scRepertoire v2 Analysis

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Loading Libraries

```
# Define required libraries
packages <- c(
  "Azimuth", "BiocParallel", "celldex", "dplyr", "ggplot2",
  "ggthemes", "harmony", "igraph", "patchwork", "RColorBrewer",
  "scDblFinder", "scGate", "scRepertoire", "scran", "Seurat",
  "SeuratData", "SingleR", "stringr", "viridis"
)

# Suppress startup messages
suppressPackageStartupMessages(
  lapply(packages, library, character.only = TRUE)
)

# Load annotation references if not already present
if (!exists("HPCA")) {
  HPCA <- celldex::HumanPrimaryCellAtlasData()
}
if (!exists("Monaco")) {
  Monaco <- celldex::MonacoImmuneData()
}

# Set options
options(future.globals.maxSize = 8000 * 1024^2)

# Define custom negate function
"%!in%" <- Negate("%in%")
```

Directory Setup

```
# Define directories
qc_dir <- "./qc"
input_dir <- "./inputs/data/GSE169440"
output_dir <- "./output"
processed_dir <- "./inputs/data/processedData"

# Create directories if they do not exist
dir.create(qc_dir, showWarnings = FALSE)
dir.create(output_dir, showWarnings = FALSE)
dir.create(processed_dir, showWarnings = FALSE)
```

Helper Functions

```
# Save plots with consistent formatting
save_plot <- function(plot, filename, height = 3, width = 3, dpi = 300) {
  ggsave(filename = filename, plot = plot, height = height, width = width,
         dpi = dpi)
}

# Calculate QC metrics
calculate_qc_metrics <- function(seurat_obj) {
  seurat_obj$nCount_RNA <- colSums(seurat_obj@assays$RNA@layers$counts)
  seurat_obj$nFeature_RNA <- colSums(seurat_obj@assays$RNA@layers$counts != 0)
  seurat_obj[["mito.genes"]] <- PercentageFeatureSet(seurat_obj,
                                                     pattern = "^MT-")
  seurat_obj[["ribo.genes"]] <- PercentageFeatureSet(seurat_obj,
                                                     pattern = "^RPS|RPL-")
  return(seurat_obj)
}

# Plot QC metrics
plot_qc_metrics <- function(seurat_obj, file_name, qc_dir) {
  p <- VlnPlot(
    object = seurat_obj,
    features = c("nCount_RNA", "nFeature_RNA", "mito.genes", "ribo.genes"),
    pt.size = 0,
    cols = "grey"
  ) +
```

```
    theme_minimal() +
    theme(legend.position = "none") +
    plot_layout(ncol = 2)

  save_plot(p,
            file.path(qc_dir, paste0(file_name, "_metrics.pdf")),
            height = 8,
            width = 8)
}

# Filter cells based on QC metrics
filter_cells <- function(seurat_obj, file_name, qc_dir) {
  standev <- sd(log(seurat_obj$nFeature_RNA)) * 2
  mean_val <- mean(log(seurat_obj$nFeature_RNA))
  cut <- round(exp(standev + mean_val))

  p <- FeatureScatter(seurat_obj,
                      feature1 = "nCount_RNA",
                      feature2 = "nFeature_RNA") +
    geom_hline(yintercept = cut)

  save_plot(p, file.path(qc_dir,
                         paste0(file_name, "_cutpoint.pdf")),
            height = 8,
            width = 8)

  seurat_obj <- subset(seurat_obj,
                       subset = mito.genes < 10 & nFeature_RNA < cut)
  return(seurat_obj)
}
```

Plot Standardization

```
custom_theme <- theme_minimal(base_size = 8) +
  theme(
    axis.title = element_text(size = 8, color = "black"),
    axis.text = element_text(size = 6, color = "black"),
    legend.text = element_text(size = 4, color = "black"),
    legend.key.size = unit(0.1, "cm"),
    legend.title = element_text(size = 4, color = "black"),
```

```
    legend.position = c(1, 1),
    legend.justification = c("right", "top"),
    legend.box.just = "right",
    legend.margin = margin(6, 6, 6, 6),
    legend.spacing.y = unit(2, "cm")
  )
```

```
Warning: A numeric `legend.position` argument in `theme()` was deprecated in ggplot2
3.5.0.
i Please use the `legend.position.inside` argument of `theme()` instead.
```

Processing Sequencing Runs

```
# Setup
dir.create("./qc", showWarnings = FALSE)
file_list <- list.files("./inputs/data/GSE169440", full.names = FALSE)

# Helper functions
calculate_qc_metrics <- function(seurat_obj) {
  seurat_obj$nCount_RNA <- colSums(seurat_obj@assays$RNA@layers$counts)
  seurat_obj$nFeature_RNA <- colSums(seurat_obj@assays$RNA@layers$counts != 0)
  seurat_obj[["mito.genes"]] <- PercentageFeatureSet(seurat_obj,
                                                     pattern = "^MT-")
  seurat_obj[["ribo.genes"]] <- PercentageFeatureSet(seurat_obj,
                                                     pattern = "^RPS|RPL-")
  return(seurat_obj)
}

plot_qc_metrics <- function(seurat_obj, file_name) {
  p <- VlnPlot(
    object = seurat_obj,
    features = c("nCount_RNA", "nFeature_RNA", "mito.genes", "ribo.genes"),
    pt.size = 0,
    cols = "grey"
  ) +
    theme(legend.position = "none") +
    plot_layout(ncol = 2)
  ggsave(paste0("./qc/", file_name, "_metrics.pdf"),
         plot = p,
         height = 8,
```

```
         width = 8)
}

filter_cells <- function(seurat_obj, file_name) {
  standev <- sd(log(seurat_obj$nFeature_RNA)) * 2
  mean_val <- mean(log(seurat_obj$nFeature_RNA))
  cut <- round(exp(standev + mean_val))

  p <- FeatureScatter(seurat_obj,
                      feature1 = "nCount_RNA",
                      feature2 = "nFeature_RNA") +
    geom_hline(yintercept = cut)
  ggsave(paste0("./qc/", file_name, "_cutpoint.pdf"),
         plot = p,
         height = 8,
         width = 8)

  seurat_obj <- subset(seurat_obj,
                       subset = mito.genes < 10 & nFeature_RNA < cut)
  return(seurat_obj)
}

annotate_with_singler <- function(sce, ref, ref_name) {
  com.res <- SingleR(sce,
                     ref = ref,
                     labels = ref$label.fine,
                     assay.type.test = 1)
  df <- data.frame("labels" = com.res$labels,
                   "pruned.labels" = com.res$pruned.labels)
  rownames(df) <- rownames(com.res)
  colnames(df) <- paste0(ref_name, ".", colnames(df))
  return(df)
}

# Download and prepare reference datasets if not already present
if (!exists("HPCA")) {
  HPCA <- celldex::HumanPrimaryCellAtlasData()
}
if (!exists("Monaco")) {
  Monaco <- celldex::MonacoImmuneData()
}
```

```r
# Main processing loop
for (file in file_list) {
  message("Processing ", file)

  # 1. Read Data and Create Seurat Object
  tmp <- Read10X(paste0("./inputs/data/GSE169440/",
                        file, "/filtered_feature_bc_matrix/"))

  SeuratObj <- CreateSeuratObject(counts = tmp,
                                  assay = "RNA",
                                  project = file) %>%
    subset(subset = nFeature_RNA > 100) # Filter out low feature cells early

  # Remove tmp to free up memory
  rm(tmp)

  # 2. Calculate QC Metrics and Rename Cells
  SeuratObj <- calculate_qc_metrics(SeuratObj)
  SeuratObj <- RenameCells(SeuratObj, new.names =
                             paste0(file, "_", colnames(SeuratObj)))

  # 3. Plot QC Metrics
  plot_qc_metrics(SeuratObj, file)

  # 4. Filter Cells
  SeuratObj <- filter_cells(SeuratObj, file)

  # 5. Estimate Doublets
  sce <- as.SingleCellExperiment(SeuratObj)
  sce <- scDblFinder(sce)
  doublets <- data.frame(db.class = sce$scDblFinder.class,
                         db.score = sce$scDblFinder.score)
  rownames(doublets) <- rownames(sce@colData)
  SeuratObj <- AddMetaData(SeuratObj, doublets)

  # 6. Azimuth Annotation
  SeuratObj <- RunAzimuth(SeuratObj, reference = "pbmcref", verbose = FALSE)

  # 7. SingleR Annotation
  SeuratObj <- AddMetaData(SeuratObj,
                           annotate_with_singler(sce, HPCA, "HPCA"))
  SeuratObj <- AddMetaData(SeuratObj,
```

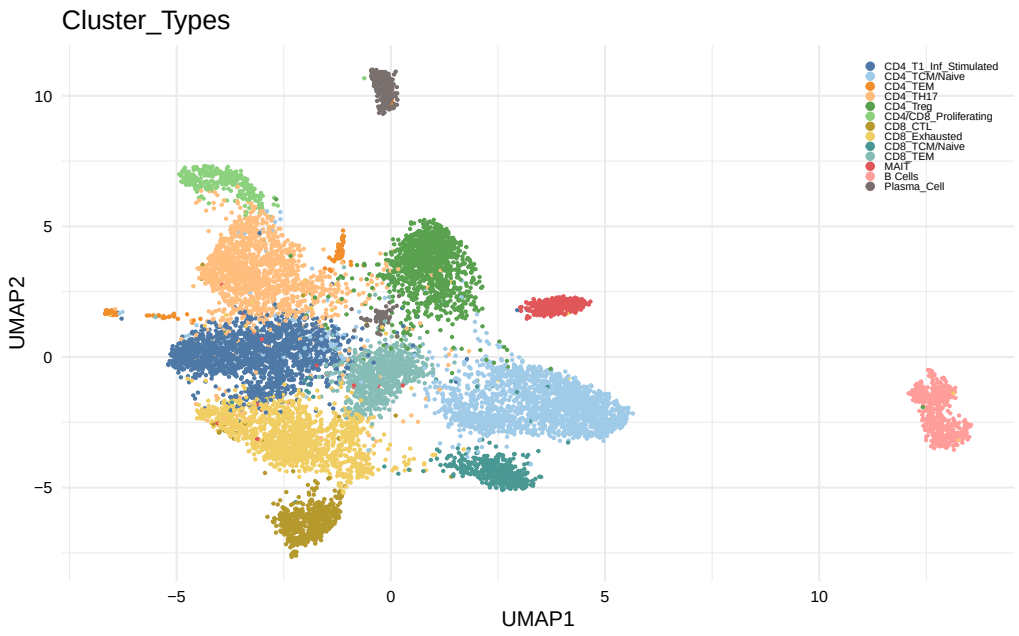
```
                          annotate_with_singler(sce, Monaco, "Monaco"))

  rm(sce)

  # 8. Add Clonal Information
  TCR.file <- list.files(paste0("./inputs/data/GSE169440/",
                                file, "/TCR"), pattern = "annotations")[1]
  TCR.file <- read.csv(paste0("./inputs/data/GSE169440/",
                              file, "/TCR/", TCR.file))
  combinedTCR <- combineTCR(TCR.file, samples = file, filterMulti = TRUE)

  BCR.file <- list.files(paste0("./inputs/data/GSE169440/",
                                file, "/BCR"), pattern = "annotations")[1]
  BCR.file <- read.csv(paste0("./inputs/data/GSE169440/",
                              file, "/BCR/", BCR.file))
  combinedBCR <- combineBCR(BCR.file, samples = file)

  SeuratObj <- combineExpression(c(combinedTCR, combinedBCR),
                                 SeuratObj,
                                 cloneCall = "strict",
                                 proportion = TRUE)

  # 9. scGate Filtering
  suppressWarnings({
    scGateModelDb <- get_scGateDB("data/scGateDB")
  })
  SeuratObj <- scGate(SeuratObj, scGateModelDb$human$generic)

  cells.to.keep <- which(SeuratObj$is.pure_Tcell == "Pure" |
                           SeuratObj$is.pure_Bcell == "Pure" |
                           SeuratObj$is.pure_PlasmaCell == "Pure")
  clones.recovered <- which(!is.na(SeuratObj$CTaa))
  cells.to.keep <- intersect(cells.to.keep, clones.recovered)
  SeuratObj <- subset(SeuratObj, cells = colnames(SeuratObj)[cells.to.keep])

  # 10. Save Preliminary Seurat Object
  saveRDS(SeuratObj, paste0("./inputs/data/processedData/", file, ".rds"))
  rm(SeuratObj)
  gc()
}
```

Integrate Cohort

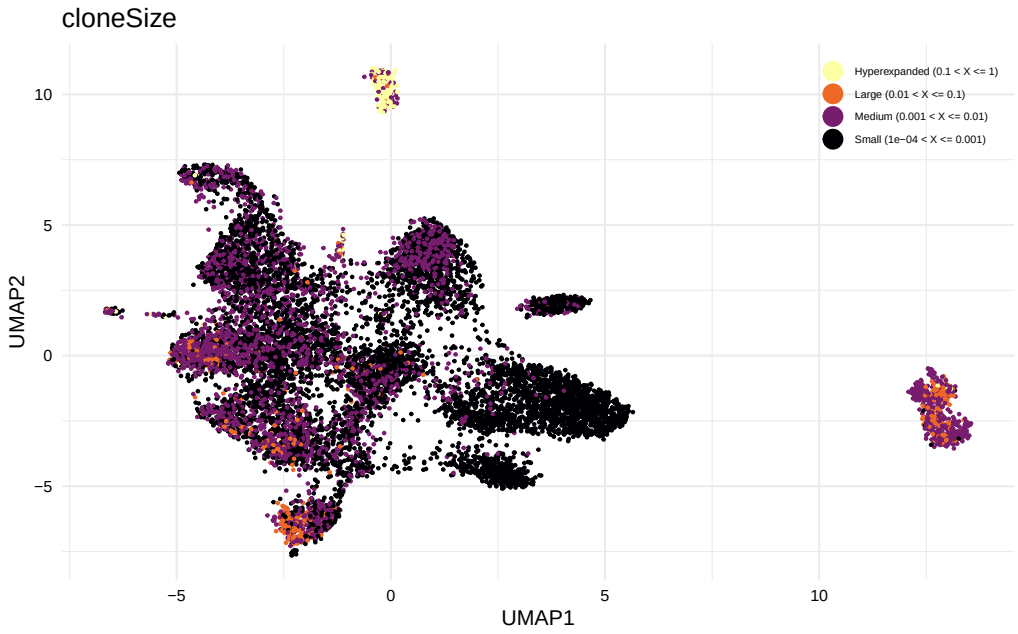
```
dir.create("./output", showWarnings = FALSE)
# 1. Read and merge data
files <- list.files("./inputs/data/processedData/", full.names = TRUE)

object.list <- lapply(files, function(file) {
  readRDS(file)
})

# Use reduce and merge for a more efficient merge
object.merge <- Reduce(function(x, y) merge(x, y), object.list)

# 2. Preprocessing and Integration
object.merge <- object.merge %>%
  NormalizeData(verbose = FALSE) %>%
  FindVariableFeatures(nfeatures = 2500, verbose = FALSE) %>%
  Ibex::quietBCRgenes() %>% # Corrected function call using quietly
  Trex::quietTCRgenes() %>% # Corrected function call using quietly
  ScaleData(verbose = FALSE,
            vars.to.regress = c("mito.genes")) %>%
  RunPCA(verbose = FALSE) %>%
  RunHarmony("orig.ident", verbose = FALSE)

# 3. Clustering and Visualization
object.merge <- object.merge %>%
  RunUMAP(reduction = "harmony",
          dims = 1:30,
          reduction.name = "umap.harmony",
          verbose = FALSE) %>%
  FindNeighbors(dims = 1:30,
                reduction = "harmony",
                verbose = FALSE) %>%
  FindClusters(algorithm = 4,
               resolution = 0.4,
               verbose = FALSE)

# 4. Defining Cell Types By Cluster
cluster.types <- c("CD4_TCM/Naive",
                   "CD4_T1_Inf_Stimulated",
                   "CD4_TH17",
                   "CD8_Exhausted",
```

```
                    "CD4_Treg",
                    "CD8_TEM",
                    "CD8_CTL",
                    "B Cells",
                    "CD8_TCM/Naive",
                    "CD4/CD8_Proliferating",
                    "MAIT",
                    "Plasma_Cell",
                    "CD4_TEM",
                    "Plasma_Cell",
                    "CD4_TEM")
cluster.types <- setNames(cluster.types, as.character(1:15))
object.merge$Cluster_Types <- recode(as.character(object.merge$seurat_clusters),
                                     !!!cluster.types)

#5. Add patient meta data
object.merge$Patient <- str_remove_all(object.merge$orig.ident, "SKN")
object.merge$Patient <- str_remove_all(object.merge$Patient, "SKL")


#6. Plotting UMAP by Cell type and Clonal Expansion
celltype.palette <- tableau_color_pal(palette = "Tableau 20")(length(unique(object.merge$Clus
object.merge$Cluster_Types <- factor(object.merge$Cluster_Types,
                                     sort(unique(object.merge$Cluster_Types))[c(2:12,1,13)])

plot1 <- DimPlot(object.merge, group.by = "Cluster_Types", pt.size = 0.1) +
                  scale_color_manual(values = celltype.palette) +
                  theme_minimal() +
                  custom_theme +
                  guides(colour = guide_legend(override.aes = list(size=1))) +
                  ylab("UMAP2") +
                  xlab("UMAP1")

plot2 <- DimPlot(object.merge, group.by = "cloneSize") +
                  scale_color_viridis(option = "inferno",
                                      discrete = TRUE,
                                      direction = -1) +
                  theme_minimal() +
                  custom_theme +
                  ylab("UMAP2") +
                  xlab("UMAP1")
```

```
plot1
```




```
ggsave("output/Figure2A.pdf", height = 3, width = 3)

plot2
```



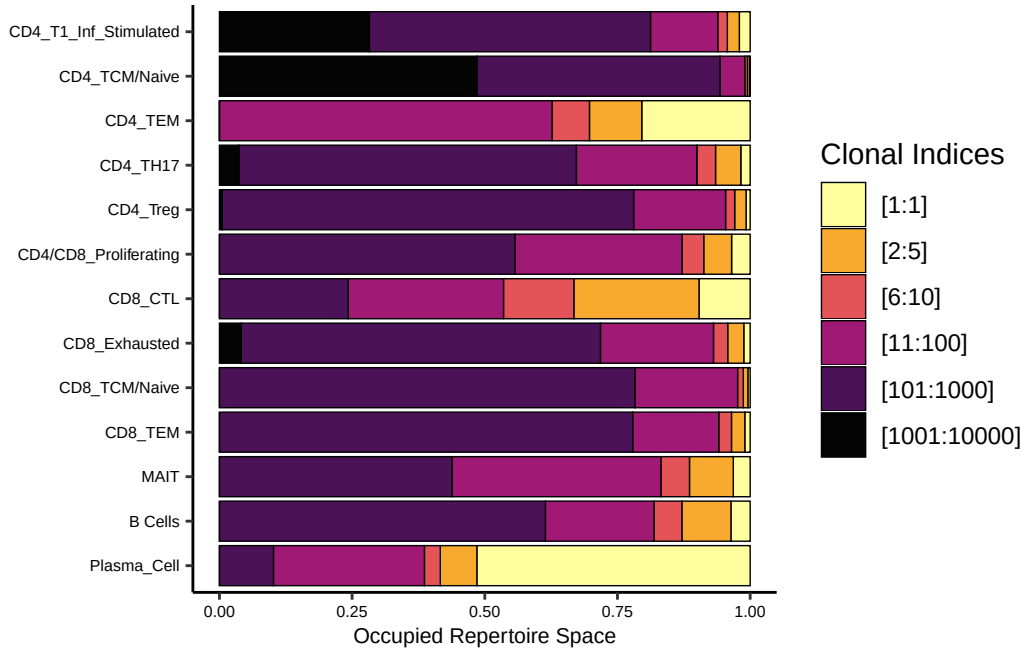

```
ggsave("output/Figure2B_P1.pdf", height = 3, width = 3, dpi = 300)

# 6. Save the integrated object
saveRDS(object.merge, "./inputs/data/IntegratedSeuratObject.rds")
```

Clonal Analysis

Clonal Proportion

```
object.merge <- readRDS("./inputs/data/IntegratedSeuratObject.rds")

plot1 <- clonalProportion(object.merge,
                          group.by = "Cluster_Types",
                  cloneCall = "strict",
                  clonalSplit = c(1, 5, 10, 100, 1000, 10000)) +
                  coord_flip() +
  scale_x_discrete(limits=rev) +
  theme(axis.title.y = element_blank(),
        axis.title = element_text(size=8, color = "black"),
        axis.text = element_text(size=6, color = "black"))
```

```
plot1
```




```
ggsave("output/Figure2B_P2.pdf", height = 2, width = 3)
```

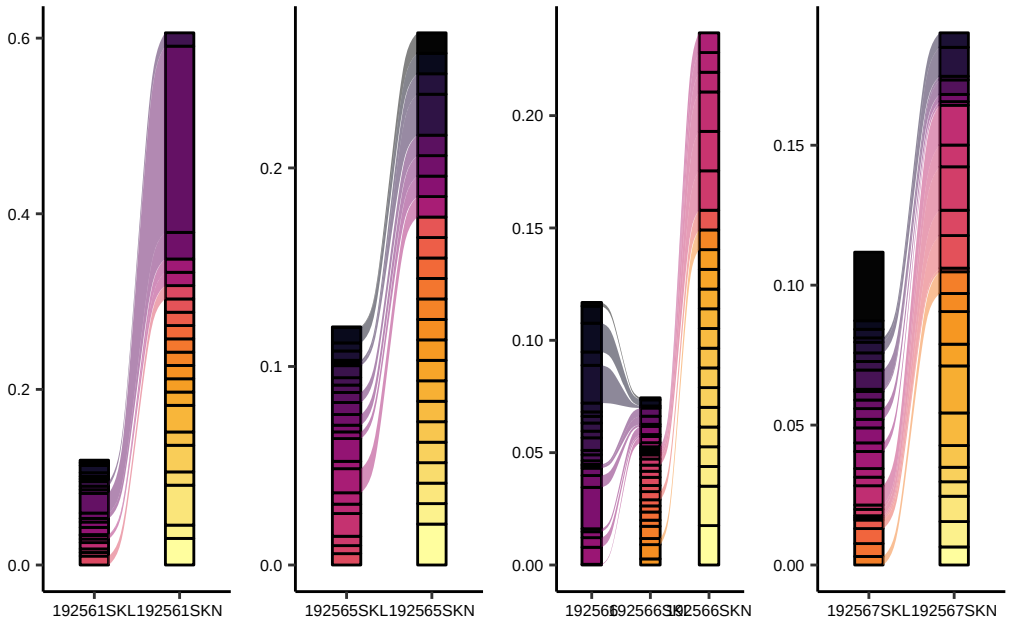
Seperating B and T Cells

```
T.types <- c("CD4_TCM/Naive", "CD4_T1_Inf_Stimulated","CD4_TH17",
             "CD8_Exhausted", "CD4_Treg", "CD8_TEM", "CD8_CTL",
             "CD8_TCM/Naive", "CD4/CD8_Proliferating", "MAIT",
             "CD4_TEM", "CD4_TEM")
object.TCells <- subset(object.merge, Cluster_Types %in% T.types)
object.BCells <- subset(object.merge, Cluster_Types %!in% T.types)
```

Comparing Clones

```
plot1 <- clonalCompare(object.TCells,
              cloneCall = "strict",
```

```
              top.clones = 20,
              samples = c("192561SKL", "192561SKN"),
              group.by = "orig.ident",
              palette = "inferno") +
  guides(fill = "none") +
  theme(axis.title = element_blank(),
        axis.text = element_text(size=6, color = "black"))

plot2 <- clonalCompare(object.TCells,
              cloneCall = "strict",
              top.clones = 20,
              samples = c("192565SKL", "192565SKN"),
              group.by = "orig.ident",
              palette = "inferno") +
  guides(fill = "none") +
  theme(axis.title = element_blank(),
        axis.text = element_text(size=6, color = "black"))

plot3 <- clonalCompare(object.TCells,
              cloneCall = "strict",
              top.clones = 20,
              samples = c("192566","192566SKL","192566SKN"),
              group.by = "orig.ident",
              palette = "inferno") +
  guides(fill = "none") +
  theme(axis.title = element_blank(),
        axis.text = element_text(size=6, color = "black"))

plot4 <- clonalCompare(object.TCells,
              cloneCall = "strict",
              top.clones = 20,
              samples = c("192567SKL", "192567SKN"),
              group.by = "orig.ident",
              palette = "inferno") +
  guides(fill = "none") +
  theme(axis.title = element_blank(),
        axis.text = element_text(size=6, color = "black"))

plot1 + plot2 + plot3 + plot4 + plot_layout(ncol = 4)
```

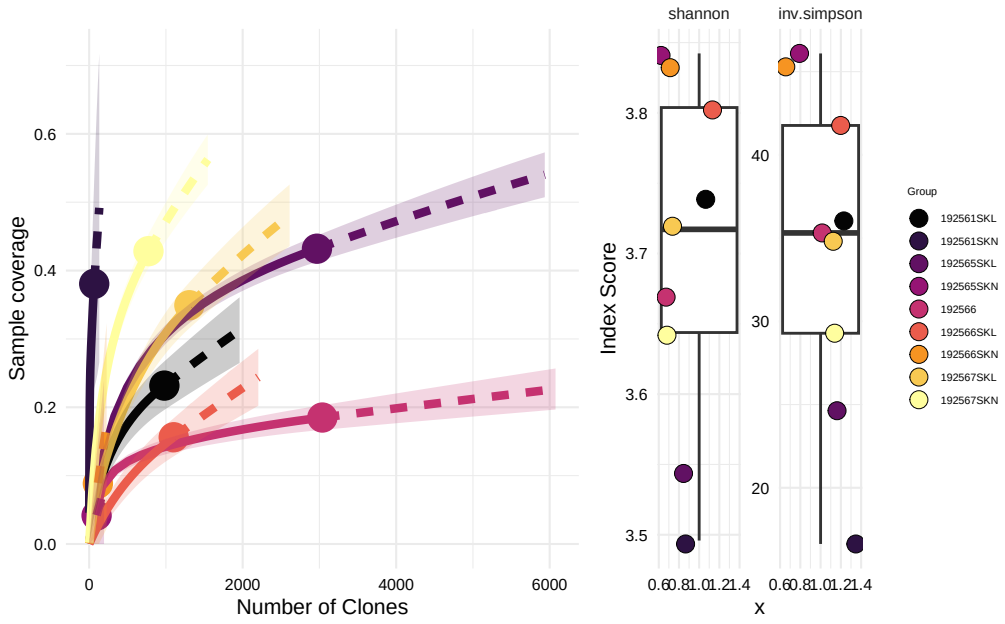



```
ggsave("output/Figure2C.pdf", height = 2, width = 4.25)
```

Clonal Rarefaction

```
plot1 <- clonalRarefaction(object.TCells,
                           group.by = "orig.ident",
                           plot.type = 2,
                           hill.numbers = 1,
                           n.boots = 10) +
                  xlab("Number of Clones") +
                  theme_minimal() +
                  custom_theme +
  guides(color = "none", fill = "none", lty = "none", shape = "none")

plot2 <- clonalDiversity(object.TCells,
                 group.by = "orig.ident",
                 metrics = c("shannon", "inv.simpson"),
                 cloneCall = "strict",
                 n.boots = 100) +
               theme_minimal() +
               custom_theme
```

```
plot1 + plot2 + plot_layout(ncol = 2, guides = "collect", widths = c(2.5, 1))
```



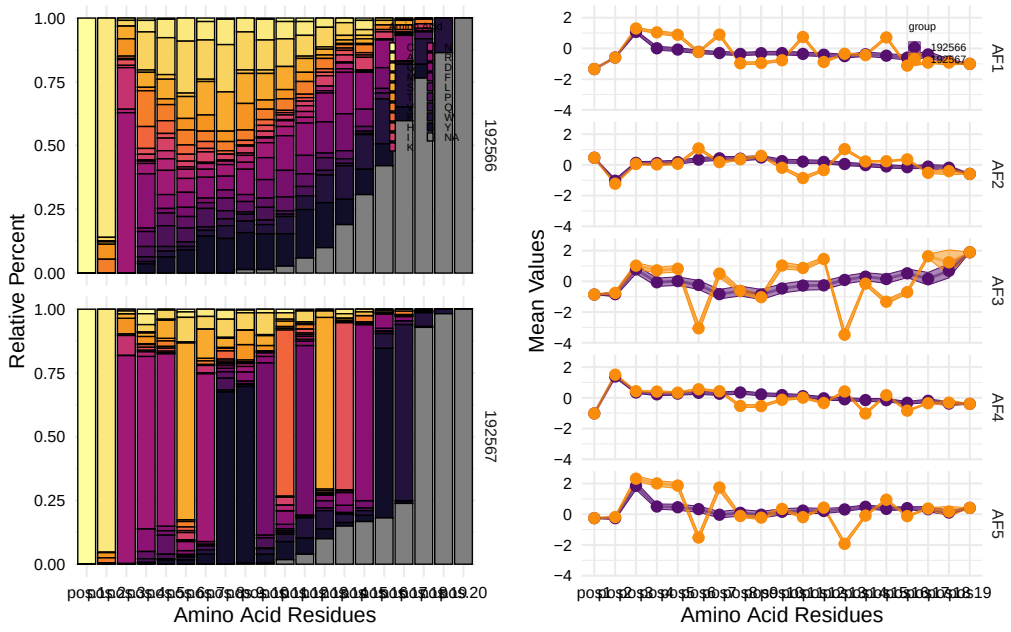

```
ggsave("output/Figure2D.pdf", height = 4, width = 8)
```

Amino Acid Summarization

```
plot1 <- percentAA(subset(object.BCells, Patient %in% c("192566", "192567")),
                        chain = "IGH",
                        group.by = "Patient") +
              custom_theme

plot2 <- positionalProperty(subset(object.BCells, Patient %in%
                                     c("192566", "192567")),
                        chain = "IGH",
                        group.by = "Patient") +
              custom_theme +
              scale_color_manual(values = viridis_pal(option = "inferno")(5)[c(2,4)]) +
              scale_fill_manual(values = viridis_pal(option = "inferno")(5)[c(2,4)])
```

```
Scale for colour is already present.
Adding another scale for colour, which will replace the existing scale.
Scale for fill is already present.
Adding another scale for fill, which will replace the existing scale.
```

```
plot1 + plot2 + plot_layout(ncol = 2)
```

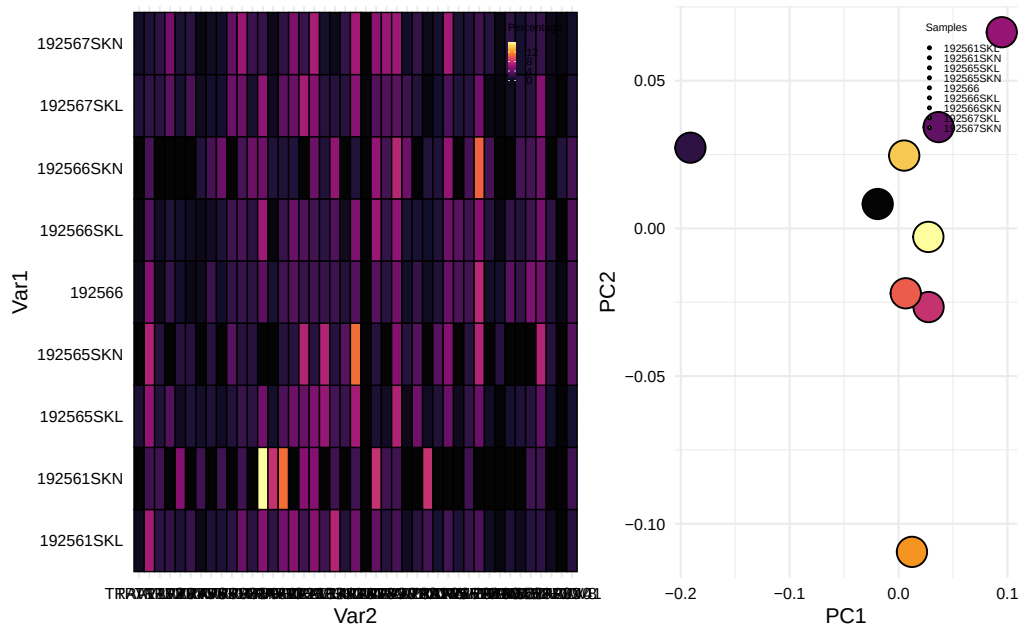



```
ggsave("output/Figure2E.pdf", height = 2, width = 8)
```

Percent Genes

```
plot1 <- percentGenes(object.TCells,
                      gene = "V",
                      chain = "TRA",
                      group.by = "orig.ident") +
             custom_theme

df.genes <- percentGenes(object.TCells,
                         gene = "V",
                         chain = "TRA",
```

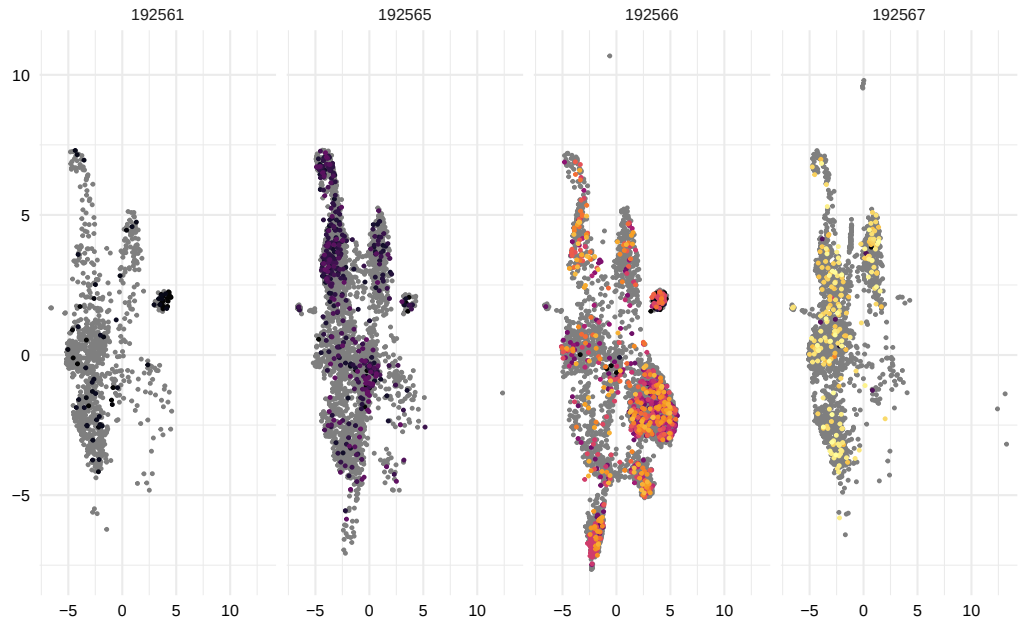
```
                          group.by = "orig.ident",
                          exportTable = TRUE)

#Performing PCA
pc <- prcomp(df.genes)

#Getting data frame to plot from
df <- as.data.frame(cbind(pc$x[,1:2], rownames(df.genes)))
df$PC1 <- as.numeric(df$PC1)
df$PC2 <- as.numeric(df$PC2)

#Plotting
plot2 <- ggplot(df, aes(x = PC1, y = PC2)) +
                 geom_point(aes(fill =df[,3]), shape = 21, size = 5) +
                 scale_fill_manual(values = hcl.colors(nrow(df), "inferno")) +
                 theme_minimal() +
                 ylab("PC2") +
                 xlab("PC1") +
                custom_theme +
                guides(fill = guide_legend(title="Samples",
                                           override.aes = list(size = 0.2)))

plot1 + plot2 + plot_layout(ncol = 2,
                                             widths = c(1.3, 1))
```




```
ggsave("output/Figure2F.pdf", height = 4, width = 9)
```

Clustering Clones

```
object.TCells <- clonalCluster(object.TCells,
                               chain = "TRA",
                               sequence = "aa",
                               threshold = 0.85,
                               group.by = "Patient")

DimPlot(object.TCells,
        group.by = "TRA_cluster",
        pt.size = 0.2,
        split.by = "Patient",
        order = TRUE) +
    scale_color_manual(values =  hcl.colors(n=length(unique(object.TCells@meta.data[,"TRA_clu
 guides(color = "none") +
  custom_theme +
  theme(plot.title = element_blank(),
        axis.title = element_blank())
```




```
ggsave("output/Figure2G_P1.pdf", height = 3, width = 9)
```

```
# Define a function for generating and plotting the network
plot_clonal_network <- function(patient_id, color_index, global_vertex_scale) {
  igraph_object <- clonalCluster(
    subset(object.TCells, Patient == patient_id),
    chain = "TRA",
    sequence = "aa",
    group.by = "Patient",
    threshold = 0.85,
    exportGraph = TRUE
  )

  # Generate color for the vertices
  col_samples <- hcl.colors(4, "inferno")[color_index]

  # Standardize vertex sizes
  vertex_sizes <- igraph::V(igraph_object)$size * global_vertex_scale

  # Plot the network with Fruchterman-Reingold layout
  plot(
    igraph_object,
    layout          = layout_with_fr(igraph_object),
```

```
    vertex.size    = vertex_sizes,
    vertex.label   = NA,
    edge.arrow.size = 0.05,
    vertex.color   = col_samples,
    main           = paste(patient_id)
  )
}

# Define patient IDs and color indices
patient_ids <- c("192561", "192565", "192566", "192567")
color_indices <- 1:4

# Calculate a global scaling factor for vertex sizes
all_sizes <- unlist(lapply(patient_ids, function(pid) {
  graph <- clonalCluster(
    subset(object.TCells, Patient == pid),
    chain = "TRA",
    sequence = "aa",
    group.by = "Patient",
    threshold = 0.85,
    exportGraph = TRUE
  )
  sqrt(igraph::V(graph)$size)
}))
global_vertex_scale <- 5 / max(all_sizes)  # Scale so max size is 5

pdf("output/Figure2G_P2.pdf", height = 3, width = 9)
# Set up the plotting grid
par(mfrow = c(1,4), mar = c(1, 1, 2, 1))
for (i in seq_along(patient_ids)) {
  plot_clonal_network(patient_ids[i], color_indices[i], global_vertex_scale)
}
dev.off()
```

```
pdf
  2
```

Conclusion

```
sessionInfo()
```

```
R version 4.4.2 (2024-10-31)
Platform: aarch64-apple-darwin20
Running under: macOS Sonoma 14.0

Matrix products: default
BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib;  L

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

time zone: America/Chicago
tzcode source: internal

attached base packages:
[1] stats4    stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] viridis_0.6.5               viridisLite_0.4.2
 [3] stringr_1.5.1               SingleR_2.8.0
 [5] pbmcref.SeuratData_1.0.0    SeuratData_0.2.2.9001
 [7] Seurat_5.1.0                SeuratObject_5.0.2
 [9] sp_2.1-4                    scran_1.34.0
[11] scuttle_1.16.0              scRepertoire_2.2.1
[13] scGate_1.6.2                scDblFinder_1.20.0
[15] SingleCellExperiment_1.28.1 RColorBrewer_1.1-3
[17] patchwork_1.3.0             igraph_2.1.2
[19] harmony_1.2.3               Rcpp_1.0.13-1
[21] ggthemes_5.1.0              ggplot2_3.5.1
[23] dplyr_1.1.4                 celldex_1.16.0
[25] SummarizedExperiment_1.36.0 Biobase_2.66.0
[27] GenomicRanges_1.58.0        GenomeInfoDb_1.42.0
[29] IRanges_2.40.0              S4Vectors_0.44.0
[31] BiocGenerics_0.52.0         MatrixGenerics_1.18.0
[33] matrixStats_1.4.1           BiocParallel_1.40.0
[35] Azimuth_0.5.0               shinyBS_0.61.1

loaded via a namespace (and not attached):
```

```
 [1] hash_2.2.6.3                ica_1.0-3
 [3] plotly_4.10.4               scater_1.34.0
 [5] zlibbioc_1.52.0             tidyselect_1.2.1
 [7] bit_4.5.0.1                 lattice_0.22-6
 [9] rjson_0.2.23                evmix_2.12
[11] blob_1.2.4                  S4Arrays_1.6.0
[13] parallel_4.4.2              seqLogo_1.68.0
[15] png_0.1-8                   cli_3.6.3
[17] ProtGenerics_1.34.0         goftest_1.2-3
[19] gargle_1.5.2                textshaping_0.4.1
[21] BiocIO_1.12.0               bluster_1.16.0
[23] purrr_1.0.2                 BiocNeighbors_2.0.0
[25] Signac_1.14.0               stringdist_0.9.14
[27] uwot_0.2.2                  curl_6.0.1
[29] mime_0.12                   evaluate_1.0.1
[31] leiden_0.4.3.1              stringi_1.8.4
[33] gsl_2.1-8                   XML_3.99-0.17
[35] httpuv_1.6.15               AnnotationDbi_1.68.0
[37] magrittr_2.0.3              rappdirs_0.3.3
[39] splines_4.4.2               RcppRoll_0.3.1
[41] ggraph_2.2.1                DT_0.33
[43] sctransform_0.4.1           ggbeeswarm_0.7.2
[45] DBI_1.2.3                   HDF5Array_1.34.0
[47] withr_3.0.2                 systemfonts_1.1.0
[49] tfruns_1.5.3                xgboost_1.7.8.1
[51] lmtest_0.9-40               tidygraph_1.3.1
[53] Trex_0.99.12                rtracklayer_1.66.0
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[61] SparseArray_1.6.0           cellranger_1.1.0
[63] annotate_1.84.0             reticulate_1.40.0
[65] zoo_1.8-12                  JASPAR2020_0.99.10
[67] XVector_0.46.0              knitr_1.49
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[83] alabaster.schemas_1.6.0     fastDummies_1.7.4
[85] lazyeval_0.2.2              yaml_2.3.10
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[111] BSgenome_1.74.0               alabaster.base_1.6.1
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[115] httr_1.4.7                    tools_4.4.2
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[121] hdf5r_1.3.11                  ExperimentHub_2.14.0
[123] shinyjs_2.1.0                 MatrixModels_0.5-3
[125] assertthat_0.2.1              digest_0.6.37
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[129] tzdb_0.4.0                    AnnotationFilter_1.26.0
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[133] glue_1.8.0                    cachem_1.1.0
[135] BiocFileCache_2.14.0          polyclip_1.10-7
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[171] cluster_2.1.8                 whisker_0.4.1
```

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[243] metapod_1.14.0                    locfit_1.5-9.10
[245] jsonlite_1.8.9
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