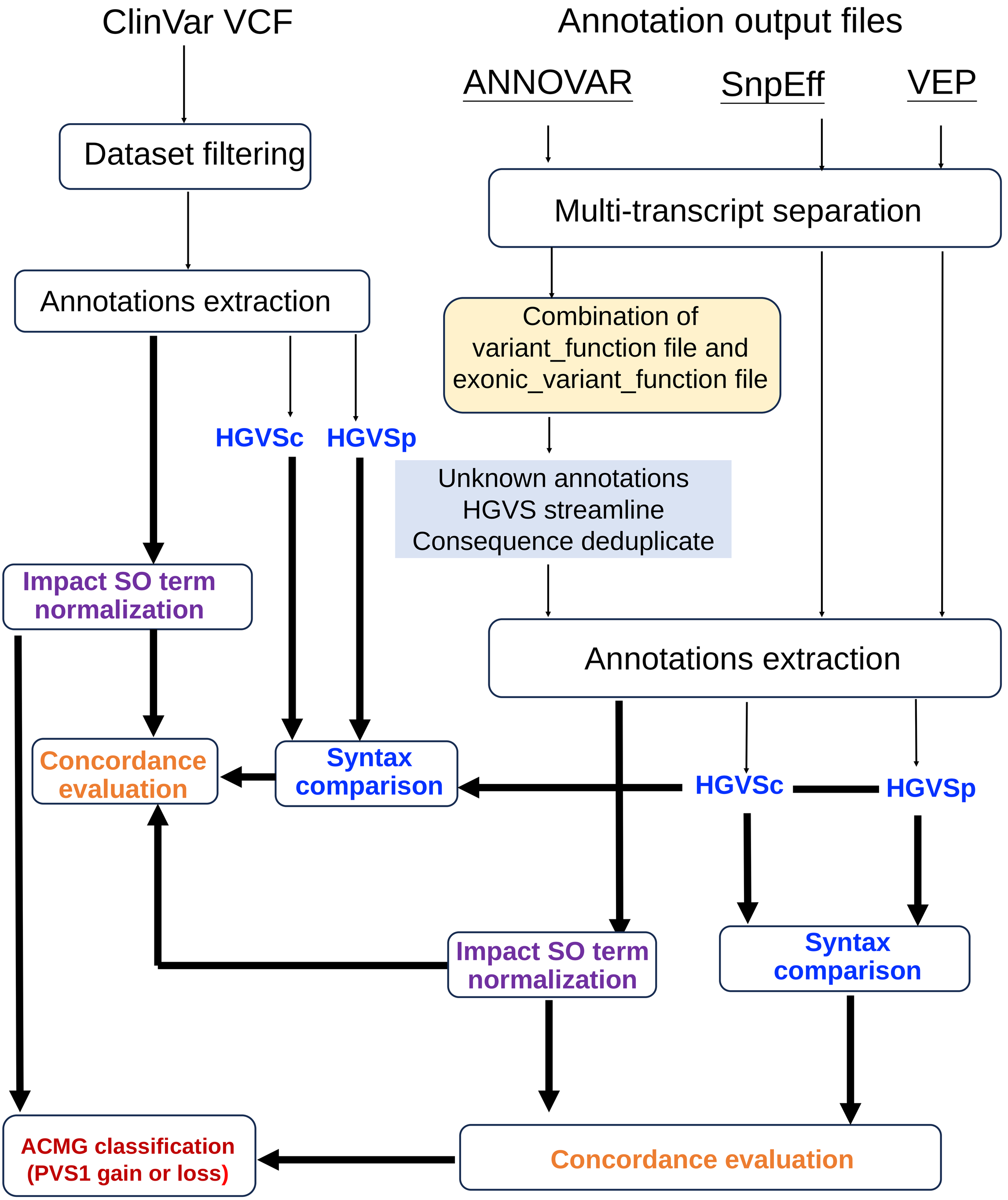
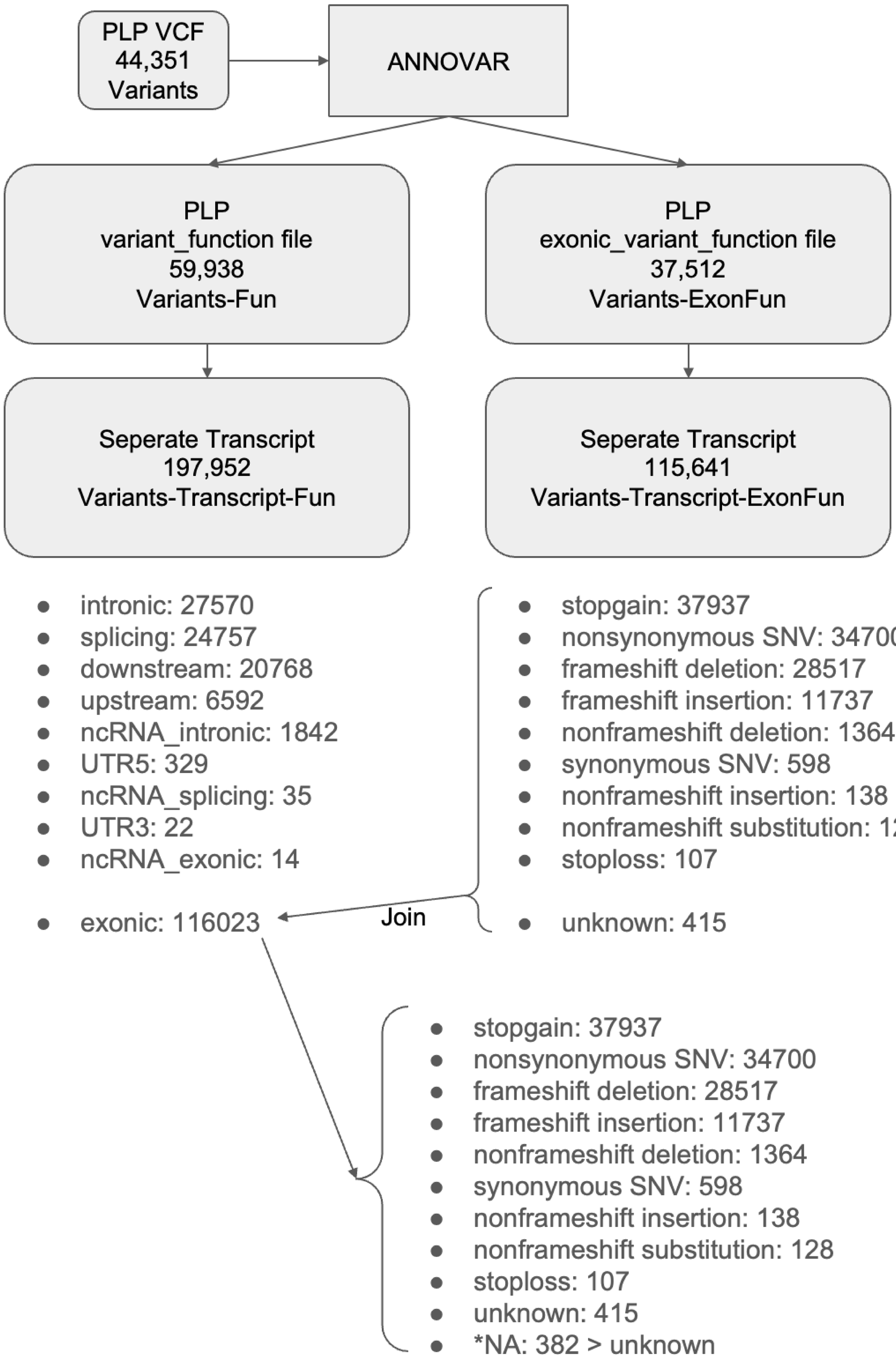
Procedure of Data Process and Evaluation



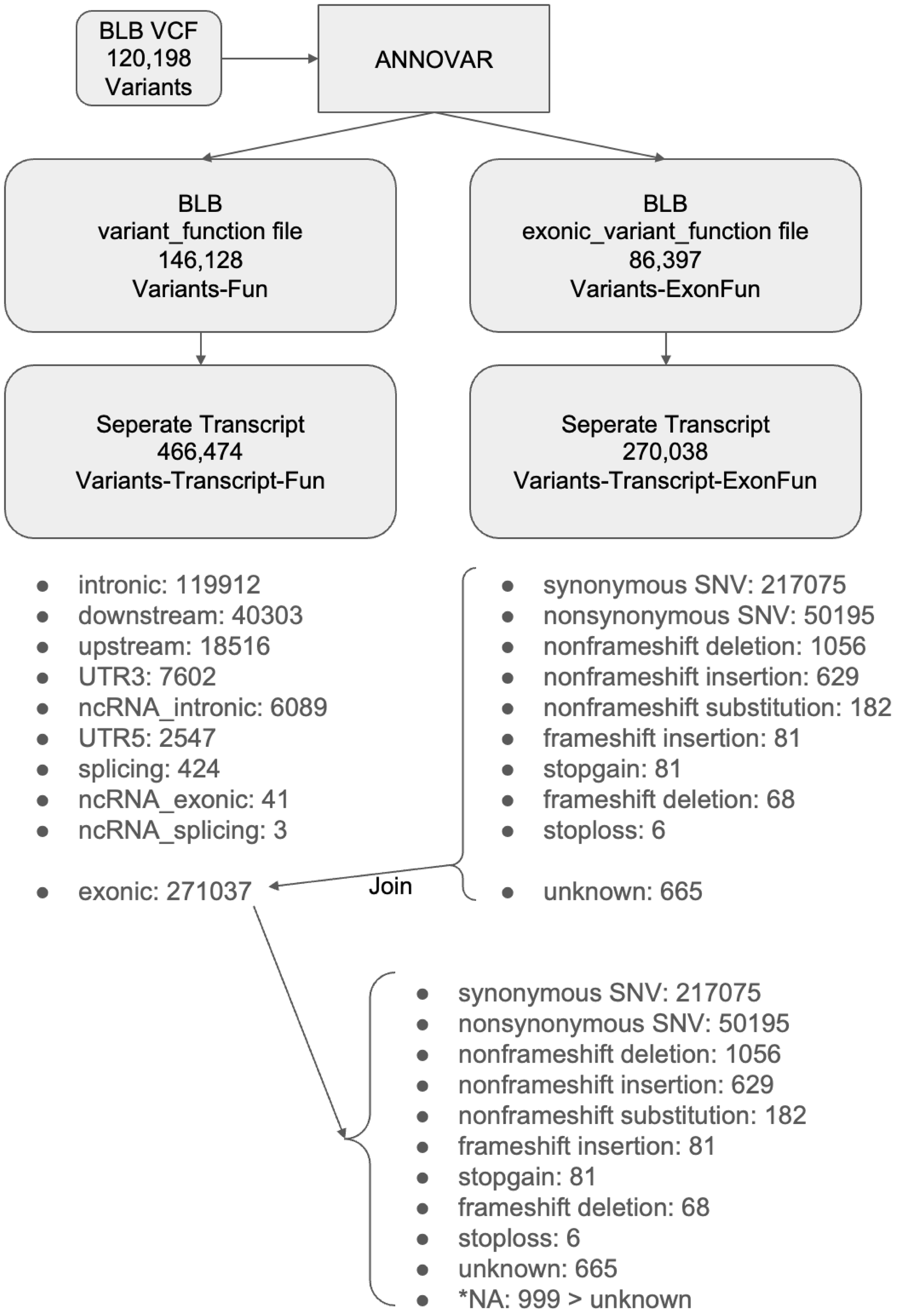
VCF: Variant Call Format; HGVSc: HGVS coding sequence name; HGVSp: HGVS protein sequence name; SO: Sequence Ontology.

ANNOVAR annotation output file process (PLP variants)



VCF: Variant Call Format; PLP: Pathogenic or Likely-Pathogenic; BLB: Benign or Likely Benign;Variants-Transcript-Fun: The functional impact of genomic variants on specific transcripts; Variants-Transcript-ExonFun: The functional impact of exonic variants on specific transcripts; NA: Not applicable

ANNOVAR annotation output file process (BLB variants)



VCF: Variant Call Format; PLP: Pathogenic or Likely-Pathogenic; BLB: Benign or Likely Benign;Variants-Transcript-Fun: The functional impact of genomic variants on specific transcripts; Variants-Transcript-ExonFun: The functional impact of exonic variants on specific transcripts; NA: Not applicable

ANNOVAR variant_function file process

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
1	183227698	G	T	intronic	NM_005562,NM_018891
3	48576771	C	G	downstream	NR_031756(dist=2131)
4	1001564	G	A	splicing	NM_000203(NM_000203:exon5:c.589+1G>A),NM_001363576(NM_001363576:exon4:c.193+1G>A)
4	110618699	T	C	upstream	NR_147203,NR_147204(dist=3241)
21	46366954	CGAGCAGA	C	exonic	NM_001315529,NM_006031

Seperate Transcript (Row)

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
1	183227698	G	T	intronic	NM_005562
1	183227698	G	T	intronic	NM_018891
3	48576771	C	G	downstream	NR_031756(dist=2131)
4	1001564	G	A	splicing	NM_000203(NM_000203:exon5:c.589+1G>A)
4	1001564	G	A	splicing	NM_001363576(NM_001363576:exon4:c.193+1G>A)
4	110618699	T	C	upstream	NR_147203
4	110618699	T	C	upstream	NR_147204(dist=3241)
21	46366954	CGAGCAGA	C	exonic	NM_001315529
21	46366954	CGAGCAGA	C	exonic	NM_006031

Extract HGVSc

Chr	Start	Ref	Alt	Transcript	Consequence	HGVSc
1	183227698	G	T	NM_005562	intronic	
1	183227698	G	T	NM_018891	intronic	
3	48576771	C	G	NR_031756	downstream	dist=2131
4	1001564	G	A	NM_000203	splicing	c.589+1G>A
4	1001564	G	A	NM_001363576	splicing	c.193+1G>A
4	110618699	T	C	NR_147203	upstream	
4	110618699	T	C	NR_147204	upstream	dist=3241
21	46366954	CGAGCAGACTTT	C	NM_001315529	exonic	
21	46366954	CGAGCAGACTTT	C	NM_006031	exonic	

Seperated variant_function file

ANNOVAR exonic_variant_function file process

Chr	Start	Ref	Alt	ExonicFunc_refGene	AAChange_refGene
21	46366954	CGAGCAGACTTT	C	frameshift deletion	PCNT:NM_001315529:exon15:c.2627_2637del:p.A877Gfs*59,PCNT:NM_006031:exon15:c.2981_2991del:p.A995Gfs*59,

Seperate Transcript (Row)

Chr	Start	Ref	Alt	ExonicFunc_refGene	AAChange_refGene
21	46366954	CGAGCAGACTTT	C	frameshift deletion	PCNT:NM_001315529:exon15:c.2627_2637del:p.A877Gfs*59
21	46366954	CGAGCAGACTTT	C	frameshift deletion	PCNT:NM_006031:exon15:c.2981_2991del:p.A995Gfs*59

Extract Symbol, HGVSc, HGVSp

Chr	Start	Ref	Alt	Transcript	Symbol	Consequence	HGVSc	HGVSp
21	46366954	CGAGCAGACTTT	C	NM_001315529	PCNT	frameshift deletion	c.2627_2637del	p.A877Gfs*59
21	46366954	CGAGCAGACTTT	C	NM_006031	PCNT	frameshift deletion	c.2981_2991del	p.A995Gfs*59

Seperated exonic_variant_function file

ANNOVAR variant_function and exonic_variant_function file merge

Chr	Start	Ref	Alt	Transcript	Consequence	HGVSc
1	183227698	G	T	NM_005562	intronic	
1	183227698	G	T	NM_018891	intronic	
3	48576771	C	G	NR_031756	downstream	dist=2131
4	1001564	G	A	NM_000203	splicing	c.589+1G>A
4	1001564	G	A	NM_001363576	splicing	c.193+1G>A
4	110618699	T	C	NR_147203	upstream	
4	110618699	T	C	NR_147204	upstream	dist=3241
21	46366954	CGAGCAGACTTT	C	NM_001315529	exonic	
21	46366954	CGAGCAGACTTT	C	NM_006031	exonic	

Seperated variant_function file

Chr	Start	Ref	Alt	Transcript	Symbol	Consequence	HGVSc	HGVSp
21	46366954	CGAGCAGACTTT	C	NM_001315529	PCNT	frameshift deletion	c.2627_2637del	p.A877Gfs*59
21	46366954	CGAGCAGACTTT	C	NM_006031	PCNT	frameshift deletion	c.2981_2991del	p.A995Gfs*59

Add Add

Seperated exonic_variant_function file

Merge

Chr	Start	Ref	Alt	Transcript	Symbol	Consequence	HGVSc	HGVSp
1	183227698	G	T	NM_005562		intronic		
1	183227698	G	T	NM_018891		intronic		
3	48576771	C	G	NR_031756		downstream	dist=2131	
4	1001564	G	A	NM_000203		splicing	c.589+1G>A	
4	1001564	G	A	NM_001363576		splicing	c.193+1G>A	
4	110618699	T	C	NR_147203		upstream		
4	110618699	T	C	NR_147204		upstream	dist=3241	
21	46366954	CGAGCAGACTTT	C	NM_001315529	PCNT	frameshift deletion	c.2627_2637del	p.A877Gfs*59
21	46366954	CGAGCAGACTTT	C	NM_006031	PCNT	frameshift deletion	c.2981_2991del	p.A995Gfs*59

ANNOVAR Merged Vatiant Table

ANNOVAR unknown annotation

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
9	128219105	C	A	exonic	NM_001005336
9	128219105	C	A	exonic	NM_001288737
9	128219105	C	A	exonic	NM_001288738
9	128219105	C	A	exonic	NM_001288739
9	128219105	C	A	exonic	NM_001374269
9	128219105	C	A	exonic	NM_004408

Seperated variant_function file

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
9	128219105	C	A	unknown	UNKNOWN,

exonic_variant_function file UNKNOWN variant

Merge

Chr	Start	Ref	Alt	Transcript	Symbol	Consequence	HGVSc	HGVSp
9	128219105	C	A	NM_001005336		unknown	UNKNOWN	
9	128219105	C	A	NM_001288737		unknown	UNKNOWN	
9	128219105	C	A	NM_001288738		unknown	UNKNOWN	
9	128219105	C	A	NM_001288739		unknown	UNKNOWN	
9	128219105	C	A	NM_001374269		unknown	UNKNOWN	
9	128219105	C	A	NM_004408		unknown	UNKNOWN	

ANNOVAR HGVS streamline

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
5	53101358	C	T	splicing	NM_004531(NM_004531:exon5:c.377+1G>A),NM_176806(NM_176806:exon5:c.564+1G>A,NM_176806:exon5:UTR3)
9	128325882	G	C	splicing	NM_001305942(NM_001305942:exon3:c.305+1G>C,NM_001305942:exon3:UTR3),NM_016035(NM_016035:exon4:c.402+1G>C)

Seperate Transcript (Row)

Chr	Start	Ref	Alt	Func_refGene	Gene_refGene
5	53101358	C	T	splicing	NM_004531(NM_004531:exon5:c.377+1G>A)
5	53101358	C	T	splicing	NM_176806(NM_176806:exon5:c.564+1G>A,~~NM_176806:exon5:UTR3~~)
9	128325882	G	C	splicing	NM_001305942(NM_001305942:exon3:c.305+1G>C,~~NM_001305942:exon3:UTR3~~)
9	128325882	G	C	splicing	NM_016035(NM_016035:exon4:c.402+1G>C)

Extract 1st. HGVSc

Chr	Start	Ref	Alt	Transcript	Symbol	Consequence	HGVSc	HGVSp
5	53101358	C	T	NM_004531		splicing	c.377+1G>A	
5	53101358	C	T	NM_176806		splicing	c.564+1G>A	
9	128325882	G	C	NM_001305942		splicing	c.305+1G>C	
9	128325882	G	C	NM_016035		splicing	c.402+1G>C	

ANNOVAR consequence deduplicate

key	chr	start	ref	alt	NM	Consequence	HGVSc	Symbol	HGVSp
167569|178004	1	145926947	G	T	NM_005105	intronic	.	.	.
167569|178004	1	145926947	G	T	NR_002328	downstream	.	.	.
167569|178004	1	145926947	G	T	NR_104033	downstream	dist=1606	.	.
167569|178004	1	145926947	G	T	NR_147182	ncRNA_intronic	.	.	.
167569|178004	1	145926947	G	T	NR_147182	ncRNA_splicing	c.116+1G>T	.	.
2049534|2107421	9	127818309	A	G	NM_000118	synonymous SNV	c.1497T>C	ENG	p.P499P
2049534|2107421	9	127818309	A	G	NM_001018078	downstream	.	.	.
2049534|2107421	9	127818309	A	G	NM_001114753	synonymous SNV	c.1497T>C	ENG	p.P499P
2049534|2107421	9	127818309	A	G	NM_001278138	synonymous SNV	c.951T>C	ENG	p.P317P
2049534|2107421	9	127818309	A	G	NM_001288803	downstream	.	.	.
2049534|2107421	9	127818309	A	G	NM_004957	downstream	dist=4228	.	.
2049534|2107421	9	127818309	A	G	NR_136302	ncRNA_intronic	.	.	.
2049534|2107421	9	127818309	A	G	NR_136302	ncRNA_splicing	c.1378-2A>G	.	.
773598|704570	18	31354311	C	T	NM_001942	synonymous SNV	c.2115C>T	DSG1	p.Y705Y
773598|704570	18	31354311	C	T	NR_110788	ncRNA_intronic	.	.	.
773598|704570	18	31354311	C	T	NR_110788	ncRNA_splicing	c.298+1G>A	.	.
773598|704570	18	31354311	C	T	NR_110789	upstream	dist=375	.	.

ClinVar
VariationID+AlleleID
As Join Key

Concordance

key	NM	Consequence	Concordance
167569|178004	NM_005105	intronic	intron_variant
167569|178004	NR_002328	downstream	genic_downstream_transcript_variant
167569|178004	NR_104033	downstream	genic_downstream_transcript_variant
167569|178004	NR_147182	ncRNA_intronic	non_coding_transcript_variant
167569|178004	NR_147182	ncRNA_splicing	non_coding_transcript_variant
2049534|2107421	NM_000118	synonymous SNV	synonymous_variant
2049534|2107421	NM_001018078	downstream	genic_downstream_transcript_variant
2049534|2107421	NM_001114753	synonymous SNV	synonymous_variant
2049534|2107421	NM_001278138	synonymous SNV	synonymous_variant
2049534|2107421	NM_001288803	downstream	genic_downstream_transcript_variant
2049534|2107421	NM_004957	downstream	genic_downstream_transcript_variant
2049534|2107421	NR_136302	ncRNA_intronic	non_coding_transcript_variant
2049534|2107421	NR_136302	ncRNA_splicing	non_coding_transcript_variant
773598|704570	NM_001942	synonymous SNV	synonymous_variant
773598|704570	NR_110788	ncRNA_intronic	non_coding_transcript_variant
773598|704570	NR_110788	ncRNA_splicing	non_coding_transcript_variant
773598|704570	NR_110789	upstream	genic_upstream_transcript_variant

DeDup

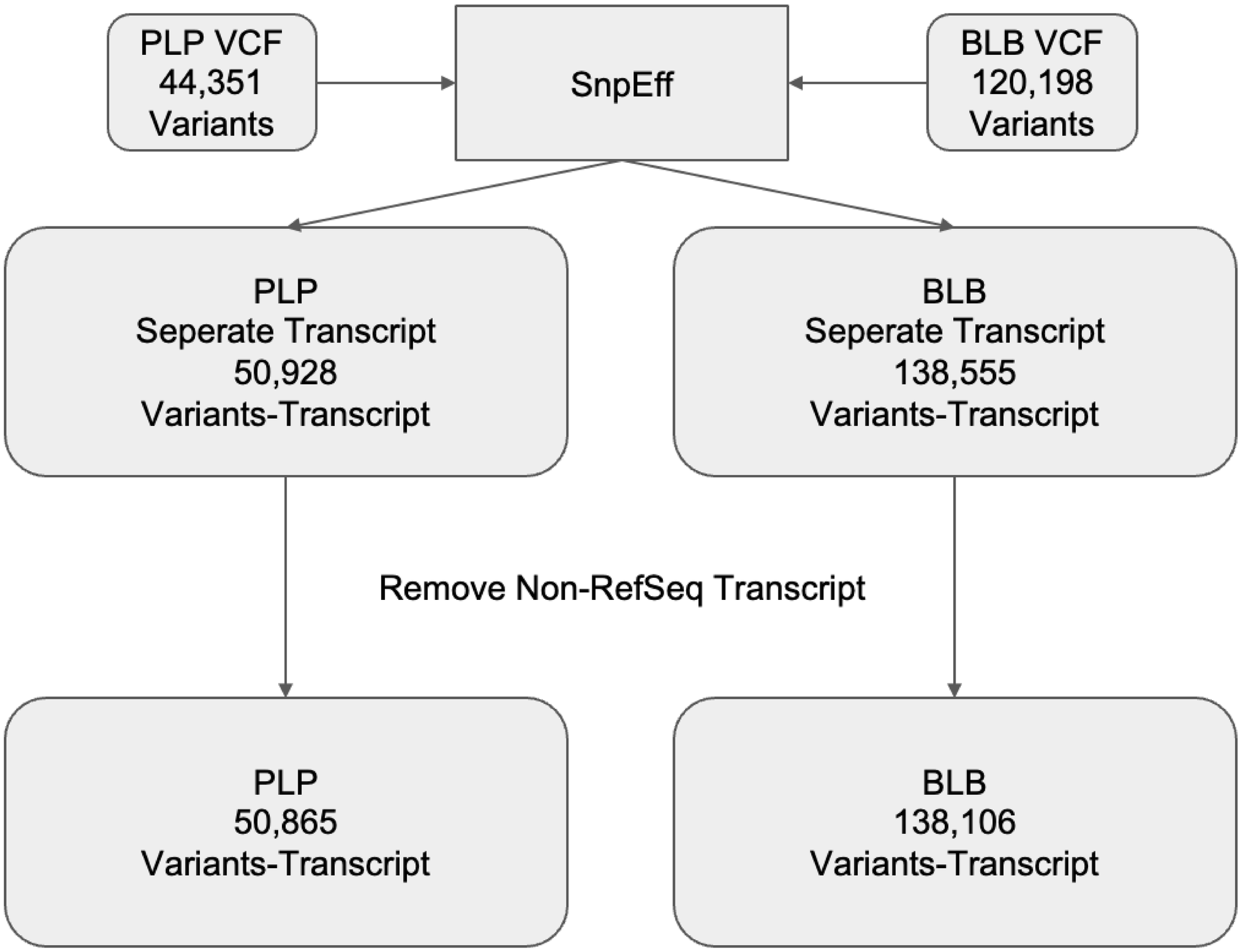
DeDup

DeDup

Concordance DeDup

key	NM	Consequence
167569|178004	NM_005105	intron_variant
167569|178004	NR_002328	genic_downstream_transcript_variant
167569|178004	NR_104033	genic_downstream_transcript_variant
167569|178004	NR_147182	non_coding_transcript_variant
167569|178004	NR_147182	non_coding_transcript_variant
2049534|2107421	NM_000118	synonymous_variant
2049534|2107421	NM_001018078	genic_downstream_transcript_variant
2049534|2107421	NM_001114753	synonymous_variant
2049534|2107421	NM_001278138	synonymous_variant
2049534|2107421	NM_001288803	genic_downstream_transcript_variant

SnpEff annotation output file process



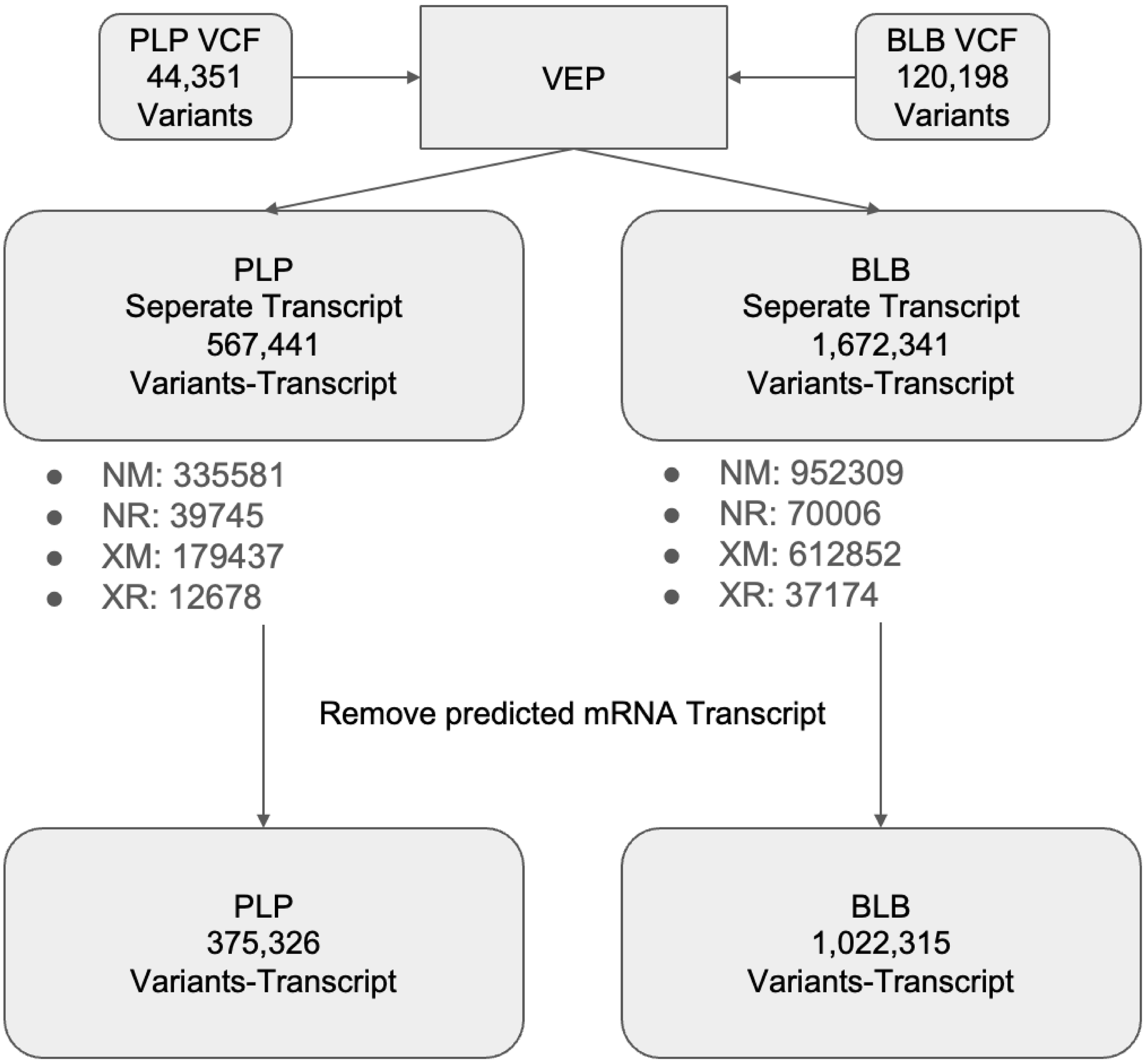
SnpEff intergenic_region variant

Note: For intergenic variant, SnpEff does not select the nearest gene as a representative but instead displays gene symbol of both genes. However, because the Feature_ID is not shown as a transcript, it cannot be used for subsequent join comparisons

Chr	Start	Ref	Alt	Gene_ID	Feature_ID	Feature_Type
7	129774728	G	A	NRF1-UBE2H	NRF1-UBE2H	intergenic_region
8	22161695	G	C	LGI3-SFTPC	LGI3-SFTPC	intergenic_region
11	34916524	GGGC	G	APIP-PDHX	APIP-PDHX	intergenic_region
11	119024925	G	A	TRAPPC4-HYOU1	TRAPPC4-HYOU1	intergenic_region
11	119025252	G	A	TRAPPC4-HYOU1	TRAPPC4-HYOU1	intergenic_region
11	119026921	C	T	TRAPPC4-HYOU1	TRAPPC4-HYOU1	intergenic_region
19	8882863	C	T	MBD3L1-OR1M1	MBD3L1-OR1M1	intergenic_region
22	50697269	T	TG	ARSA-ACR	ARSA-ACR	intergenic_region
22	50706113	C	T	ARSA-ACR	ARSA-ACR	intergenic_region
X	74421488	G	A	ZCCHC13-SLC16A2	ZCCHC13-SLC16A2	intergenic_region

VCF: Variant Call Format; PLP: Pathogenic or Likely-Pathogenic; BLB: Benign or Likely Benign;Variants-Transcript: The variants on specific transcripts

VEP annotation output file process



VCF: Variant Call Format; PLP: Pathogenic or Likely-Pathogenic; BLB: Benign or Likely Benign;Variants-Transcript: The variants on specific transcripts