Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

Sergej Ruff, AG Jung, 01.06.2022

Forschungskonzeption: single-cell RNA-seq analysis

Inhalt

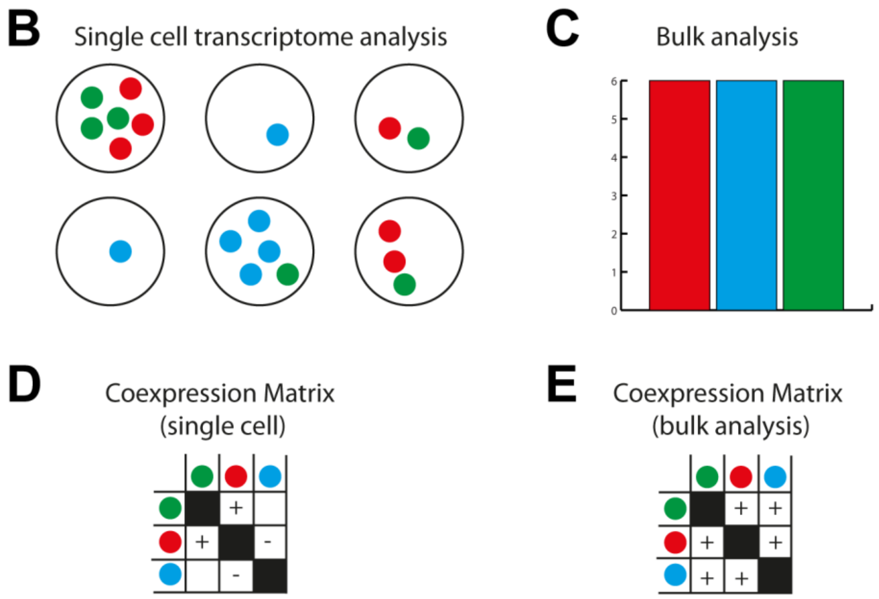
-Einleitung: Droplet und Information zum Paper

- Methoden und Daten: Datenanalyse in Seurat

-Forschungshypothesen für die Bachelorarbeit

Wozu Single-Cell RNA-seq?

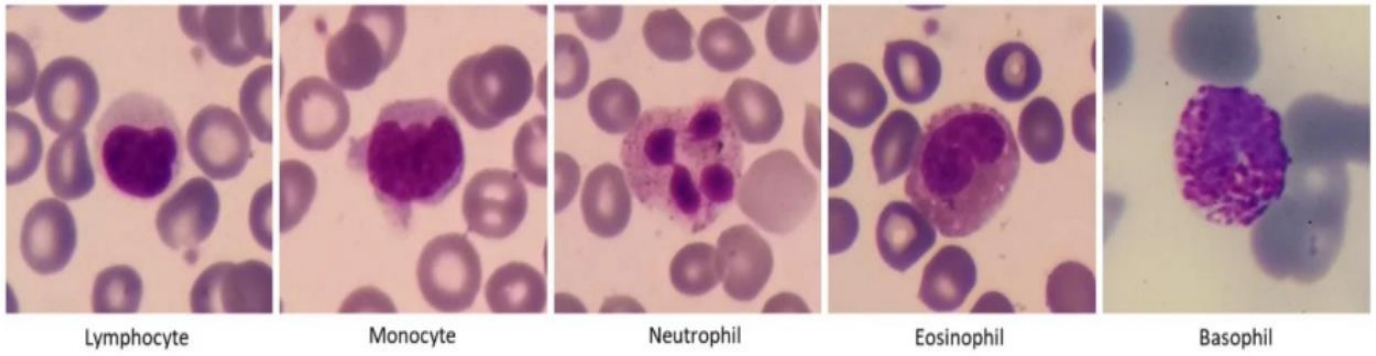
- Heterogene Zellpopulationen
  - Charakterisierung
  - Identifizierung
  - Krankheiten
- Untersuchung von Co-Expression-Mustern
- Expressionsunterschiede
- Untersuchung seltener Zellpopulationen



	🟢	🔴	🔵
🟢	■	+	
🔴	+	■	-
🔵		-	■

	🟢	🔴	🔵
🟢	■	+	+
🔴	+	■	+
🔵	+	+	■

Macaulay IC, Voet T (2014) Single Cell Genomics: Advances and Future Perspectives. PLoS Genet 10(1): e1004126. https://doi.org/10.1371/journal.pgen.1004126

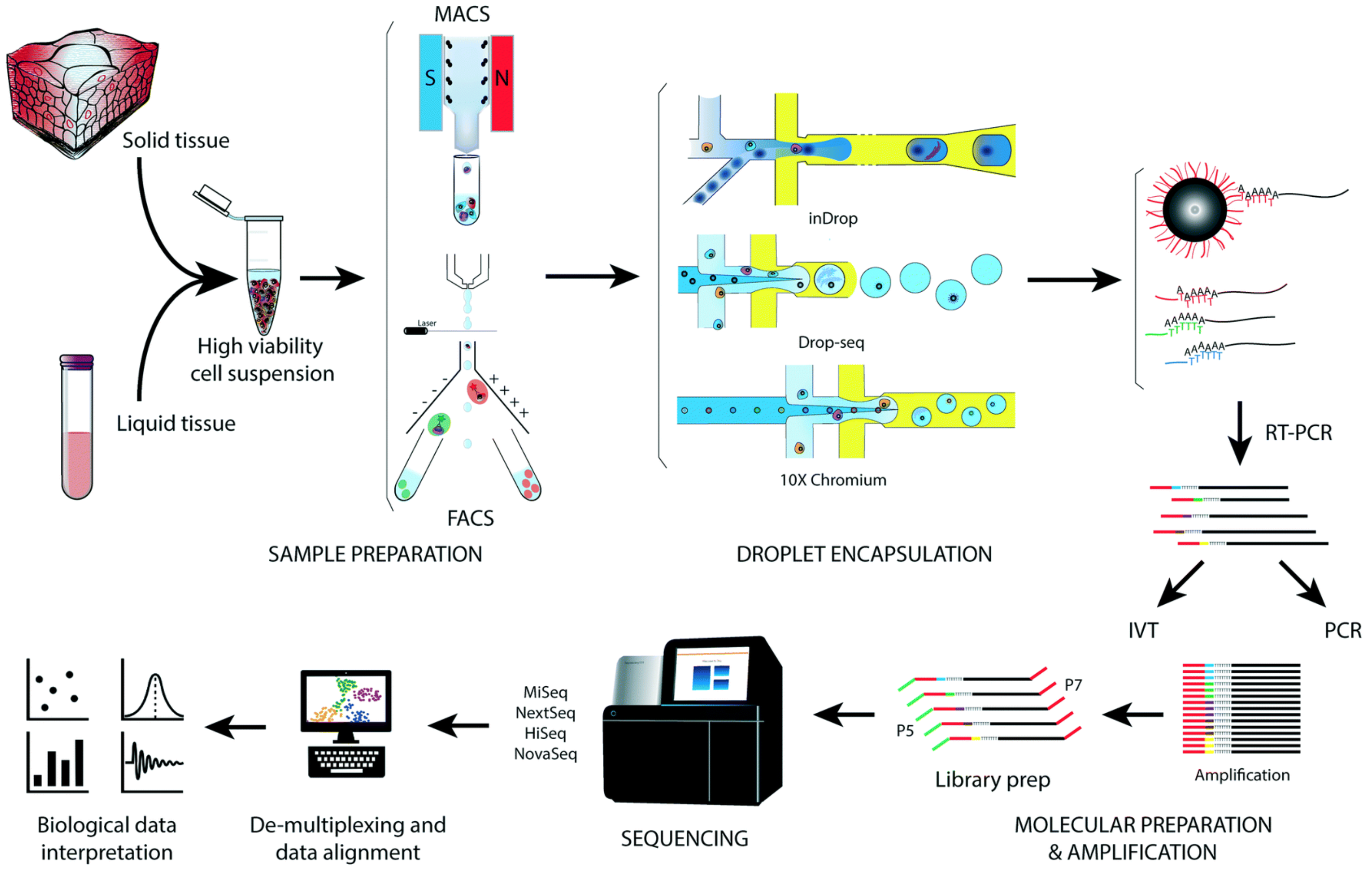


Five types of white blood cells in the normal peripheral blood.

Kouzehkanan, Z.M., Saghari, S., Tavakoli, S. *et al.* A large dataset of white blood cells containing cell locations and types, along with segmented nuclei and cytoplasm. *Sci Rep* **12,** 1123 (2022). https://doi.org/10.1038/s41598-021-04426-x

Droplet basierte Methoden

1. Vorbereitung der Zellsuspension
2. Zellsortierung
3. Einkapseln einzelner Zellen in Droplets
4. CDNA-Synthese und Amplifikation
5. Bibliothek
6. Sequenzierung, data alignment und Interpretation



Salomon, Robert, et al. "Droplet-based single cell RNAseq tools: a practical guide." *Lab on a Chip* 19.10 (2019): 1706-1727.

Beads

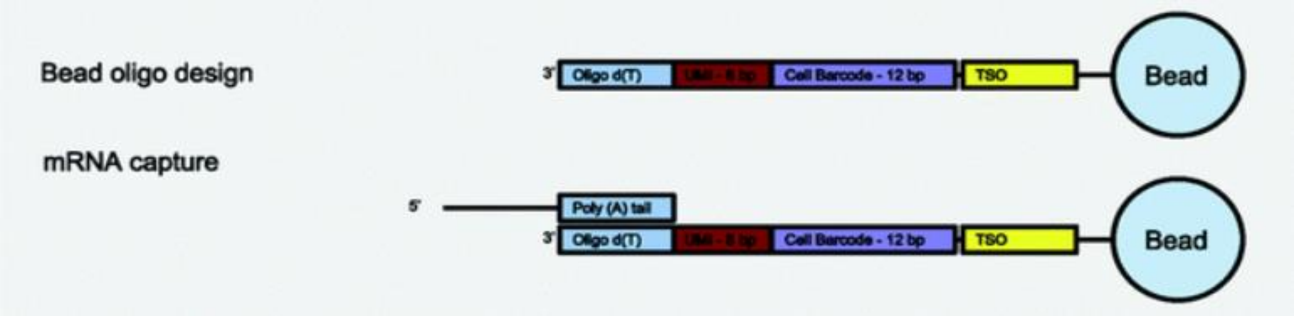
Oligo-d(T) Primer bindet den Poly-A-Schwanz der RNA.

Unique Molecular Identifiers (UMI): Zufällige, einzigartige Sequenzen auf den Beads, die als Tags die einzelnen Moleküle markieren.

- Transcript counting, normalisation of amplification artifacts

Cell Barcode: identische, mehrmals auftauchende Sequenz auf den Beads. Für Identifikation einzelner Zellen.

Primer Region für Amplifikationen - **Template Switching Oligonucleotides (TSO)**



Salomon, Robert, et al. "Droplet-based single cell RNAseq tools: a practical guide." *Lab on a Chip* 19.10 (2019): 1706-1727.

Entwicklung eines Forschungskonzepts

Information zum Paper

iScience. 2021 Nov 19; 24(11): 103325.
Published online 2021 Oct 23. doi: 10.1016/j.isci.2021.103325

PMCID: PMC8536484
PMID: 34723157

Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19

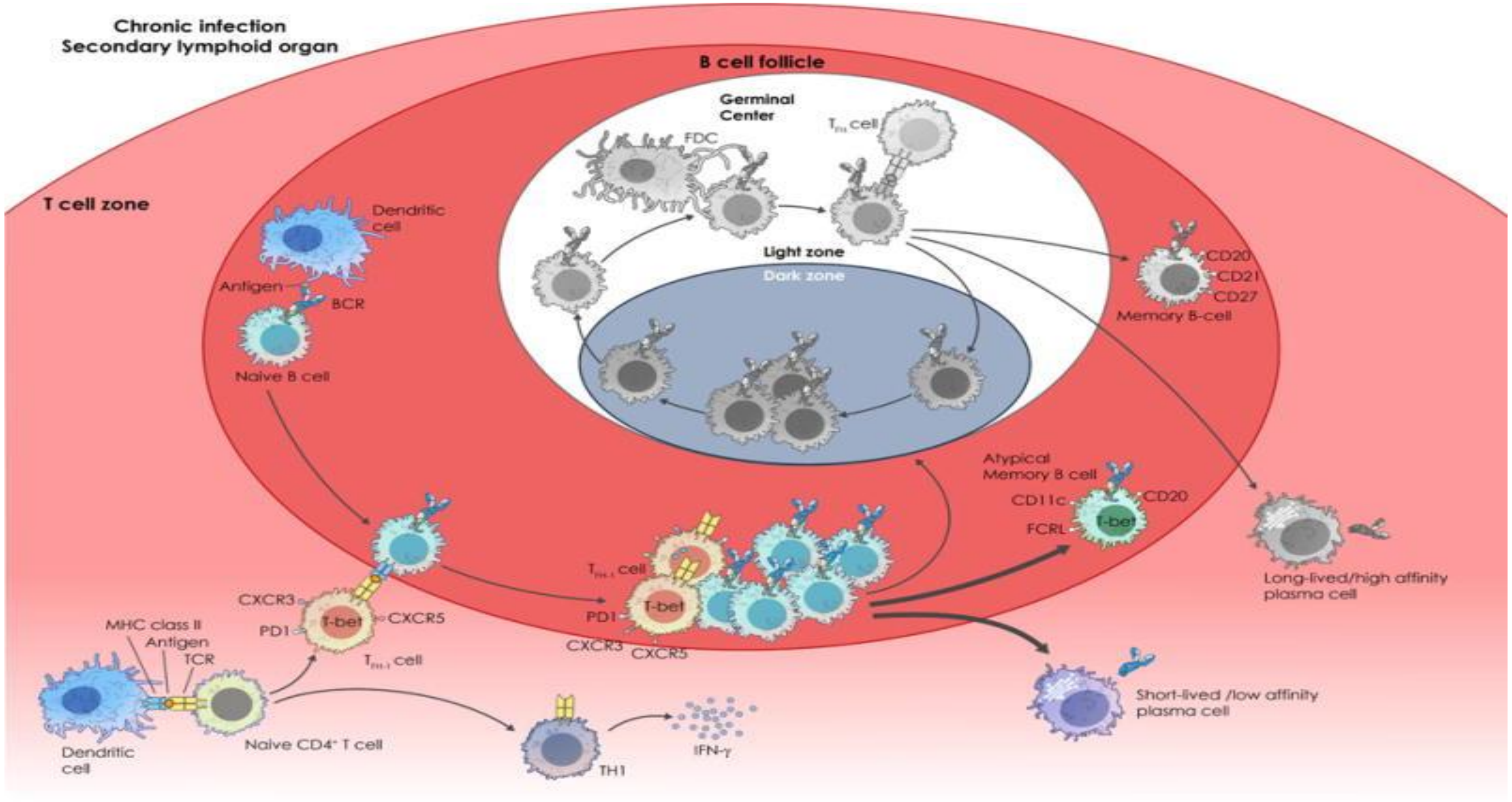
Christoph Schultheiß,[1] Lisa Paschold,[1] Edith Willscher,[1] Donjete Simnica,[1] Anna Wöstemeier,[2] Franziska Muscate,[2] Maxi Wass,[1] Stephan Eisenmann,[3] Jochen Dutzmann,[4] Gernot Keyßer,[5] Nicola Gagliani,[2,6,7] and Mascha Binder[1,8,*]

- doi: 10.1016/j.isci.2021.103325
- Veröffentlichung: 19.11.2021
- Online Veröffentlicht: 23.10.2021

-ArrayExpress: E-MTAB-11011

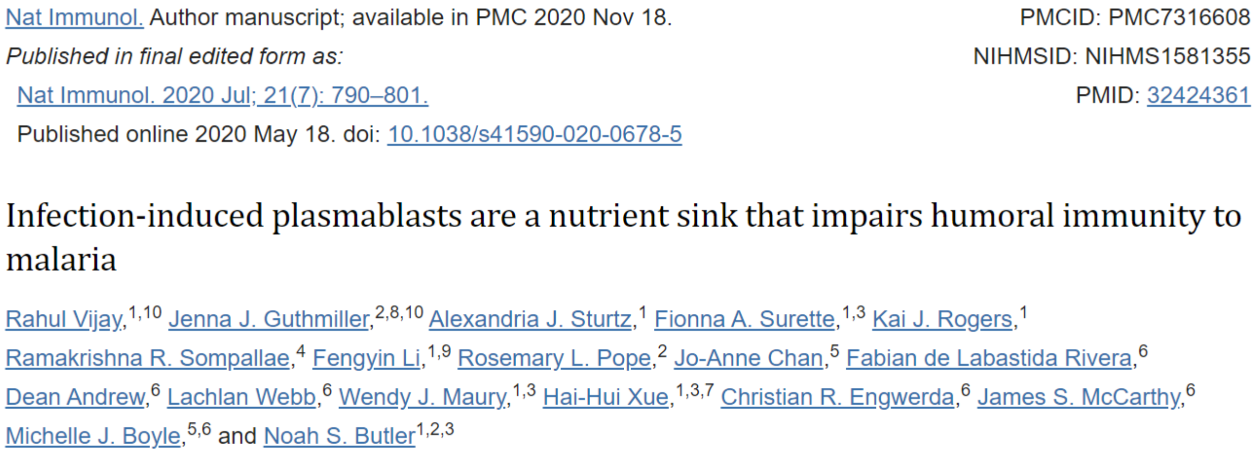
Schultheiß, Christoph, et al. "Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19." *Iscience* 24.11 (2021): 103325.

A model for the generation of T cell-dependent B cell memory



Portugal, Silvia et al. "Atypical memory B cells in human chronic infectious diseases: An interim report." *Cellular immunology* vol. 321 (2017): 18-25. doi:10.1016/j.cellimm.2017.07.003

Wozu?



Nat Immunol. Author manuscript; available in PMC 2020 Nov 18. PMCID: PMC7316608

Published in final edited form as: NIHMSID: NIHMS1581355

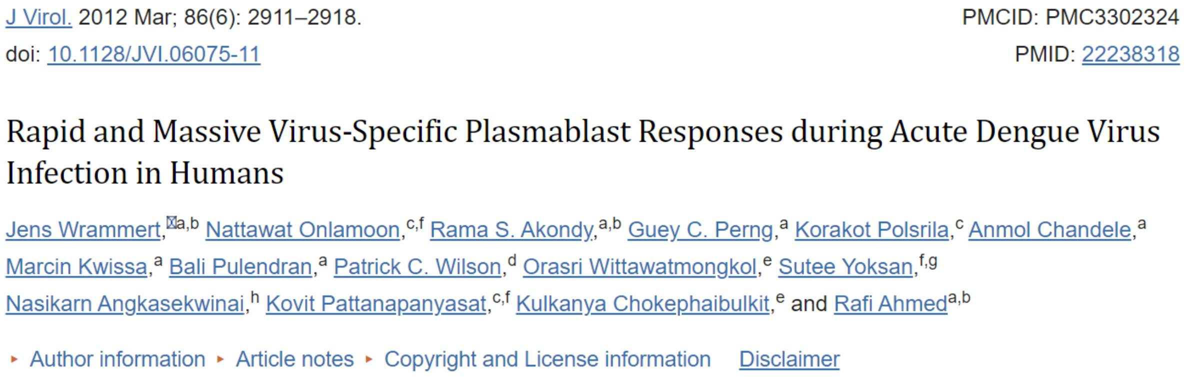
Nat Immunol. 2020 Jul; 21(7): 790–801. PMID: 32424361

Published online 2020 May 18. doi: 10.1038/s41590-020-0678-5

Infection-induced plasmablasts are a nutrient sink that impairs humoral immunity to malaria

Rahul Vijay,[1,10] Jenna J. Guthmiller,[2,8,10] Alexandria J. Sturtz,[1] Fionna A. Surette,[1,3] Kai J. Rogers,[1] Ramakrishna R. Sompallae,[4] Fengyin Li,[1,9] Rosemary L. Pope,[2] Jo-Anne Chan,[5] Fabian de Labastida Rivera,[6] Dean Andrew,[6] Lachlan Webb,[6] Wendy J. Maury,[1,3] Hai-Hui Xue,[1,3,7] Christian R. Engwerda,[6] James S. McCarthy,[6] Michelle J. Boyle,[5,6] and Noah S. Butler[1,2,3]

Vijay, Rahul et al. “Infection-induced plasmablasts are a nutrient sink that impairs humoral immunity to malaria.” *Nature immunology* vol. 21,7 (2020): 790-801. doi:10.1038/s41590-020-0678-5



J Virol. 2012 Mar; 86(6): 2911–2918. PMCID: PMC3302324

doi: 10.1128/JVI.06075-11 PMID: 22238318

Rapid and Massive Virus-Specific Plasmablast Responses during Acute Dengue Virus Infection in Humans

Jens Wrammert,⊠[a,b] Nattawat Onlamoon,[c,f] Rama S. Akondy,[a,b] Guey C. Perng,[a] Korakot Polsrila,[c] Anmol Chandele,[a] Marcin Kwissa,[a] Bali Pulendran,[a] Patrick C. Wilson,[d] Orasri Wittawatmongkol,[e] Sutee Yoksan,[f,g] Nasikarn Angkasekwinai,[h] Kovit Pattanapanyasat,[c,f] Kulkanya Chokephaibulkit,[e] and Rafi Ahmed[a,b]

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Wrammert, Jens et al. “Rapid and massive virus-specific plasmablast responses during acute dengue virus infection in humans.” *Journal of virology* vol. 86,6 (2012): 2911-8. doi:10.1128/JVI.06075-11

Wozu wurde Single Cell im Paper genutzt?

COVID-19 als Modell:

- Kein Exposure= kein Memory.

- Frühere Verweise auf...

  ... *Vermeidung von GC-Reaktionen.*

  ... *Hohe Konzentration an PB.*

Modell, um B-Zell-Antworten und ihre Konsequenzen auf das Immunologische Gedächtnis und Immunpathologie zu untersuchen.

- Untersuchung der Zellpopulationen!

Schultheiß, Christoph, et al. "Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19." *Iscience* 24.11 (2021): 103325.

Daten

Beschreibung

Sc-Seq Daten von B-Lymphozyten aus PBMC
für gesunde Patienten („HD“), "active" COVID-19 Patienten und "recovered" Patienten.

Datenset

E-MTAB-11011

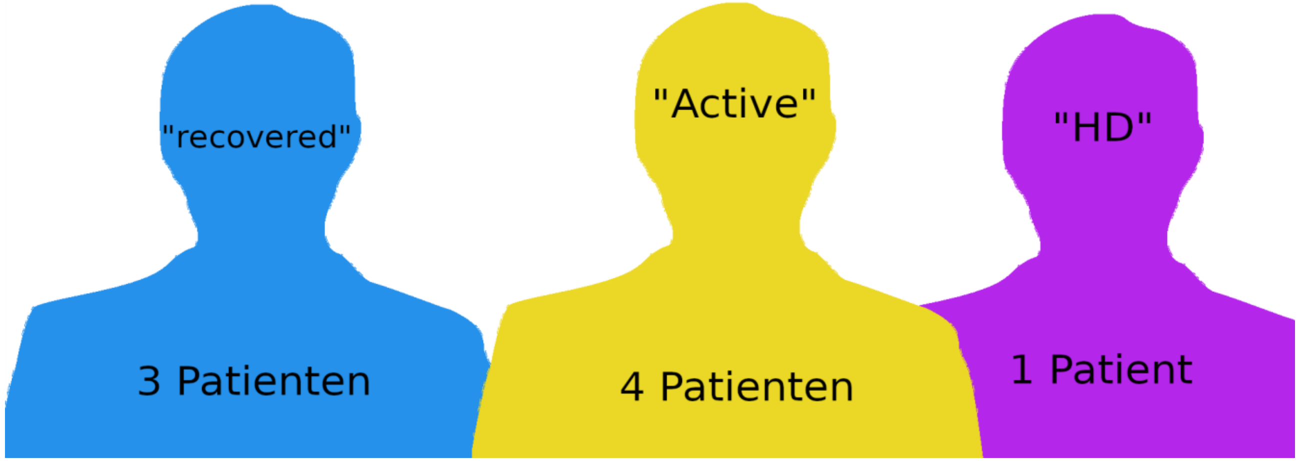
Ordner

E-MTAB-11011.processed.1

Counts (Pre-Processed)

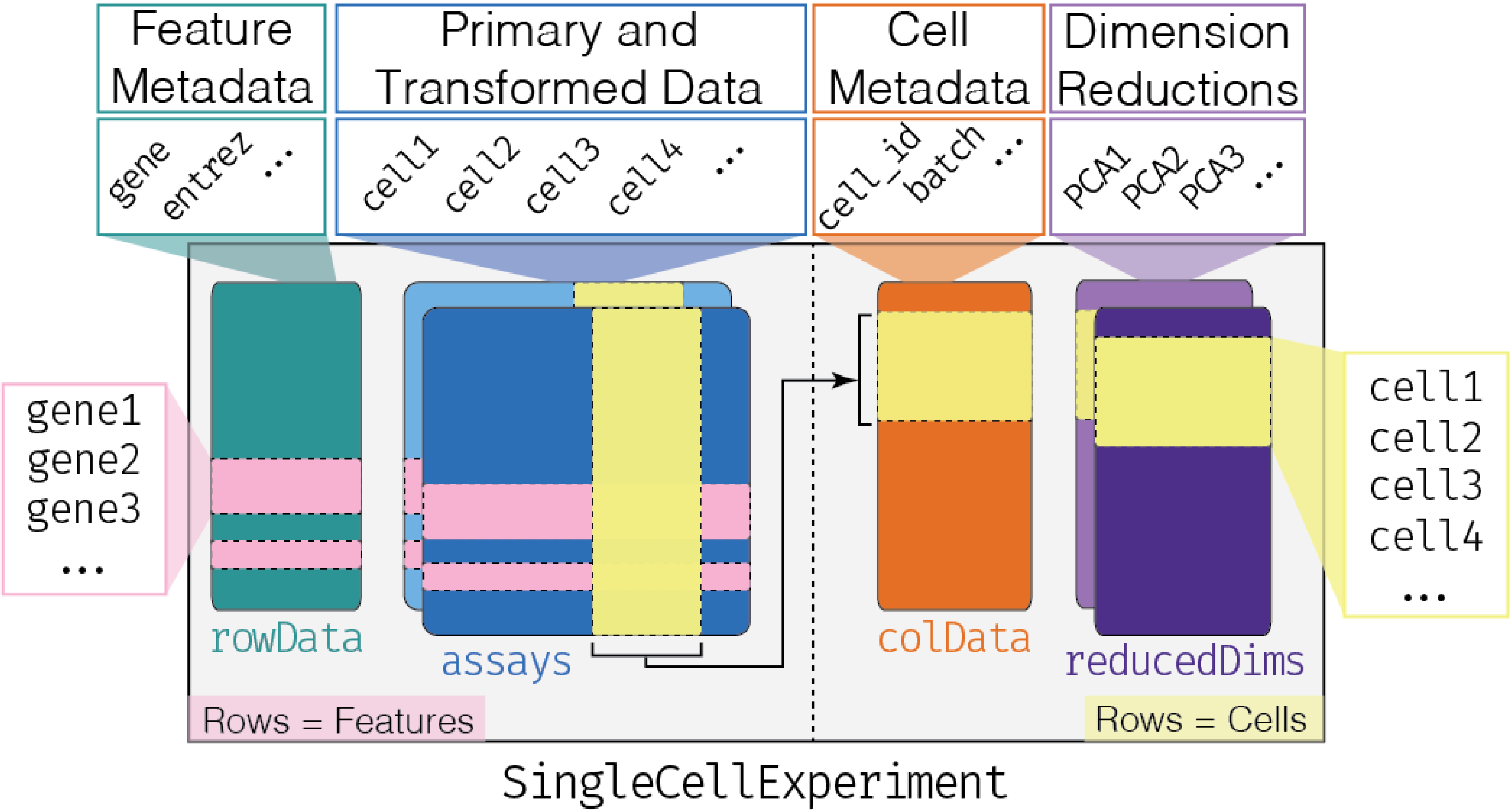
pbmc.HD_gex_and_vdj.rds
pbmc.active.2.5.3.8_gex_and_vdj.rds
pbmc.recovered.14.16.26_gex_and_vdj.rds

***Patienten für scRNA-seq*.**



Schultheiß, Christoph, et al. "Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19." *Iscience* 24.11 (2021): 103325.

SingleCellExperiment Class (Bioconductor)



Slots

Assays: Primäre Daten (Matrix mit Seq.-Counts).

rowData: Information zu den Genen (Reihen des Assays)

ColData: Information zu den Zellen (Spalten des Assays).

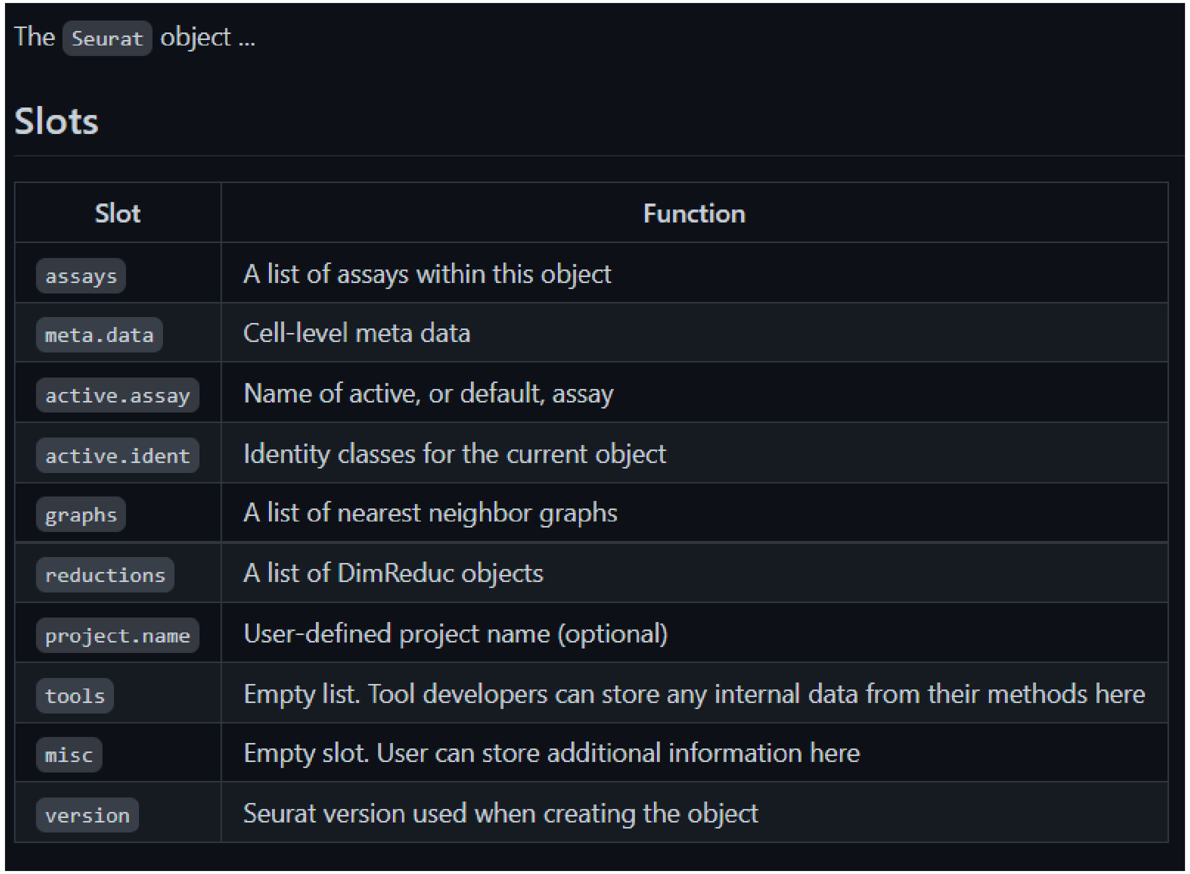
Besonderheiten:

reducedDims: dimensionale Reduktionen.

Alternative Experimente

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." *Nature methods* 17.2 (2020): 137-145.

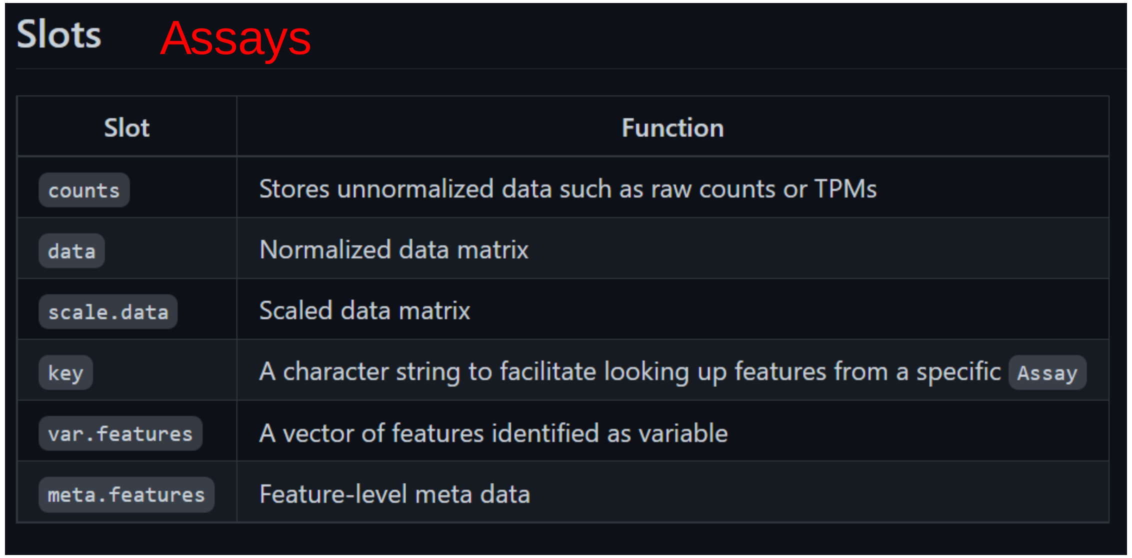
Seurat



The `Seurat` object ...

Slots

Slot	Function
`assays`	A list of assays within this object
`meta.data`	Cell-level meta data
`active.assay`	Name of active, or default, assay
`active.ident`	Identity classes for the current object
`graphs`	A list of nearest neighbor graphs
`reductions`	A list of DimReduc objects
`project.name`	User-defined project name (optional)
`tools`	Empty list. Tool developers can store any internal data from their methods here
`misc`	Empty slot. User can store additional information here
`version`	Seurat version used when creating the object

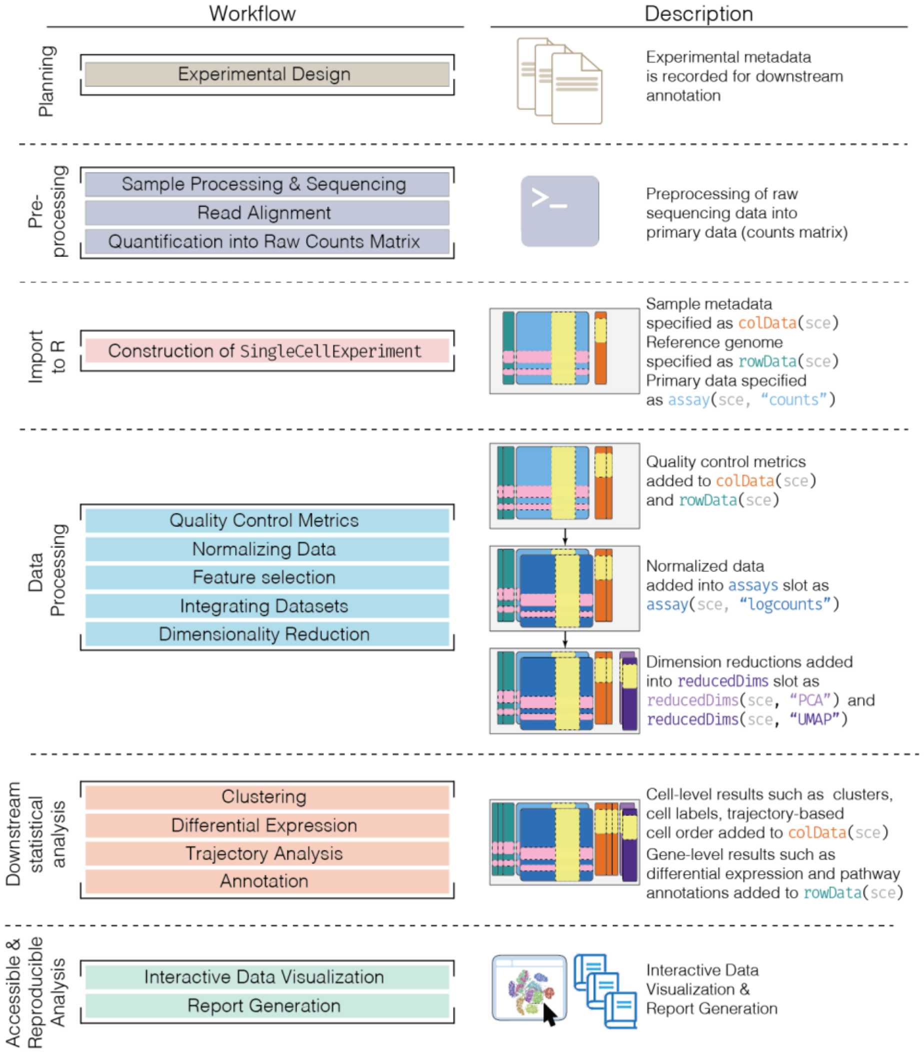


Slots Assays

Slot	Function
`counts`	Stores unnormalized data such as raw counts or TPMs
`data`	Normalized data matrix
`scale.data`	Scaled data matrix
`key`	A character string to facilitate looking up features from a specific `Assay`
`var.features`	A vector of features identified as variable
`meta.features`	Feature-level meta data

https://github.com/satijalab/seurat/wiki/Assay (Letzter Zugriff: 23.05.2022, 13:50)

Basic Pipeline



Workflow

Description

Planning

Experimental Design

Experimental metadata is recorded for downstream annotation

Pre-processing

Sample Processing & Sequencing

Read Alignment

Quantification into Raw Counts Matrix

Preprocessing of raw sequencing data into primary data (counts matrix)

Import to R

Construction of SingleCellExperiment

Sample metadata specified as colData(sce)
Reference genome specified as rowData(sce)
Primary data specified as assay(sce, "counts")

Data Processing

Quality Control Metrics

Normalizing Data

Feature selection

Integrating Datasets

Dimensionality Reduction

Quality control metrics added to colData(sce) and rowData(sce)

Normalized data added into assays slot as assay(sce, "logcounts")

Dimension reductions added into reducedDims slot as reducedDims(sce, "PCA") and reducedDims(sce, "UMAP")

Downstream statistical analysis

Clustering

Differential Expression

Trajectory Analysis

Annotation

Cell-level results such as clusters, cell labels, trajectory-based cell order added to colData(sce)
Gene-level results such as differential expression and pathway annotations added to rowData(sce)

Accessible & Reproducible Analysis

Interactive Data Visualization

Report Generation

Interactive Data Visualization & Report Generation

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." *Nature methods* 17.2 (2020): 137-145.

Packages

```
library("Seurat")
library("celldex")
library("SingleR")
library("biomaRt")
library("clusterProfiler")
library("org.Hs.eg.db")

library("SingleCellExperiment")
```

Import in R

Daten importieren

readSparseCounts(): *scuttle* package

read10xCounts(): *DropletUtils* package

readRDS()

readH5AD(): *zellkonverter* package

Erschaffen eines sce-Objektes oder Seurat-Objektes

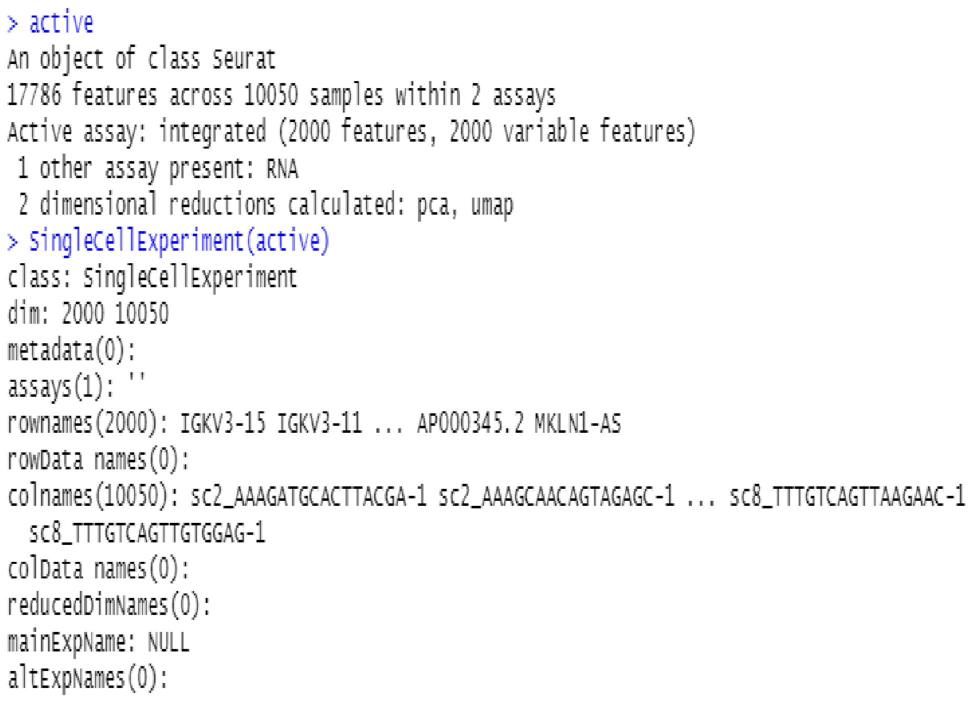
SingleCellExperiment():*SingleCellExperiment* package

as.SingleCellExperiment(): *Seurat* package

As.Seurat: *Seurat* package

Datenzugriff

Counts(sce),assay(sce,"counts"),assays(sce)



```
> active
An object of class Seurat
17786 features across 10050 samples within 2 assays
Active assay: integrated (2000 features, 2000 variable features)
 1 other assay present: RNA
 2 dimensional reductions calculated: pca, umap
> SingleCellExperiment(active)
class: SingleCellExperiment
dim: 2000 10050
metadata(0):
assays(1): ''
rownames(2000): IGKV3-15 IGKV3-11 ... AP000345.2 MKLN1-AS
rowData names(0):
colnames(10050): sc2_AAAGATGCACTTACGA-1 sc2_AAAGCAACAGTAGAGC-1 ... sc8_TTTGTCAGTTAAGAAC-1
  sc8_TTTGTCAGTTGTGGAG-1
colData names(0):
reducedDimNames(0):
mainExpName: NULL
altExpNames(0):
```

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." Nature methods 17.2 (2020): 137-145

Quality Control

Motivation

Finden und Entfernen von low-quality libraries

Gründe für Low-quality Libraries

Zellschäden (Mitochondriale RNA "↑",endogene RNA↓)

Fehler in der Reverse Transkriptase oder PCR-Amplifikation

Wie beeinflussen Sie die Analyse?

Bilden eigene Cluster

Clustern verschiedene Zelltypen zusammen (induzierte Expressionsprofile)

Einfluss auf PCA

QC-Metrics

-Anzahl einzigartiger Gene pro Zelle
-Spike-Ins
-Anreicherung an mt-RNA

Bsp. Aus Tutorial pbmc= Seurat-Objekt

```
pbmc[["percent.mt"]] <-PercentageFeatureSet(pbmc, pattern = "^MT-")
```

"The calculation here is simply the column sum of the matrix present in the counts slot for features belonging to the set divided by the column sum for all features times 100."

```
##                  orig.ident nCount_RNA nFeature_RNA percent.mt
## AAACATACAACCAC-1     pbmc3k       2419          779  3.0177759
## AAACATTGAGCTAC-1     pbmc3k       4903         1352  3.7935958
## AAACATTGATCAGC-1     pbmc3k       3147         1129  0.8897363
## AAACCGTGCTTCCG-1     pbmc3k       2639          960  1.7430845
## AAACCGTGTATGCG-1     pbmc3k        980          521  1.2244898
```

Visualisierung der QC-Metrics als Violin-Plot und Scatter

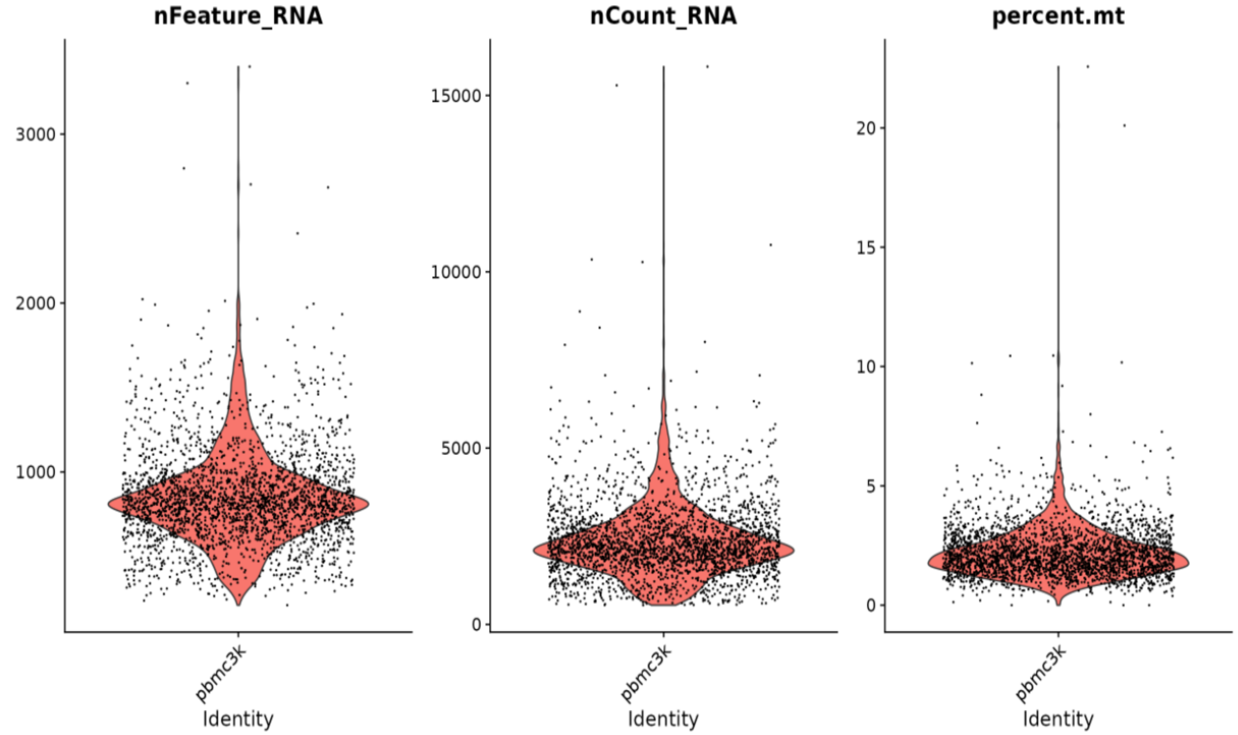
```
VlnPlot(pbmc, features = c("nFeature_RNA", "nCount_RNA",
"percent.mt"), ncol = 3)

plot2 <- FeatureScatter(pbmc, feature1 = "nCount_RNA", feature2 =
"nFeature_RNA")
```

```
pbmc <- subset(pbmc, subset = nFeature_RNA > 200 & nFeature_RNA < 2500 &
percent.mt < 5)
```

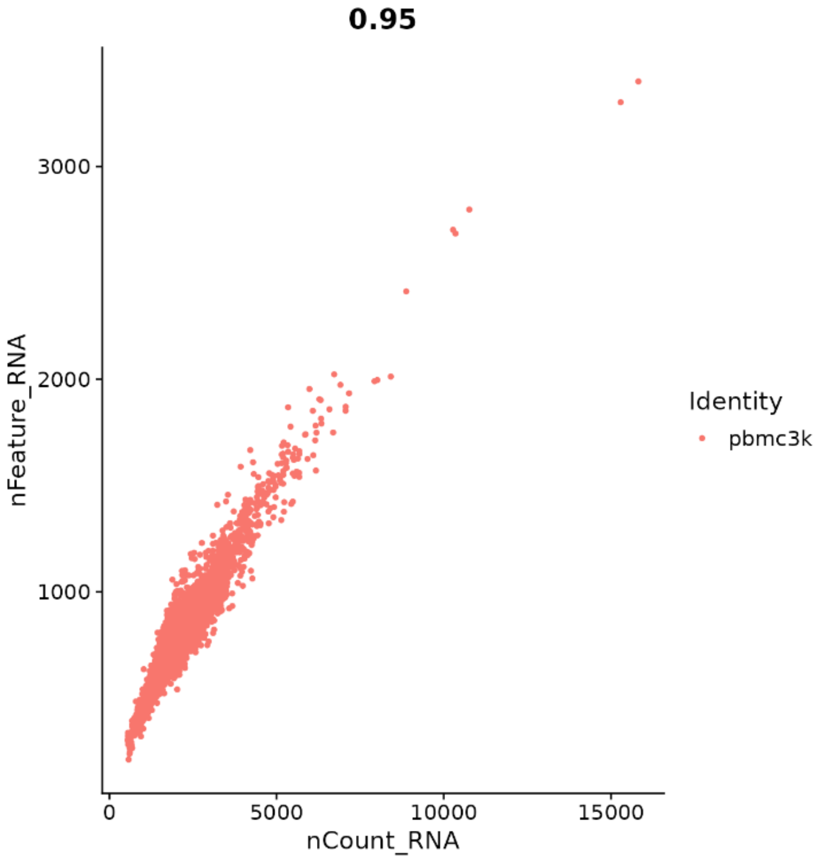
Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." Nature methods 17.2 (2020): 137-145

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

Quality Control (2)



Violinplot



Featurescatter
Median absolute Deviation (MAD) für QC.
3*MAD=Ausreißer

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." Nature methods 17.2 (2020): 137-145

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

Eine weitere Qualitätskontrolle: SingleR

Verunreinigung durch andere Zelltypen?

Lösung: Zelltyp-Annotation mit *SingleR* package!

Automatisches Annotationsmethode

Abgleich mit Referenz-Datenset mit bekannten Labeln

hpca.se=celldex::HumanPrimaryCellAtlasData()#reference data

Braucht ein SummarizedExperiment-Object

sce = as.SingleCellExperiment(SeuratObj) #convert Seurat to SingleCellExperiment

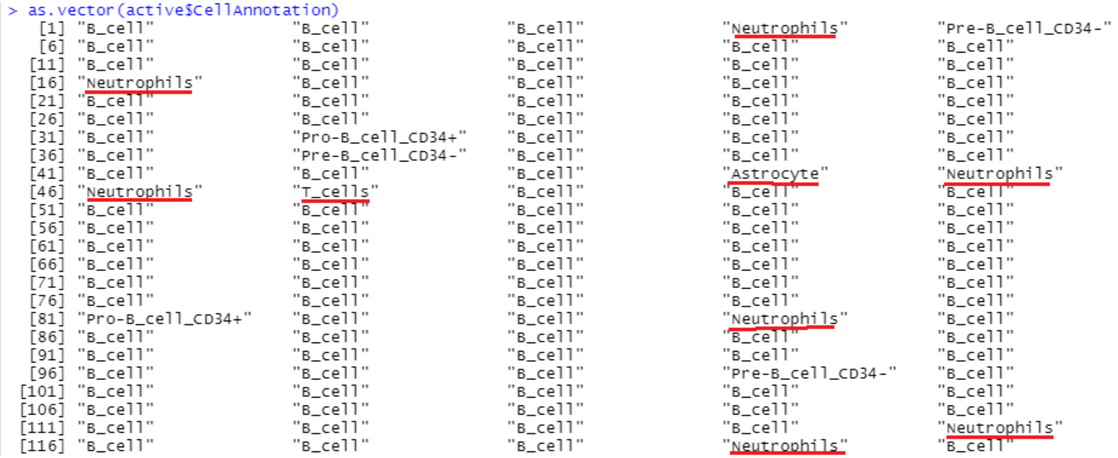
 se = as(sce, "SummarizedExperiment") #convert SingleCellExperiment to SummarizedExperiment

SingleR

pred = SingleR(test = se, ref = hpca.se, assay.type.test=1,

 labels = hpca.se$label.main)

Entfernen der falschen Zellen



```
> as.vector(active$CellAnnotation)
  [1] "B_cell"           "B_cell"           "B_cell"           "Neutrophils"      "Pre-B_cell_CD34-"
  [6] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [11] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [16] "Neutrophils"      "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [21] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [26] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [31] "B_cell"           "Pro-B_cell_CD34+" "B_cell"           "B_cell"           "B_cell"
 [36] "B_cell"           "Pre-B_cell_CD34-" "B_cell"           "B_cell"           "B_cell"
 [41] "B_cell"           "B_cell"           "B_cell"           "Astrocyte"        "Neutrophils"
 [46] "Neutrophils"      "T_cells"          "B_cell"           "B_cell"           "B_cell"
 [51] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [56] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [61] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [66] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [71] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [76] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [81] "Pro-B_cell_CD34+" "B_cell"           "B_cell"           "Neutrophils"      "B_cell"
 [86] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [91] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
 [96] "B_cell"           "B_cell"           "B_cell"           "Pre-B_cell_CD34-" "B_cell"
[101] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
[106] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "B_cell"
[111] "B_cell"           "B_cell"           "B_cell"           "B_cell"           "Neutrophils"
[116] "B_cell"           "B_cell"           "B_cell"           "Neutrophils"      "B_cell"
```

```
> active = remWC(active)
[1] "Percentage B-cells: 92.22 %"
> recovered = remWC(recovered)
[1] "Percentage B-cells: 99.34 %"
> hd = remWC(hd)
[1] "Percentage B-cells: 100 %"
```

https://bioconductor.org/packages/release/bioc/html/SingleR.html Letzter Zugriff: 23.05.2022 19:31

Daten Normalisierung

Motivation

Entfernen von systematischen, nicht-biologischen Variationen

Fehlerquellen

Fehler in cDNA-capture und PCR-amp.

Methoden

Library Size Normalisierung

Spike-In Normalisierung

Deconvolution

Aus Tutorial

```
pbmc <- NormalizeData(pbmc, normalization.method = "LogNormalize", scale.factor = 10000)
```

- "LogNormalize: Feature counts for each cell are divided by the total counts for that cell and multiplied by the scale.factor. This is then natural-log transformed using log1p."

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." Nature methods 17.2 (2020): 137-145

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

Identifizieren von Hoch-Variablen Features und Scaling

Motivation

Auswahl an Genen, die nützliche Information über die Biologie des Systems enthalten für Downstreamanalysen

Hoch-Variable Gene suchen

```
SeuratObj = FindVariableFeatures(SeuratObj, selection.method = "vst", nfeatures = 2000)
```

Skalierung der Daten

```
all.genes_SeuratObj = rownames(SeuratObj)
SeuratObj = ScaleData(SeuratObj, features = all.genes_SeuratObj)
```

- "Shifts the expression of each gene, so that the mean expression across cells is 0"
- "Scales the expression of each gene, so that the variance across cells is 1"

➔ "This step gives equal weight in downstream analyses, so that highly-expressed genes do not dominate"

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

Amezquita, Robert A., et al. "Orchestrating single-cell analysis with Bioconductor." Nature methods 17.2 (2020): 137-145

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

PCA, Clustering und UMAP

Nutzen skalierte, selektierte variable Features für PCA.

SeuratObj = RunUMAP(SeuratObj, dims = 1:10)

Clustering

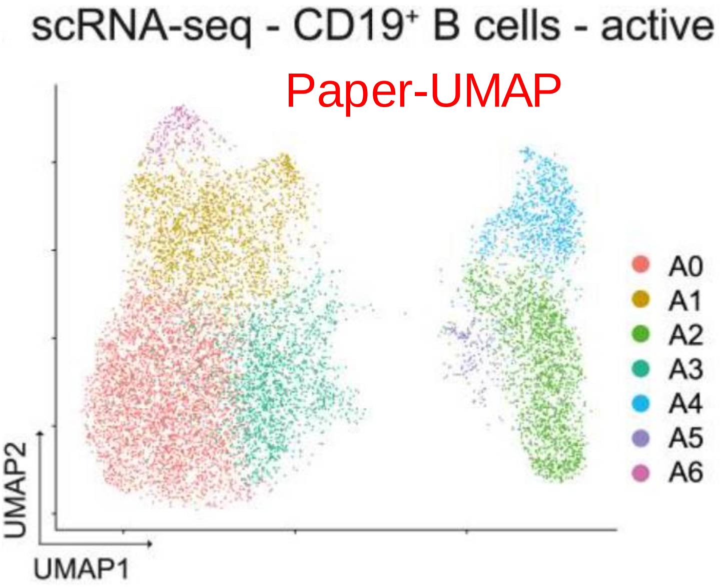
SeuratObj = FindNeighbors(SeuratObj, dims = 1:10)
SeuratObj = FindClusters(SeuratObj, resolution = 0.5)

UMAP

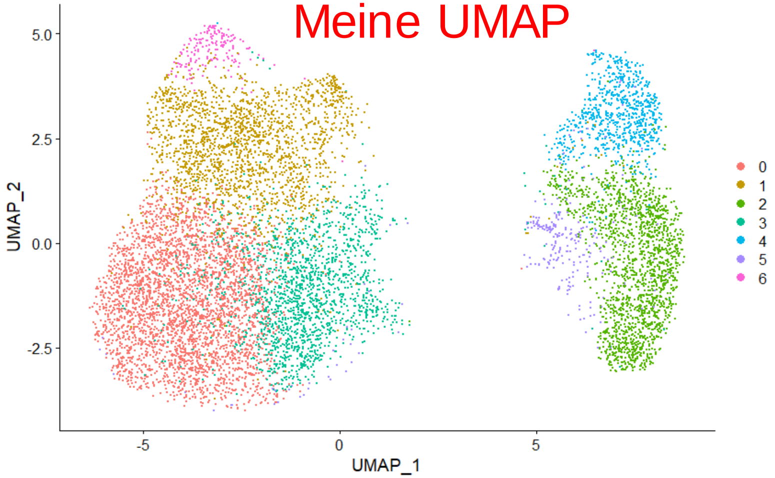
SeuratObj = RunUMAP(SeuratObj, dims = 1:10)

Visualisierung

dp=DimPlot(SeuratObj, reduction = "umap")



Paper-UMAP



Meine UMAP

Schultheiß, Christoph, et al. "Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19." *Iscience* 24.11 (2021): 103325

https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

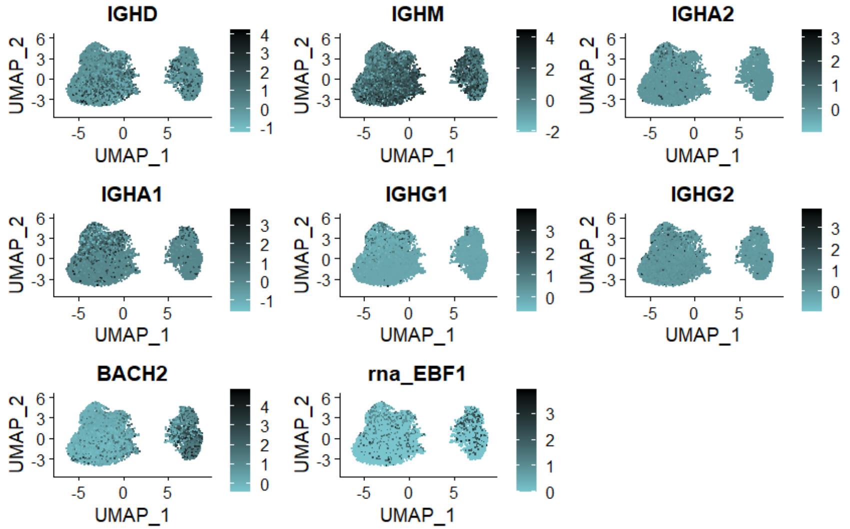
Detektion von Markern

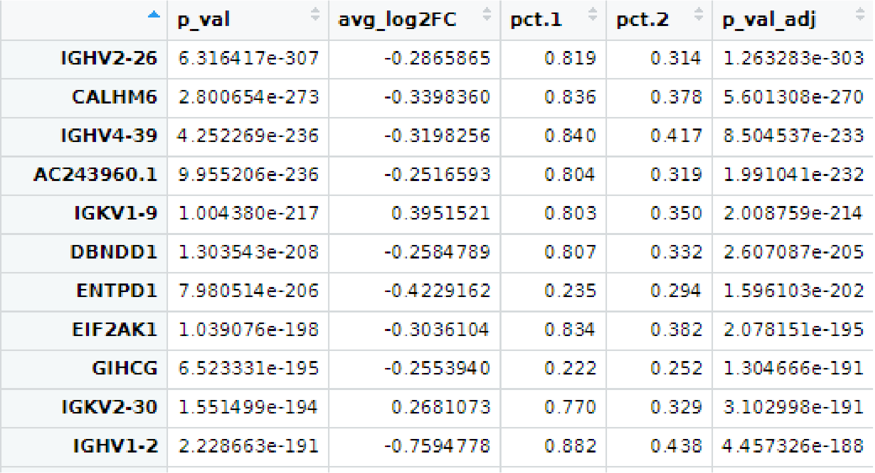
Motivation

Suche nach Markern, die Cluster durch eine differenzielle Expression definieren

```
Cluster0.active <- FindMarkers(active, ident.1 = 0, min.pct = 0.25)
#findet alle Marker des Clusters 0 der active Covid Patienten
```

Visualisierung FeaturePlot()





	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
IGHV2-26	6.316417e-307	-0.2865865	0.819	0.314	1.263283e-303
CALHM6	2.800654e-273	-0.3398360	0.836	0.378	5.601308e-270
IGHV4-39	4.252269e-236	-0.3198256	0.840	0.417	8.504537e-233
AC243960.1	9.955206e-236	-0.2516593	0.804	0.319	1.991041e-232
IGKV1-9	1.004380e-217	0.3951521	0.803	0.350	2.008759e-214
DBNDD1	1.303543e-208	-0.2584789	0.807	0.332	2.607087e-205
ENTPD1	7.980514e-206	-0.4229162	0.235	0.294	1.596103e-202
EIF2AK1	1.039076e-198	-0.3036104	0.834	0.382	2.078151e-195
GIHCG	6.523331e-195	-0.2553940	0.222	0.252	1.304666e-191
IGKV2-30	1.551499e-194	0.2681073	0.770	0.329	3.102998e-191
IGHV1-2	2.228663e-191	-0.7594778	0.882	0.438	4.457326e-188

Marker von Active_Cluster_2

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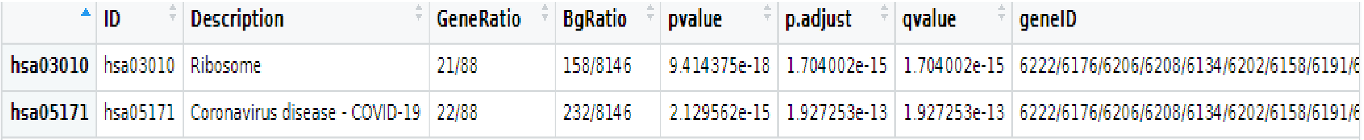
Gene Enrichment (KEGG)

Packages

library("biomaRt")
library("clusterProfiler")
library("org.Hs.eg.db")

Entrez-IDs

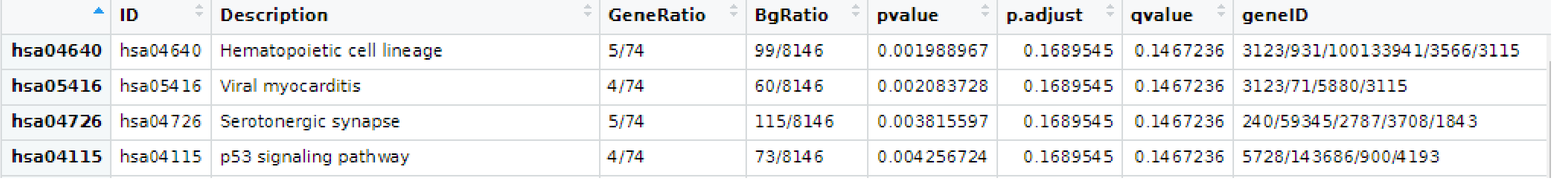
Log-fold Changes



	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID
hsa03010	hsa03010	Ribosome	21/88	158/8146	9.414375e-18	1.704002e-15	1.704002e-15	6222/6176/6206/6208/6134/6202/6158/6191/6
hsa05171	hsa05171	Coronavirus disease - COVID-19	22/88	232/8146	2.129562e-15	1.927253e-13	1.927253e-13	6222/6176/6206/6208/6134/6202/6158/6191/6

Active_kegg_1

kegg = gseKEGG(geneList= ngl,organism= "hsa",minGSSize= 5,
pvalueCutoff= pval,verbose= TRUE)



	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	geneID
hsa04640	hsa04640	Hematopoietic cell lineage	5/74	99/8146	0.001988967	0.1689545	0.1467236	3123/931/100133941/3566/3115
hsa05416	hsa05416	Viral myocarditis	4/74	60/8146	0.002083728	0.1689545	0.1467236	3123/71/5880/3115
hsa04726	hsa04726	Serotonergic synapse	5/74	115/8146	0.003815597	0.1689545	0.1467236	240/59345/2787/3708/1843
hsa04115	hsa04115	p53 signaling pathway	4/74	73/8146	0.004256724	0.1689545	0.1467236	5728/143686/900/4193

→ pt2: Cardiomyopathy

Active_kegg_2

Forschungshypothesen für die Bachelorarbeit

Nachdem ich die Daten auf ihre Reproduzierbarkeit überprüft habe, teste ich die Stabilität der Daten.

Aufgabe der Bachelorarbeit wäre es jetzt die Stabilität der Resultate zu überprüfen.

Dafür würden Bootstrapanalysen und Parameteranpassung in Frage kommen.

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-https://satijalab.org/seurat/articles/pbmc3k_tutorial.html Letzter Zugriff: 23.05.22 17:28

-https://bioconductor.org/packages/release/bioc/html/SingleR.html Letzter Zugriff: 23.05.2022 19:31