SNV examination

Nadzeya Boyeva

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Imports

Import VCF files: gen_vcf1 – VCF retrieved from genomic data, sample 1; gen_vcf2 – VCF retrieved from genomic data, sample 2; gen_merged_vcf – genomic data, sample 1 and 2 merged on alignment step; nas_vcf1 – nascent RNA data, sample 1; nas_vcf2 – nascent RNA data, sample 2; nas_vcf3 – nascent RNA data, sample 3; nas_vcf_merged – nascent RNA data, sample 1, 2 and 3 merged on alignment step.

```
fd <- '/home/nadzeya/praktika/'
gen_vcf1_path = file.path(fd, "gen1_snps_f.vcf.gz")
gen_vcf2_path = file.path(fd, "gen2_snps_f.vcf.gz")
gen_merged_vcf_path <- file.path(fd, "gen_merged_snps_f.vcf.gz")
nas_vcf1_path <- file.path(fd, "nas1_snps_f.vcf.gz")
nas_vcf2_path <- file.path(fd, "nas2_snps_f.vcf.gz")
nas_vcf3_path <- file.path(fd, "nas3_2_snps_f.vcf.gz")
nas_merged_vcf_path <- file.path(fd, "nas_merged_snps_f.vcf.gz")

filepaths <- c(gen_vcf1_path,
               gen_vcf2_path,
               gen_merged_vcf_path,
               nas_vcf1_path,
               nas_vcf2_path,
               nas_vcf3_path,
               nas_merged_vcf_path)

labels <- c('Gen 1',
            'Gen 2',
            'Gen Merged',
            'Nas 1',
            'Nas 2',
            'Nas 3',
            'Nas Merged')
```

Comparison of SNV quality metrics across files

Get the dataframe of QC metrics available and their descriptions.

```
gen1 <- readVcf(gen_vcf1_path, "hg38")
```

```
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
```

```r
descriptions <- info(header(gen1))
descriptions_df <- data.frame(Description = descriptions$Description,
                              row.names = rownames(descriptions))
descriptions_df
```

```
##
## AC                                                                                  Allele coun
## AF                                                                                           Al
## AN
## BaseQRankSum                                                                                 Z-
## DP
## ExcessHet
## FS                                                                                         Phre
## InbreedingCoeff                    Inbreeding coefficient as estimated from the genotype likelihoods
## MLEAC              Maximum likelihood expectation (MLE) for the allele counts (not necessarily the sa
## MLEAF           Maximum likelihood expectation (MLE) for the allele frequency (not necessarily the sa
## MQ
## MQRankSum                                                                             Z-score Fr
## QD
## ReadPosRankSum                                                                            Z-scor
## SOR                                                                                         Symm
```

Create information dataframes with QC metrics for all VCF files and plot these metrics densities.

```r
full_info_table <- create_full_info_table(filepaths)
```

```
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
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## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
```
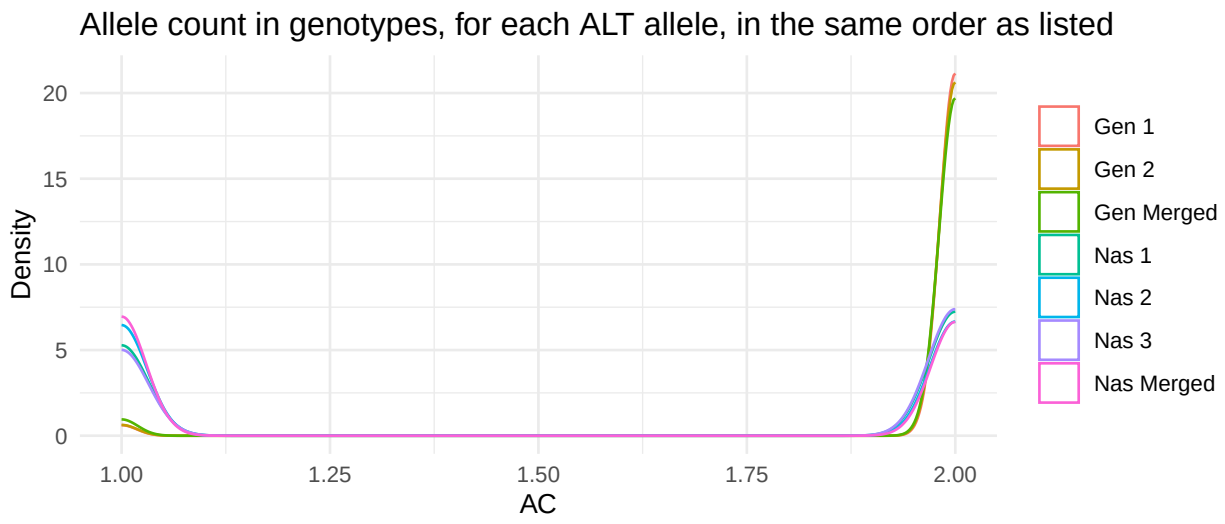
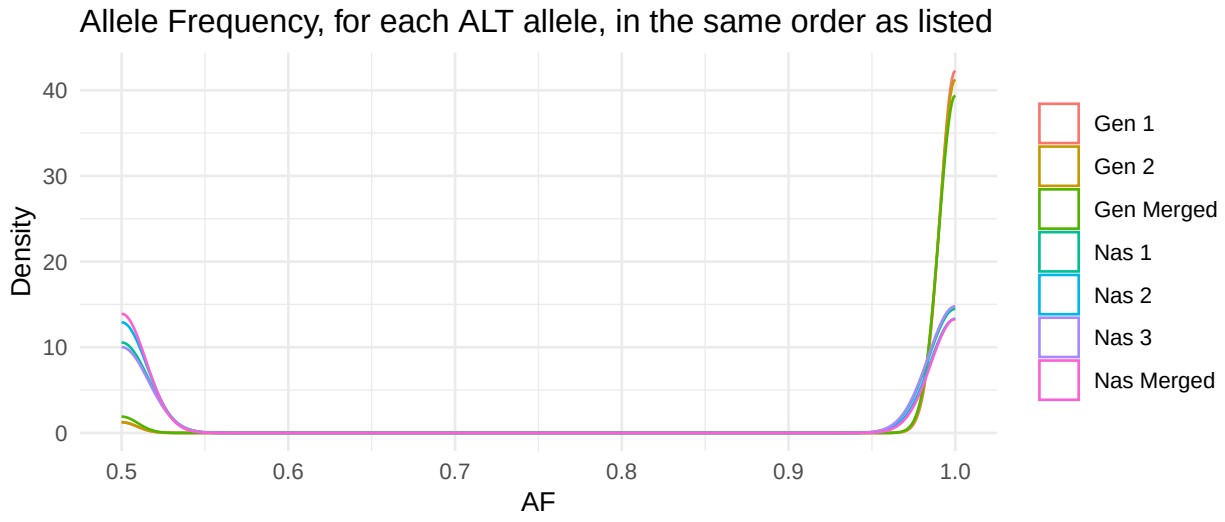
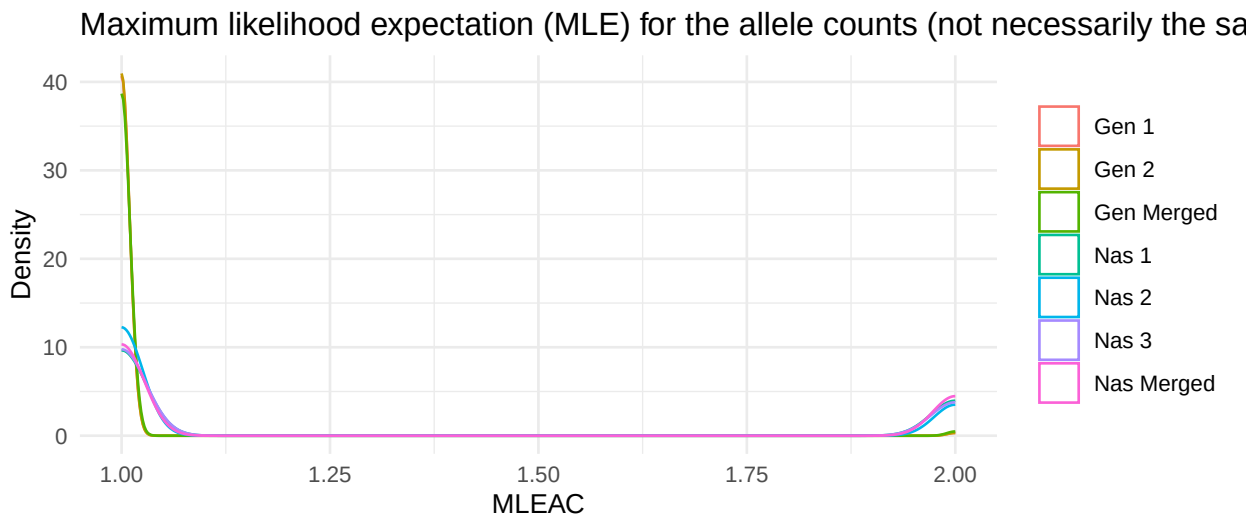

```r
plot_density(full_info_table, "AC", labels, descriptions)
```



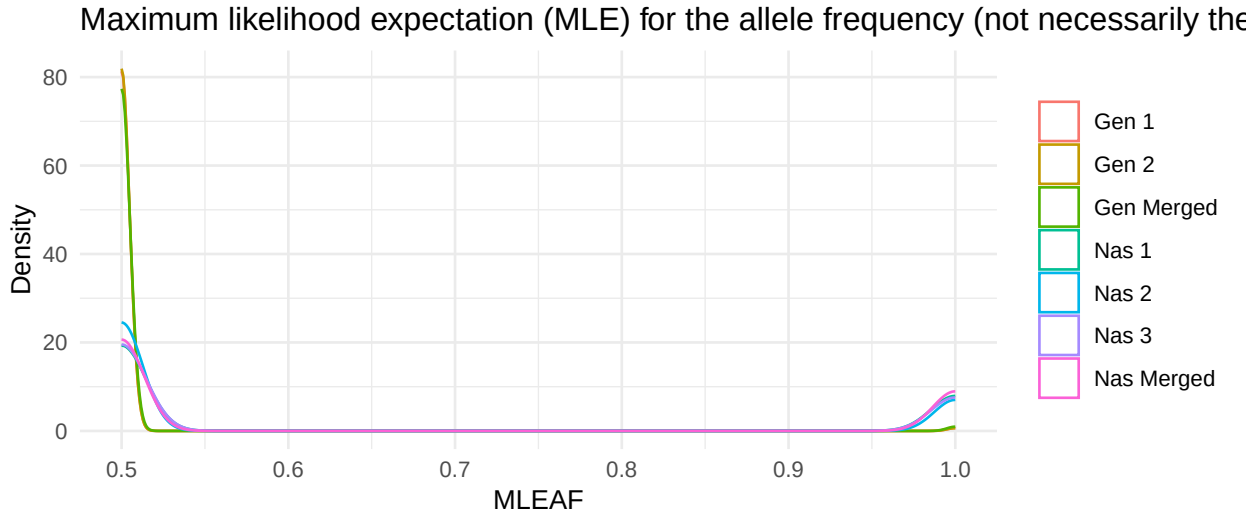

```
plot_density(full_info_table, "AF", labels, descriptions)
```



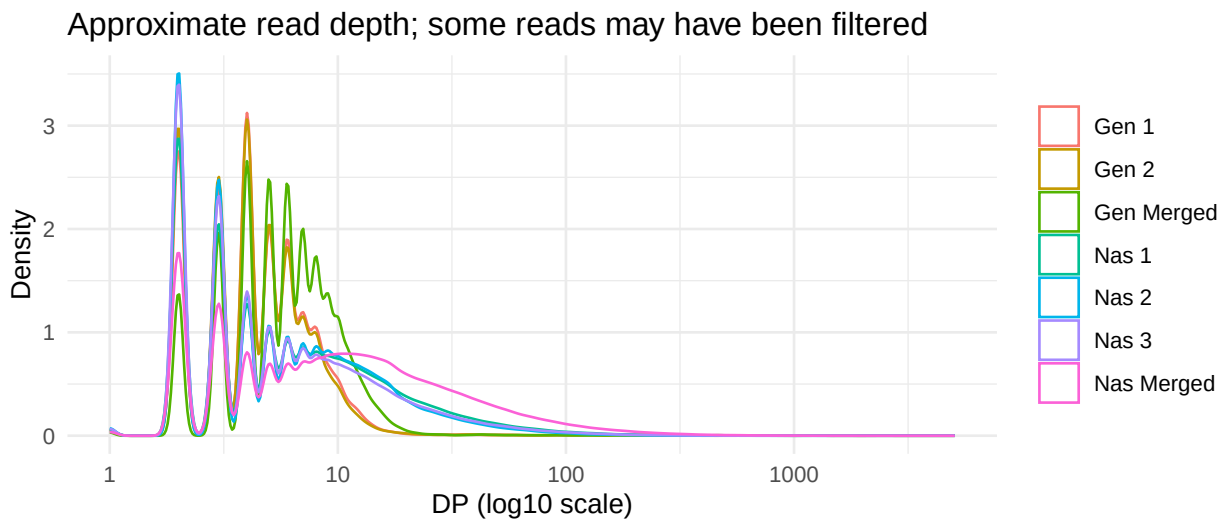

```
plot_density(full_info_table, "MLEAC", labels, descriptions)
```



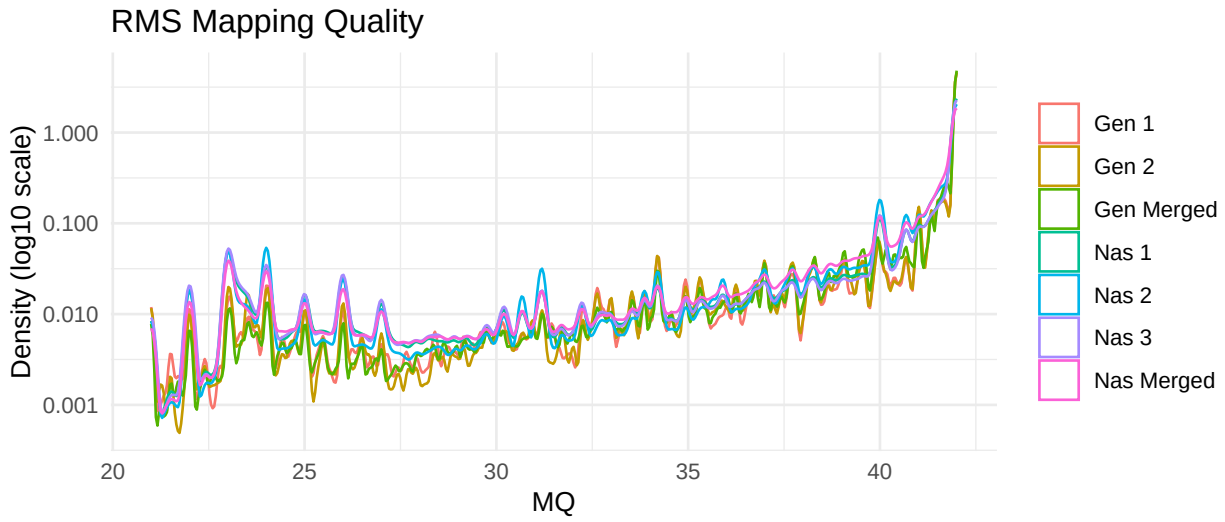

```
plot_density(full_info_table, "MLEAF", labels, descriptions)
```



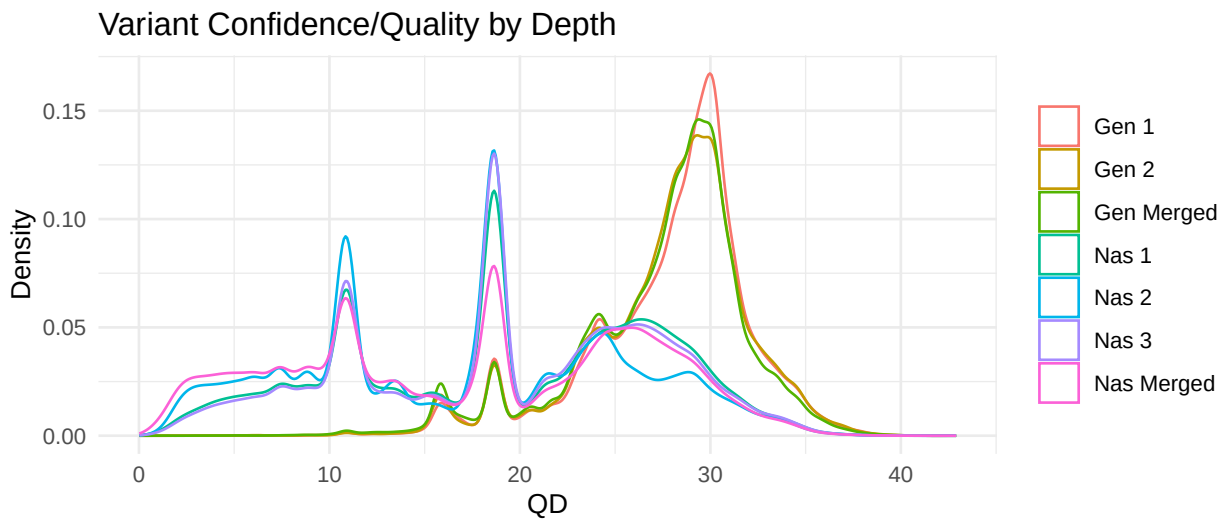

```
plot_density(full_info_table, 'DP', labels, descriptions, x_log10=TRUE)
```



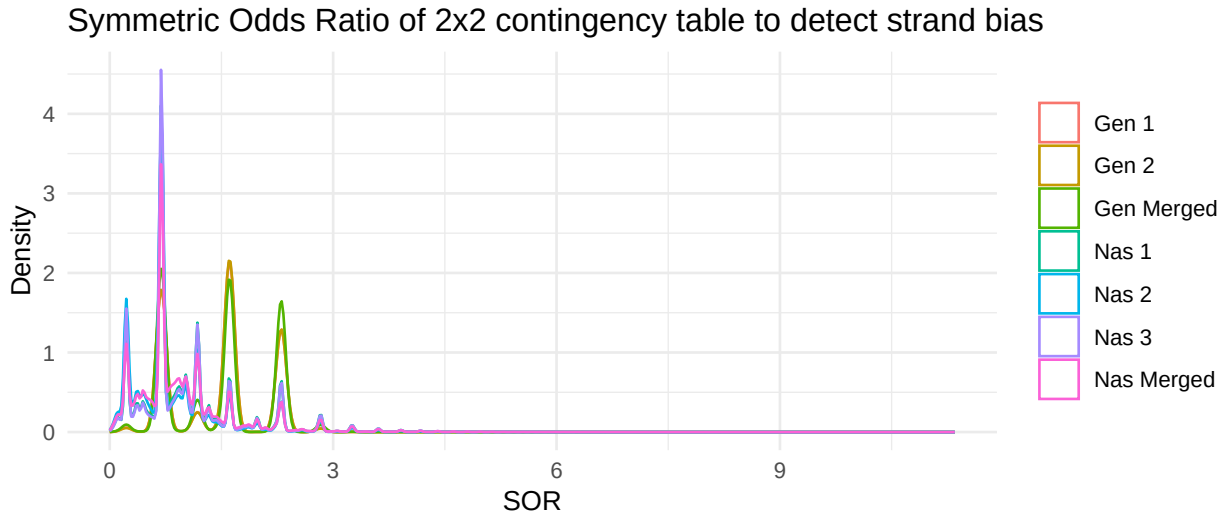

```
plot_density(full_info_table, 'MQ', labels, descriptions, y_log10=TRUE)
```




```
plot_density(full_info_table, 'QD', labels, descriptions)
```




```
plot_density(full_info_table, 'SOR', labels, descriptions)
```



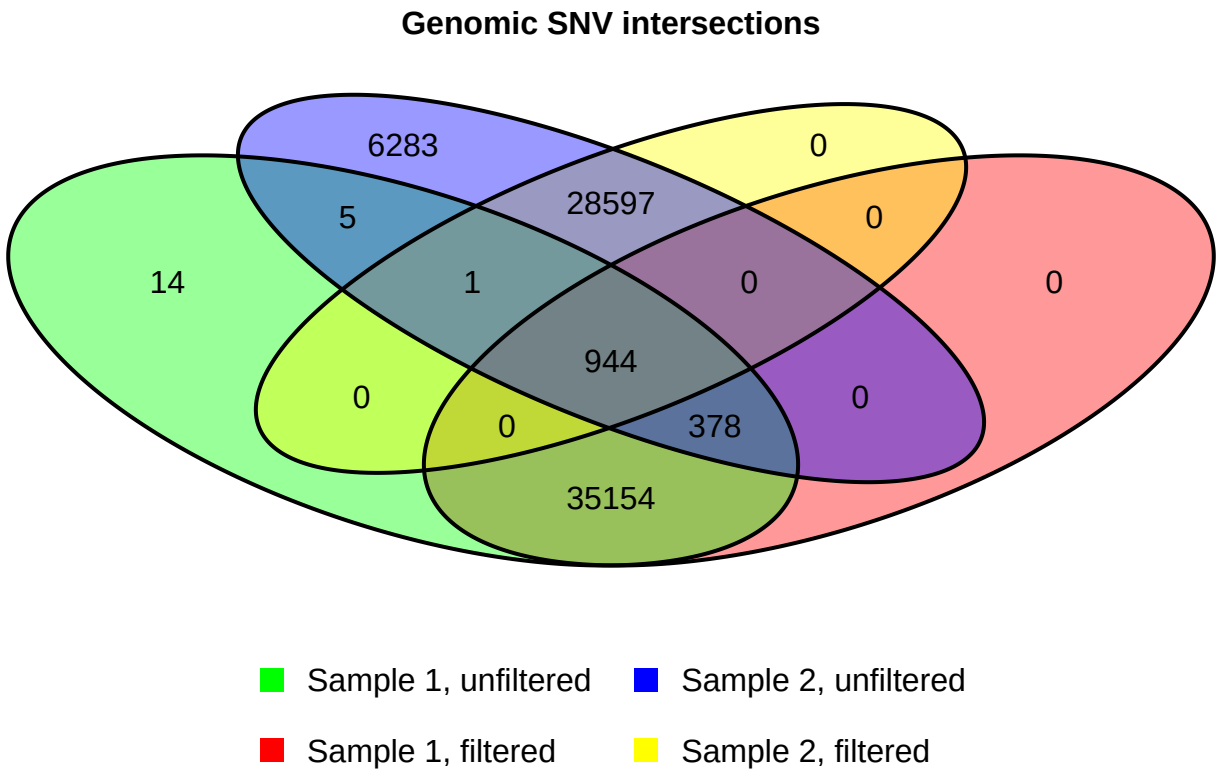
Analysis of intersections

```
gen1_uf <- load_vcf(gen_vcf1_path, hardfilter=FALSE)
gen2_uf <- load_vcf(gen_vcf2_path, hardfilter=FALSE)
gen_merged_uf <- load_vcf(gen_merged_vcf_path, hardfilter=FALSE)
nas1_uf <- load_vcf(nas_vcf1_path, hardfilter=FALSE)
nas2_uf <- load_vcf(nas_vcf2_path, hardfilter=FALSE)
nas3_uf <- load_vcf(nas_vcf3_path, hardfilter=FALSE)
nas_merged_uf <- load_vcf(nas_merged_vcf_path, hardfilter=FALSE)

gen1 <- load_vcf(gen_vcf1_path)
gen2 <- load_vcf(gen_vcf2_path)
gen_merged <- load_vcf(gen_merged_vcf_path)
```

```
nas1 <- load_vcf(nas_vcf1_path)
nas2 <- load_vcf(nas_vcf2_path)
nas3 <- load_vcf(nas_vcf3_path)
nas_merged <- load_vcf(nas_merged_vcf_path)
```

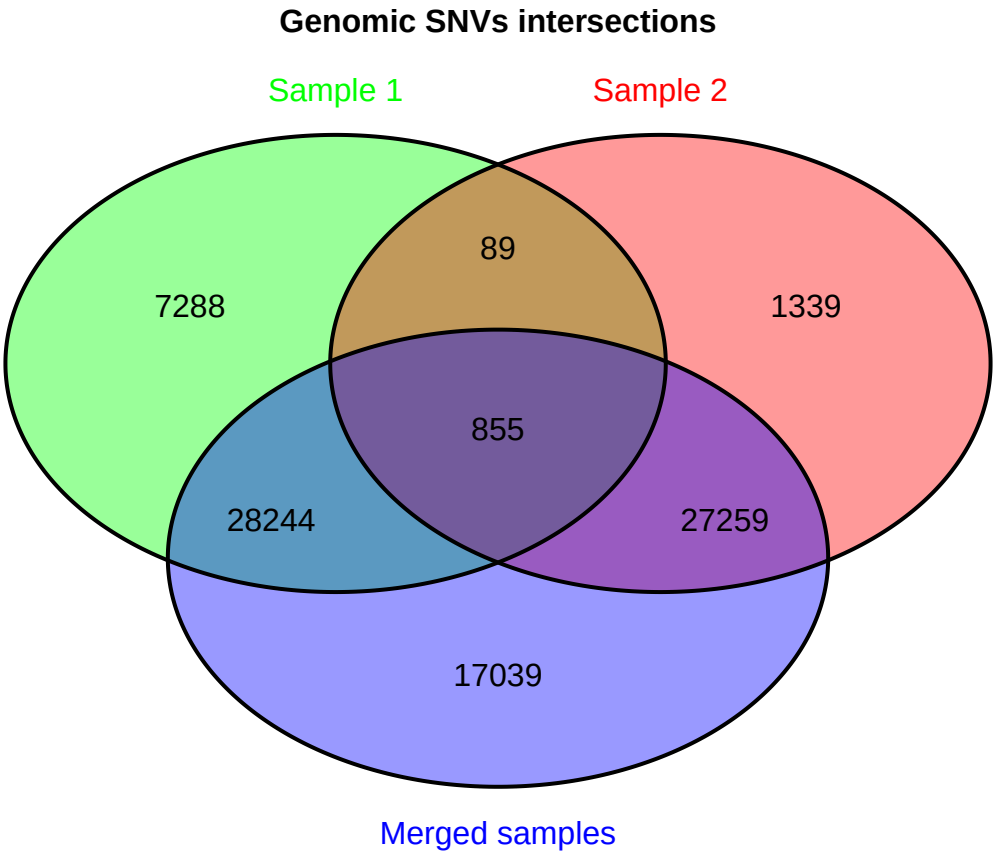
Genomic SNVs

```
plot_venn_4sets(data_list=list(gen1_uf,
                               gen1,
                               gen2_uf,
                               gen2),
                param='All SNPs',
                labels=c("Sample 1, unfiltered",
                         "Sample 1, filtered",
                         "Sample 2, unfiltered",
                         "Sample 2, filtered"),
                title="Genomic SNV intersections")
```



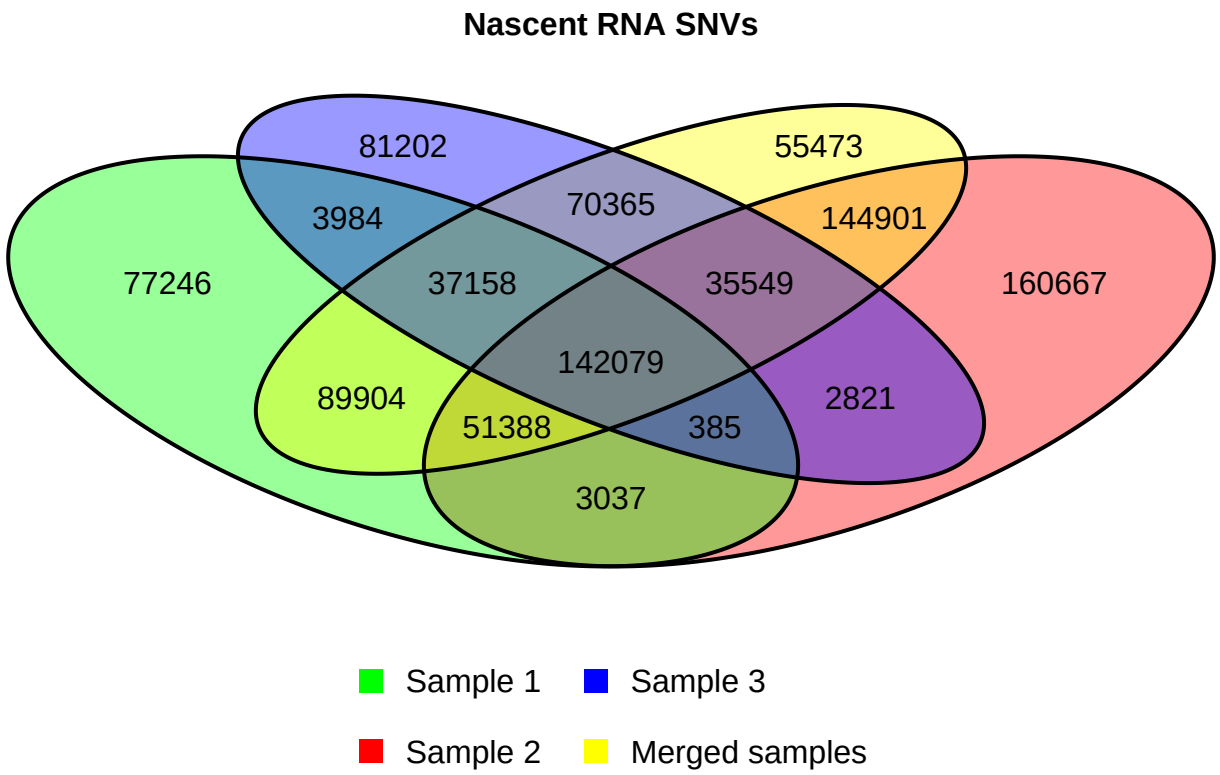

```
plot_venn_3sets(data_list=list(gen1,
                               gen2,
                               gen_merged),
                param='All SNPs',
                labels=c("Sample 1",
```

```
                  "Sample 2",
                  "Merged samples"),
          title="Genomic SNVs intersections")
```



Nascent SNVs

```
plot_venn_4sets(data_list=list(nas1,
                               nas2,
                               nas3,
                               nas_merged),
          param='All SNPs',
          labels=c("Sample 1",
                   "Sample 2",
                   "Sample 3",
                   "Merged samples"),
          title="Nascent RNA SNVs")
```



Genomic and nascent SNVs

Out of 73 thousand SNVs detected in merged genomic data and 627 thousands of SNVs detected in merged nascent RNA data only 12 thousands SNVs are common:

```
length(gen_merged@ranges@NAMES)
```

```
## [1] 73397
```

```
length(nas_merged@ranges@NAMES)
```

```
## [1] 626817
```

```
length(intersect(gen_merged@ranges@NAMES, nas_merged@ranges@NAMES))
```

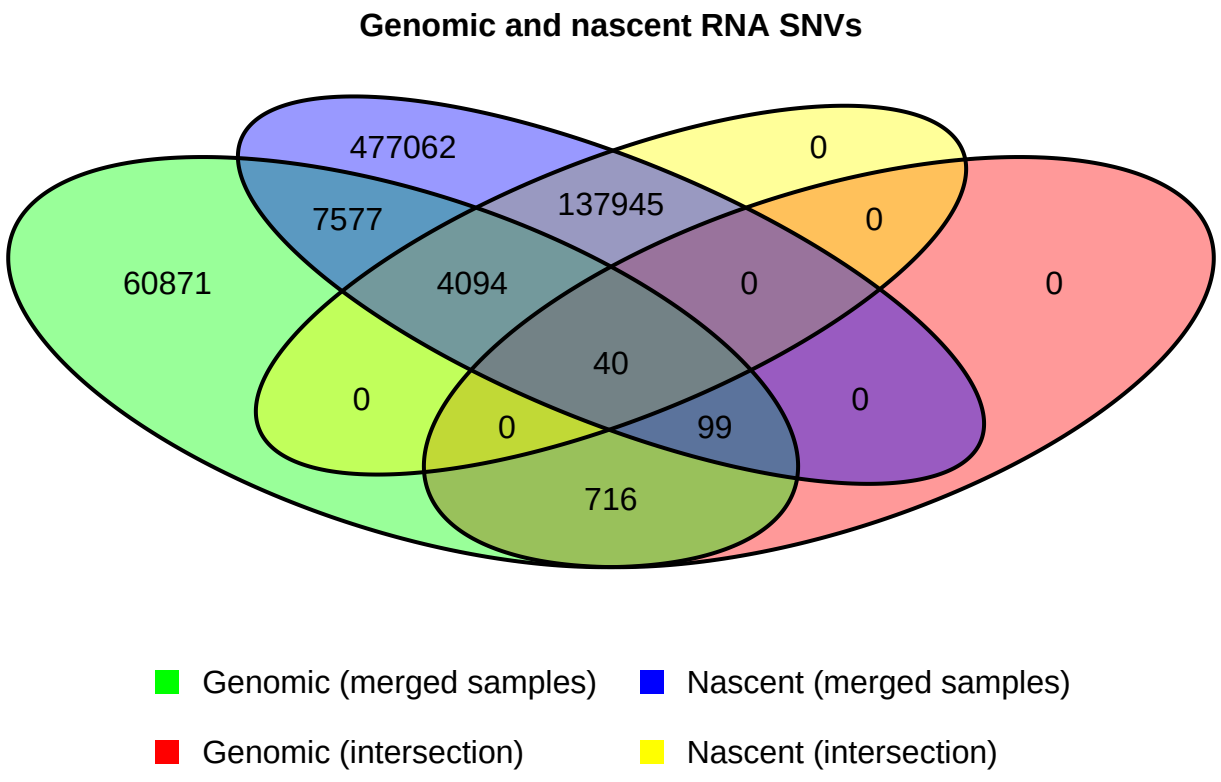
```
## [1] 11810
```

Only 40 SNVs are common between all VCF files used:

```
gen_common <- gen_merged[gen_merged@ranges@NAMES %in%
                           Reduce(intersect, list(gen1@ranges@NAMES,
                                                  gen2@ranges@NAMES,
```

```
                                                gen_merged@ranges@NAMES))]

nas_common <- nas_merged[nas_merged@ranges@NAMES %in%
                         Reduce(intersect, list(nas1@ranges@NAMES,
                                                nas2@ranges@NAMES,
                                                nas3@ranges@NAMES,
                                                nas_merged@ranges@NAMES))]
```

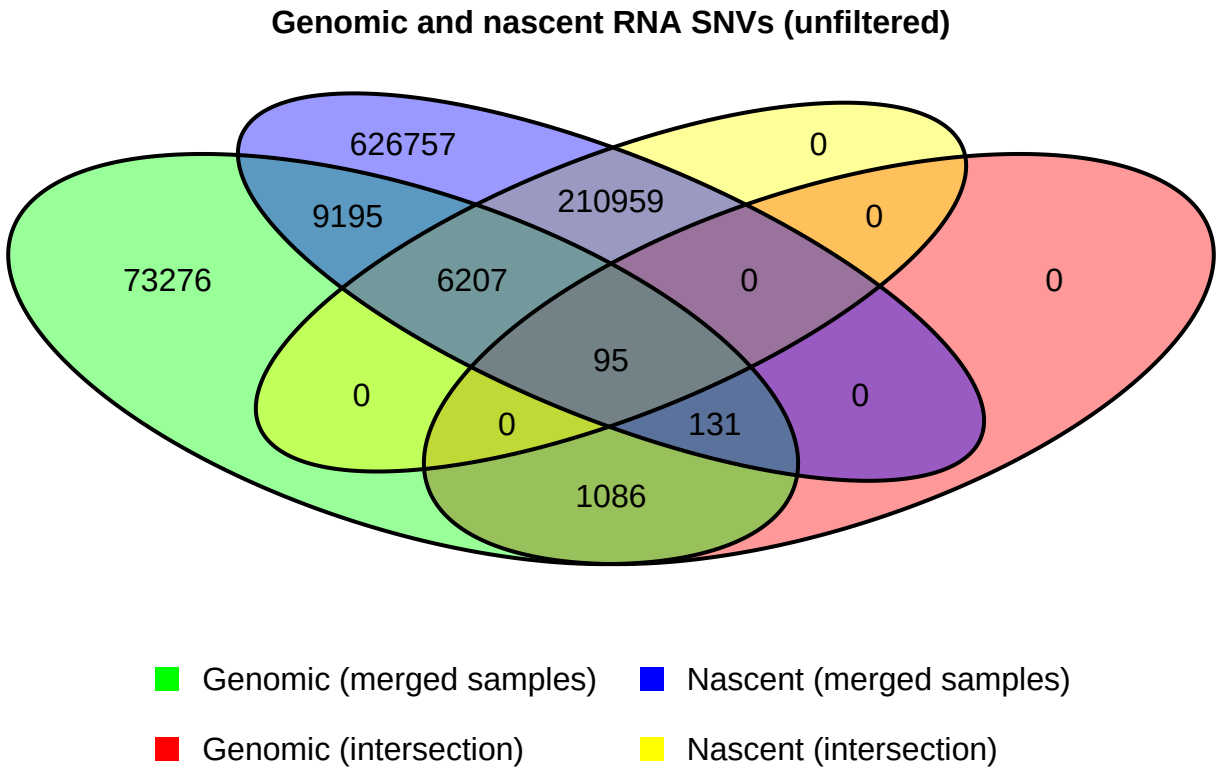
```
plot_venn_4sets(data_list=list(gen_merged,
                               gen_common,
                               nas_merged,
                               nas_common),
          param='All SNPs',
          labels=c("Genomic (merged samples)",
                   "Genomic (intersection)",
                   "Nascent (merged samples)",
                   "Nascent (intersection)"),
          title="Genomic and nascent RNA SNVs")
```



In case of unfiltered data, this intersection was only 95 SNVs:

```
gen_common_uf <- gen_merged_uf[gen_merged_uf@ranges@NAMES %in%
                         Reduce(intersect, list(gen1_uf@ranges@NAMES,
                                                gen2_uf@ranges@NAMES,
                                                gen_merged_uf@ranges@NAMES))]
```

```
nas_common_uf <- nas_merged_uf[nas_merged_uf@ranges@NAMES %in%
                           Reduce(intersect, list(nas1_uf@ranges@NAMES,
                                                  nas2_uf@ranges@NAMES,
                                                  nas3_uf@ranges@NAMES,
                                                  nas_merged_uf@ranges@NAMES))]

plot_venn_4sets(data_list=list(gen_merged_uf,
                               gen_common_uf,
                               nas_merged_uf,
                               nas_common_uf),
          param='All SNPs',
          labels=c("Genomic (merged samples)",
                   "Genomic (intersection)",
                   "Nascent (merged samples)",
                   "Nascent (intersection)"),
          title="Genomic and nascent RNA SNVs (unfiltered)")
```



Therefore, SNVs which are common in all nascent RNA VCF files will be used further.

```
common_snps <- Reduce(intersect, list(nas1@ranges@NAMES,
                                      nas2@ranges@NAMES,
                                      nas3@ranges@NAMES,
                                      nas_merged@ranges@NAMES))
```

Comparison of unique and intersecting SNVs

Define subsets of SNVs which are unique for all the files (e.g. SNVs which are present in gen_vcf1, but absent in gen_vcf2, gen_merged, nas_vcf1, nas_vcf2, nas_vcf3, nas_merged)

```r
# Extract INFO tables and SNV positions (i.e. SNV identifiers)
info_tables <- lapply(filepaths, extract_info_and_positions)
```

```
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
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## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
## Warning in .bcfHeaderAsSimpleList(header): duplicate keys in header will be
## forced to unique rownames
```
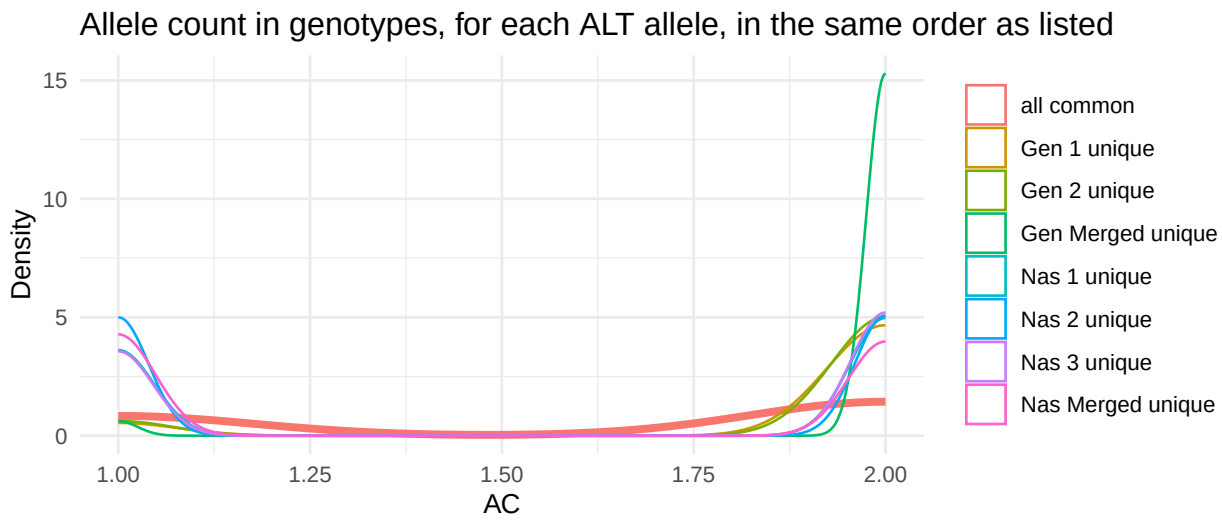
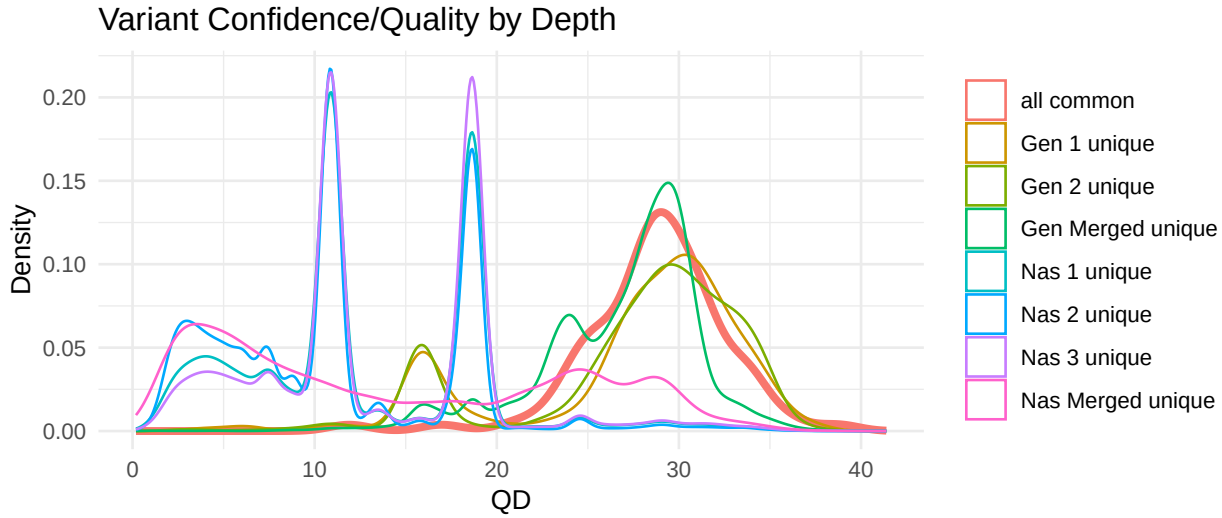

```r
# Identify unique and common SNV sets based on positions
snv_set_positions <- identify_snv_sets_positions(lapply(info_tables, function(x) x$positions),
                                                  labels)
```

```r
param_name <- "AC"
snv_set_with_metric <- extract_metric_from_info_tables(info_tables, param_name, snv_set_positions)
plot_ac <- plot_snv_density(snv_set_with_metric, param_name, descriptions_df)
print(plot_ac)
```

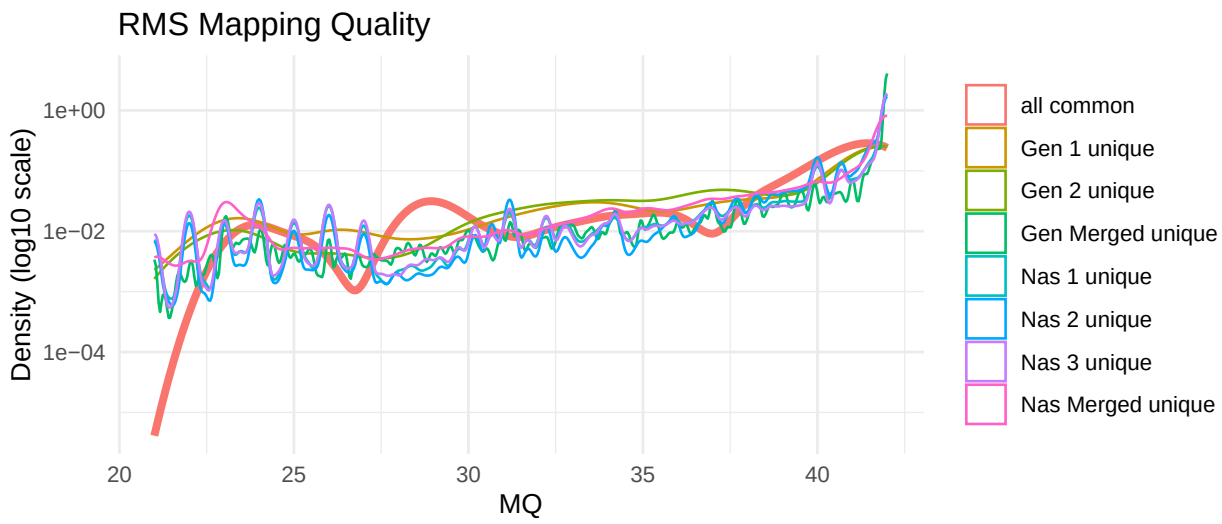
```
## Warning: Using ‘size‘ aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use ‘linewidth‘ instead.
## This warning is displayed once every 8 hours.
## Call ‘lifecycle::last_lifecycle_warnings()‘ to see where this warning was
## generated.
```




```
param_name <- "QD"
snv_set_with_metric <- extract_metric_from_info_tables(info_tables, param_name, snv_set_positions)
plot_qd <- plot_snv_density(snv_set_with_metric, param_name, descriptions_df)
print(plot_qd)
```




```
param_name <- "MQ"
snv_set_with_metric <- extract_metric_from_info_tables(info_tables, param_name, snv_set_positions)
plot_mq <- plot_snv_density(snv_set_with_metric, param_name, descriptions_df, x_log10 = FALSE, y_log10 =
print(plot_mq)
```




```
param_name <- "SOR"
snv_set_with_metric <- extract_metric_from_info_tables(info_tables, param_name, snv_set_positions)
plot_sor <- plot_snv_density(snv_set_with_metric, param_name, descriptions_df, x_log10 = FALSE, y_log10
print(plot_sor)
```