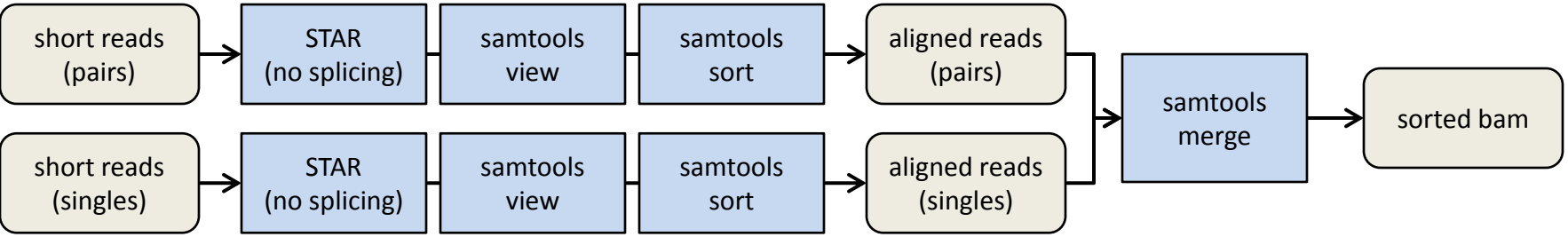
Short read alignment

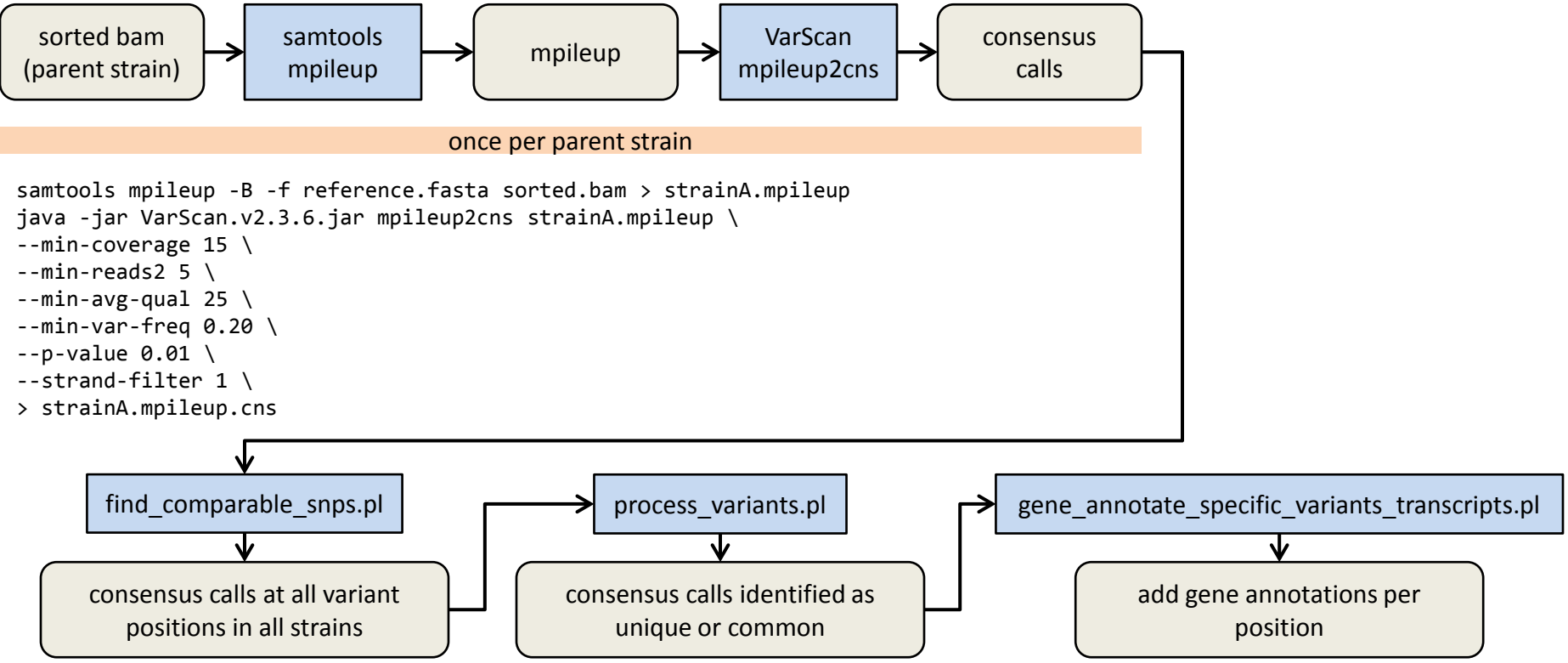



```
## Align paired reads
STAR \
--runMode alignReads \
--genomeLoad LoadAndKeep \
--genomeDir ~/stardb/O.latipes_cDNA \
--readFilesIn /path/to/reads/reads_1.fastq /path/to/reads/reads_2.fastq \
--runThreadN $THREADS \
--outFileNamePrefix paired/ \
--outFilterMismatchNoverLmax 0.05 \
--outFilterMultimapNmax 10  \
--alignIntronMax 20 \ # these two together will
--alignIntronMin 21 \ # disable splicing in STAR
--outSAMattributes All \
--outStd SAM | \
samtools view -S -h -b -u - | samtools sort -@ $THREADS - paired/paired.sorted

## Align single reads
STAR \
--runMode alignReads \
--genomeLoad LoadAndKeep \
--genomeDir ~/stardb/O.latipes_cDNA \
--readFilesIn /path/to/reads/reads_S.fastq \
--runThreadN $THREADS \
--outFileNamePrefix single/ \
--outFilterMismatchNoverLmax 0.05 \
--outFilterMultimapNmax 10  \
--alignIntronMax 20 \ # these two together will
--alignIntronMin 21 \ # disable splicing in STAR
--outSAMattributes All \
--outStd SAM | \
samtools view -S -h -b -u - | samtools sort -@ $THREADS - single/single.sorted

## Merge sorted aligned reads into one bam file
samtools merge merged.sorted.bam paired/paired.sorted.bam single/single.sorted.bam
```

Identify SNPs



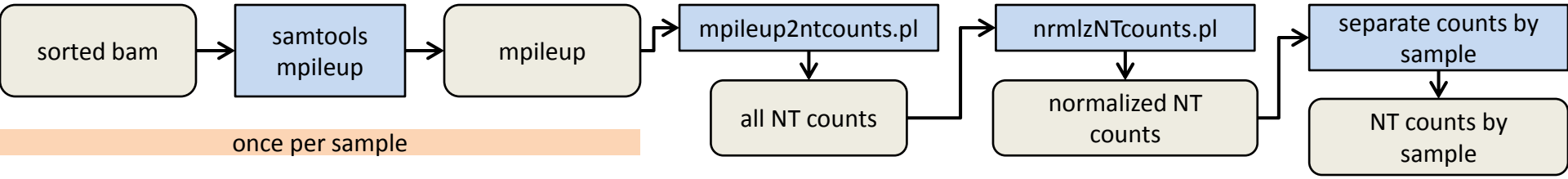

```
samtools mpileup -B -f reference.fasta sorted.bam > strainA.mpileup
java -jar VarScan.v2.3.6.jar mpileup2cns strainA.mpileup \
--min-coverage 15 \
--min-reads2 5 \
--min-avg-qual 25 \
--min-var-freq 0.20 \
--p-value 0.01 \
--strand-filter 1 \
> strainA.mpileup.cns
```

```
# Identify variants in all strains and consensus calls (as IUPAC ambiguity codes) at the same position in all parental strains
find_comparable_snps.pl reference.fasta \
strainA strainA.mpileup.cns \
strainB strainB.mpileup.cns \
strainC strainC.mpileup.cns \
> strainA_strainB_strainC.comp.cns

# Determine whether variants are uniquely found in one or more strains or whether the observed nucleotide at a given
# position and in a given strain are also seen in that position in other strains.
process_variants.pl --consensus-file=strainA_strainB_strainC.comp.cns > strainA_strainB_strainC.specific.variants

# Add annotations identifying ENSEMBL gene ID from ENSEMBL gene annotation in GTF format
gene_annotate_specific_variants_transcripts.pl genes.gtf strainA_strainB_strainC.specific.variants \
> strainA_strainB_strainC.ENSG.specific.variants
```

Normalize for sample size



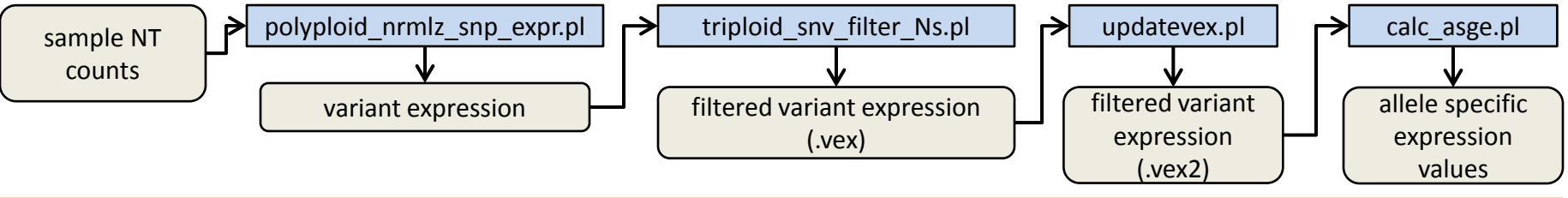

```
# Once for each sample: use samtools to generate an mpileup file from the sorted bam
samtools mpileup -B -f refseq.fasta sample.sorted.bam > sample.mpileup

# Count occurrences of each nucleotide at each position in all samples and report as large tabular matrix
mpileup2ntcounts.pl strainA.mpileup strainB.mpileup strainC.mpileup \
triploidA.mpileup triploidB.mpileup triploidC.mpileup > all.ntcounts

# Normalize counts for sample size
nrmlzNTCounts.pl all.ntcounts > all_nrml_ntcounts2

#Separate normalized counts by sample
perl -e '$a=`head -n 1 all_nrml_ntcounts2`;
chomp $a;
@b=split/\t/,$a;
$n=(@b-2)/4-1;
for $i (0..$n) {
             $s=$i*4+3;
             $e=$s+3;
             $name=$b[$s-1];
             $name=~s/A\.(\S+)/$1/;
             @args=("cut -f ".join(",",1,2,$s..$e)." all_nrml_ntcounts2 > $name.nrml.ntcounts");
             system @args;
}'
```

Integrate normalized NT counts to calculate ASE



once per sample

```
# Determine the expression attributable to each allele from the normalized nucleotide counts and expected nucleotides
#   at each dSNP position identified
polyploid_nrmlz_snp_expr.pl --variants= strainA_strainB_strainC.specific.variants --ntcounts=sample.nrml.ntcounts

# Filter out less informative nucleotide positions (0 counts, completely ambiguous, all NTs same, no expression expected)
triploid_snv_filter_Ns.pl --N --nonrep-zero --uncertain --all-same sample.vex > sample.f.vex

# Convert format
updatevex.pl sample.f.vex > sample.f.vex2

# Combine data from useful variant sites into per-gene allele specific expression values.
calc_asge.pl --vex2=sample.f.vex2
```