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An introduction to the SingleCellExperiment class

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4 January 2019

Package

SingleCellExperiment 1.4.1

1 The SingleCellExperiment class definition

The SingleCellExperiment class is a light-weight container for single-cell genomics data. lt extends the RangedSummarizedExperiment class and follows similar conventions, i.e., rows should represent features (genes, transcripts, genomic regions) and columns should represent cells. addition to the slots already present RangedSummarizedExperiment, the SingleCellExperiment class contains:

- int_elementMetadata, to hold internal metadata for each row.
- int colData, to hold internal metadata for each column.
- int_metadata, to hold internal metadata for the entire

object.

reducedDims, to hold dimensionality reduction results.

The int_ prefix describes *internal* slots that are **not** meant for direct manipulation by the user. Instead, they are set by other user-visible methods, which will be discussed in more detail below. Package developers should see the other vignette for how to define methods to access these internal fields.

2 Creating SingleCellExperiment instances

SingleCellExperiment objects can be created via the constructor of the same name:

```
library(SingleCellExperiment)
counts <- matrix(rpois(100, lambda = 10), ncol=10, nrow=10)</pre>
sce <- SingleCellExperiment(assays = list(counts = counts))</pre>
## class: SingleCellExperiment
## dim: 10 10
## metadata(0):
## assays(1): counts
## rownames: NULL
## rowData names(0):
## colnames: NULL
## colData names(0):
## reducedDimNames(0):
## spikeNames(0):
An
       alternative
                     approach
                                   is
                                         via
                                                coercion
                                                            from
SummarizedExperiment objects.
se <- SummarizedExperiment(list(counts=counts))</pre>
as(se, "SingleCellExperiment")
## class: SingleCellExperiment
## dim: 10 10
## metadata(0):
## assays(1): counts
## rownames: NULL
## rowData names(0):
## colnames: NULL
## colData names(0):
## reducedDimNames(0):
## spikeNames(0):
```

To demonstrate the use of the class, we will use a subset of the allen data set from the scRNAseq (https://bioconductor.org/packages/3.8/scRNAseq) package. These data are stored as a SummarizedExperiment, so we will first coerce it into a

SingleCellExperiment.

```
library(scRNAseq)
data(allen)
allen
## class: SummarizedExperiment
## dim: 20908 379
## metadata(2): SuppInfo which_qc
## assays(4): tophat counts cufflinks fpkm rsem counts rsem
## rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zz
z3
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
## colData names(22): NREADS NALIGNED ... Animal.ID passes_q
c checks s
sce <- as(allen, "SingleCellExperiment")</pre>
sce
## class: SingleCellExperiment
## dim: 20908 379
## metadata(2): SuppInfo which qc
## assays(4): tophat counts cufflinks fpkm rsem counts rsem
## rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zz
z3
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
## colData names(22): NREADS NALIGNED ... Animal.ID passes q
c_checks_s
## reducedDimNames(0):
## spikeNames(0):
```

3 Adding spike-in information

One of the main additions to SummarizedExperiment is the ability for the user to specify the rows corresponding to spike-in transcripts. This is done with the method isSpike, using an appropriate name for the spike-in set.

```
isSpike(sce, "ERCC") <- grepl("^ERCC-", rownames(sce))
sce</pre>
```

```
## class: SingleCellExperiment
## dim: 20908 379
## metadata(2): SuppInfo which_qc
## assays(4): tophat_counts cufflinks_fpkm rsem_counts rsem_
tpm
## rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zz
z3
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
39336
## colData names(22): NREADS NALIGNED ... Animal.ID passes_q
c_checks_s
## reducedDimNames(0):
## spikeNames(1): ERCC
```

The identities of the spike-in rows can be easily retrieved using the name of the spike-in set, as shown below. The names of currently available spike-in sets can also be returned with the spikeNames method.

```
table(isSpike(sce, "ERCC"))

##

## FALSE TRUE

## 20816 92

spikeNames(sce)

## [1] "ERCC"
```

While most experimental designs use a single set of spike-ins, the class has the flexibility of including more than one set of spikes. Let us pretend that the members of the *Adam* gene family have been spiked-in as external genes in these data.

```
isSpike(sce, "Adam") <- grepl("^Adam[0-9]", rownames(sce))
sce</pre>
```

```
## class: SingleCellExperiment
## dim: 20908 379
## metadata(2): SuppInfo which_qc
## assays(4): tophat_counts cufflinks_fpkm rsem_counts rsem_
## rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zz
z3
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
## colData names(22): NREADS NALIGNED ... Animal.ID passes_q
c_checks_s
## reducedDimNames(0):
## spikeNames(2): ERCC Adam
table(isSpike(sce, "Adam"))
##
## FALSE TRUE
## 20875
            33
spikeNames(sce)
## [1] "ERCC" "Adam"
If isSpike is used without specifying any name, it will return the
union of all spike-in sets.
table(isSpike(sce))
##
## FALSE TRUE
## 20783
          125
Running isSpike<- with NULL will clear the specified set from the
SingleCellExperiment, while running clearSpikes will delete all
spike-in information.
temp <- sce
isSpike(temp, "Adam") <- NULL</pre>
spikeNames(temp)
## [1] "ERCC"
temp <- clearSpikes(temp)</pre>
spikeNames(temp)
## character(0)
```

Note that the isSpike and isSpike<- methods get and set columns in int_elementMetadata and int_metadata. This information is only relevant to package developers and not necessary for routine use of this class.

4 Adding size factors

One can also store size factors in the SingleCellExperiment object. For illustration, we simply compute the total number of reads as size factors here. Note that more sophisticated methods for computing size factors are available (see, e.g., scran (https://bioconductor.org/packages/3.8/scran)).

```
sizeFactors(sce) <- colSums(assay(sce))
head(sizeFactors(sce))

## SRR2140028 SRR2140022 SRR2140055 SRR2140083 SRR2139991 SR
R2140067
## 5173863 6445002 2343379 5438526 4757468
2364851</pre>
```

We can compute multiple size factors and store them in the object, by providing a name to sizeFactors. This does *not* affect the values of the unnamed size factors.

```
sizeFactors(sce, "ERCC") <- colSums(assay(sce)[isSpike(sce,</pre>
"ERCC"),])
head(sizeFactors(sce, "ERCC"))
## SRR2140028 SRR2140022 SRR2140055 SRR2140083 SRR2139991 SR
R2140067
       224648
                  186208
                              162370
                                                    278034
##
                                         512991
64975
head(sizeFactors(sce)) # same as before
## SRR2140028 SRR2140022 SRR2140055 SRR2140083 SRR2139991 SR
R2140067
                 6445002
                            2343379
##
      5173863
                                        5438526
                                                   4757468
2364851
```

5 Extracting colData and rowData

The colData and rowData methods can be used to retrieve the stored sample- and gene-level metadata. By default, this will only return the user-visible metadata fields, i.e., not including the fields stored in the <code>int_*</code> slots.

https://bioconductor.riken.jp/packages/3.8/bioc/vi...

## DataFrame \	ui+h 270 ra	owe and 22	columns		
## Datarrame \	NREADS	NALIGNED		TOTAL DUP	PR
IMER	MINEAUS	NALIGNED	IVALION	TOTAL_DOT	111
##	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<nume< td=""></nume<>
ric>					
## SRR2140028	13743900	13011100	94.6681	51.11	0.014
8148					
## SRR2140022	14078700	13521900	96.0454	55.9157	0.0085
3083					
## SRR2140055	5842930	5135980	87.9008	59.1126	0.035
6112					
## SRR2140083	16784400	15585800	92.8587	55.3076	0.020
9695					
## SRR2139991	11558600	10864300	93.9929	50.2258	0.01
6408					
##				• • • •	
## SRR2139325	12875700	11307000	87.8172	70.3564	0.045
## SRR2139325 3119	128/5/00	11307000	87.8172	70.3304	0.045
## SRR2139373	0600/00	8964140	92.4196	45.5249	0.021
6694	3033400	0304140	32.4130	73.3279	0.021
## SRR2139379	6175660	5728080	92.7526	45.2652	0.021
7132	01/3000	3720000	3217320	1312032	0.021
## SRR2139341	28038500	26320000	93.8711	65.1959	0.027
0482					
## SRR2139336	7878700	7467200	94.7772	56.9675	0.019
0784					
##	PCT_RIBOS(MAI BASES	DCT CODING	DACEC DO	IITR R
" "),	I CI_CODIN	J_DASES FC	_0111_5
ASES		5. I. L	TCI_CODING	J_DASES FC	_011\
ASES ##		<numeric></numeric>	_	umeric>	<nume< td=""></nume<>
ASES ## ric>		- <numeric></numeric>	- <nu< td=""><td>_ umeric></td><td><nume< td=""></nume<></td></nu<>	_ umeric>	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028		_	- <nu< td=""><td></td><td></td></nu<>		
ASES ## ric> ## SRR2140028 5609		- <numeric> le-06</numeric>	- <nu< td=""><td>_ umeric> .216848</td><td><nume< td=""></nume<></td></nu<>	_ umeric> .216848	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022		- <numeric></numeric>	- <nu< td=""><td>_ umeric></td><td><nume< td=""></nume<></td></nu<>	_ umeric>	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332		- <numeric> le-06</numeric>	- <ni 0</ni 	 umeric> .216848 .263052	<nume 0.26="" 0.31<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055		- <numeric> le-06</numeric>	- <ni 0</ni 	_ umeric> .216848	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241		- <numeric> le-06 0 3e-06</numeric>	- <nu 0 0</nu 	umeric> .216848 .263052	<nume 0.26="" 0.31="" 0.32<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083		- <numeric> le-06</numeric>	- <nu 0 0</nu 	 umeric> .216848 .263052	<nume 0.26="" 0.31<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681		- <numeric> le-06 0 3e-06</numeric>	- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083			- <ni 0 0 0</ni 	umeric> .216848 .263052	<nume 0.26="" 0.31="" 0.32<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991			- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831			- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
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ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ##			- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></ni<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373			- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></nu<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625			- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729 </td><td><pre></pre></td></ni<>	.216848 .263052 .207086 .129243 .257729 	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139373 4625 ## SRR2139379			- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></ni<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924			- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541</td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341			- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 </td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341 7713			- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996</td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996	<pre></pre>
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ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341 7713			- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996</td><td></td></ni<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996	

NA BASES		
##	<numeric></numeric>	<numeric></numeric>
<numeric></numeric>		
## SRR2140028		0.148033
0.482457		
## SRR2140022	0.290329	0.136288
0.573384		
## SRR2140055	0.291128	0.174542
0.534327		
## SRR2140083	0.444594	0.172481
0.382924		
## SRR2139991	0.323493	0.141946
0.53456		
##		
## SRR2139325	0.34636	0.173333
0.480295		
## SRR2139373	0.385409	0.139423
0.475167		
## SRR2139379	0.3254	0.13068
0.54392		
## SRR2139341	0.190673	0.144421
0.664906		
## SRR2139336	0.077377	0.14587
0.776752		
##	MEDIAN_CV_COVERAGE	MEDIAN_5PRIME_BIAS MEDIAN_3
PRIME_BIAS		
##	<numeric></numeric>	<numeric></numeric>
<numeric></numeric>		
## SRR2140028	0.507749	0.14181
0.409045		
## SRR2140022	0.488182	0.145024
0.41916	0 700074	0.000046
## SRR2140055	0.729874	0.069846
0.54856	0.701070	0
## SRR2140083	0.781878	0
0.697916 ## SBB2120001	0.48292	0 160644
## SRR2139991 0.413018	0.40292	0.160644
##		
##		•••
## SRR2139325	0.754565	0.049809
0.45433	0.754505	0.043003
## SRR2139373	0.504545	0.162197
0.518013	01301313	0.102137
## SRR2139379	0.521732	0.136669
0.458149	0.022702	0.2000
## SRR2139341	0.491965	0.155719
0.551382		
## SRR2139336	0.517592	0.148329
0.445874		-
##	MEDIAN_5PRIME_TO 3F	PRIME_BIAS driver_1_s d
issection_s		_
##		<numeric> <character></character></numeric>
<character></character>		
<cli>cliaracter></cli>		

			1001000,,,	
## SRR2140028		0.425234	Scnn1a-Tg3	·Cre
L4 ## SRR2140022		0.41926	Scnn1a-Tg3	-Cre
L4 ## SRR2140055		0.257657	Scnn1a-Tg3	-Cre
All ## SRR2140083		0.01825	Scnn1a-Tg3-	·Cre
L4			_	
## SRR2139991 L4		0.4621/1	Scnn1a-Tg3	·Cre
##				• • •
## SRR2139325 L6a		0.413165	Ntsr1-Cre_GN	1220
## SRR2139373		0.356451	Ntsr1-Cre_GN	N220
L6a ## SRR2139379		0.367889	Ntsr1-Cre_GN	N220
L6a ## SRR2139341		0.330835	Ntsr1-Cre_GN	1220
L6a ## SRR2139336		0 390592	Ntsr1-Cre_GN	1220
L6a			_	
## l.ID	Core.Type	Primary.Type Se	condary.Type	Anima
##	<character></character>	<character></character>	<character></character>	<inte< td=""></inte<>
ger> ## SRR2140028	Intermediate	L4 Scnn1a	L4 Ctxn3	13
3632			2. 00,0	
## SRR2140022 3632	Core	L4 Scnn1a		13
	Intermediate	L5a Tcerg1l	L5a Batf3	15
## SRR2140083	NA	NA	NA	
NA ## SRR2139991	Intermediate	L4 Scnn1a	L4 Ctxn3	12
6846	Tirecrinediate	L4 Sciiila	L4 CCAIIS	12
##	• • • •			
## SRR2139325	Intermediate	L6a Sla	L6a Mgp	17
5613 ## SRR2139373	Core	L6a Sla		13
2856 ## SRR2139379	Core	L6a Sla		13
2856 ## SRR2139341	Core	L6a Sla		13
2856	Core	Lua Sta		13
## SRR2139336 2856	Core	L6a Sla		13
##	passes_qc_che	_		
## ## SRR2140028	<chara< td=""><td>ncter> Y</td><td></td><td></td></chara<>	ncter> Y		
## SRR2140020 ## SRR2140022		Ϋ́		
## SRR2140055		Y		
## SRR2140083		N		
## SRR2139991		Y		

An introduction to the SingleCellExperiment class

https://bioconductor.riken.jp/packages/3.8/bioc/vi...

rowData(sce)

DataFrame with 20908 rows and 0 columns

However, it is sometimes useful to retrieve a DataFrame with the internal fields, i.e., spike-in and size factor information. This can be achieved by specifying internal=TRUE.

colData(sce, internal=TRUE)

## DataErama	4+h 270 m	ous and 24	columns		
## DataFrame \ ##		NALIGNED		TOTAL DUP	PR
## IMER	NNLADS	NALIGNED	RALIGN	TOTAL_DOF	ΓN
##	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<nume< td=""></nume<>
ric>	\Tullet IC>	<iidilici 10=""></iidilici>	<iiiiiici 1c=""></iiiiiici>	<pre></pre>	\Trullic
## SRR2140028	13743900	13011100	94.6681	51.11	0.014
8148	137 13300	13011100	3110001	31.11	0.011
## SRR2140022	14078700	13521900	96.0454	55.9157	0.0085
3083	11070700	13321300	30.0.3.	33.3137	0.0005
## SRR2140055	5842930	5135980	87.9008	59.1126	0.035
6112	33.233	3133333	07.1000	331111	0.000
## SRR2140083	16784400	15585800	92.8587	55.3076	0.020
9695					
## SRR2139991	11558600	10864300	93.9929	50.2258	0.01
6408					
##					
## SRR2139325	12875700	11307000	87.8172	70.3564	0.045
3119					
## SRR2139373	9699400	8964140	92.4196	45.5249	0.021
6694					
## SRR2139379	6175660	5728080	92.7526	45.2652	0.021
7132					
## SRR2139341	28038500	26320000	93.8711	65.1959	0.027
0482					
## SRR2139336	7878700	7467200	94.7772	56.9675	0.019
0784					
##	PCT_RIBOSO	DMAL_BASES	PCT_CODING	G_BASES PC	_UTR_B
ASES	PCT_RIBOS(_	_		Γ_UTR_B
ASES ##	PCT_RIBOSO	OMAL_BASES	_	G_BASES PCT	T_UTR_B <nume< td=""></nume<>
ASES ## ric>	PCT_RIBOS(- <numeric></numeric>	- <nu< td=""><td>_ umeric></td><td><nume< td=""></nume<></td></nu<>	_ umeric>	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028	PCT_RIBOS(_	- <nu< td=""><td></td><td></td></nu<>		
ASES ## ric> ## SRR2140028 5609	PCT_RIBOSO	- <numeric> le-06</numeric>	- <nu< td=""><td>_ umeric> .216848</td><td><nume< td=""></nume<></td></nu<>	_ umeric> .216848	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022	PCT_RIBOS(- <numeric></numeric>	- <nu< td=""><td>_ umeric></td><td><nume< td=""></nume<></td></nu<>	_ umeric>	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332	PCT_RIBOS(- <numeric> le-06</numeric>	- <ni 0</ni 	 umeric> .216848 .263052	<nume 0.26="" 0.31<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055	PCT_RIBOSO	- <numeric> le-06</numeric>	- <ni 0</ni 	_ umeric> .216848	<nume< td=""></nume<>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241	PCT_RIBOSO	- <numeric> le-06 0 3e-06</numeric>	- <nu 0 0</nu 	umeric> .216848 .263052	<nume 0.26="" 0.31="" 0.32<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083	PCT_RIBOS(- <numeric> le-06</numeric>	- <nu 0 0</nu 	 umeric> .216848 .263052	<nume 0.26="" 0.31<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681	PCT_RIBOSO		- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991	PCT_RIBOSO	- <numeric> le-06 0 3e-06</numeric>	- <ni 0 0 0</ni 	umeric> .216848 .263052	<nume 0.26="" 0.31="" 0.32<="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831	PCT_RIBOSO		- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ##	PCT_RIBOSO		- <ni 0 0 0</ni 	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ##	PCT_RIBOSO		- <ni< td=""><td>umeric> .216848 .263052 .207086 .129243</td><td><nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume></td></ni<>	umeric> .216848 .263052 .207086 .129243	<nume 0.25<="" 0.26="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ##	PCT_RIBOSO		- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></ni<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325	PCT_RIBOSO		- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></nu<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041	PCT_RIBOSO		- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></nu<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373	PCT_RIBOSO		- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729</td><td><nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume></td></ni<>	.216848 .263052 .207086 .129243 .257729	<nume 0.25="" 0.26="" 0.27<="" 0.31="" 0.32="" td=""></nume>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625	PCT_RIBOSO		- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729 </td><td><pre></pre></td></ni<>	.216848 .263052 .207086 .129243 .257729 	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139373 4625 ## SRR2139379	PCT_RIBOSO		- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 </td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341 7713	PCT_RIBOSO		- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996</td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341 7713 ## SRR2139336	PCT_RIBOSO		- <nu< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541</td><td><pre></pre></td></nu<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541	<pre></pre>
ASES ## ric> ## SRR2140028 5609 ## SRR2140022 0332 ## SRR2140055 7241 ## SRR2140083 3681 ## SRR2139991 6831 ## ## SRR2139325 9041 ## SRR2139373 4625 ## SRR2139379 9924 ## SRR2139341 7713			- <ni< td=""><td>.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996</td><td></td></ni<>	.216848 .263052 .207086 .129243 .257729 .211253 .220541 .253996	

NA_BASES ##	<numeric></numeric>	<numeric></numeric>
<numeric> ## SRR2140028</numeric>	0.369509	0.148033
0.482457 ## SRR2140022	0.290329	0.136288
0.573384 ## SRR2140055 0.534327	0.291128	0.174542
## SRR2140083 0.382924	0.444594	0.172481
## SRR2139991 0.53456	0.323493	0.141946
##		
## SRR2139325 0.480295	0.34636	0.173333
## SRR2139373 0.475167	0.385409	0.139423
## SRR2139379 0.54392	0.3254	0.13068
## SRR2139341 0.664906		
## SRR2139336 0.776752	0.077377	0.14587
## PRIME_BIAS	MEDIAN_CV_COVERAGE	MEDIAN_5PRIME_BIAS MEDIAN_3
##	<numeric></numeric>	<numeric></numeric>
<pre><numeric> ## SRR2140028 0 400045</numeric></pre>	0.507749	0.14181
0.409045 ## SRR2140022 0.41916	0.488182	0.145024
## SRR2140055 0.54856	0.729874	0.069846
## SRR2140083 0.697916	0.781878	0
## SRR2139991 0.413018	0.48292	0.160644
##		
## SRR2139325 0.45433	0.754565	0.049809
## SRR2139373 0.518013	0.504545	0.162197
## SRR2139379 0.458149	0.521732	0.136669
## SRR2139341 0.551382	0.491965	0.155719
## SRR2139336 0.445874	0.517592	0.148329
## issection_s	MEDIAN_5PRIME_TO_3P	PRIME_BIAS driver_1_s d
## <character></character>		<numeric> <character></character></numeric>

## SRR2140028 L4		0.425234	Scnnla-Tg3-	Cre
## SRR2140022		0.41926	Scnn1a-Tg3-	Cre
L4 ## SRR2140055		0.257657	7 Scnn1a-Tg3-	Cre
All ## SRR2140083		0.01825	Scnn1a-Tg3-	Cre
L4 ## SRR2139991		0.462171	l Scnn1a-Tg3-	Cre
L4 ##				
 ## SRR2139325		0.413165	5 Ntsr1-Cre_GN	220
L6a			_	
## SRR2139373 L6a		0.356451	l Ntsr1-Cre_GN	220
## SRR2139379 L6a		0.367889	O Ntsr1-Cre_GN	220
## SRR2139341 L6a		0.330835	5 Ntsr1-Cre_GN	220
## SRR2139336 L6a		0.390592	2 Ntsr1-Cre_GN	220
##	Core.Type	Primary.Type Se	econdary.Type	Anima
l.ID ##	<character></character>	<character></character>	<character> ·</character>	<inte< td=""></inte<>
ger>				
## SRR2140028 3632	Intermediate	L4 Scnn1a	L4 Ctxn3	13
## SRR2140022 3632	Core	L4 Scnnla		13
	Intermediate	L5a Tcerg1l	L5a Batf3	15
## SRR2140083	NA	NA	NA	
NA				
## SRR2139991	Intermediate	L4 Scnn1a	L4 Ctxn3	12
6846 ##				
##	• • •			
## SRR2139325 5613	Intermediate	L6a Sla	L6a Mgp	17
## SRR2139373 2856	Core	L6a Sla		13
## SRR2139379	Core	L6a Sla		13
2856 ## SRR2139341	Core	L6a Sla		13
2856 ## SRR2139336	Core	L6a Sla		13
2856	core	Lua Sta		13
##	passes qc che	ecks_s size_fact	or size facto	r ERC
С		_ _	_	_
## >	<chara< td=""><td>acter> <numeri< td=""><td>Lc> <nui< td=""><td>meric</td></nui<></td></numeri<></td></chara<>	acter> <numeri< td=""><td>Lc> <nui< td=""><td>meric</td></nui<></td></numeri<>	Lc> <nui< td=""><td>meric</td></nui<>	meric
## SRR2140028 8		Y 51738	363	22464
## SRR2140022		Y 64450	002	18620

8			
## SRR2140055	Υ	2343379	16237
## SRR2140083	N	5438526	51299
## SRR2139991 4	Υ	4757468	27803
##			
## SRR2139325 5	Υ	4377966	33195
## SRR2139373 4	Υ	3393227	7952
## SRR2139379 1	Υ	2529501	3702
## SRR2139341 0	Υ	13972642	43958
## SRR2139336 4	Υ	4838243	16341

rowData(sce, internal=TRUE)

```
## DataFrame with 20908 rows and 3 columns
                 is_spike_ERCC is_spike is_spike_Adam
##
                      <logical> <logical>
                                              <logical>
## 0610007P14Rik
                         FALSE
                                    FALSE
                                                  FALSE
## 0610009B22Rik
                         FALSE
                                    FALSE
                                                   FALSE
## 0610009L18Rik
                         FALSE
                                    FALSE
                                                   FALSE
## 0610009020Rik
                         FALSE
                                    FALSE
                                                   FALSE
## 0610010F05Rik
                         FALSE
                                    FALSE
                                                   FALSE
## ...
                                                     . . .
## Zyg11a
                         FALSE
                                    FALSE
                                                   FALSE
## Zyg11b
                         FALSE
                                    FALSE
                                                   FALSE
## Zyx
                          FALSE
                                    FALSE
                                                   FALSE
## Zzef1
                          FALSE
                                    FALSE
                                                   FALSE
## Zzz3
                                                   FALSE
                          FALSE
                                    FALSE
```

See below for some discussion of why an internal storage mechanism is used here.

6 Adding low-dimensional representations

For simplicity and speed, we work on a subset of 100 genes. To avoid ending up with only uninteresting genes, we extract the 100 genes with maximal variance in the log-transformed counts.

```
library(magrittr)
assay(sce) %>% log1p %>% rowVars -> vars
names(vars) <- rownames(sce)</pre>
vars <- sort(vars, decreasing = TRUE)</pre>
sce_sub <- sce[names(vars[1:100]),]</pre>
sce_sub
## class: SingleCellExperiment
## dim: 100 379
## metadata(2): SuppInfo which_qc
## assays(4): tophat_counts cufflinks_fpkm rsem_counts rsem_
## rownames(100): Lamp5 Fam19a1 ... Rnf2 Zfp35
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
39336
## colData names(22): NREADS NALIGNED ... Animal.ID passes_q
c checks s
## reducedDimNames(0):
## spikeNames(2): ERCC Adam
```

We obtain the PCA and t-SNE representations of the data and add them to the object with the reducedDims method.

```
library(Rtsne)
set.seed(5252)
pca_data <- prcomp(t(log1p(assay(sce_sub))))</pre>
tsne data <- Rtsne(pca data$x[,1:50], pca = FALSE)
reducedDims(sce_sub) <- SimpleList(PCA=pca_data$x, TSNE=tsne
data$Y)
sce_sub
## class: SingleCellExperiment
## dim: 100 379
## metadata(2): SuppInfo which qc
## assays(4): tophat counts cufflinks fpkm rsem counts rsem
tpm
## rownames(100): Lamp5 Fam19a1 ... Rnf2 Zfp35
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
## colData names(22): NREADS NALIGNED ... Animal.ID passes q
c checks s
## reducedDimNames(2): PCA TSNE
## spikeNames(2): ERCC Adam
```

The stored coordinates can be retrieved by name or by numerical index. Each row of the coordinate matrix is assumed to correspond to a cell, while each column represents a dimension.

```
reducedDims(sce_sub)
## List of length 2
## names(2): PCA TSNE
reducedDimNames(sce_sub)
## [1] "PCA" "TSNE"
head(reducedDim(sce_sub, "PCA")[,1:2])
##
                    PC1
                               PC2
## SRR2140028 17.557295 -7.717162
## SRR2140022 21.468975 -1.198212
## SRR2140055 4.303756 -11.360330
## SRR2140083 21.440479 -9.435868
## SRR2139991 15.592089 -11.043989
## SRR2140067 16.539336 -9.831779
head(reducedDim(sce_sub, "TSNE")[,1:2])
##
                   [,1]
                             [,2]
## SRR2140028 -7.305172 -13.21667
## SRR2140022 -5.571360 -12.47031
## SRR2140055 1.730797 -14.54116
## SRR2140083 -3.774831 -14.21703
## SRR2139991 -2.702975 -13.59155
## SRR2140067 -2.529361 -15.59027
Any subsetting by column of sce_sub will also lead to subsetting
of the dimensionality reduction results by cell.
dim(reducedDim(sce_sub, "PCA"))
## [1] 379 100
dim(reducedDim(sce_sub[,1:10], "PCA"))
## [1] 10 100
```

7 Convenient access to named assays

In the SingleCellExperiment, users can assign arbitrary names to entries of assays. To assist interoperability between packages, we provide some suggestions for what the names should be for particular types of data:

- counts: Raw count data, e.g., number of reads or transcripts for a particular gene.
- normcounts: Normalized values on the same scale as the original counts. For example, counts divided by cell-specific size factors that are centred at unity.
- logcounts: Log-transformed counts or count-like values. In most cases, this will be defined as log-transformed normcounts, e.g., using log base 2 and a pseudo-count of 1.
- cpm: Counts-per-million. This is the read count for each gene in each cell, divided by the library size of each cell in millions.
- tpm: Transcripts-per-million. This is the number of transcripts for each gene in each cell, divided by the total number of transcripts in that cell (in millions).

Each of these suggested names has an appropriate getter/setter method for convenient manipulation of the SingleCellExperiment. For example, we can take the (very specifically named) tophat_counts name and assign it to counts instead:

```
counts(sce) <- assay(sce, "tophat_counts")</pre>
## class: SingleCellExperiment
## dim: 20908 379
## metadata(2): SuppInfo which_qc
## assays(5): tophat_counts cufflinks_fpkm rsem_counts rsem_
tpm counts
## rownames(20908): 0610007P14Rik 0610009B22Rik ... Zzef1 Zz
z3
## rowData names(0):
## colnames(379): SRR2140028 SRR2140022 ... SRR2139341 SRR21
## colData names(22): NREADS NALIGNED ... Animal.ID passes_q
c_checks_s
## reducedDimNames(0):
## spikeNames(2): ERCC Adam
dim(counts(sce))
## [1] 20908
               379
```

This means that functions expecting count data can simply call counts() without worrying about package-specific naming conventions.

8 Session Info

sessionInfo()

```
## R version 3.5.2 (2018-12-20)
## Platform: x86 64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.5 LTS
##
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.8-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.8-bioc/R/lib/libRlapack.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8
                                  LC NUMERIC=C
## [3] LC_TIME=en_US.UTF-8
                                  LC COLLATE=C
## [5] LC MONETARY=en US.UTF-8
                                  LC MESSAGES=en US.UTF-8
## [7] LC_PAPER=en_US.UTF-8
                                  LC NAME=C
## [9] LC ADDRESS=C
                                  LC TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
## attached base packages:
## [1] parallel stats4
                          stats
                                    graphics grDevices uti
       datasets
## [8] methods base
##
## other attached packages:
## [1] Rtsne_0.15
                                   magrittr 1.5
## [3] scRNAseq_1.8.0
                                   SingleCellExperiment_1.
4.1
## [5] SummarizedExperiment 1.12.0 DelayedArray 0.8.0
## [7] BiocParallel 1.16.5
                                   matrixStats 0.54.0
## [9] Biobase_2.42.0
                                   GenomicRanges_1.34.0
## [11] GenomeInfoDb_1.18.1
                                   IRanges 2.16.0
## [13] S4Vectors_0.20.1
                                   BiocGenerics_0.28.0
## [15] BiocStyle_2.10.0
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.0
                              knitr_1.21
                                                     XVecto
r 0.22.0
## [4] zlibbioc_1.28.0
                              lattice_0.20-38
                                                     string
r_1.3.1
## [7] tools_3.5.2
                              grid 3.5.2
                                                     xfun
0.4
## [10] htmltools 0.3.6
                              yaml 2.2.0
                                                     digest
_0.6.18
## [13] bookdown_0.9
                              Matrix_1.2-15
                                                     Genome
InfoDbData_1.2.0
## [16] BiocManager_1.30.4
                              bitops_1.0-6
                                                     RCurl
1.95-4.11
## [19] evaluate 0.12
                               rmarkdown 1.11
                                                     string
i 1.2.4
## [22] compiler_3.5.2
```