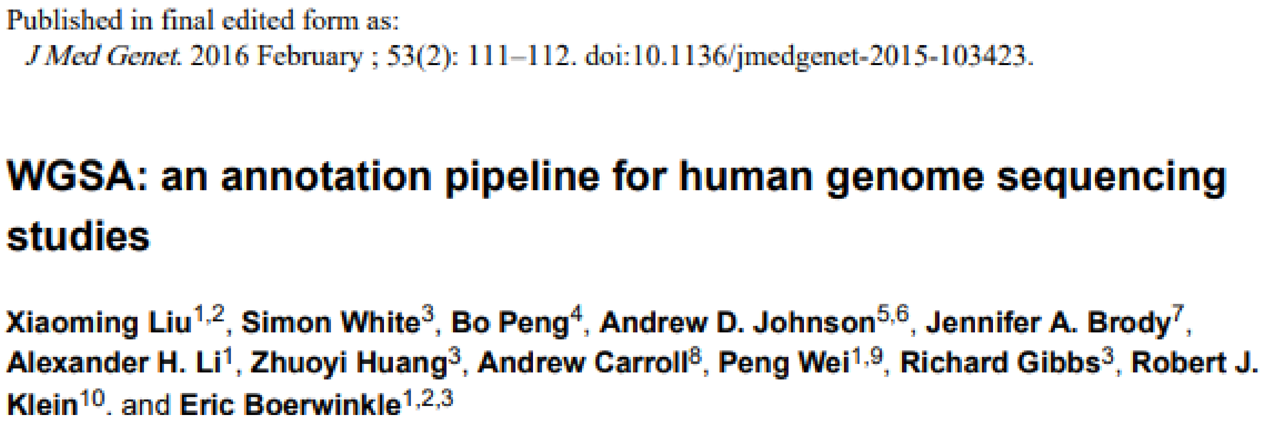
- Lots of resources!

Annotation can be generated by any lab,center or consortium

- NCBI
- Ensemble
- UCSC
- ENCylopedia Of DNA Elements (ENCODE)
- Roadmap Epigenomics Consortium (REMC)
- Functional annotation of Mammalian genome (FANTOM5)
- dbSNP
- …

Whole Genome Sequence Annotator (WGSA)

- WGSA gathers annotations from various sources
- WGSA is provided both as
  - an Amazon Machine Image (AMI) ready to run out-of-the-box and
  - a downloadable version
- Licenses are required for non-academic usage for some of the resources
- Website: https://sites.google.com/site/jpopgen/wgsa/



Published in final edited form as:
J Med Genet. 2016 February ; 53(2): 111–112. doi:10.1136/jmedgenet-2015-103423.

WGSA: an annotation pipeline for human genome sequencing studies

Xiaoming Liu[1,2], Simon White[3], Bo Peng[4], Andrew D. Johnson[5,6], Jennifer A. Brody[7], Alexander H. Li[1], Zhuoyi Huang[3], Andrew Carroll[8], Peng Wei[1,9], Richard Gibbs[3], Robert J. Klein[10], and Eric Boerwinkle[1,2,3]

WGSA has over a *lot* of annotations!

- Gene based location and consequence
  - Softwares : SnpEff, ANNOVAR, VEP
  - Gene models: Ensembl ,RefSeq ,UCSC
- Transcript-specific annotation (transcript name, consequence etc.)
- Loss-of-function annotations (eg: LOFTEE)
- Deleteriousness predictions(CADD, FATHMM,MetaSVM, ssSNV etc)
- Allele frequencies (1000G, UK10K, EXAC, gnomAD etc)
- Regulatory annotations (ENCODE, Roadmap, FANTOM5)
- Conservation scores (GERP etc)
- Mappability scores
- rsIDs
- Many more …. (~2000)

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How to prepare aggregated variant sets for association testing

STEP1: Define genomic regions over which variants will be grouped

- Example : TSS to TES of a gene

STEP2: Decide on filtering criteria

- Example : keep only missense variants

Goal is to create aggregated sets of variants across genome which can be used for variants association tests (example Burden,SKAT, SKAT-O tests)

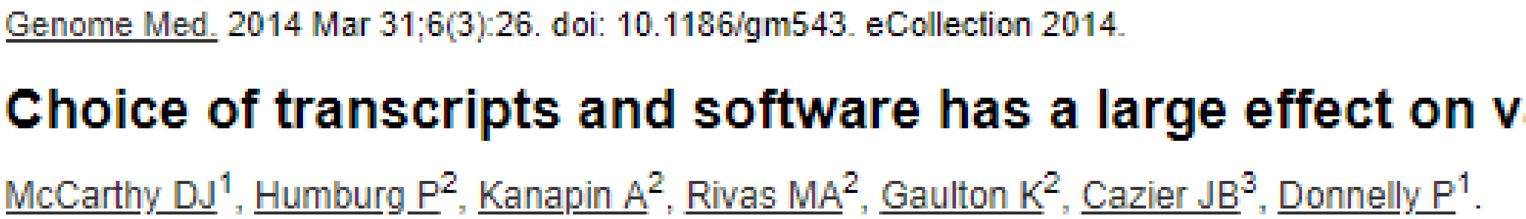
TSS: transcription start site
TES: transcription End site

STEP1: Define genomic regions over which variants will be grouped

Gene is one of the fundamental units of biology and gene-based aggregation units are frequently used in rare variant association testing so we will go over these in detail

Gene based aggregation units

- Gene and/or gene related elements are the unit of aggregation
- Multiple gene models available
  - GENCODE/Ensembl, RefSeq and UCSC
- Multiple releases of a given gene model are available for same genome build
  - GENCODE v24,v26 etc. on same genome build
- Gene models and gene model specific annotations may change a given gene model version
  - Track gene model and its specific version for reproducible analyses



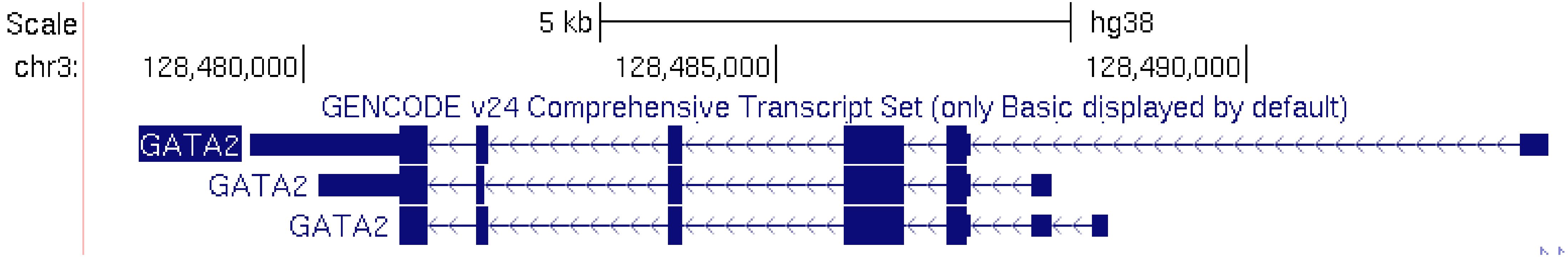
Genome Med. 2014 Mar 31;6(3):26. doi: 10.1186/gm543. eCollection 2014.

Choice of transcripts and software has a large effect on variant annotation.

McCarthy DJ[1], Humburg P[2], Kanapin A[2], Rivas MA[2], Gaulton K[2], Cazier JB[3], Donnelly P[1].

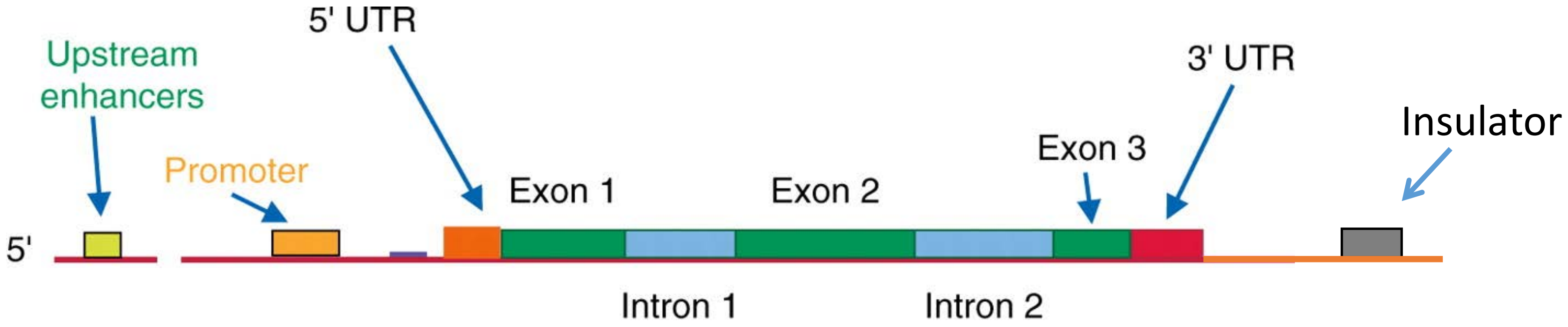
Gene as the aggregation unit

- Gene is the contiguous genomic region spanning all the transcripts of a gene


Scale
5 kb
hg38
chr3:
128,480,000
128,485,000
128,490,000
GENCODE v24 Comprehensive Transcript Set (only Basic displayed by default)
GATA2
GATA2
GATA2

Biotype	Unique Ensembl identifier	Genomic coordinates
Gene	ENSG00000179348	Chromosome 3: 128,479,427-128,493,185
Transcript	ENST00000341105	Chromosome 3: 128,479,427-128,493,185
Transcript	ENST0000043026	Chromosome 3: 128,480,146-128,487,916
Transcript	ENST00000487848	Chromosome 3: 128,481,019-128,488,530

Gene is regulated by non-coding regulatory elements



Functional gene unit = transcript + its regulatory elements

Modified from R. Searle and P. M. Hopkins, 2009

Biochemical signatures typically associated with non-coding functional elements

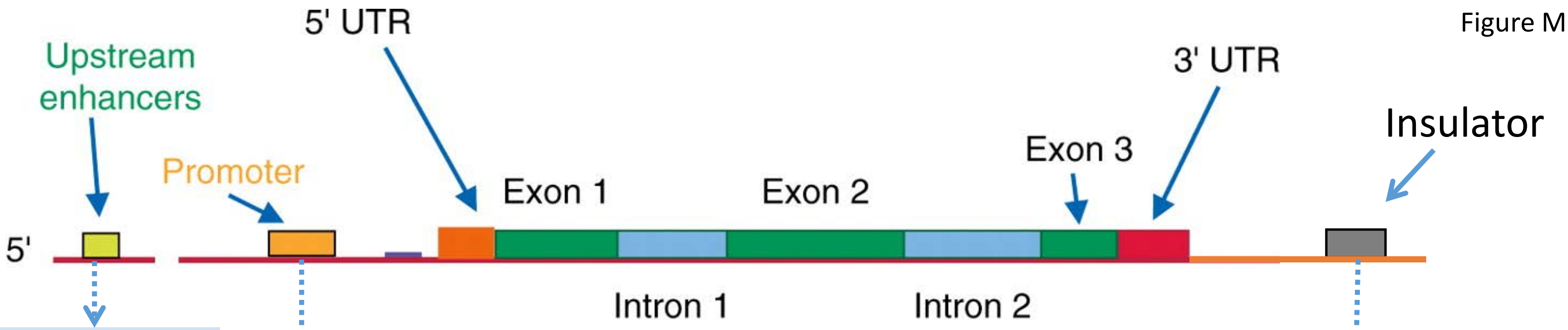


Figure Modified from R. Searle and P. M. Hopkins, 2009

DnaseI[#]/ATAC-seq[^]
H3k4me1 mark
H3k27ac mark
TF[*] binding

DnaseI[#]/ATAC-seq[^]
H3k4me3 mark
H3k27ac mark
Polymerase II
TF[*] binding

DnaseI[#]/ATAC-seq[^]
CTCF binding

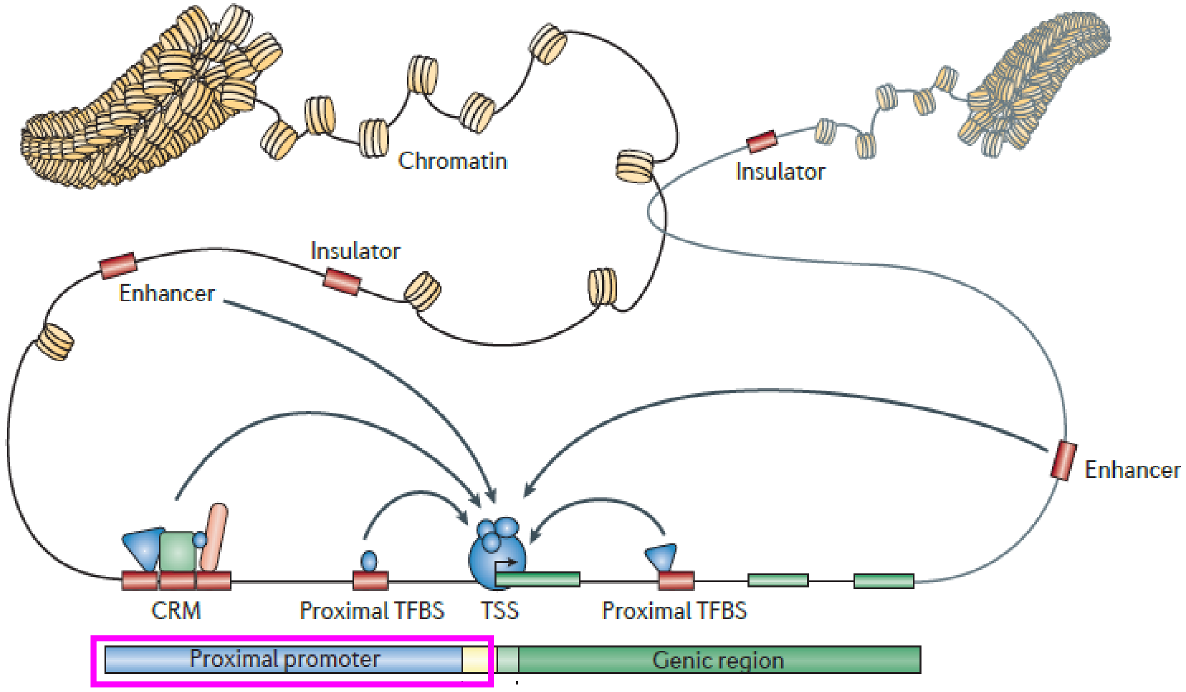
Enhancer : Interacts with promoter can be involved in repression or induction of a gene
Promoter : Genomic element where the transcription machinery assembles
UTR : Untranslated region
EXON : Coding part of a transcript (mRNA)
INTRON : Non-Coding part of a transcript (mRNA)
Insulator : Barriers that protect genes from influence of outside enhancers or inactivating chromatin structures

Patterns of these biochemical marks can be cell- or tissue-specific . These may also show temporal and treatment specific variations within a cell/tissue type

- [*] TF : transcription factor,
- [#] DNaseI Hypersensitivity, which is an indicator of chromatin accessibility
- [^] ATAC-seq: Assay for Transposase-Accessible Chromatin using sequencing. Also an indicator of chromatin accessibility

Defining promoter & proximal promoter elements boundaries

- Promoter and proximal promoter region boundaries are hard to define. Some approaches to define them are
  - Some distance upstream from TSS (typically 5Kb)
  - 5Kb upstream overlapping with H3K4me3 and or H3K27ac mark
  - 5Kb upstream overlaps with DNaseI hypersensitive regions/ATAC-Seq regions
  - 5Kb upstream that overlaps with CAGE peaks[1]

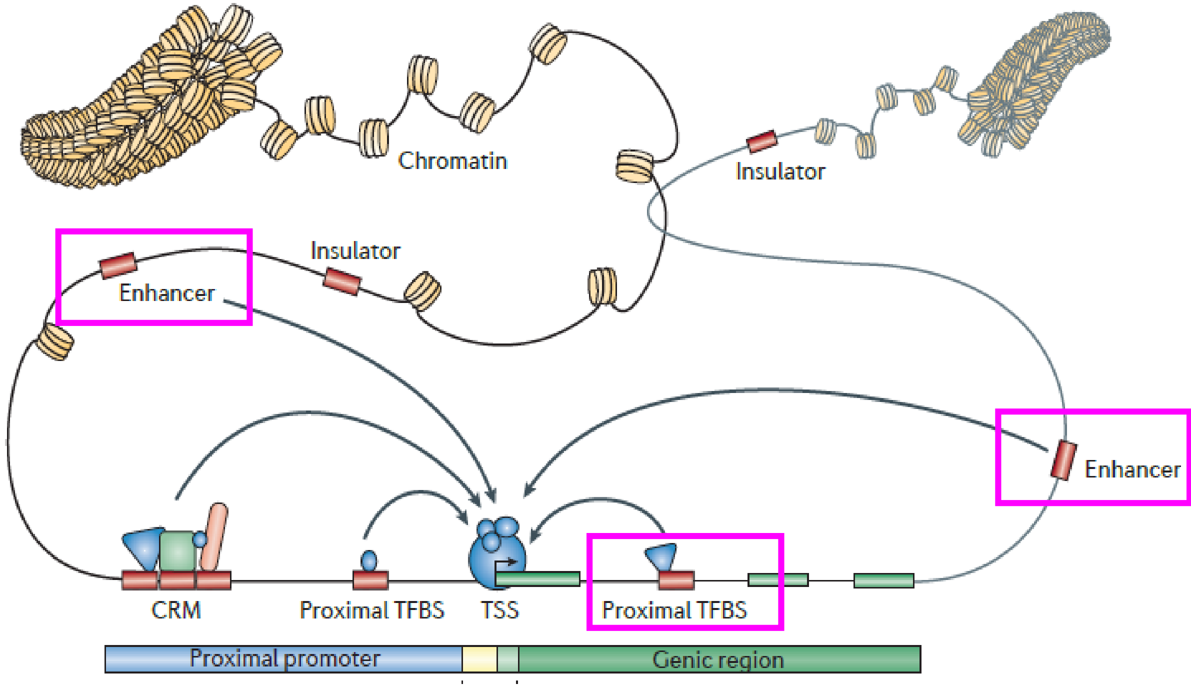


TSS: Transcription Start site, **TFBSs** : transcription factor binding sites, **CRM**: cis-regulatory modules are clusters of TFBS
CAGE: Cap analysis gene expression is a technique that accurately identify transcriptional start sites

[1] Morrison AC, Huang Z, Yu B, et al. Practical Approaches for Whole-Genome Sequence Analysis of Heart- and Blood-Related Traits. Am J Hum Genet. 2017;100(2):205-215
Figure from Lenhard B, Sandelin A, Carninci P.Metazoan promoters: emerging characteristics and insights into transcriptional regulation, Nat Rev Genet. 2012

Defining enhancer element boundaries

- Enhancer region boundaries are hard to define. Some approaches to define them are
  - Flanking regions overlapping with H4K4me1 and or H3K27ac
  - Flanking regions overlapping with DNaseI hypersensitive regions
  - Enhancer-gene link predictions[1,2, 3]
  - Chromosome conformation capture (3C,4C,Hi-C etc.)



TSS: Transcription Start site, **TFBSs** : transcription factor binding sites, **CRM**: cis-regulatory modules are clusters of TFBS
CAGE: Cap analysis gene expression is a technique that accurately identify transcriptional start sites

[1] *Thurman RE et.al Nature. 2012 Sep 6; 489(7414):75-82.*

[2] *Forrest AR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507(7493):462-70.*

[3] *Fishilevich et.al. GeneHancer: genome-wide integration of enhancers and target genes in GeneCards, Database, 2017*

Figure from Lenhard B, Sandelin A, Carninci P.Metazoan promoters: emerging characteristics and insights into transcriptional regulation, Nat Rev Genet. 2012

Example gene-based aggregation units

- Gene
- Gene + flanking regions
- Gene + enhancer(s) + promoter
- UTR’s+ enhancer(s) + promoter
- Promoter of a gene
- First intron of a transcript

Other approaches for aggregating variants

Aggregation units are defined based on genomic positions and they can be :

1. Contiguous units of aggregation
   - Moving window
   - Gene
   - Transcript
   - Exons
   - introns
   - Regulatory regions
     - Promoters
     - Enhancers
     - DNAse hypersensitive site (DNAse sites)
     - ATAC-Seq sites
     - Transcription factor binding sites (TFBSs)
     - ChromHMM states

   - Topologically associated domains (TAD’s)

2. Non-contiguous units of aggregation
   - Gene/Transcript + its associated regulatory regions
   - Domains of interacting proteins
   - Genes in a pathway

Any other biologically motivated unit of your choice …

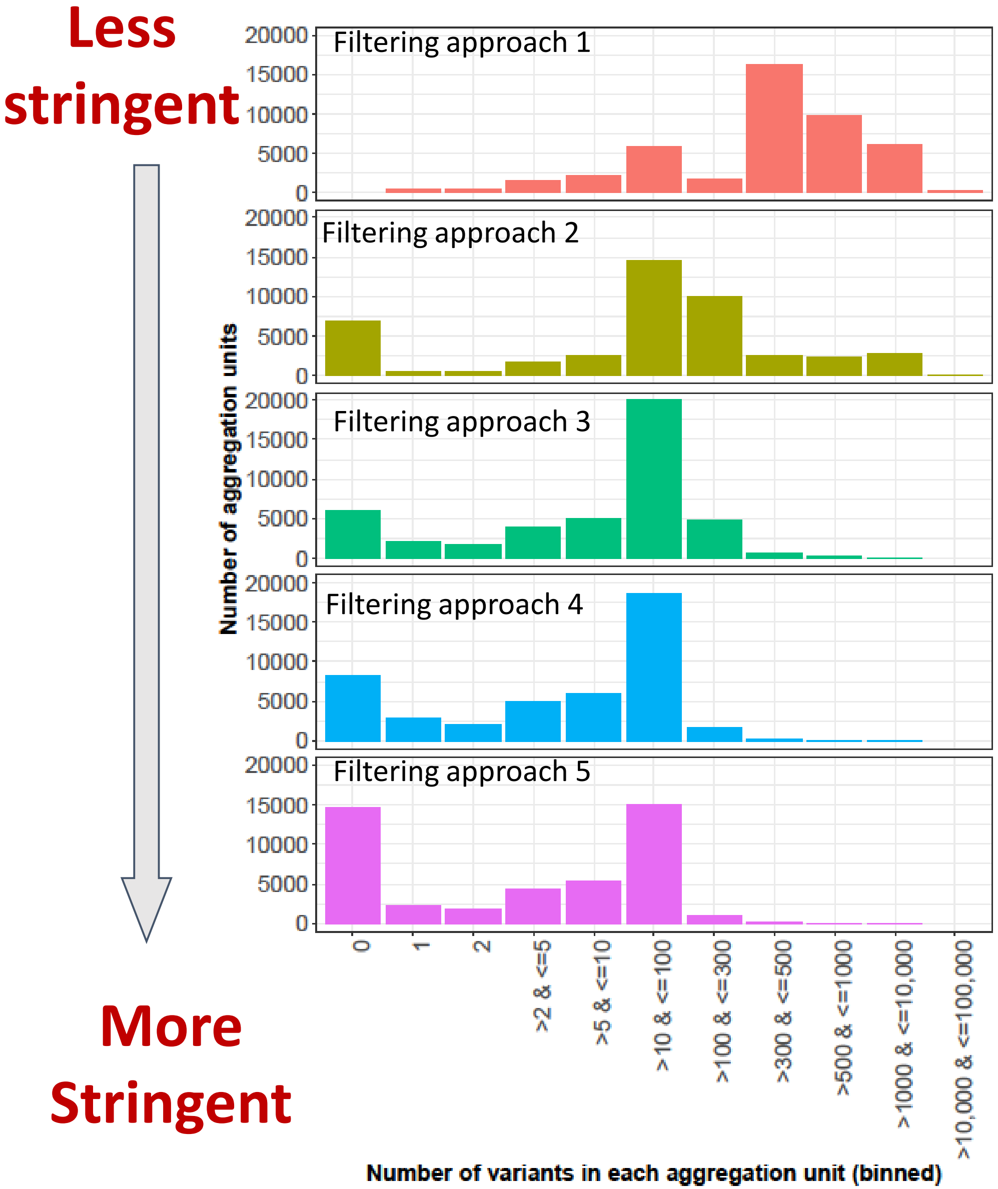
STEP2: Filtering aggregation units

Filtering variants

- Good filtering strategy increases the signal to noise ratio & increases power to detect an association
- Typically, one or a combination of annotations are used for filtering
  - Example: Within a gene keep variants that
    a) Are frameshift mutations or
    b) Overlap Genomic Evolutionary Rate Profiling (GERP) score > 0 or
    c) Overlap with transcript factor binding sites in blood cells
- Quantitative variants can be used as weights
- It is hard to predict the best filtering strategy
- A very active area of research

Filtering variants

- Generate variants sets with one or more filtering approach
- Evaluate the distributions of variants within each aggregation unit
- Can choose a less-, medium- and more-stringent filtered set



Less stringent

More Stringent

Example coding variant based aggregation & filtering strategy

- Grouping unit
  - GENCODE genes

- Filters:
  - Keep only loss of function variants (Frameshift, stop-gain, stop-loss, splice variants)
  - FATHMM-MKL>=0.5

- Functional Analysis Through Hidden Markov Models -Multiple Kernel Learning (FATHMM-MKL)
  - FATHMM-MKL generates scores predicting functional consequences of both coding and non-coding sequence. FATHMM-MKL is a machine learning approach that integrates functional annotations from ENCODE with nucleotide based sequence conservation measures variants.
  - FATHMM-XF (FATHMM with eXtended Features) represents a substantial improvement over FATHMM-MKL

Dong C, et.al. Hum Mol Genet, 2015
Shihab HA, et.al, Bioinformatics, 2015

Example coding+non-coding variant based aggregation & filtering strategy – tissue agnostic

- Grouping unit
  - GENCODE genes
  - Variants in the enhancer(s) linked to the gene using GeneHancer
  - Variants in the promoter of the gene defined as the region 5kb upstream of TSS^ & GeneHancer

- Filters:
  - Keep only loss of function variants (Frameshift, stop-gain, stop-loss, splice variants) or
  - Variants with FATHMM-MKL>=0.5 and MAF<=1% or
  - Overlaps with “Ensembl_Regulatory_Build_Overviews”

Ensembl_Regulatory_Build_Overviews

- genome segment prediction based on 17 cell types from ENCODE and Roadmap.
- ctcf - CTCF binding sites,
- distal - Predicted enhancers
- open - Unannotated open chromatin regions
- proximal - Predicted promoter flanking regions
- tfbs - Unannotated transcription factor binding sites
- tss - Predicted promoters

Example coding+non-coding variant based aggregation & filtering strategy – tissue specific

- Grouping unit
  - GENCODE genes
  - Variants in the enhancer(s) linked to the gene using GeneHancer
  - Variants in the promoter of the gene defined as the region 5kb upstream of TSS^ & GeneHancer

- Filters:
  - Keep only loss of function variants (Frameshift, stop-gain, stop-loss, splice variants) or
  - Variants with FATHMM-MKL>=0.5 & Overlaps with either H3K4me3 or H3K4me1 enriched regions) & DNaseI hypersensitivity sites in k562 cells

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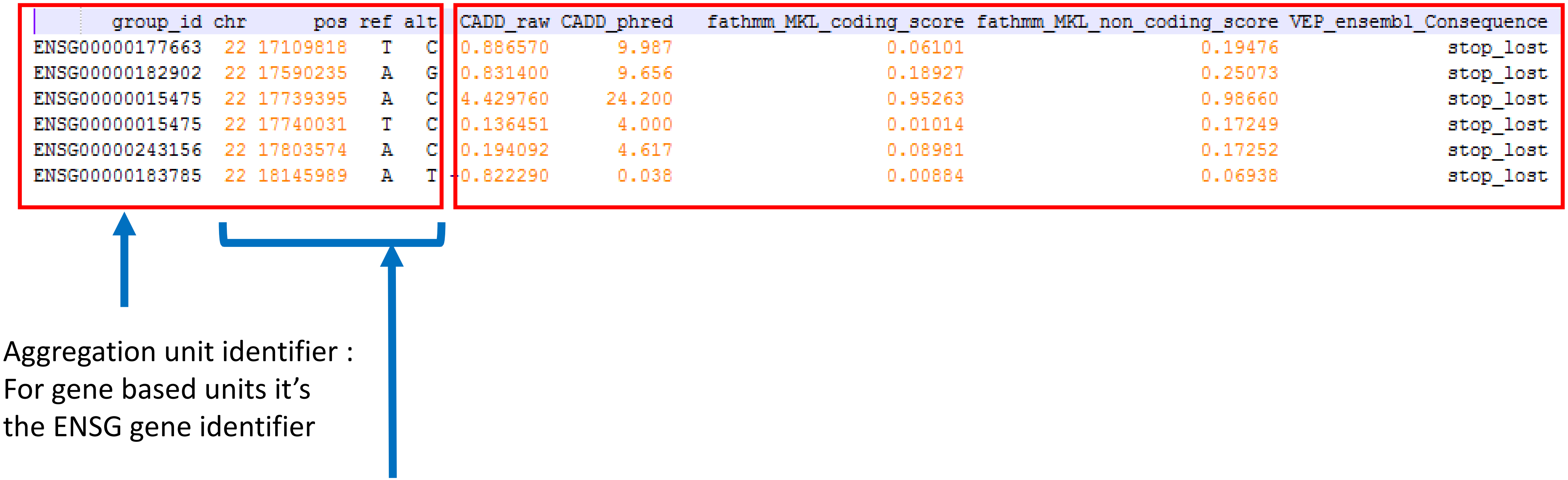
Grouped sets of filtered list of variants are stored in “variant grouping files”

- Variant-level grouping file example from the TOPMed DCC pipeline
- Variants aggregated over gene and filtered to keep Loss of Function variants

Required fields

Additional annotation fields



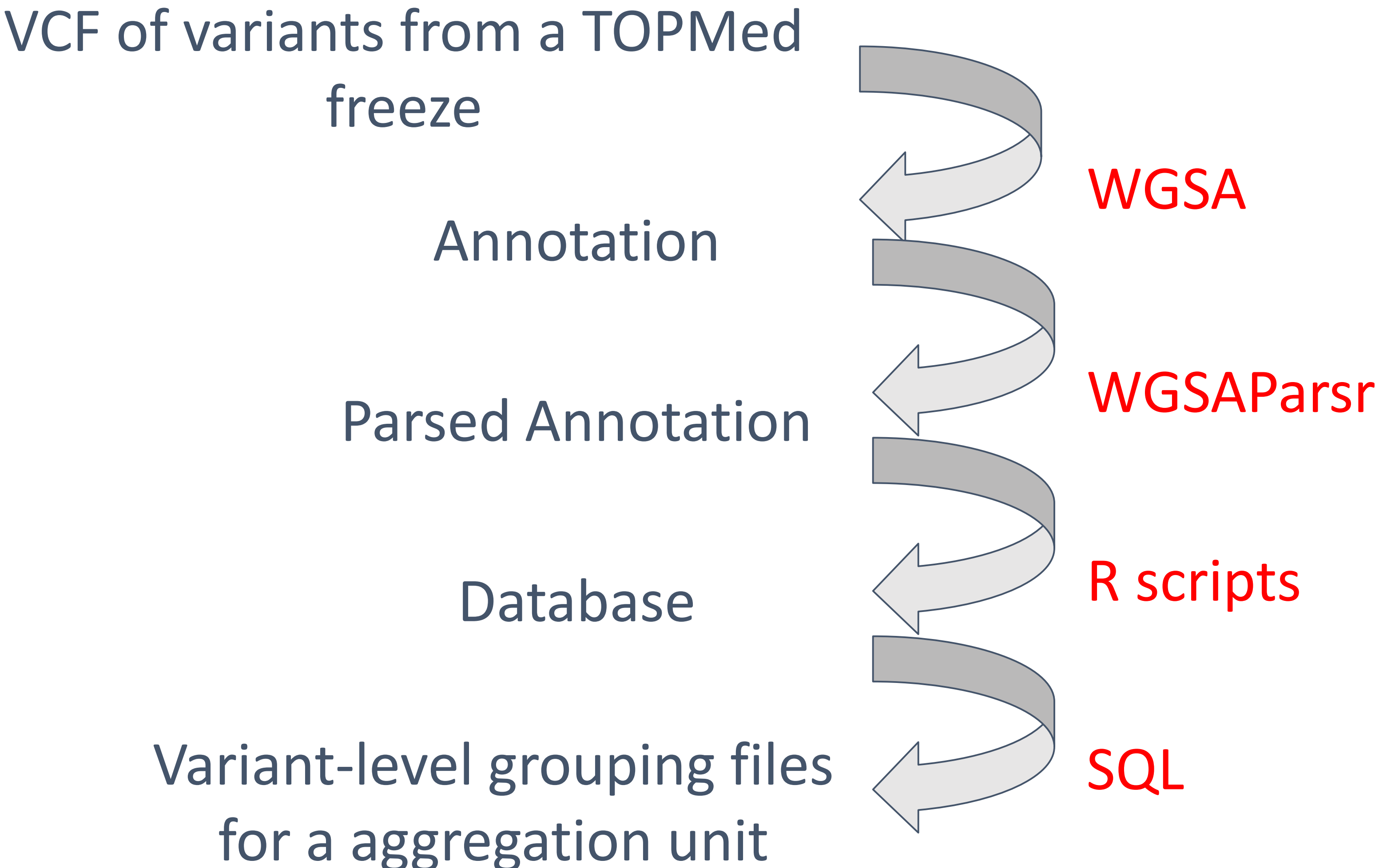
group_id	chr	pos	ref	alt	CADD_raw	CADD_phred	fathmm_MKL_coding_score	fathmm_MKL_non_coding_score	VEP_ensembl_Consequence
ENSG00000177663	22	17109818	T	C	0.886570	9.987	0.06101	0.19476	stop_lost
ENSG00000182902	22	17590235	A	G	0.831400	9.656	0.18927	0.25073	stop_lost
ENSG00000015475	22	17739395	A	C	4.429760	24.200	0.95263	0.98660	stop_lost
ENSG00000015475	22	17740031	T	C	0.136451	4.000	0.01014	0.17249	stop_lost
ENSG00000243156	22	17803574	A	C	0.194092	4.617	0.08981	0.17252	stop_lost
ENSG00000183785	22	18145989	A	T	0.822290	0.038	0.00884	0.06938	stop_lost

Aggregation unit identifier :
For gene based units it’s
the ENSG gene identifier

Variant information

Workflow for generating variant grouping files in TOPMed

- In the TOPMed DCC pipeline filtered aggregation units are passed to the pipeline as variant grouping files



VCF of variants from a TOPMed freeze

WGSA

Annotation

WGSAParsr

Parsed Annotation

R scripts

Database

SQL

Variant-level grouping files for a aggregation unit

Working with variant-level grouping files

TOPMed DCC analysis Pipeline:

- The variant-level grouping file are .Rdata files which can be used with the DCC analysis pipeline[1] directly
- File name is passed as a value to the config parameter “variant_group_file”

GENESIS :

- variant-level grouping files can be processed using the function TopmedPipeline[2]::aggregateGRangesList to produce a format suitable for GENESIS[3] function assocTestAggregate
- See scripts below for an example of implementation
    - https://github.com/UW-GAC/analysis_pipeline/blob/devel/R/aggregate_list.R
    - https://github.com/UW-GAC/analysis_pipeline/blob/devel/R/assoc_aggregate.R

[1] https://github.com/UW-GAC/analysis_pipeline,

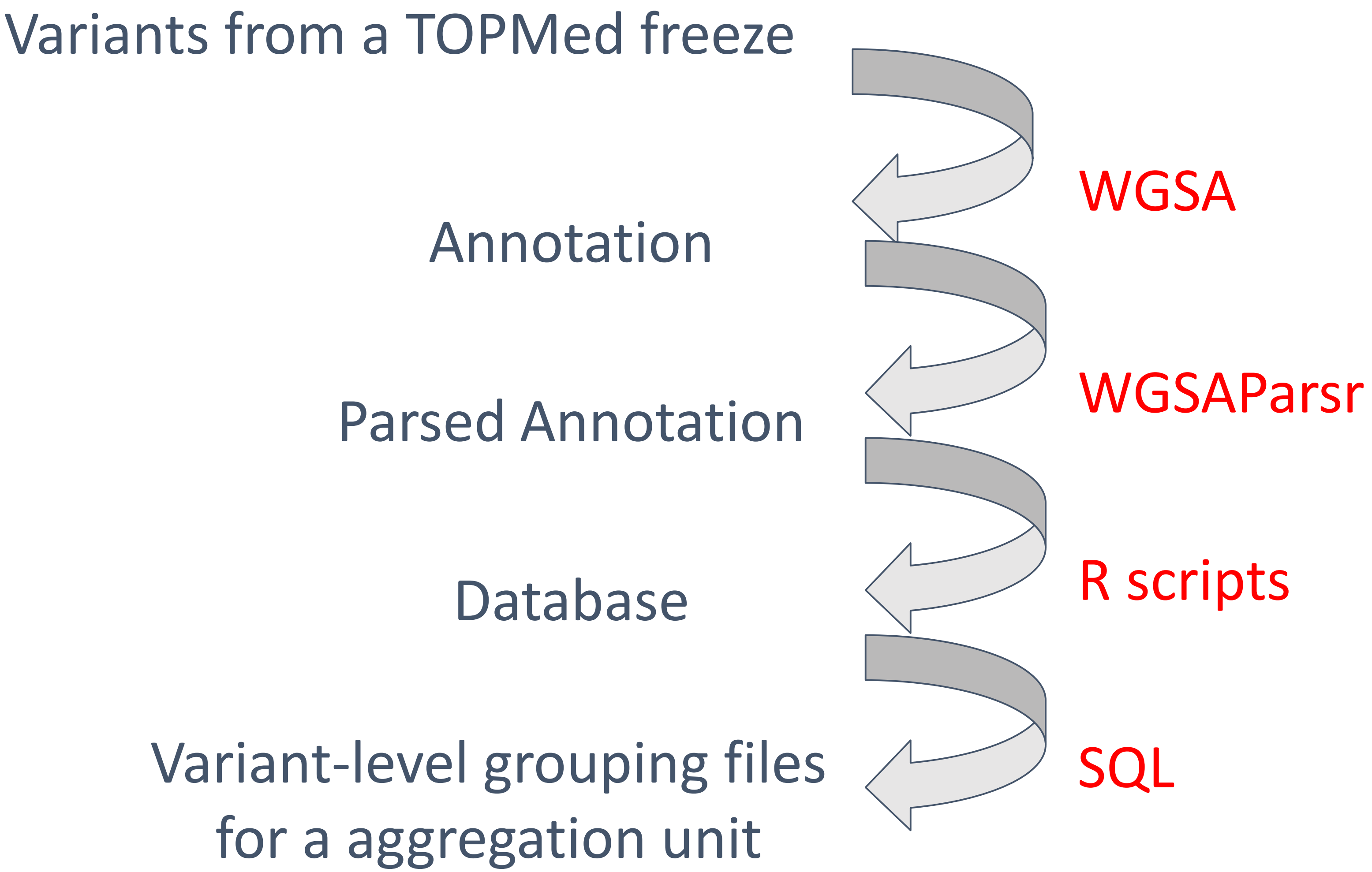
[2] https://github.com/UW-GAC/analysis_pipeline/tree/devel/TopmedPipeline,

[3] https://github.com/smgogarten/GENESIS

Section I : Outline

- What are variant annotations?
- Applications of annotations in rare variant association testing
- How to obtain annotations ?
- Approaches for aggregating and filtering variants for rare variant association testing
- Generating variant grouping files for conducting rare-variant aggregate test
- WGSAparsr

Workflow for generating variant grouping files in TOPMed



Variants from a TOPMed freeze

WGSA

Annotation

WGSAParsr

Parsed Annotation

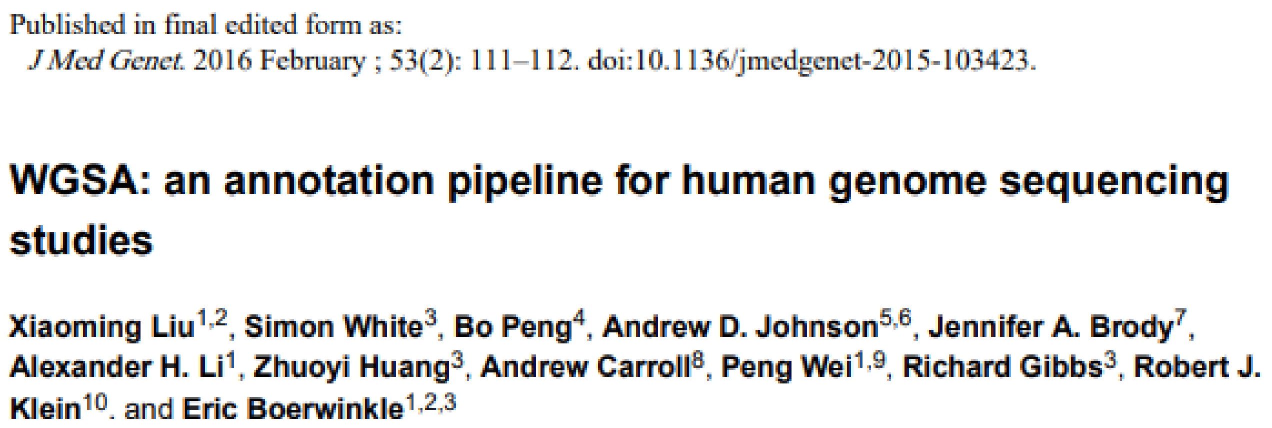
R scripts

Database

SQL

Variant-level grouping files
for a aggregation unit

WGSA



Published in final edited form as:
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WGSA: an annotation pipeline for human genome sequencing studies

Xiaoming Liu[1,2], Simon White[3], Bo Peng[4], Andrew D. Johnson[5,6], Jennifer A. Brody[7], Alexander H. Li[1], Zhuoyi Huang[3], Andrew Carroll[8], Peng Wei[1,9], Richard Gibbs[3], Robert J. Klein[10], and Eric Boerwinkle[1,2,3]

- Built and maintained by Xiaoming Liu
- https://sites.google.com/site/jpopgen/wgsa
- The latest TOPMed freeze has ~1 billion variants
- The WGSA files are 1 TB

WGSA annotations are complex

- Several annotations are compound entries
  - Example : **VEP_ensembl_Transcript_ID**
    - ENST00000456328|ENST00000488147|ENST00000438504|ENST00000515242|ENST00000541675|ENST00000423562|ENST00000450305|ENST00000538476|ENST00000518655
- Some annotations for indels are derived from pseudo-SNVs & have multiple values
  - Example : GERP score
    - indel: chr1:12729:GAGAGT: G
    - GERP score : .{1}-0.824{1}-0.943{1}0.472{2}
- We often only work with only subset of the annotations

Gene-based annotations in WGSA output are at transcript level

chr:10273 T>C

VEP_ensembl_Transcript_ID

ENST00000456328|ENST00000488147|ENST00000438504|ENST00000515242|ENST00000541675|ENST00000423562|ENST00000450305|ENST00000538476|ENST00000518655

VEP_ensembl_Consequence

upstream_gene_variant|downstream_gene_variant|downstream_gene_variant|upstream_gene_variant|downstream_gene_variant|downstream_gene_variant|upstream_gene_variant|downstream_gene_variant|*splice_region_variant*

VEP_ensembl_Gene_Name

DDX11L1|WASH7P|WASH7P|DDX11L1|WASH7P|WASH7P|DDX11L1|WASH7P|DDX11L1

VEP_ensembl_Gene_ID

ENSG00000223972|ENSG00000227232|ENSG00000227232|ENSG00000223972|ENSG00000227232|ENSG00000227232|ENSG00000223972|ENSG00000227232|ENSG00000223972

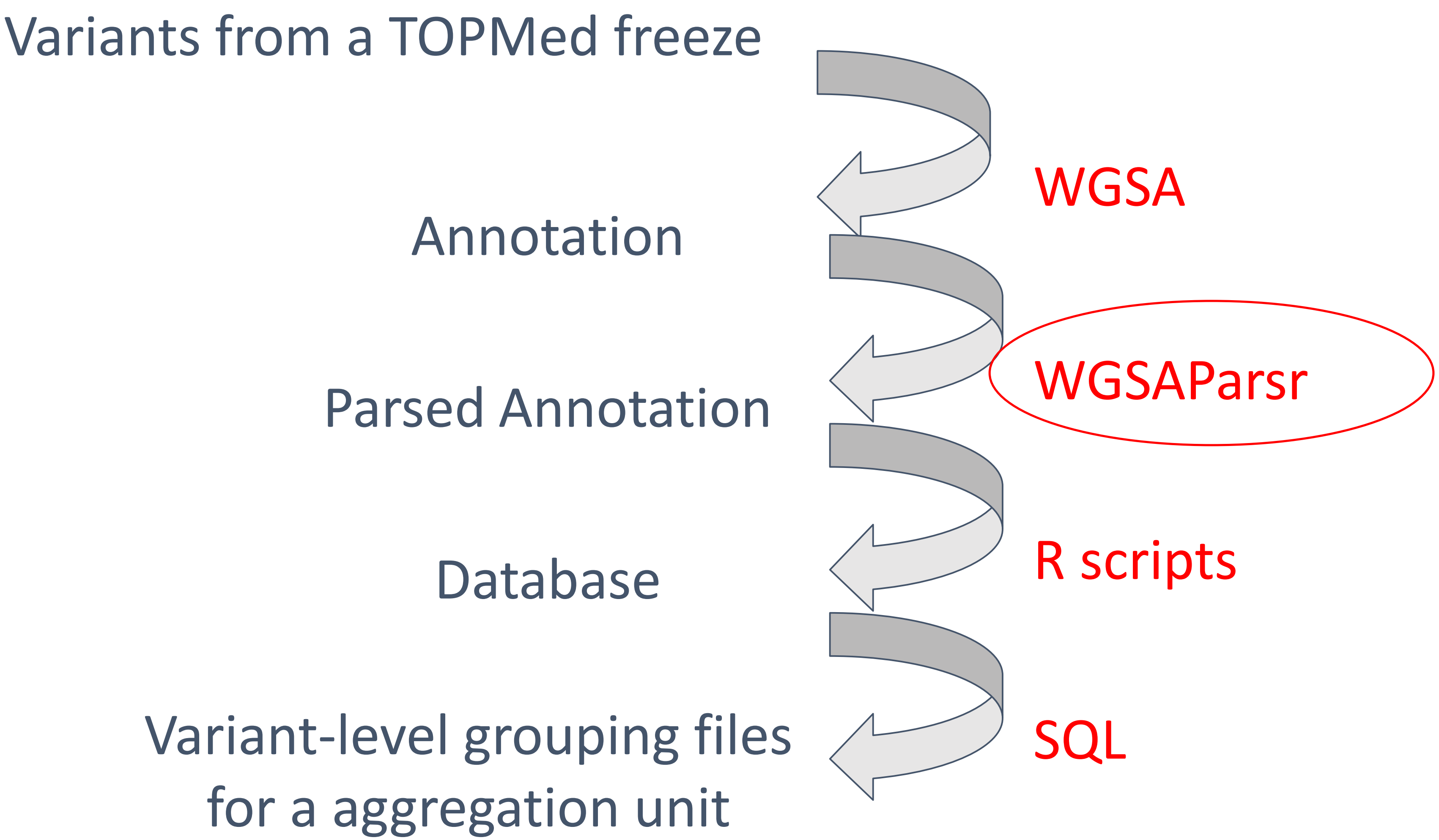
Ensembl_Regulatory_Build_Overviews

ctcf

VEP_ensembl_LoF

.|.|.|.|.|.|.|.|HC

Workflow for generating variant grouping files in TOPMed



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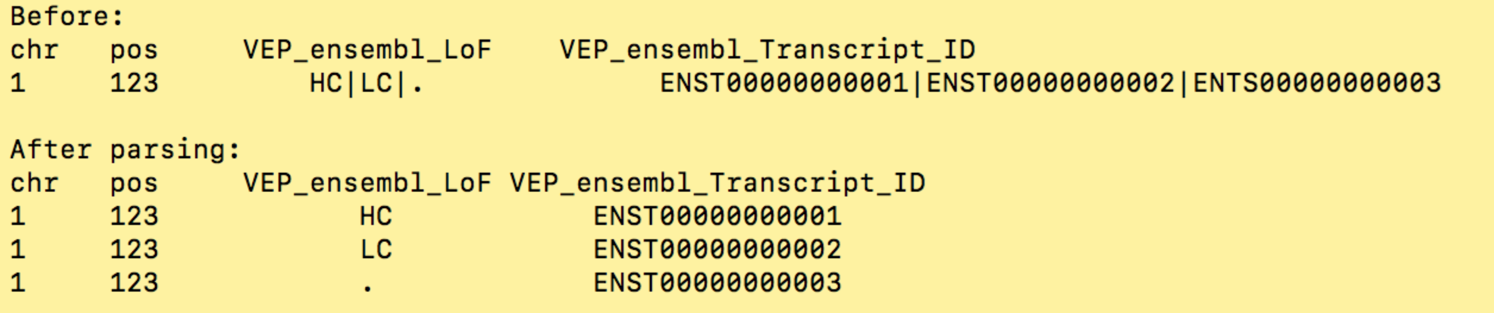
WGSAparsr

- We need to simplify the WGSA output so that we can easily parse these complex annotation files

- **WGSAparsr**: an R package by the Ben Heavner (TOPMed DCC)
  - https://github.com/UW-GAC/wgsaparsr

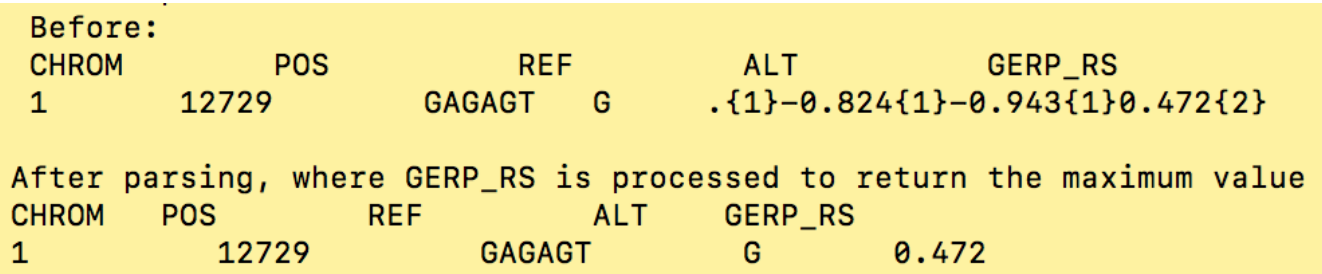
WGSAParsr operations

1. Selecting fields
2. Renaming fields
3. Simplifying fields
   a. Pivoting



```
Before:
chr    pos      VEP_ensembl_LoF     VEP_ensembl_Transcript_ID
1      123          HC|LC|.               ENST00000000001|ENST00000000002|ENTS00000000003

After parsing:
chr    pos      VEP_ensembl_LoF VEP_ensembl_Transcript_ID
1      123               HC             ENST00000000001
1      123               LC             ENST00000000002
1      123               .              ENST00000000003
```

   a. Selecting values

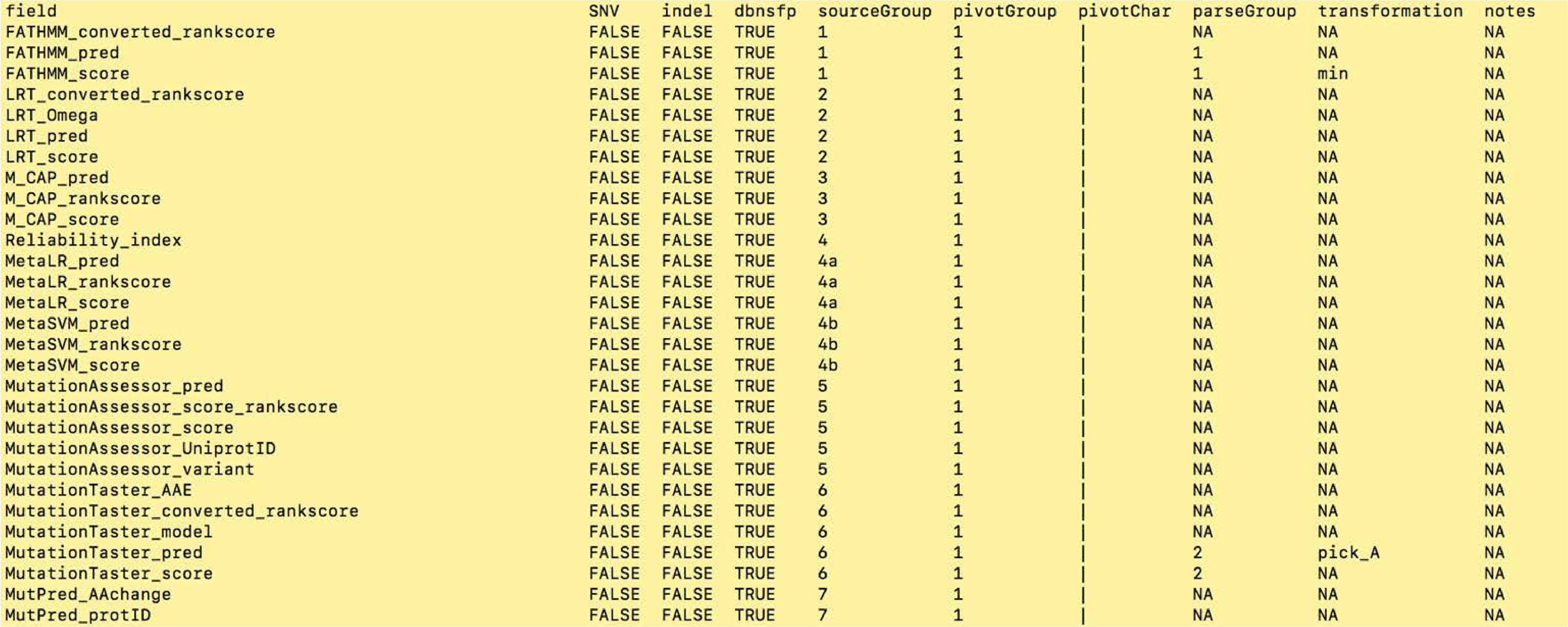


```
 Before:
 CHROM          POS           REF          ALT           GERP_RS
 1        12729        GAGAGT    G      .{1}-0.824{1}-0.943{1}0.472{2}

After parsing, where GERP_RS is processed to return the maximum value
CHROM    POS          REF          ALT      GERP_RS
1           12729          GAGAGT          G         0.472
```

Using WGSAParsr

```
# parse snv and dbnsfp:

parse_to_file(source_file = snv_source_file,

             destination = snv_destination,

             dbnsfp_destination = dbnsfp_destination,

             config = config,

             freeze = 5,

             chunk_size = 1000,

             verbose = TRUE)
```

Using WGSAParsr - Configuration file



```
field                               SNV    indel  dbnsfp  sourceGroup  pivotGroup  pivotChar  parseGroup  transformation  notes
FATHMM_converted_rankscore          FALSE  FALSE  TRUE    1            1           |          NA          NA              NA
FATHMM_pred                         FALSE  FALSE  TRUE    1            1           |          1           NA              NA
FATHMM_score                        FALSE  FALSE  TRUE    1            1           |          1           min             NA
LRT_converted_rankscore             FALSE  FALSE  TRUE    2            1           |          NA          NA              NA
LRT_Omega                           FALSE  FALSE  TRUE    2            1           |          NA          NA              NA
LRT_pred                            FALSE  FALSE  TRUE    2            1           |          NA          NA              NA
LRT_score                           FALSE  FALSE  TRUE    2            1           |          NA          NA              NA
M_CAP_pred                          FALSE  FALSE  TRUE    3            1           |          NA          NA              NA
M_CAP_rankscore                     FALSE  FALSE  TRUE    3            1           |          NA          NA              NA
M_CAP_score                         FALSE  FALSE  TRUE    3            1           |          NA          NA              NA
Reliability_index                   FALSE  FALSE  TRUE    4            1           |          NA          NA              NA
MetaLR_pred                         FALSE  FALSE  TRUE    4a           1           |          NA          NA              NA
MetaLR_rankscore                    FALSE  FALSE  TRUE    4a           1           |          NA          NA              NA
MetaLR_score                        FALSE  FALSE  TRUE    4a           1           |          NA          NA              NA
MetaSVM_pred                        FALSE  FALSE  TRUE    4b           1           |          NA          NA              NA
MetaSVM_rankscore                   FALSE  FALSE  TRUE    4b           1           |          NA          NA              NA
MetaSVM_score                       FALSE  FALSE  TRUE    4b           1           |          NA          NA              NA
MutationAssessor_pred               FALSE  FALSE  TRUE    5            1           |          NA          NA              NA
MutationAssessor_score_rankscore    FALSE  FALSE  TRUE    5            1           |          NA          NA              NA
MutationAssessor_score              FALSE  FALSE  TRUE    5            1           |          NA          NA              NA
MutationAssessor_UniprotID          FALSE  FALSE  TRUE    5            1           |          NA          NA              NA
MutationAssessor_variant            FALSE  FALSE  TRUE    5            1           |          NA          NA              NA
MutationTaster_AAE                  FALSE  FALSE  TRUE    6            1           |          NA          NA              NA
MutationTaster_converted_rankscore  FALSE  FALSE  TRUE    6            1           |          NA          NA              NA
MutationTaster_model                FALSE  FALSE  TRUE    6            1           |          NA          NA              NA
MutationTaster_pred                 FALSE  FALSE  TRUE    6            1           |          2           pick_A          NA
MutationTaster_score                FALSE  FALSE  TRUE    6            1           |          2           NA              NA
MutPred_AAchange                    FALSE  FALSE  TRUE    7            1           |          NA          NA              NA
MutPred_protID                      FALSE  FALSE  TRUE    7            1           |          NA          NA              NA
```

Documented in ?wgsaparsr::load_config()

Overview of variant annotation session

Section I : Thursday + Friday (instructional part)

- What are variant annotations?
- Applications of annotations in rare variant association testing
- How to obtain annotations ?
- Approaches for aggregating and filtering variants for rare variant association testing
- Generating variant grouping files for conducting rare-variant aggregate test
- WGSAparsr

Section II: Friday (hands–on part)

- Parsing WGSA files using WGSAparsr
- Generate variant grouping files
- Association testing in aggregation units using variant grouping files

Key libraries and functions

<table>
<tr><th colspan="2">Parse the WGSA annotation file</th></tr>
<tr><td>library (wgsaparsr)</td><td>Package for working with WGSA output files</td></tr>
<tr><td>get_fields()</td><td>List the annotation fields available in a WGSA output file</td></tr>
<tr><td>parse_to_file()</td><td>Parses WGSA output files using selection and transformation defined in configuration file</td></tr>
<tr><td>load_config()</td><td>Load a configuration file to an R data frame</td></tr>
<tr><th colspan="2">Aggregate variants by genic units and create input file for association testing</th></tr>
<tr><td colspan="2">- R code for the workshop
- DCC uses a MySQL server for creating and filtering variant list in aggregation units using WGSA annotations</td></tr>
</table>