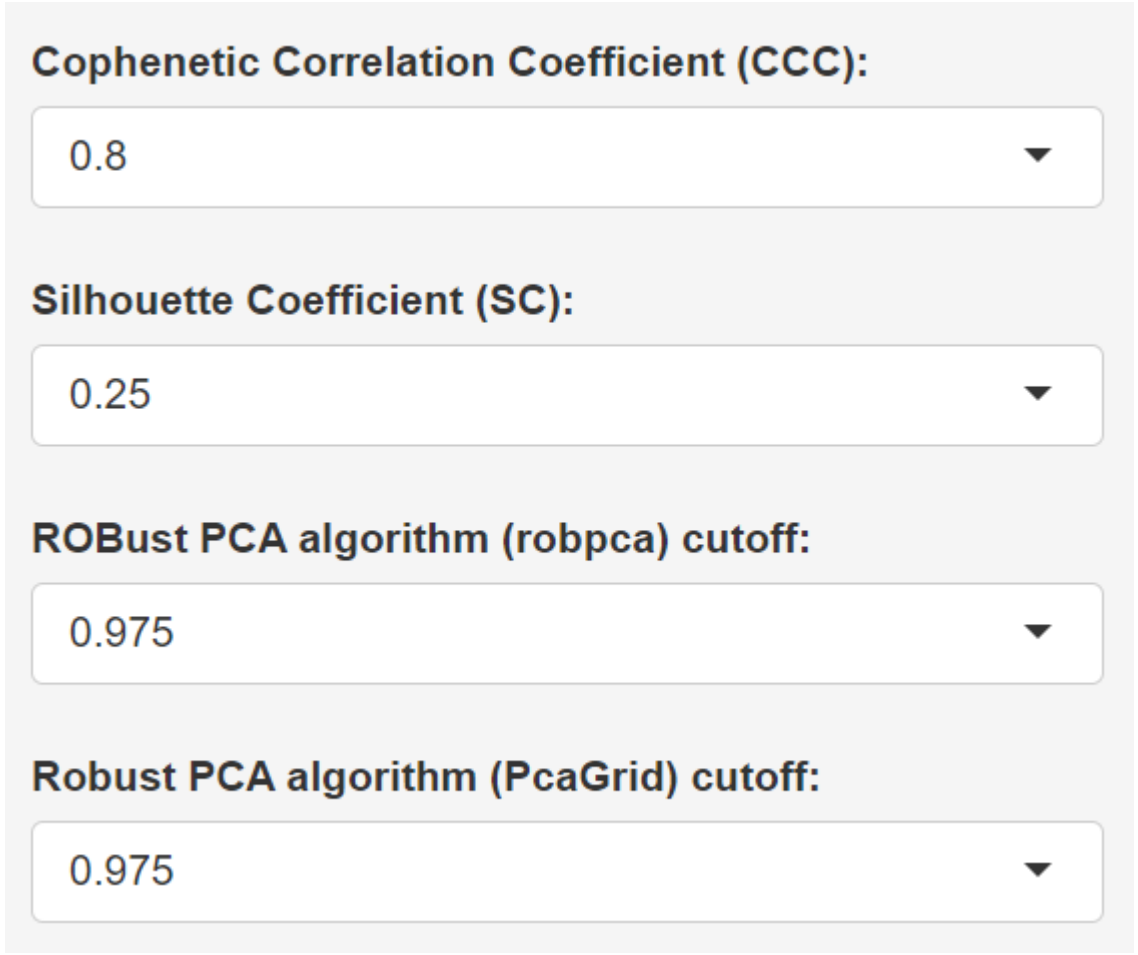
EnsMOD Monte Carlo (EnsMOD-MC)

EnsMOD is used for Omics Sample Outlier Detection

The user can set four threshold parameters:



Descriptive Statistic

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Statistical Test (assumes normality)

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An overall robust probability value was not calculated for the detected outlier(s)

Monte Carlo Methods

Monte Carlo methods are a broad class of algorithms that rely on repeated random sampling to obtain a numerical result(s).

Monte Carlo methods can be used to robustly solve any problem having a probabilistic interpretation.

Monte Carlo methods tend to follow a particular pattern:

1. Define the domain of possible input values
2. Generate inputs randomly
3. Perform a deterministic computation using each input to produce a result
4. Aggregate the results

https://en.wikipedia.org/wiki/Monte_Carlo_method

False Positive Rate (FPR)

		Predicted condition	
	Total population = P + N	Positive (PP)	Negative (PN)
Actual condition	Positive (P)	True positive (TP), hit	False negative (FN), type II error, miss, underestimation
	Negative (N)	False positive (FP), type I error, false alarm, overestimation	True negative (TN), correct rejection

$$\mathrm{FPR} = \frac{\mathrm{FP}}{\mathrm{TN} + \mathrm{FP}}$$

https://en.wikipedia.org/wiki/Sensitivity_and_specificity

If one or more outliers are detected using EnsMOD, then EnsMOD-MC can be used to reanalyze the dataset to estimate the FPR of the outlier detection

EnsMOD-MC does not make any assumptions about the user's dataset
(e.g., normal variance is not assumed)

EnsMOD-MC is 100% separate from EnsMOD, and it is available as an open-source, freely available, stand-alone script at https://github.com/niaid/EnsMOD

Use of EnsMOD-MC:

1. Delete the outliers detected by EnsMOD from the input dataset
2. Optional: Append "_fixed" to the end of column headers to prevent sampling
3. Optional: Adjust the number of MC simulations and/or the random seed
4. Use exactly the same threshold parameters used for EnsMOD

EnsMOD-MC

1. Randomly samples (without replacement) the non-outlier data
2. Performs EnsMOD outlier detection (detected outliers are false positives)
3. Loops to Step 1 to perform N simulations

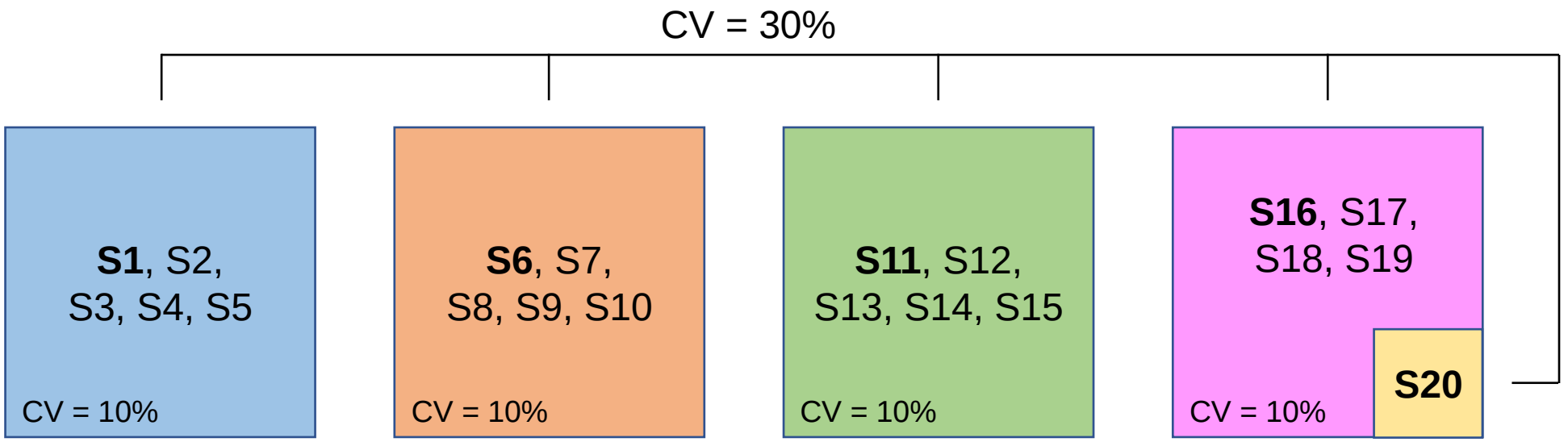
$$FPR = \frac{FP}{TN + FP} = \frac{FP}{Loops}$$

Note that $P = TP + FN = 0$

Simulated Proteomics Dataset

S1, S6, S11, S16, S20 = Gaussian random sampling
μ = 1,000,000, CV = 30%, N=100 proteins

Remaining Samples: CV = 10%



EnsMOD-MC Example: Simulated Proteomics Dataset

Original simulated proteomics dataset used for EnsMOD (s_20 is the simulated outlier).

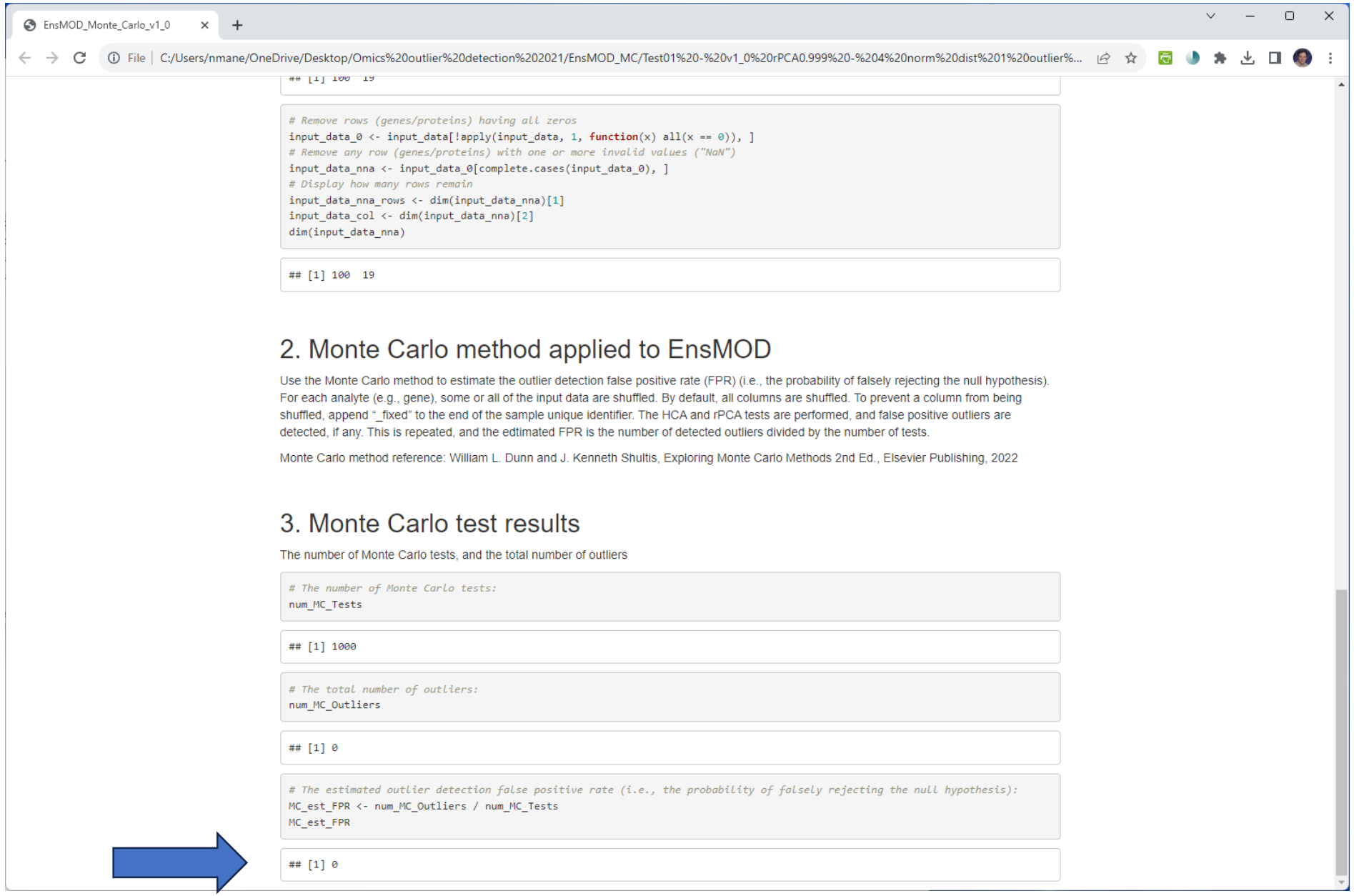
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	s_01	s_02	s_03	s_04	s_05	s_06	s_07	s_08	s_09	s_10	s_11	s_12	s_13	s_14	s_15	s_16	s_17	s_18	s_19	s_20	
2	1.74E+06	1.61E+06	1.76E+06	2.10E+06	1.75E+06	1.28E+06	1.48E+06	1.44E+06	1.21E+06	1.31E+06	1.17E+06	9.34E+05	1.08E+06	1.17E+06	1.04E+06	1.20E+06	1.36E+06	1.16E+06	1.27E+06	8.69E+05	
3	1.14E+06	1.26E+06	1.21E+06	1.38E+06	9.95E+05	7.07E+05	7.26E+05	6.21E+05	8.27E+05	7.02E+05	1.45E+06	1.43E+06	1.40E+06	1.29E+06	1.37E+06	1.28E+06	1.22E+06	1.35E+06	1.18E+06	1.31E+06	
4	9.53E+05	1.06E+06	9.32E+05	8.94E+05	8.20E+05	1.23E+06	1.30E+06	1.34E+06	1.19E+06	1.13E+06	8.15E+05	8.49E+05	8.83E+05	9.52E+05	8.95E+05	1.13E+06	1.08E+06	1.03E+06	1.19E+06	7.49E+05	
5	8.94E+05	8.74E+05	8.89E+05	7.87E+05	9.52E+05	1.34E+06	1.45E+06	1.32E+06	1.61E+06	1.46E+06	9.41E+05	9.50E+05	1.00E+06	1.09E+06	8.97E+05	1.04E+06	9.87E+05	9.40E+05	1.03E+06	1.37E+06	
6	1.32E+06	1.18E+06	1.54E+06	1.30E+06	1.18E+06	1.17E+06	1.24E+06	1.20E+06	9.94E+05	1.24E+06	1.23E+06	1.46E+06	1.42E+06	1.20E+06	1.02E+06	1.22E+06	1.34E+06	9.84E+05	1.10E+06	1.11E+06	

Simulated proteomics dataset used for EnsMOD_MC. Sample s_20 was deleted, and only the fourth experimental condition (it was s_16, s_17, s_18, s_19, s_20) was shuffled.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	s_01_fixe	s_02_fixe	s_03_fixe	s_04_fixe	s_05_fixe	s_06_fixe	s_07_fixe	s_08_fixe	s_09_fixe	s_10_fixe	s_11_fixe	s_12_fixe	s_13_fixe	s_14_fixe	s_15_fixe	s_16	s_17	s_18	s_19	
2	1.74E+06	1.61E+06	1.76E+06	2.10E+06	1.75E+06	1.28E+06	1.48E+06	1.44E+06	1.21E+06	1.31E+06	1.17E+06	9.34E+05	1.08E+06	1.17E+06	1.04E+06	1.20E+06	1.36E+06	1.16E+06	1.27E+06	
3	1.14E+06	1.26E+06	1.21E+06	1.38E+06	9.95E+05	7.07E+05	7.26E+05	6.21E+05	8.27E+05	7.02E+05	1.45E+06	1.43E+06	1.40E+06	1.29E+06	1.37E+06	1.28E+06	1.22E+06	1.35E+06	1.18E+06	
4	9.53E+05	1.06E+06	9.32E+05	8.94E+05	8.20E+05	1.23E+06	1.30E+06	1.34E+06	1.19E+06	1.13E+06	8.15E+05	8.49E+05	8.83E+05	9.52E+05	8.95E+05	1.13E+06	1.08E+06	1.03E+06	1.19E+06	
5	8.94E+05	8.74E+05	8.89E+05	7.87E+05	9.52E+05	1.34E+06	1.45E+06	1.32E+06	1.61E+06	1.46E+06	9.41E+05	9.50E+05	1.00E+06	1.09E+06	8.97E+05	1.04E+06	9.87E+05	9.40E+05	1.03E+06	
6	1.32E+06	1.18E+06	1.54E+06	1.30E+06	1.18E+06	1.17E+06	1.24E+06	1.20E+06	9.94E+05	1.24E+06	1.23E+06	1.46E+06	1.42E+06	1.20E+06	1.02E+06	1.22E+06	1.34E+06	9.84E+05	1.10E+06	

EnsMOD-MC Example: Simulated Proteomics Dataset --> Estimated FPR = 0%

```
# Set the number of Mont
# Note that ~1000 or mor
num_MC_Tests <- 1000

# Set the initial seed f
MC_seed <- 90421

# Set the Cophenetic Cor
# The CCC is a measure o
# between the original u
CCC_min <- 0.8

# Set the Silhouette Coe
# The SC is a measure of
SC_max <- 0.25

# Set the Robust PCA alg
# For normally distribut
iers.
robpca_prob <- 0.999

# Set the Robust PCA alg
# For normally distribut
iers.
PcaGrid_prob <- 0.999
```



Spleen Phosphoproteomics of Mice with Anthrax

			Toxin−, capsule−, asymptomatic, abortive infection	Toxin+, capsule−, lethal in 2-4d
Prepared:	5 mice	15 mice	15 mice	25 mice

Time	No Injection	Vehicle	ΔSterne	Sterne
0 h	5 mice			
24 h		5 mice	5 mice	5 mice
48 h		5 mice	5 mice	5 mice
72 h		5 mice	5 mice	1 mouse

The Sterne 72h sample functions as a pseudo-outlier.

Spleens, TiO_2 phospho-enrichment, Label-Free, LTQ-Orbitrap Classic

EnsMOD-MC Example: Anthrax Phosphoproteomics --> Estimated FPR = 1.4%

```r
# Set the number of Monte Carlo tests
# Note that ~1000 or more might take mo
num_MC_Tests <- 500

# Set the initial seed for reproducible
MC_seed <- 90421

# Set the Cophenetic Correlation Coeffi
# The CCC is a measure of how faithfull
# between the original unmodeled data p
CCC_min <- 0.8

# Set the Silhouette Coefficient (SC) c
# The SC is a measure of how similar ar
SC_max <- 0.25

# Set the Robust PCA algorithm (robpca)
# For normally distributed data, this w
iers.
robpca_prob <- 0.999

# Set the Robust PCA algorithm (PcaGrid
# For normally distributed data, this w
iers.
PcaGrid_prob <- 0.999
```