Stefano Mangiola

Maria Doyle

Tidy Transcriptomics for Single-cell RNA Sequencing Analyses

WEHI brighter together

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

Resources for #tidytranscriptomics

The blog about tidy transcriptomics

Home Archives Tags Categories About

Tidy-transcriptomics manifesto

2021-10-13·tidytrancriptomics

Read more...

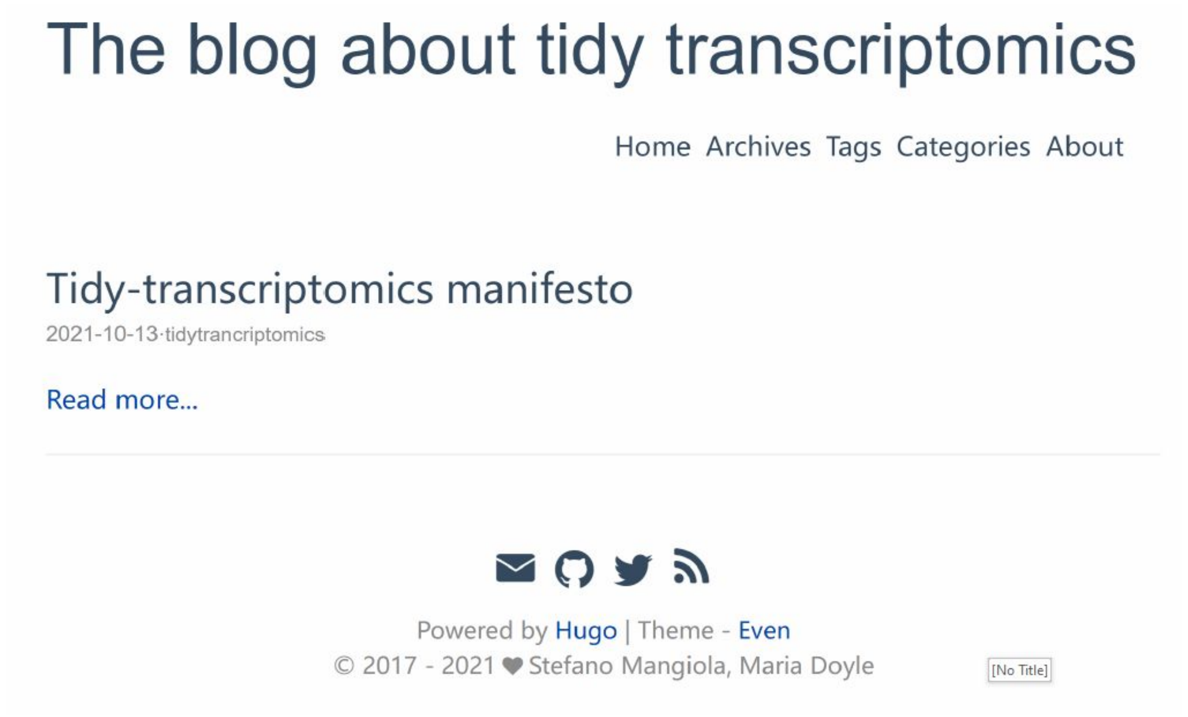
Powered by Hugo | Theme - Even

© 2017 - 2021 ♥ Stefano Mangiola, Maria Doyle

[No Title]

https://stemangiola.github.io/tidytranscriptomics/

Resources for #tidytranscriptomics



The blog about tidy transcriptomics

Home Archives Tags Categories About

Tidy-transcriptomics manifesto

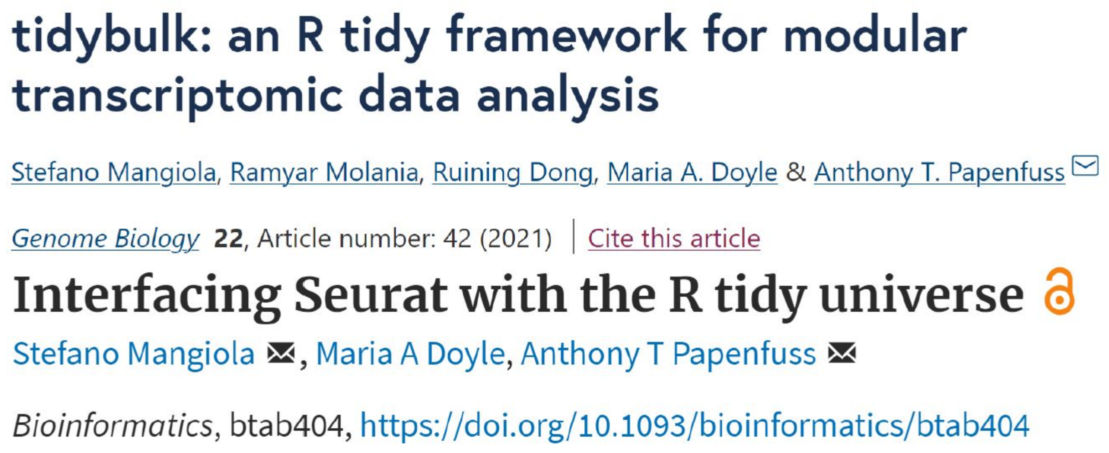
2021-10-13·tidytrancriptomics

Read more...

Powered by Hugo | Theme - Even

© 2017 - 2021 Stefano Mangiola, Maria Doyle

[No Title]



tidybulk: an R tidy framework for modular transcriptomic data analysis

Stefano Mangiola, Ramyar Molania, Ruining Dong, Maria A. Doyle & Anthony T. Papenfuss

Genome Biology **22**, Article number: 42 (2021) | Cite this article

Interfacing Seurat with the R tidy universe

Stefano Mangiola, Maria A Doyle, Anthony T Papenfuss

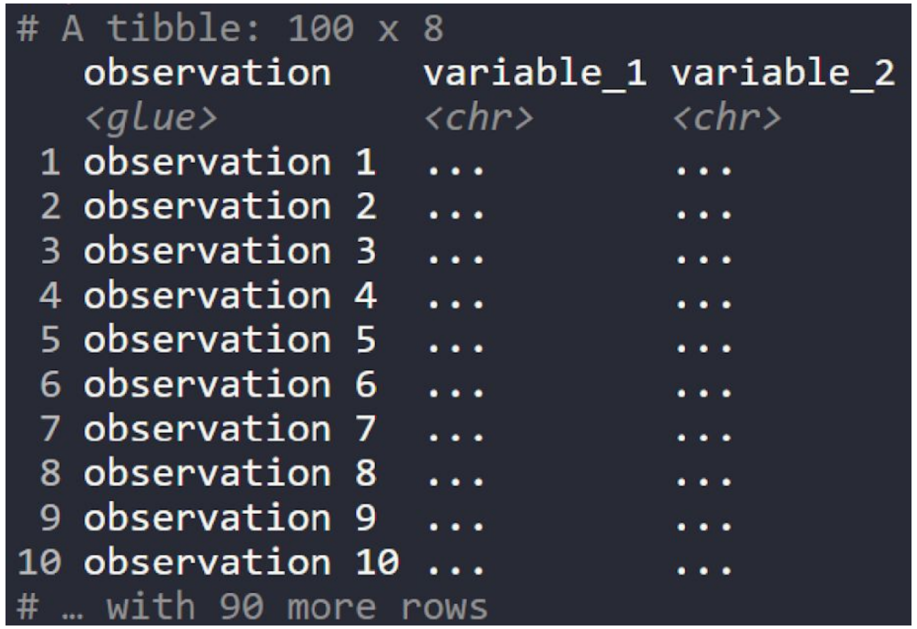
Bioinformatics, btab404, https://doi.org/10.1093/bioinformatics/btab404

https://stemangiola.github.io/tidytranscriptomics/

Tidy R tools

There are four basic principles to a tidy API:

- Reuse existing data structures.
- Compose simple functions with the pipe.
- Embrace functional programming.
- Design for humans.

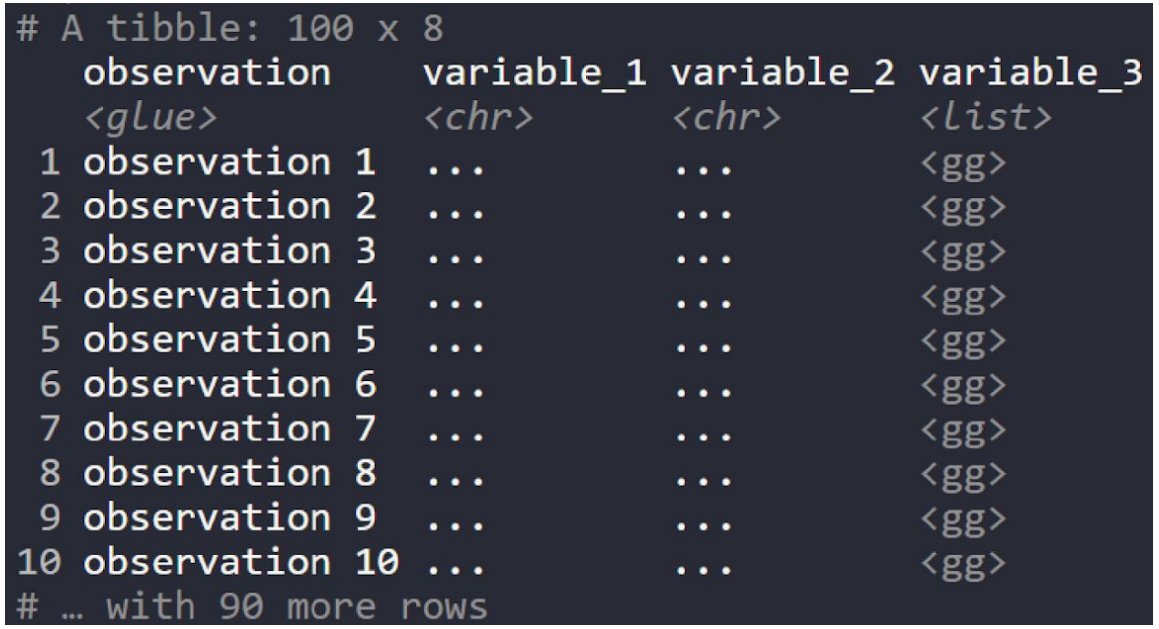


```
# A tibble: 100 x 8
   observation    variable_1 variable_2
   <glue>         <chr>      <chr>
 1 observation 1  ...        ...
 2 observation 2  ...        ...
 3 observation 3  ...        ...
 4 observation 4  ...        ...
 5 observation 5  ...        ...
 6 observation 6  ...        ...
 7 observation 7  ...        ...
 8 observation 8  ...        ...
 9 observation 9  ...        ...
10 observation 10 ...        ...
# … with 90 more rows
```

Tidy R tools

There are four basic principles to a tidy API:

- Reuse existing data structures.
- Compose simple functions with the pipe.
- Embrace functional programming.
- Design for humans.

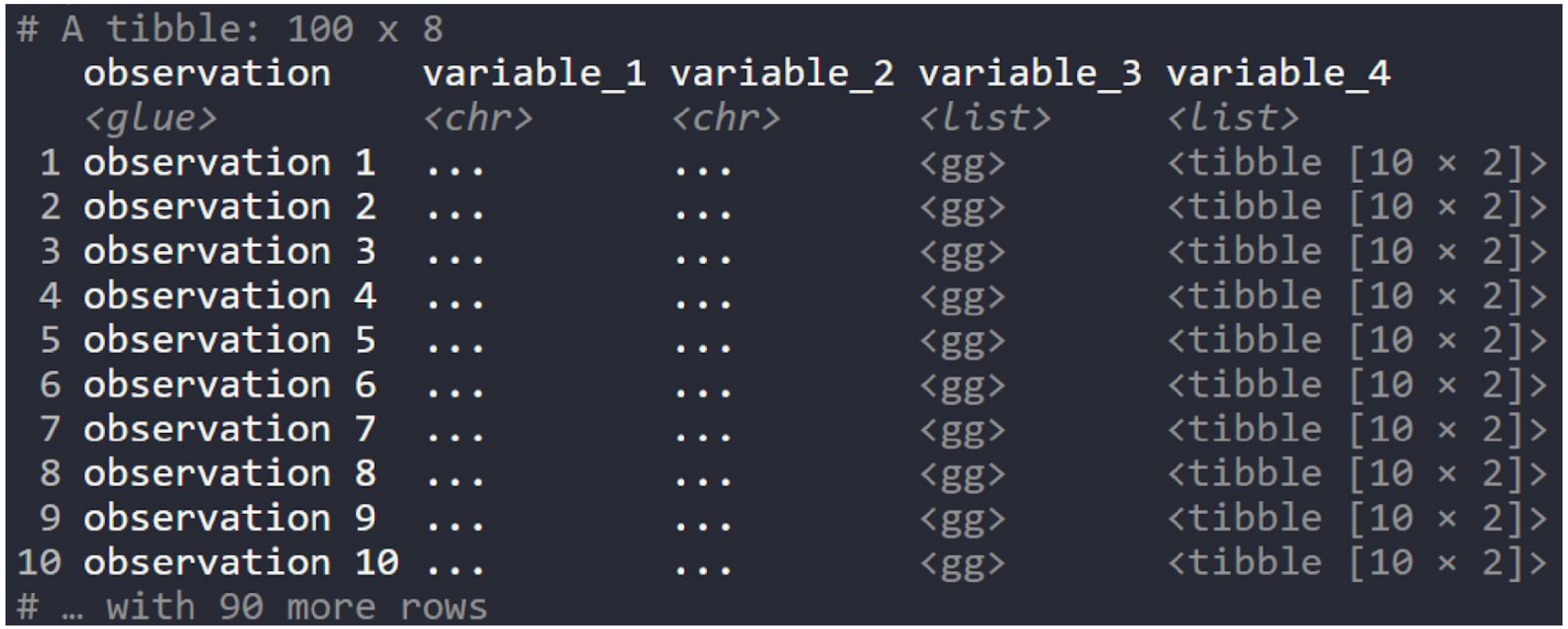


```
# A tibble: 100 x 8
   observation    variable_1 variable_2 variable_3
   <glue>         <chr>      <chr>      <list>
 1 observation 1  ...        ...        <gg>
 2 observation 2  ...        ...        <gg>
 3 observation 3  ...        ...        <gg>
 4 observation 4  ...        ...        <gg>
 5 observation 5  ...        ...        <gg>
 6 observation 6  ...        ...        <gg>
 7 observation 7  ...        ...        <gg>
 8 observation 8  ...        ...        <gg>
 9 observation 9  ...        ...        <gg>
10 observation 10 ...        ...        <gg>
# … with 90 more rows
```

Tidy R tools

There are four basic principles to a tidy API:

- Reuse existing data structures.
- Compose simple functions with the pipe.
- Embrace functional programming.
- Design for humans.

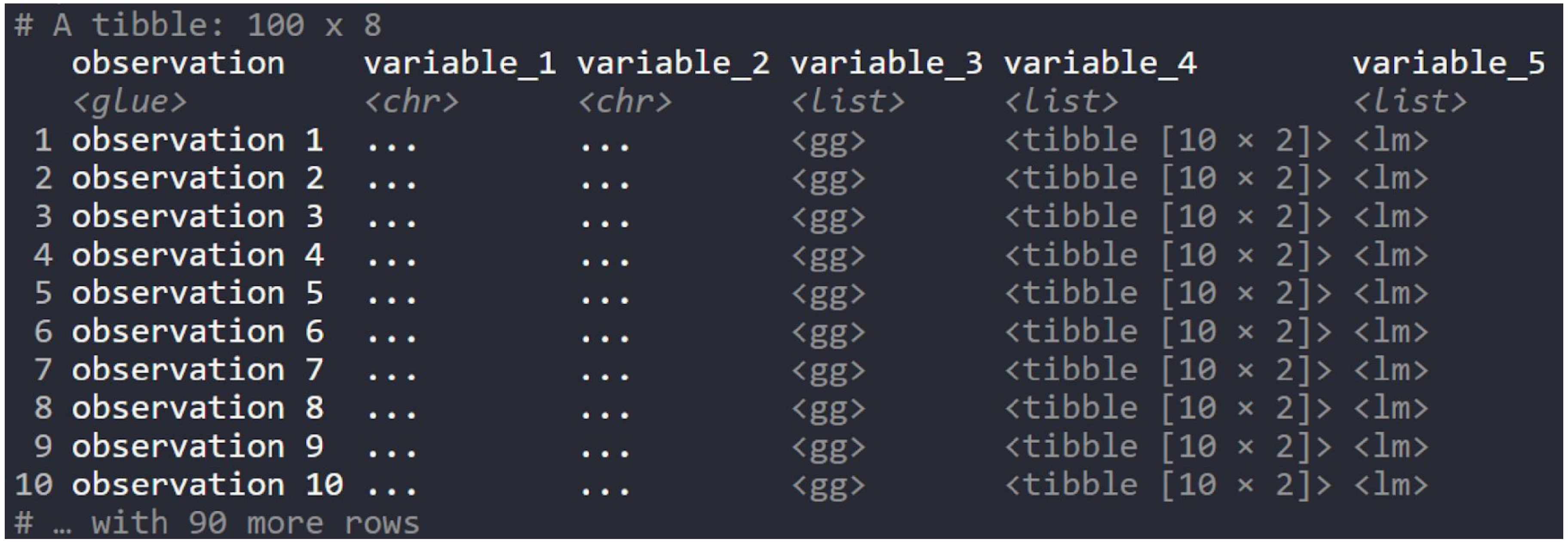


```
# A tibble: 100 x 8
   observation     variable_1 variable_2 variable_3 variable_4
   <glue>          <chr>      <chr>      <list>     <list>
 1 observation 1   ...        ...        <gg>       <tibble [10 × 2]>
 2 observation 2   ...        ...        <gg>       <tibble [10 × 2]>
 3 observation 3   ...        ...        <gg>       <tibble [10 × 2]>
 4 observation 4   ...        ...        <gg>       <tibble [10 × 2]>
 5 observation 5   ...        ...        <gg>       <tibble [10 × 2]>
 6 observation 6   ...        ...        <gg>       <tibble [10 × 2]>
 7 observation 7   ...        ...        <gg>       <tibble [10 × 2]>
 8 observation 8   ...        ...        <gg>       <tibble [10 × 2]>
 9 observation 9   ...        ...        <gg>       <tibble [10 × 2]>
10 observation 10  ...        ...        <gg>       <tibble [10 × 2]>
# … with 90 more rows
```

Tidy R tools

There are four basic principles to a tidy API:

- Reuse existing data structures.
- Compose simple functions with the pipe.
- Embrace functional programming.
- Design for humans.

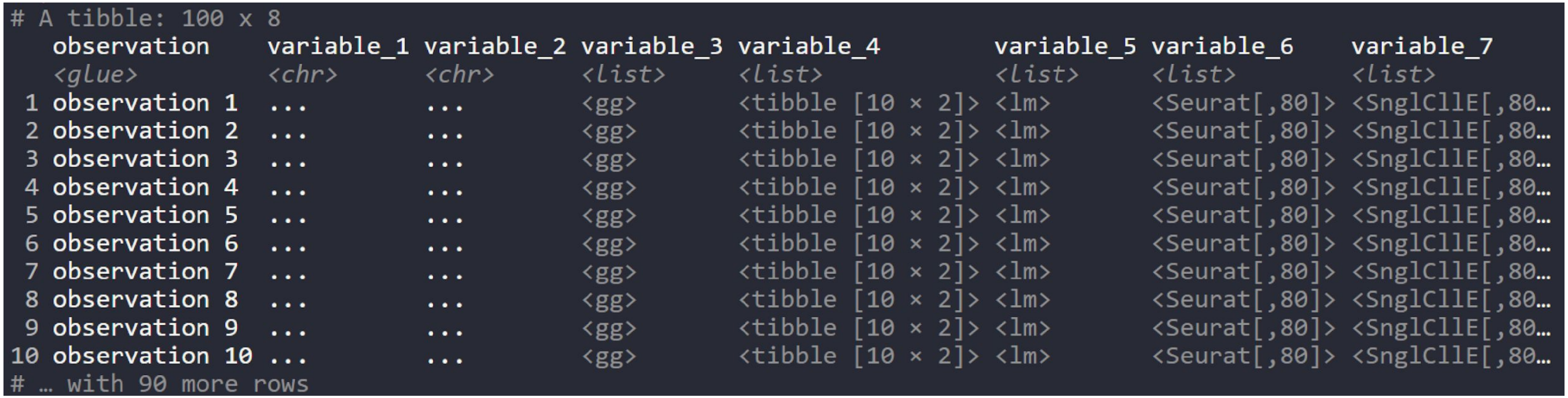


```
# A tibble: 100 x 8
   observation     variable_1 variable_2 variable_3 variable_4         variable_5
   <glue>          <chr>      <chr>      <list>     <list>             <list>
 1 observation 1  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 2 observation 2  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 3 observation 3  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 4 observation 4  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 5 observation 5  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 6 observation 6  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 7 observation 7  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 8 observation 8  ...        ...        <gg>       <tibble [10 × 2]> <lm>
 9 observation 9  ...        ...        <gg>       <tibble [10 × 2]> <lm>
10 observation 10 ...        ...        <gg>       <tibble [10 × 2]> <lm>
# … with 90 more rows
```

Tidy R tools

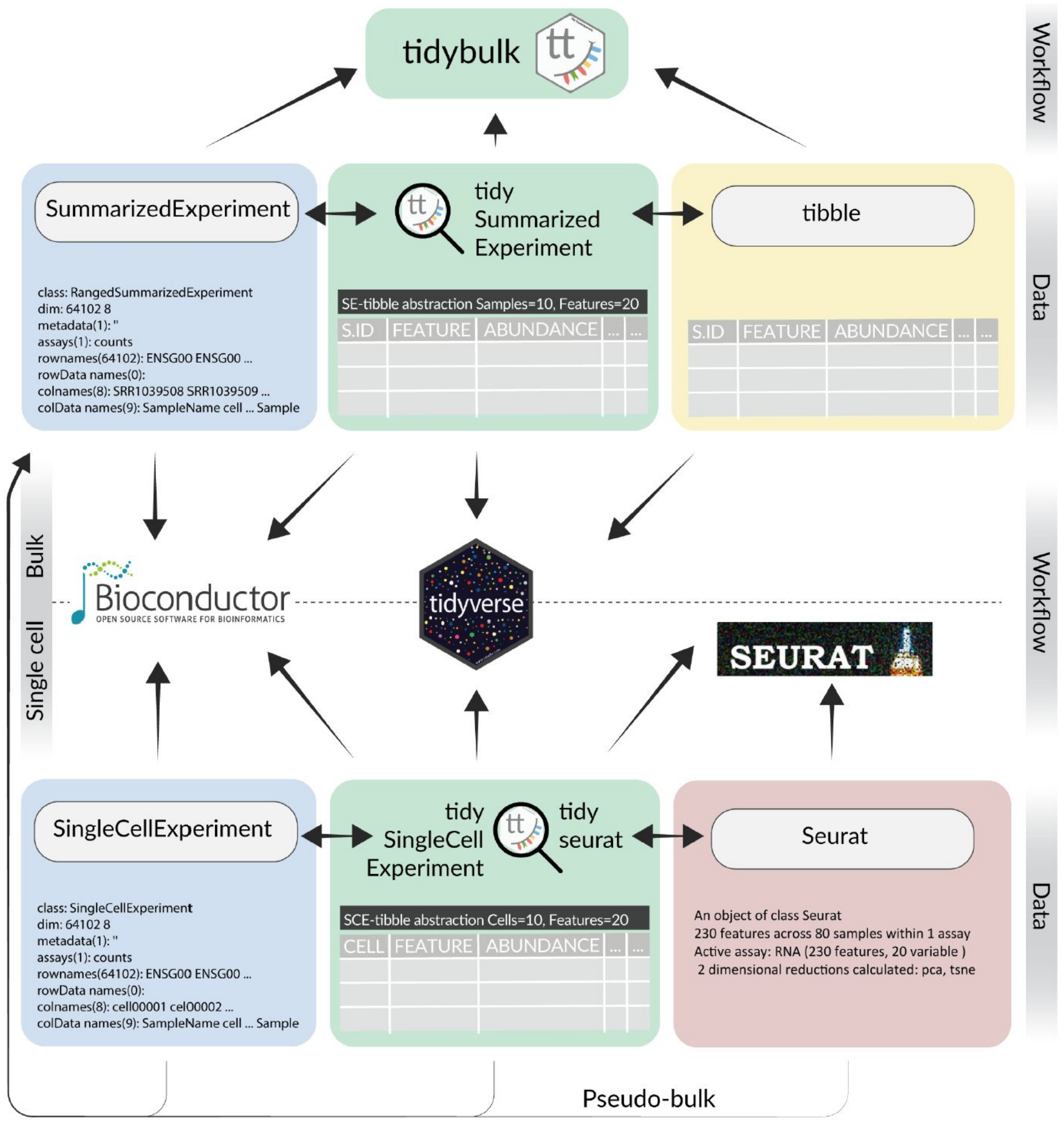
There are four basic principles to a tidy API:

- Reuse existing data structures.
- Compose simple functions with the pipe.
- Embrace functional programming.
- Design for humans.

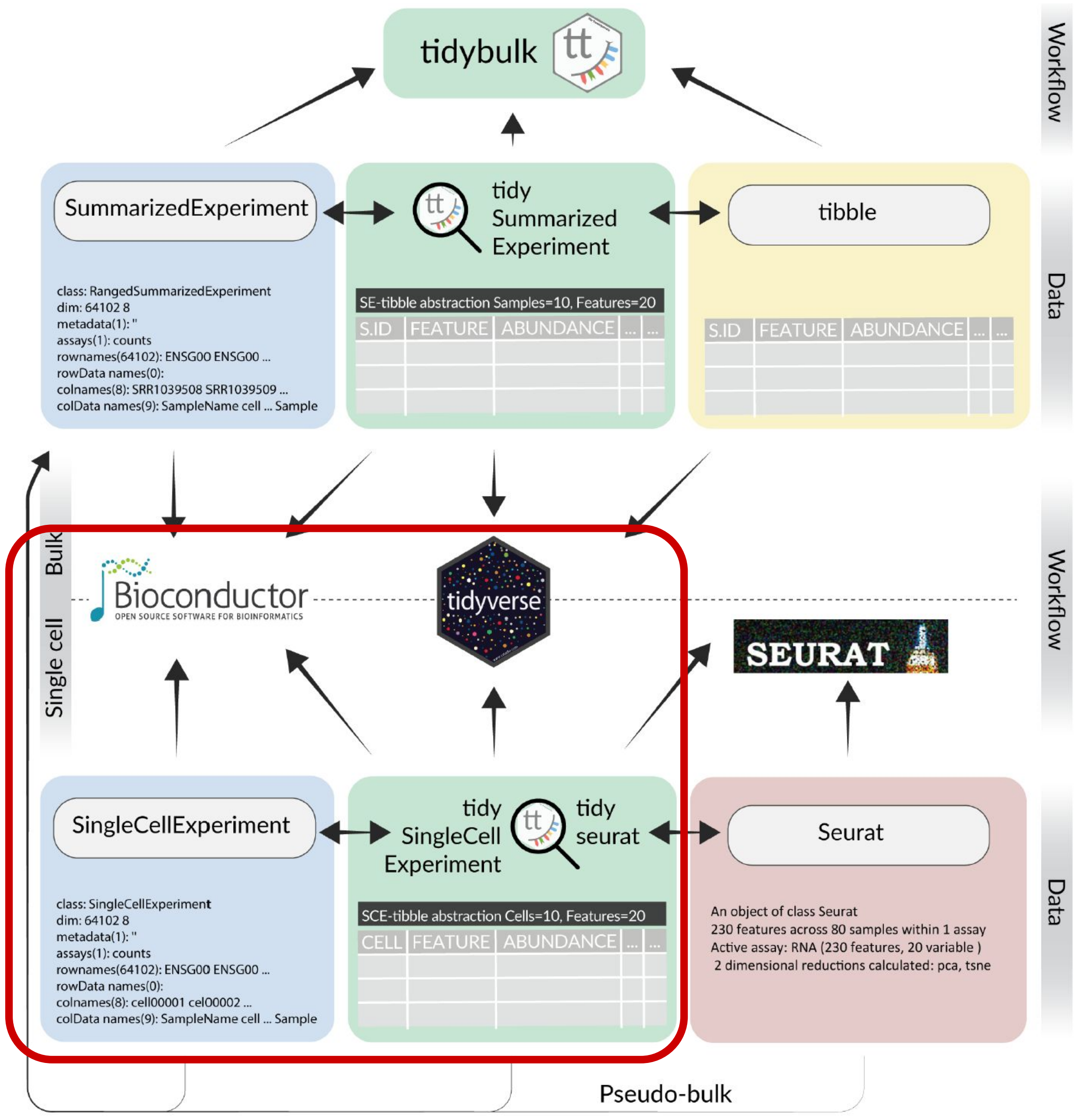


```
# A tibble: 100 x 8
   observation    variable_1 variable_2 variable_3 variable_4        variable_5 variable_6     variable_7
   <glue>         <chr>      <chr>      <list>     <list>            <list>     <list>         <list>
 1 observation 1  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 2 observation 2  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 3 observation 3  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 4 observation 4  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 5 observation 5  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 6 observation 6  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 7 observation 7  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 8 observation 8  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
 9 observation 9  ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
10 observation 10 ...        ...        <gg>       <tibble [10 × 2]> <lm>       <Seurat[,80]>  <SnglCllE[,80…
# … with 90 more rows
```

The big picture


tidybulk
Workflow
SummarizedExperiment
tidy Summarized Experiment
tibble
Data
class: RangedSummarizedExperiment
dim: 64102 8
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00 ENSG00 ...
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ...
colData names(9): SampleName cell ... Sample
SE-tibble abstraction Samples=10, Features=20
S.ID
FEATURE
ABUNDANCE
Bulk
Single cell
Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS
tidyverse
SEURAT
Workflow
SingleCellExperiment
tidy SingleCell Experiment
tidy seurat
Seurat
Data
class: SingleCellExperiment
dim: 64102 8
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00 ENSG00 ...
rowData names(0):
colnames(8): cell00001 cel00002 ...
colData names(9): SampleName cell ... Sample
SCE-tibble abstraction Cells=10, Features=20
CELL
FEATURE
ABUNDANCE
An object of class Seurat
230 features across 80 samples within 1 assay
Active assay: RNA (230 features, 20 variable)
2 dimensional reductions calculated: pca, tsne
Pseudo-bulk

The big picture


tidybulk
Workflow
SummarizedExperiment
tidy Summarized Experiment
tibble
Data
SE-tibble abstraction Samples=10, Features=20
S.ID | FEATURE | ABUNDANCE | ... | ...
S.ID | FEATURE | ABUNDANCE | ... | ...
Bulk
Single cell
Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS
tidyverse
SEURAT
Workflow
SingleCellExperiment
tidy SingleCell Experiment
tidy seurat
Seurat
Data
SCE-tibble abstraction Cells=10, Features=20
CELL | FEATURE | ABUNDANCE | ... | ...
Pseudo-bulk

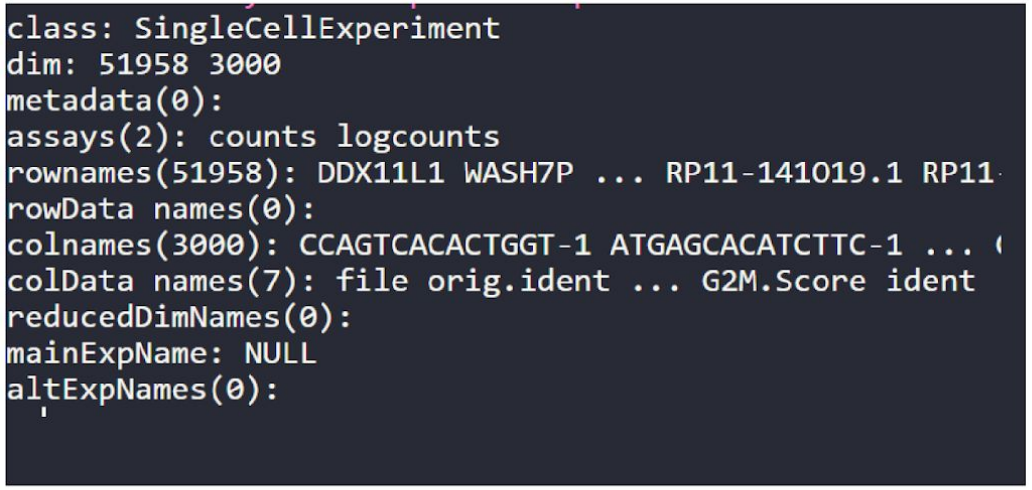
```
class: RangedSummarizedExperiment
dim: 64102 8
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00 ENSG00 ...
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ...
colData names(9): SampleName cell ... Sample
```

```
class: SingleCellExperiment
dim: 64102 8
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00 ENSG00 ...
rowData names(0):
colnames(8): cell00001 cel00002 ...
colData names(9): SampleName cell ... Sample
```

```
An object of class Seurat
230 features across 80 samples within 1 assay
Active assay: RNA (230 features, 20 variable )
 2 dimensional reductions calculated: pca, tsne
```

Analysis infrastructure for single-cell data

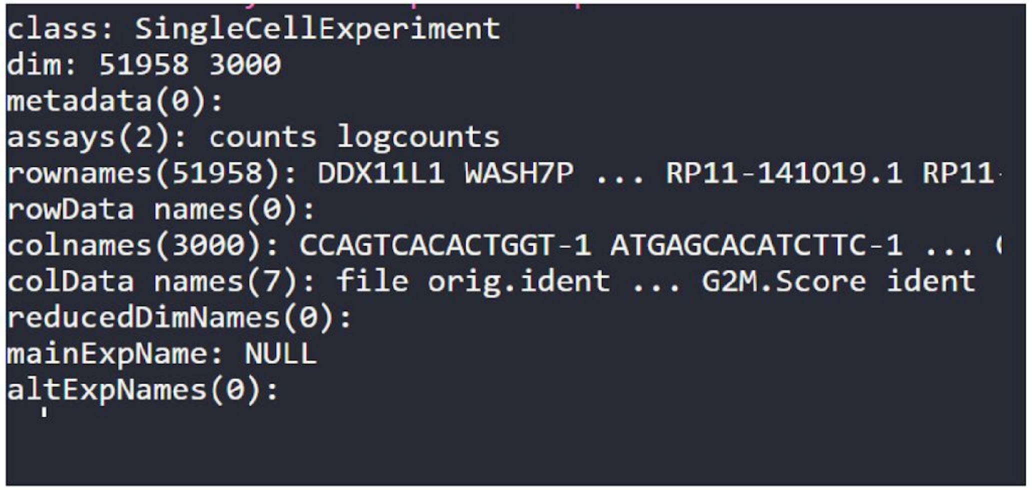
Data container



```
class: SingleCellExperiment
dim: 51958 3000
metadata(0):
assays(2): counts logcounts
rownames(51958): DDX11L1 WASH7P ... RP11-141O19.1 RP11-
rowData names(0):
colnames(3000): CCAGTCACACTGGT-1 ATGAGCACATCTTC-1 ... (
colData names(7): file orig.ident ... G2M.Score ident
reducedDimNames(0):
mainExpName: NULL
altExpNames(0):
```

Analysis infrastructure for single-cell data

Data container



```
class: SingleCellExperiment
dim: 51958 3000
metadata(0):
assays(2): counts logcounts
rownames(51958): DDX11L1 WASH7P ... RP11-141O19.1 RP11
rowData names(0):
colnames(3000): CCAGTCACACTGGT-1 ATGAGCACATCTTC-1 ...
colData names(7): file orig.ident ... G2M.Score ident
reducedDimNames(0):
mainExpName: NULL
altExpNames(0):
```

Analysis

Bioconductor community

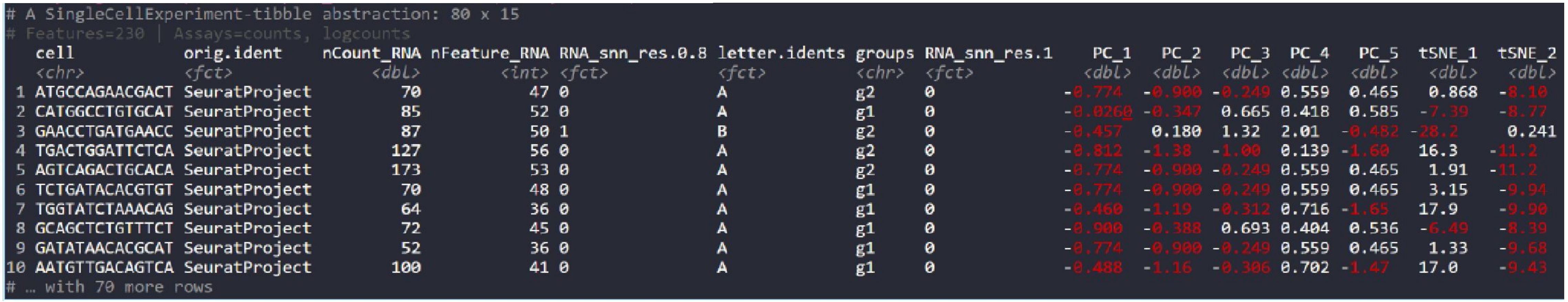
scran/scater

Manipulation

```
colData(data)
reducedDims(data, “umap”)
subset(data, , class==”A”)
data$info = info

data =    cbind( data,cohort_info[
    match(data$sample, cohort_info$sample)
    ,])
subset(data, , !is.na(sample_id))
```

Tidy data representation

Data container

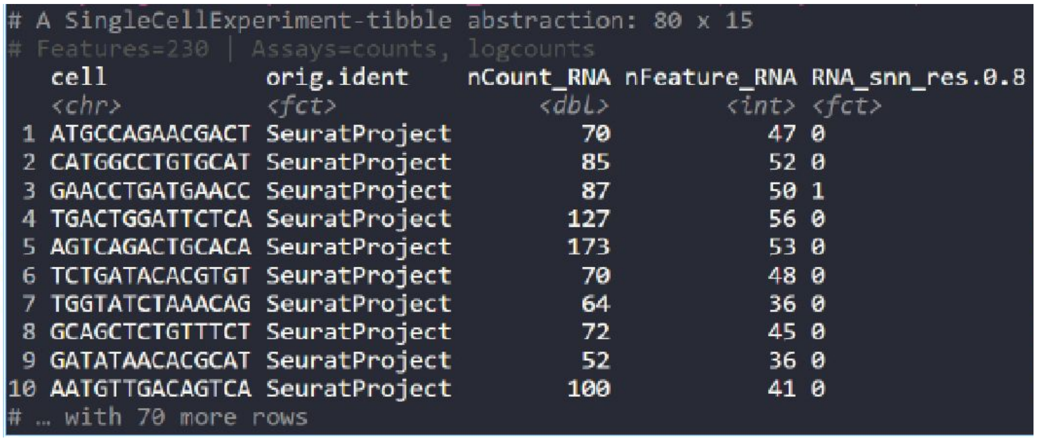


```
# A SingleCellExperiment-tibble abstraction: 80 x 15
# Features=230 | Assays=counts, logcounts
   cell         orig.ident    nCount_RNA nFeature_RNA RNA_snn_res.0.8 letter.idents groups RNA_snn_res.1    PC_1   PC_2   PC_3  PC_4   PC_5 tSNE_1 tSNE_2
   <chr>        <fct>              <dbl>        <int> <fct>           <fct>         <chr>  <fct>           <dbl>  <dbl>  <dbl> <dbl>  <dbl>  <dbl>  <dbl>
 1 ATGCCAGAACGACT SeuratProject       70           47 0               A             g2     0             -0.774  -0.900 -0.249 0.559  0.465  0.868  -8.10
 2 CATGGCCTGTGCAT SeuratProject       85           52 0               A             g1     0             -0.0260 -0.347  0.665 0.418  0.585 -7.39   -8.77
 3 GAACCTGATGAACC SeuratProject       87           50 1               B             g2     0             -0.457   0.180  1.32  2.01  -0.482 -28.2    0.241
 4 TGACTGGATTCTCA SeuratProject      127           56 0               A             g2     0             -0.812  -1.38  -1.00  0.139 -1.60   16.3   -11.2
 5 AGTCAGACTGCACA SeuratProject      173           53 0               A             g2     0             -0.774  -0.900 -0.249 0.559  0.465   1.91  -11.2
 6 TCTGATACACGTGT SeuratProject       70           48 0               A             g1     0             -0.774  -0.900 -0.249 0.559  0.465   3.15   -9.94
 7 TGGTATCTAAACAG SeuratProject       64           36 0               A             g1     0             -0.460  -1.19  -0.312 0.716 -1.65   17.9    -9.90
 8 GCAGCTCTGTTTCT SeuratProject       72           45 0               A             g1     0             -0.900  -0.388  0.693 0.404  0.536  -6.49   -8.39
 9 GATATAACACGCAT SeuratProject       52           36 0               A             g1     0             -0.774  -0.900 -0.249 0.559  0.465   1.33   -9.68
10 AATGTTGACAGTCA SeuratProject      100           41 0               A             g1     0             -0.488  -1.16  -0.306 0.702 -1.47   17.0    -9.43
# … with 70 more rows
```

Tidy analysis infrastructure

Data container

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS



```
# A SingleCellExperiment-tibble abstraction: 80 x 15
# Features=230 | Assays=counts, logcounts
   cell          orig.ident    nCount_RNA nFeature_RNA RNA_snn_res.0.8
   <chr>         <fct>              <dbl>        <int> <fct>
 1 ATGCCAGAACGACT SeuratProject        70           47 0
 2 CATGGCCTGTGCAT SeuratProject        85           52 0
 3 GAACCTGATGAACC SeuratProject        87           50 1
 4 TGACTGGATTCTCA SeuratProject       127           56 0
 5 AGTCAGACTGCACA SeuratProject       173           53 0
 6 TCTGATACACGTGT SeuratProject        70           48 0
 7 TGGTATCTAAACAG SeuratProject        64           36 0
 8 GCAGCTCTGTTTCT SeuratProject        72           45 0
 9 GATATAACACGCAT SeuratProject        52           36 0
10 AATGTTGACAGTCA SeuratProject       100           41 0
# … with 70 more rows
```

Analysis

Bioconductor community

Manipulation

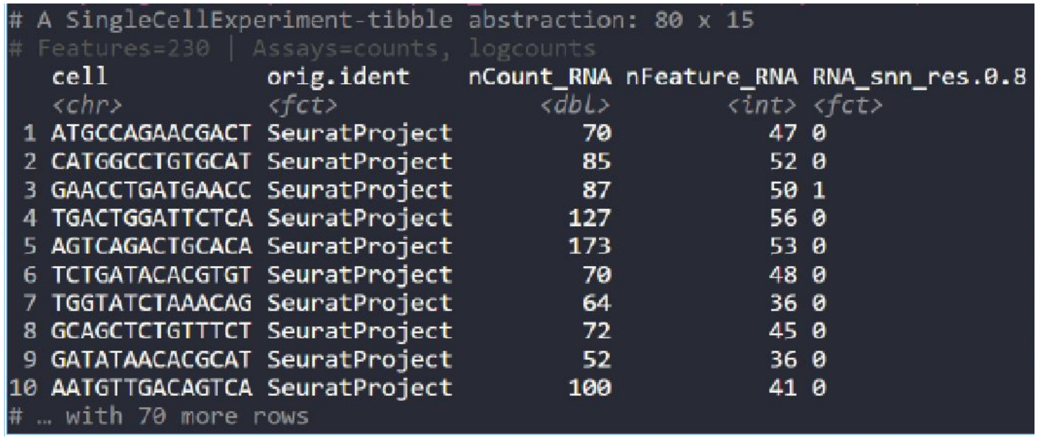
```
data
data |> select(contains("UMAP"))
data |> filter(class=="A")
data |> mutate(info = info)

data |> inner_join(cohort_info, by="sample")
```

Tidyseurat and tidySingleCellExperiment

Data container

Analysis

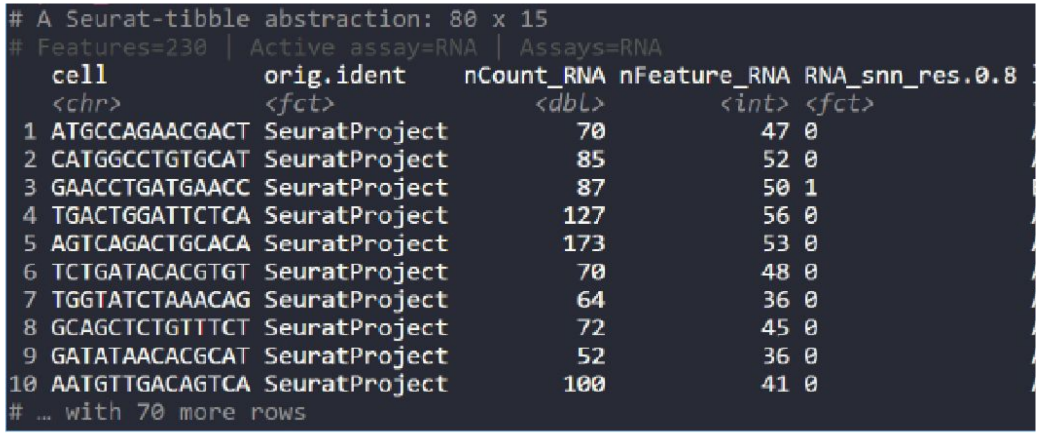
Manipulation



```
# A SingleCellExperiment-tibble abstraction: 80 x 15
# Features=230 | Assays=counts, logcounts
   cell         orig.ident     nCount_RNA nFeature_RNA RNA_snn_res.0.8
   <chr>        <fct>               <dbl>        <int> <fct>
 1 ATGCCAGAACGACT SeuratProject        70           47 0
 2 CATGGCCTGTGCAT SeuratProject        85           52 0
 3 GAACCTGATGAACC SeuratProject        87           50 1
 4 TGACTGGATTCTCA SeuratProject       127           56 0
 5 AGTCAGACTGCACA SeuratProject       173           53 0
 6 TCTGATACACGTGT SeuratProject        70           48 0
 7 TGGTATCTAAACAG SeuratProject        64           36 0
 8 GCAGCTCTGTTTCT SeuratProject        72           45 0
 9 GATATAACACGCAT SeuratProject        52           36 0
10 AATGTTGACAGTCA SeuratProject       100           41 0
# … with 70 more rows
```

Bioconductor community

```
data
data |> select(contains("UMAP"))
data |> filter(class=="A")
data |> mutate(info = info)

data |> inner_join(cohort_info, by="sample")
```



```
# A Seurat-tibble abstraction: 80 x 15
# Features=230 | Active assay=RNA | Assays=RNA
   cell         orig.ident     nCount_RNA nFeature_RNA RNA_snn_res.0.8
   <chr>        <fct>               <dbl>        <int> <fct>
 1 ATGCCAGAACGACT SeuratProject        70           47 0
 2 CATGGCCTGTGCAT SeuratProject        85           52 0
 3 GAACCTGATGAACC SeuratProject        87           50 1
 4 TGACTGGATTCTCA SeuratProject       127           56 0
 5 AGTCAGACTGCACA SeuratProject       173           53 0
 6 TCTGATACACGTGT SeuratProject        70           48 0
 7 TGGTATCTAAACAG SeuratProject        64           36 0
 8 GCAGCTCTGTTTCT SeuratProject        72           45 0
 9 GATATAACACGCAT SeuratProject        52           36 0
10 AATGTTGACAGTCA SeuratProject       100           41 0
# … with 70 more rows
```

Seurat SeuratWrappers community

```
data
data |> select(contains("UMAP"))
data |> filter(class=="A")
data |> mutate(info = info)

data |> inner_join(cohort_info, by="sample")
```

Tidy operators available

as_tibble()
mutate()
bind_rows()
left_join() inner_join() *_join()
select() **distinct()**
count() add_count() **summarise()**
pull() slice()
filter() sample_n() sample_frac()
rename()
separate() unite() extract()
nest() unnest() map_*()
pivot_longer()
join_features()
ggplot()
plotly()

What tidy data frameworks are and what are not

NO: data containers
NO: analysis tools

YES: data interface
YES: manipulation, integration, visualisation tools

Therefore, the question "**can we go from tidyseurat to Seurat and vice versa**" is not relevant, as we never leave **Seurat**.