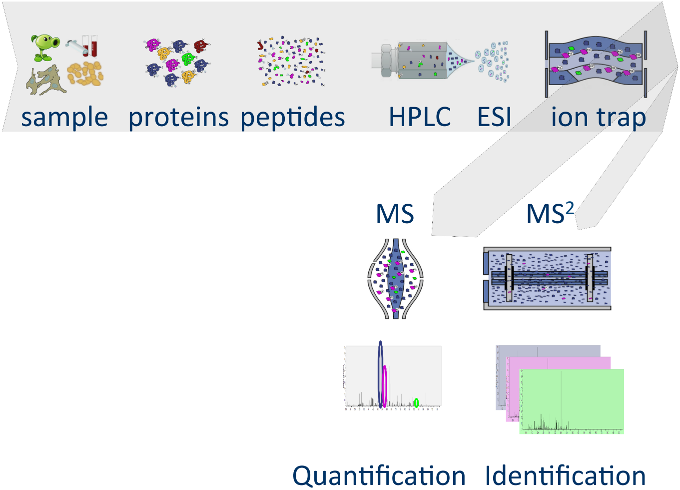
Statistical Methods for Quantitative MS-Based Proteomics:
1. Identification

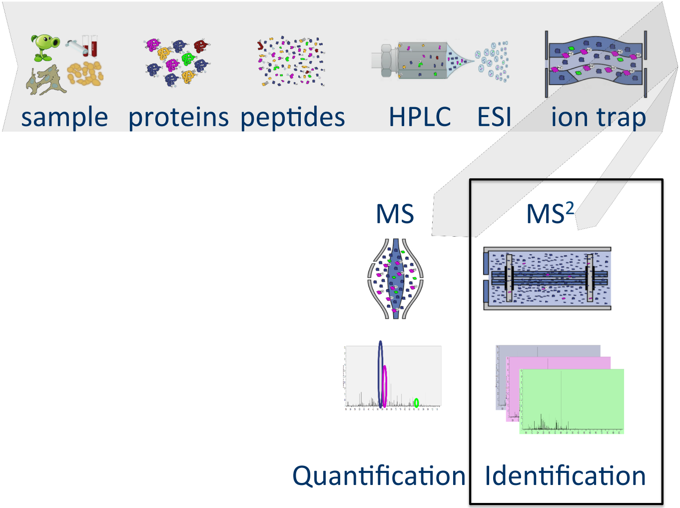
Lieven Clement

Statistics and Genomics Seminar, UCBerkeley, California

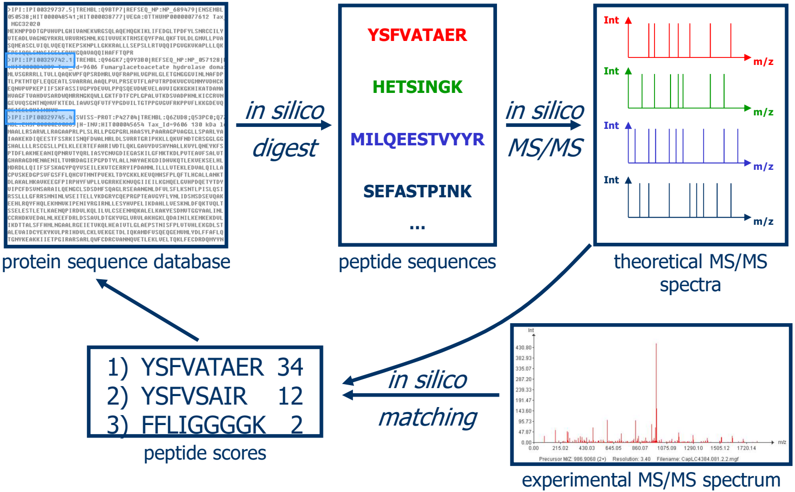
Challenges in Label Free MS-based Quatitative proteomics



Challenges in Label Free MS-based Quatitative proteomics

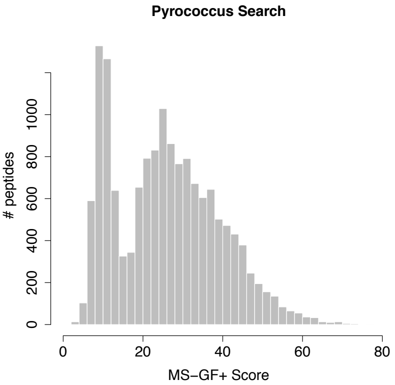


Identification



(slide courtesy to Lennart Martens)

Search engines return score that discriminates good from bad matches



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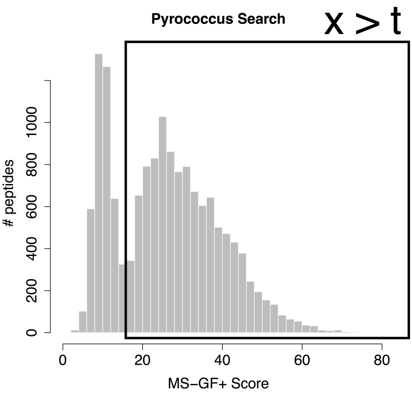

Score threshold t?

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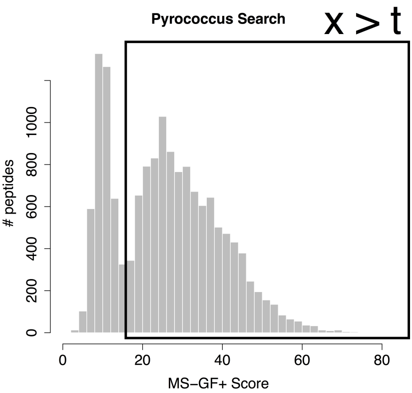

Score threshold t?

$f(x) = \pi_0 f_0(x) + (1 - \pi_0) f_1(x)$

$\text{FDR}(t) = Pr\left[FP|x \geq t\right]$

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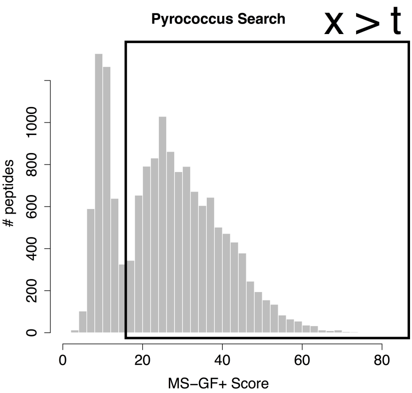

Score threshold t?

$$f(x) = \pi_0 f_0(x) + (1-\pi_0) f_1(x)$$

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$$\int_{-\infty}^{x=t} f(x) = \pi_0 \int_{-\infty}^{x=t} f_0(x) + (1-\pi_0) \int_{-\infty}^{x=t} f_1(x)$$

$$F(x) = \pi_0 F_0(t) + (1-\pi_0) F_1(t)$$

$$\mathrm{FDR}(t) = \frac{\pi_0[1-F_0(t)]}{1-F(t)}$$

Link to Benjamini Hochberg FDR

- Bayesian FDR

$$\text{FDR}(t) = \frac{\pi_0\left[1 - F_0(t)\right]}{1 - F(t)}$$

- BH for one sided test

$$FDR(t) = \frac{mp(t)}{\#t_i \geq t}$$

with

- m the number of tests
- $p(t)$ the p-value corresponding to a test statistic with value t

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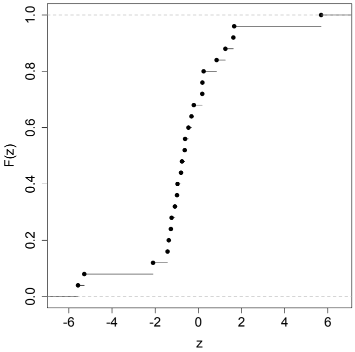
- BH for one sided test

$$FDR(t) = \frac{mp(t)}{\#t_i \geq t} = \frac{1 - F_0(t)}{\frac{\#t_i \geq t}{m}} = \frac{1 - F_0(t)}{1 - F(t)}$$

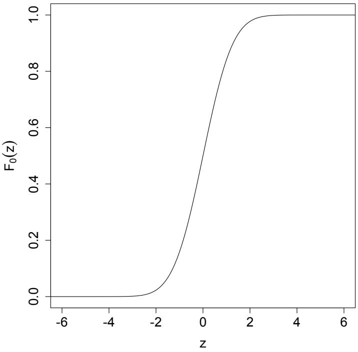
  - Use theoretical distribution for $p(t) = 1 - F_0(t)$
  - Conservative estimate $\pi_0 = 1$
  - ECDF: $1 - F(t) = \frac{\#t_i \geq t}{m}$

- Benjamini Hochberg 1995:

$F(t)$ using the ECDF



$F_0(t) theoreticalCDF$



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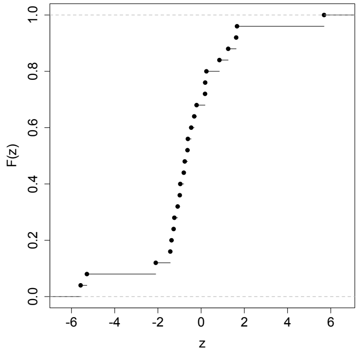

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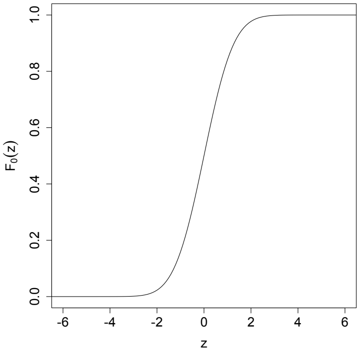
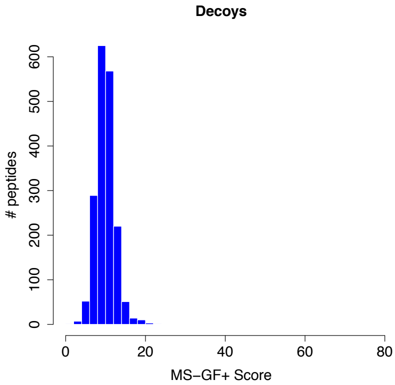


- How to define $F_0(t)$ in proteomics?

Target-Decoy approach to establish null distribution



- Searching against decoy databases to generate representative bad hits
- Reversed databases are a popular choice

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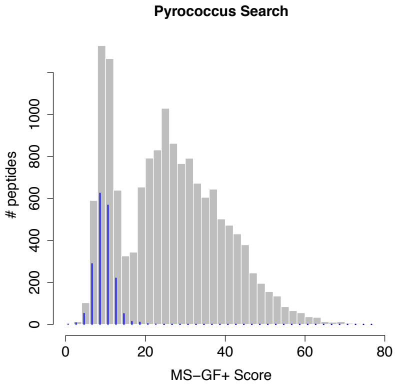

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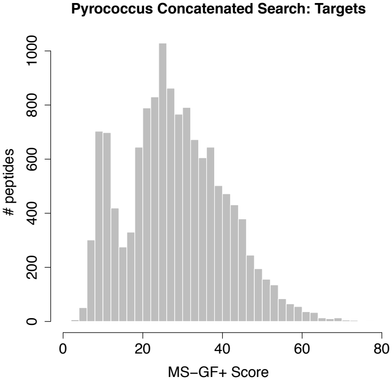

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- Assumption that bad hits have an equal probability to map on forward (target) and reverse database (decoy)

$$\hat{\pi}_0 = \frac{\#decoys}{\#targets}$$

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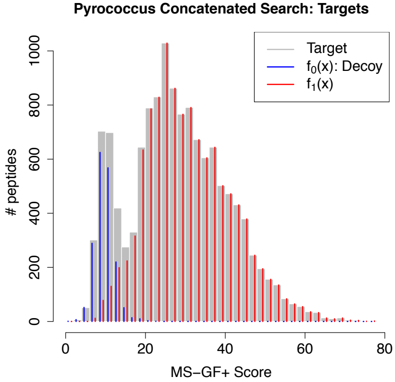

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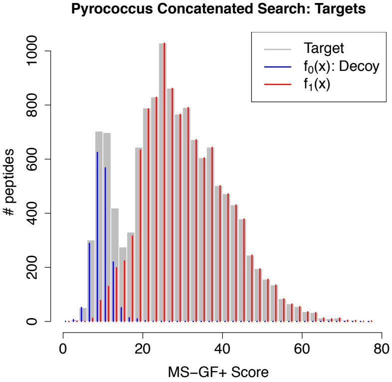

- Score cuttoff?
- Competitive Target - decoy:

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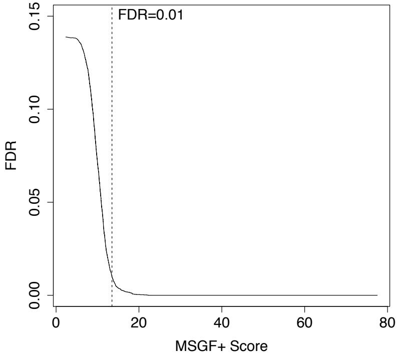

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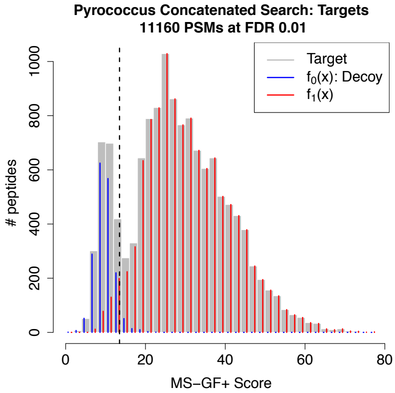

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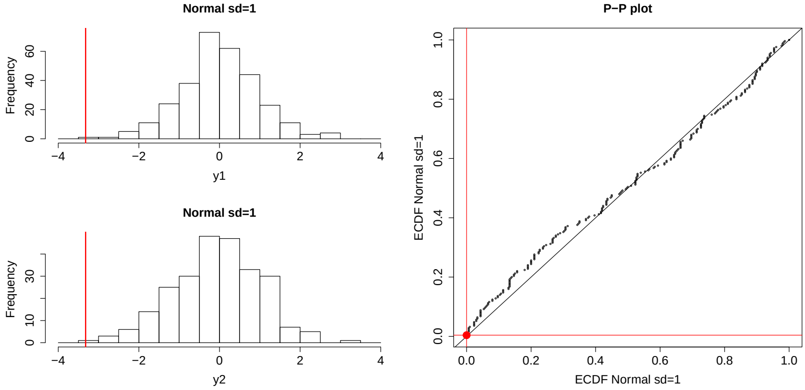
We have to evaluate that

- The decoys are good simulations of the targets: compare $\bar{F}_0(x)$ with $\bar{F}(x)$
- $\hat{\pi}_0 = \frac{\#decoys}{\#targets}$ are a good estimator for π_0.
- We will use Probability-Probability-plots for this purpose.
- They plot the ECDFs from two samples in function of each other.

PP-plot

PP-plots have the property that they show a straight 45 degree line through the origin if and only if both distributions are equivalent.



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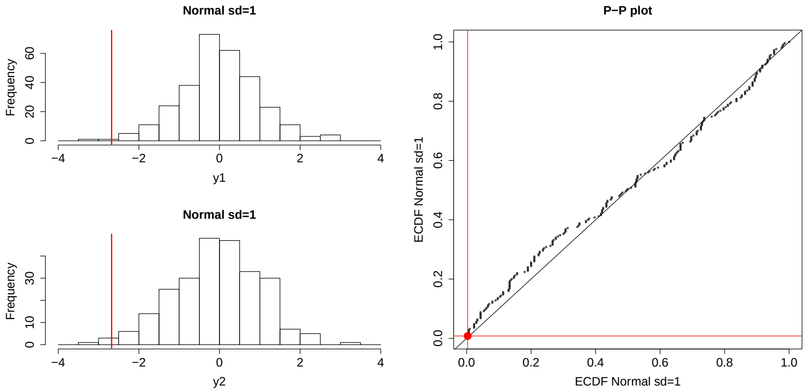

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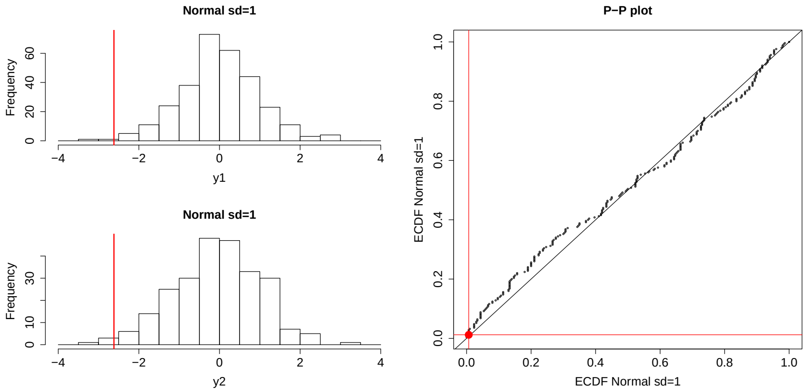

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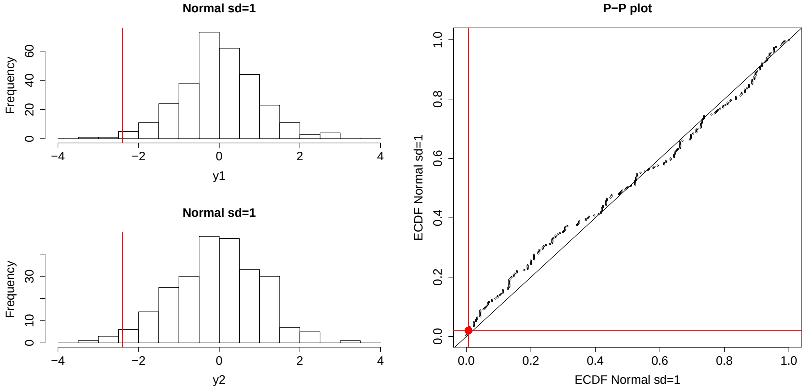

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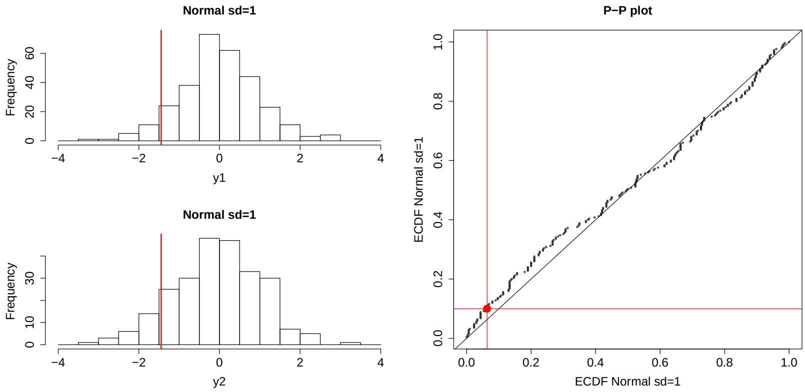

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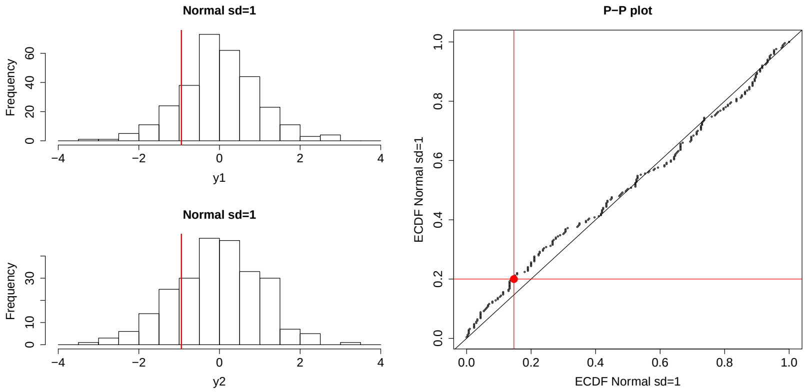

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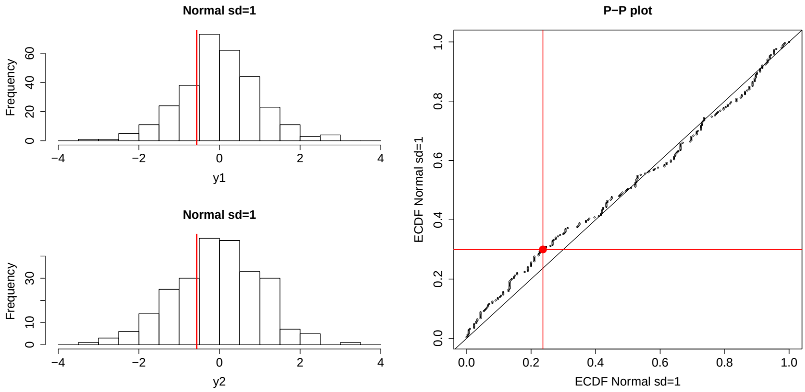

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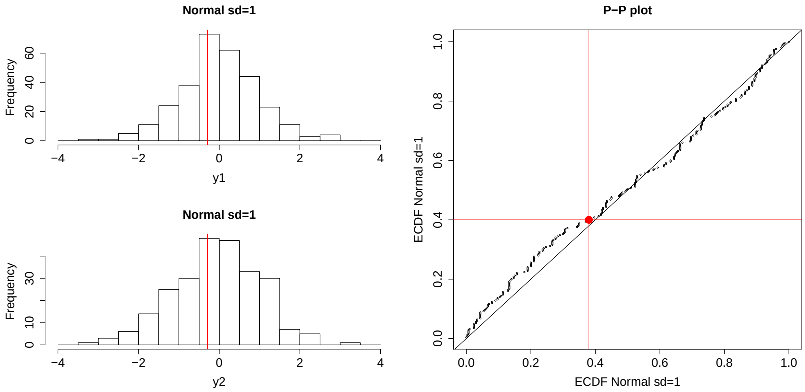

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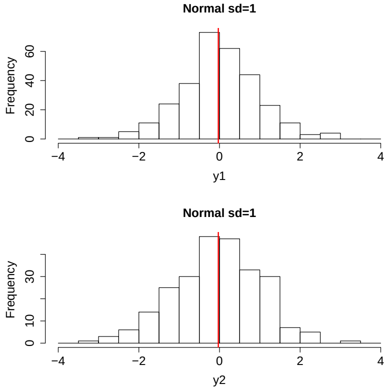

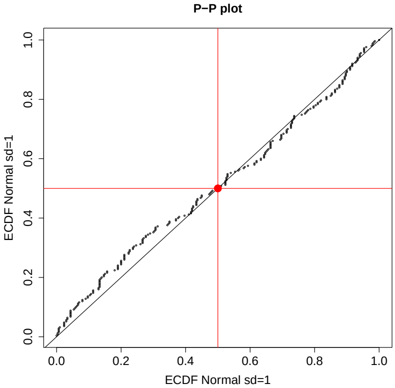

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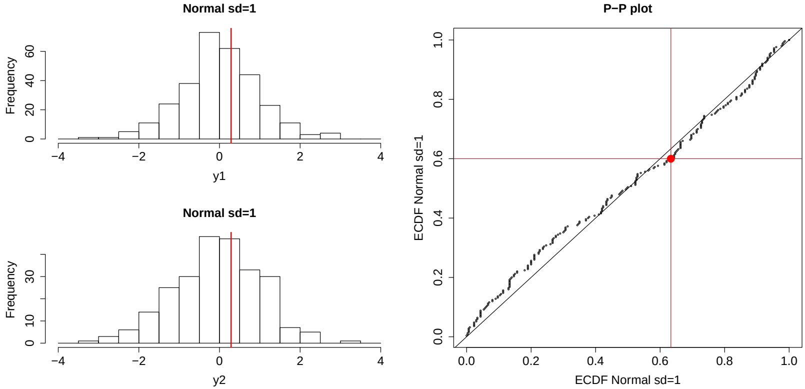

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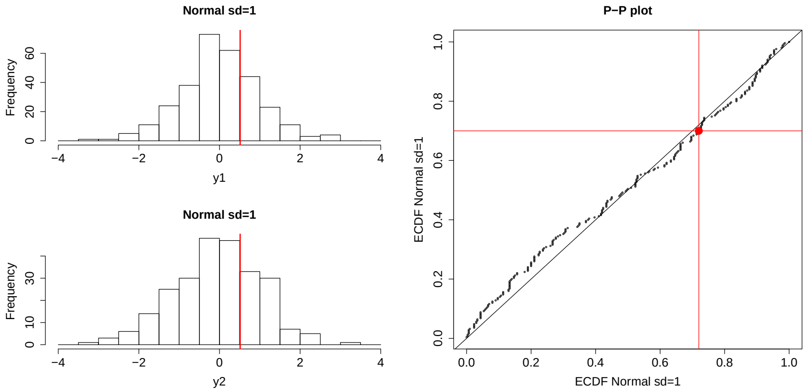

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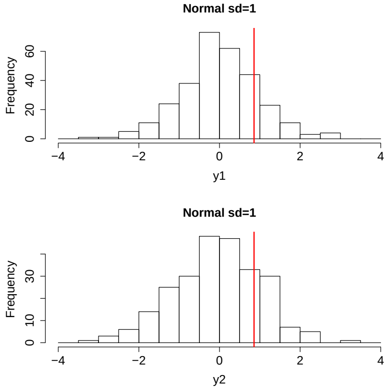

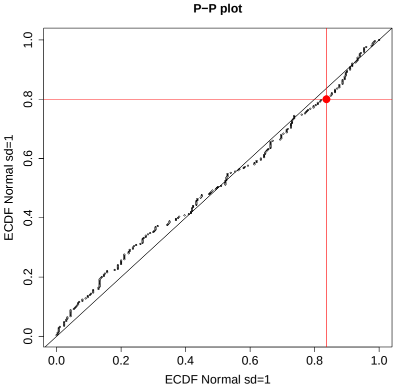

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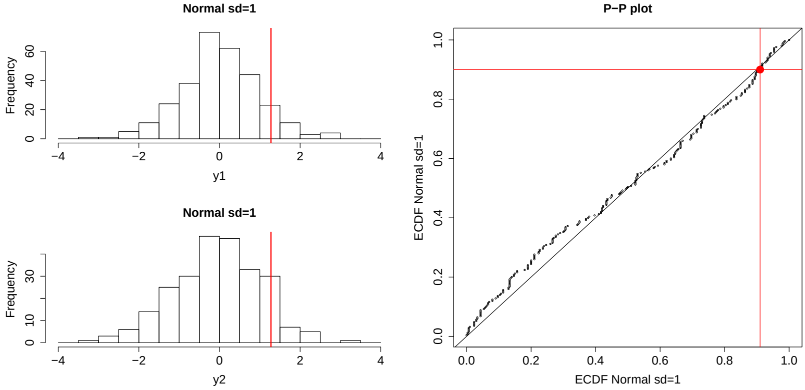

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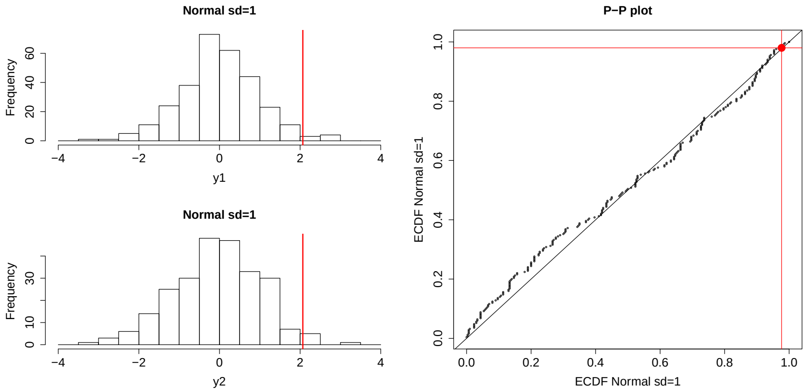

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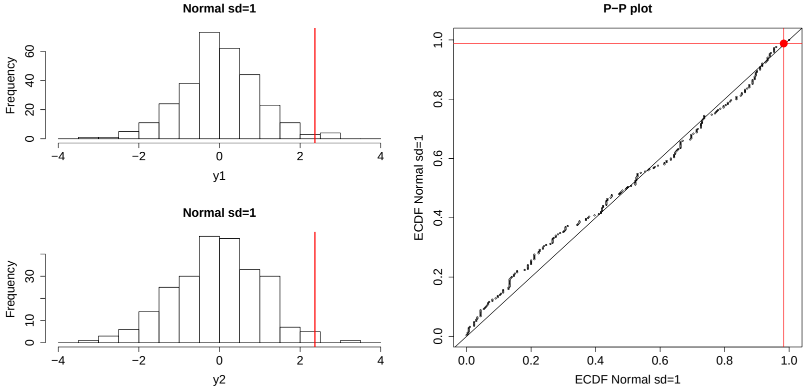

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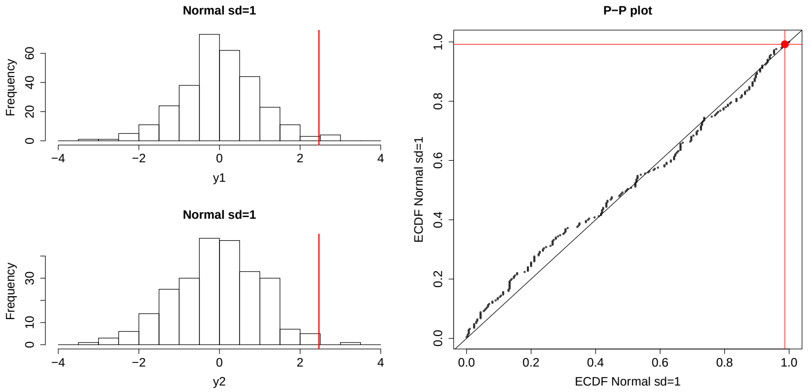

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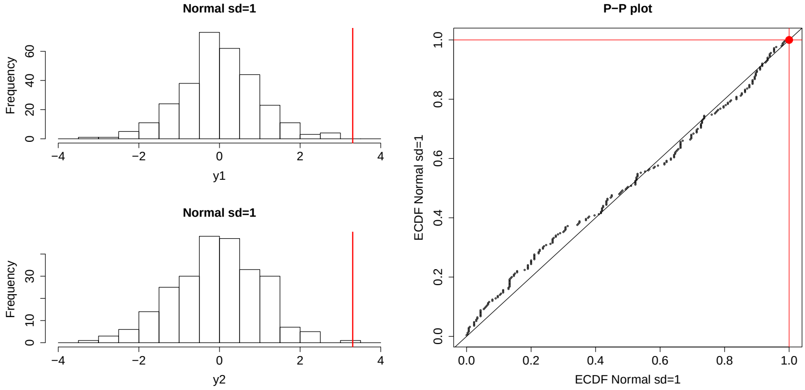

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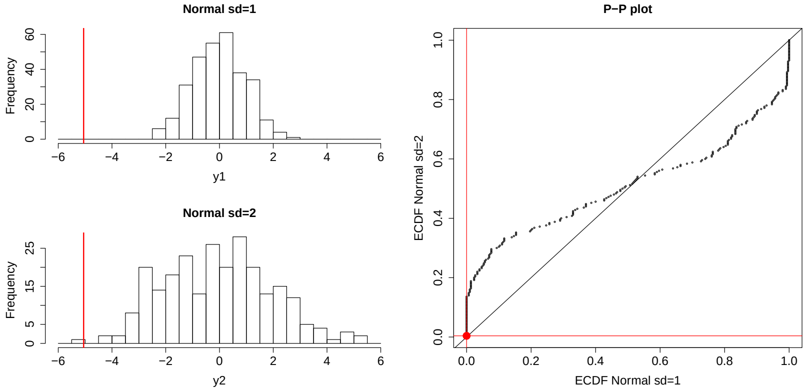
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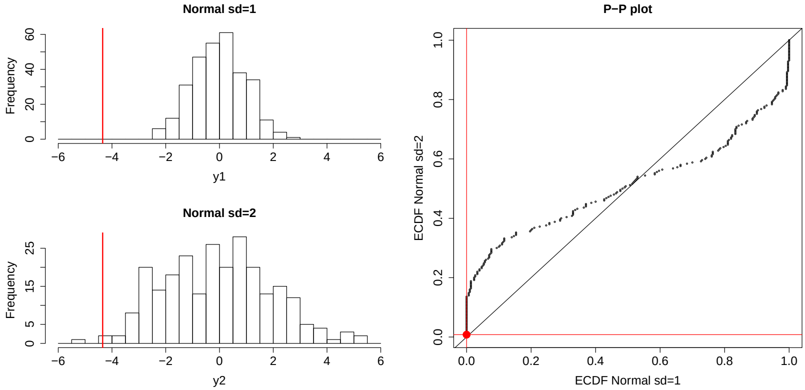
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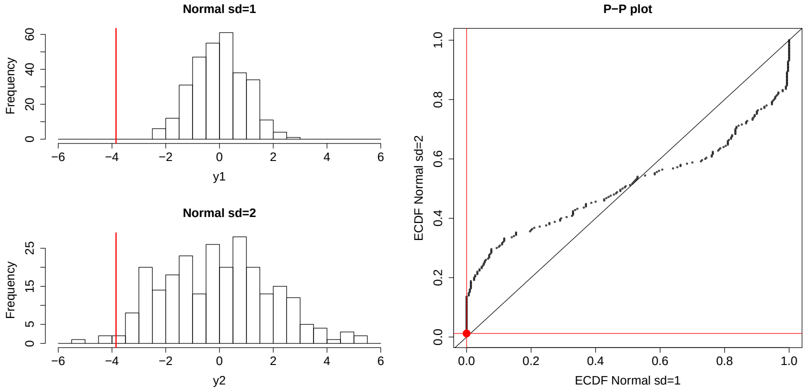
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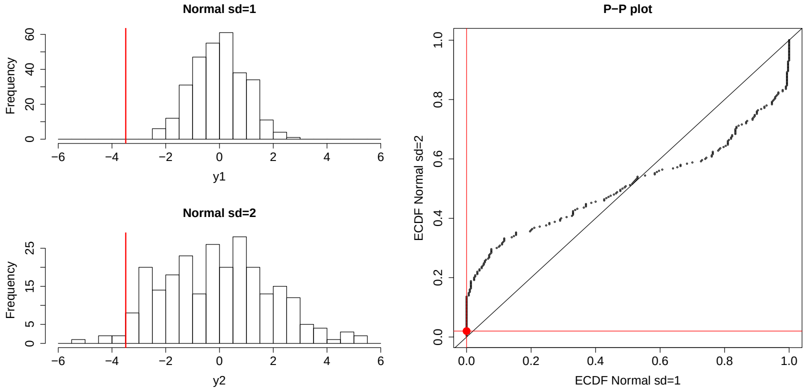
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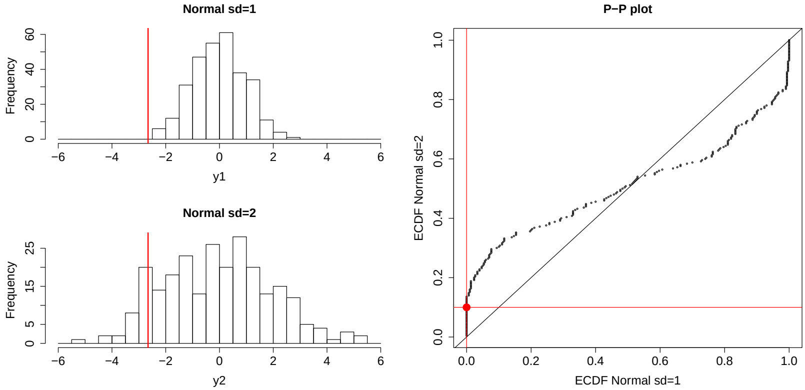
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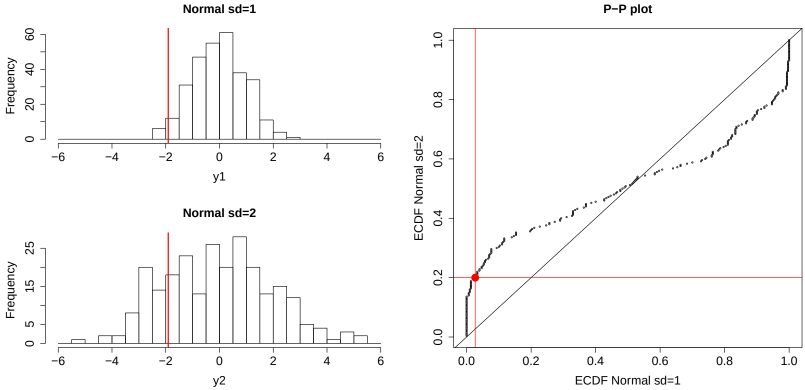
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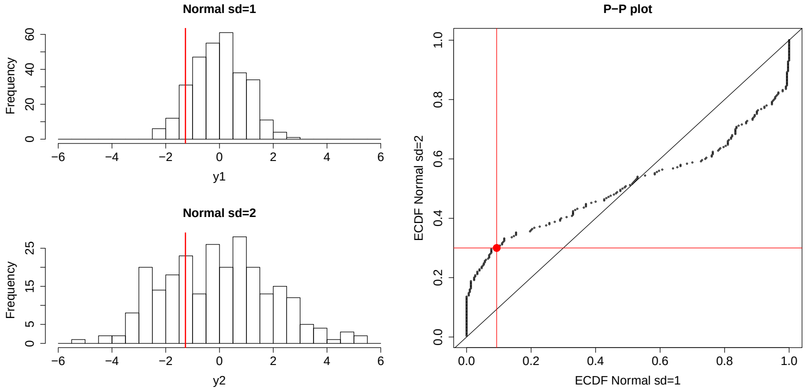
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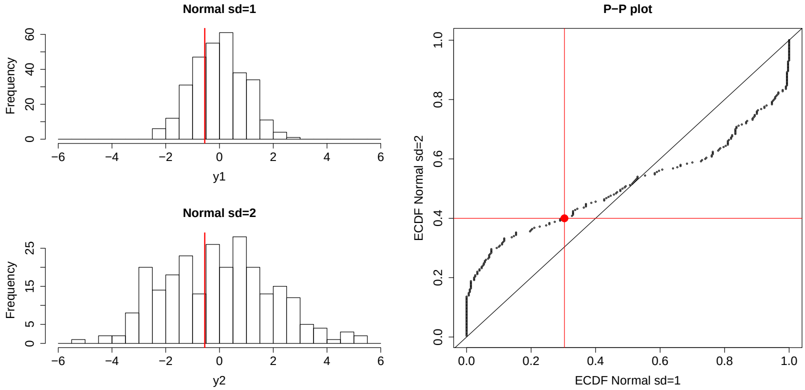
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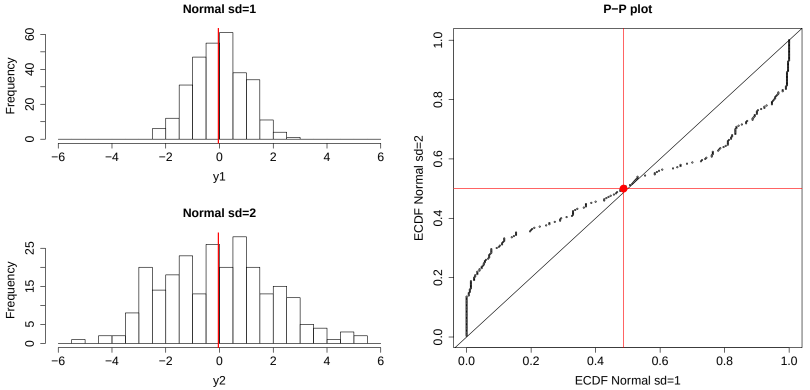
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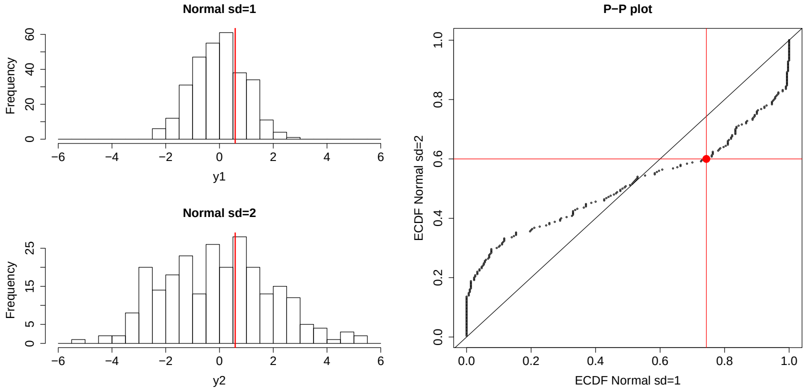
ECDF Normal sd=1

PP-plot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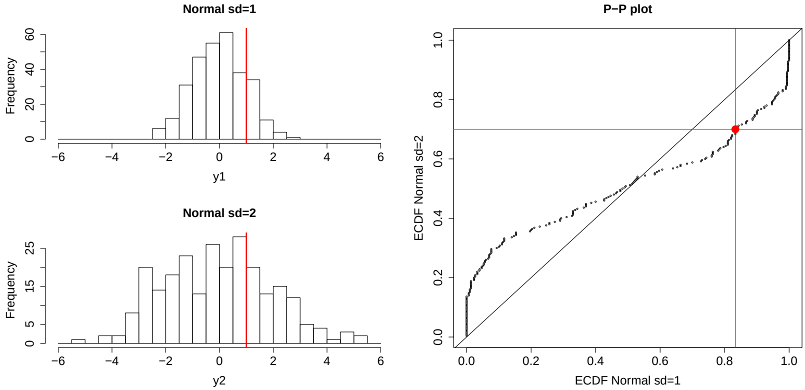
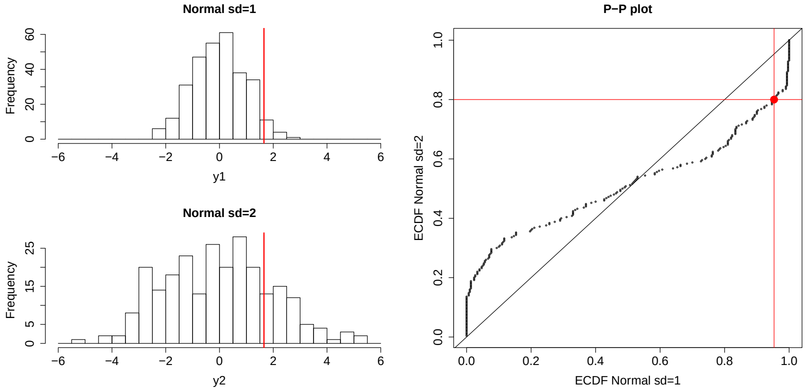
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

PP-plot



Normal sd=1
Frequency
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20
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60
−6
−4
−2
0
2
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
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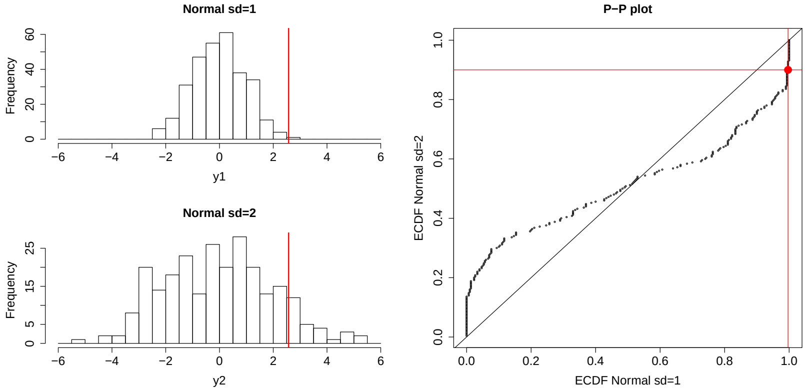
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

PP-plot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−2
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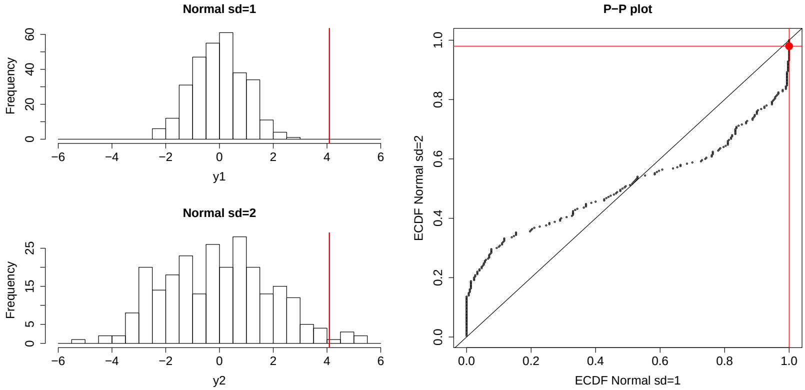
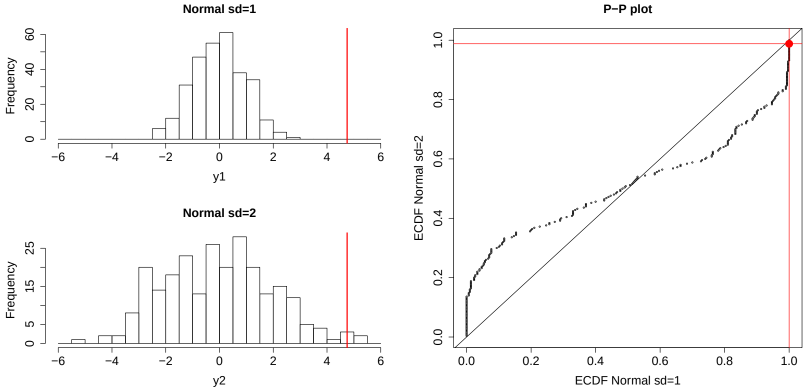
2
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

PP-plot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y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
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1.0
0.0
0.2
0.4
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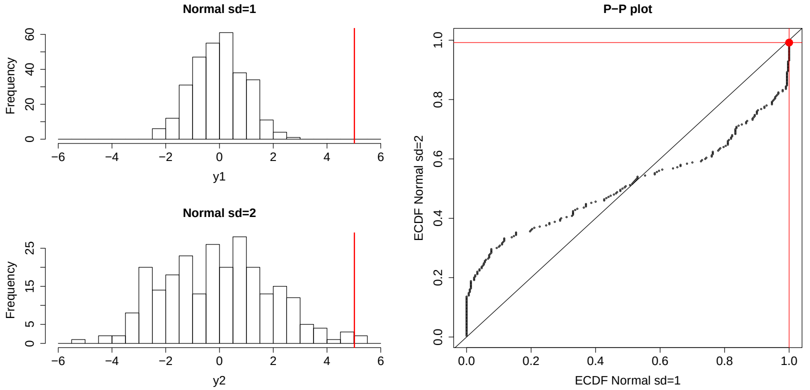
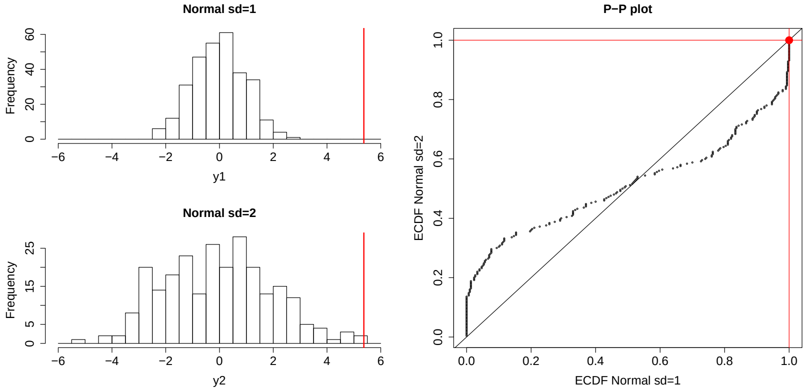
0.8
1.0
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
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4
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y1
Normal sd=2
Frequency
25
15
5
0
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
1.0
0.8
0.6
0.4
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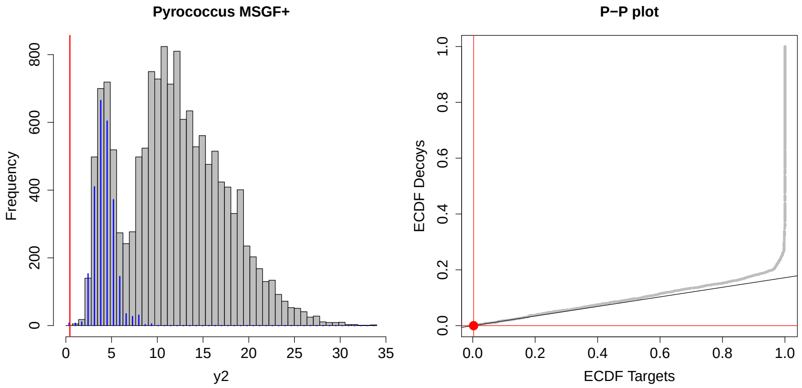
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

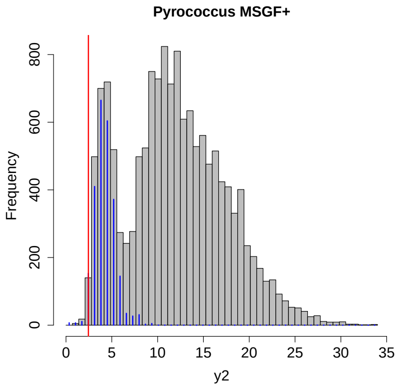
PP-plot: pyrococcus



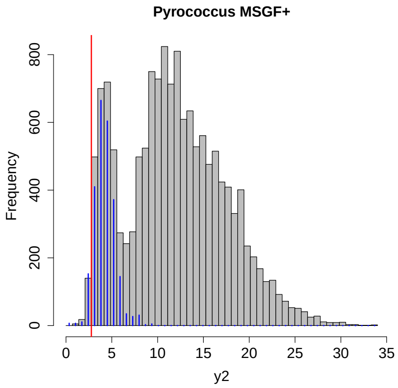
PP-plot: pyrococcus

What about π_0?

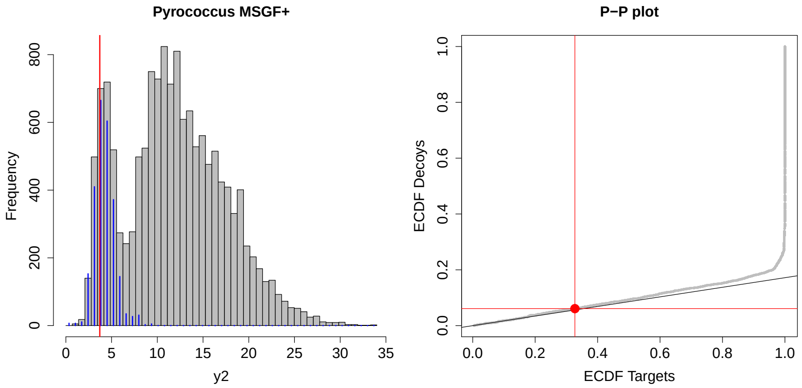
PP-plot: pyrococcus



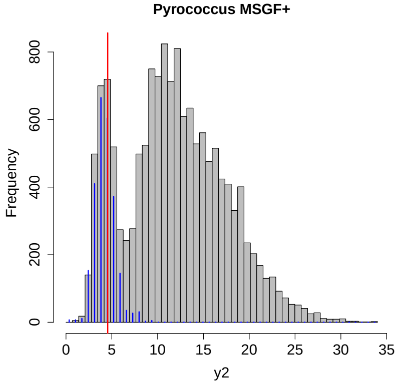
PP-plot: pyrococcus

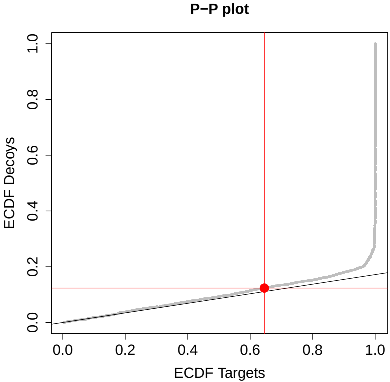


PP-plot: pyrococcus

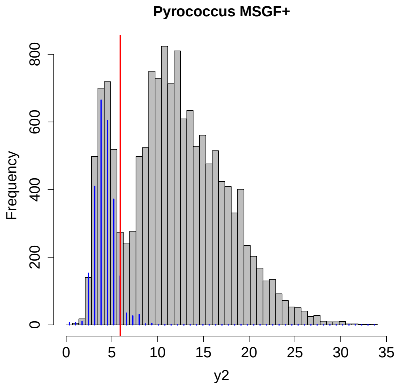


PP-plot: pyrococcus





PP-plot: pyrococcus



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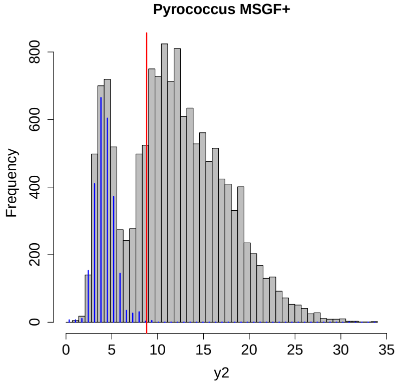

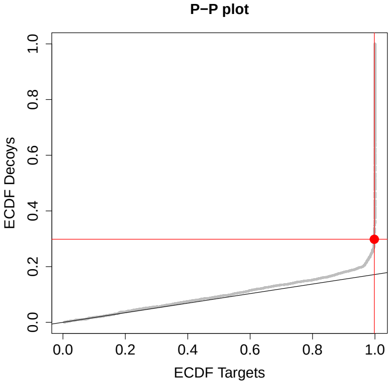

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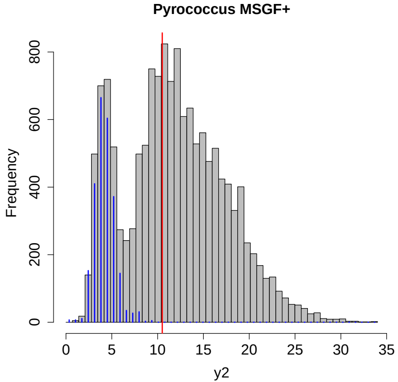

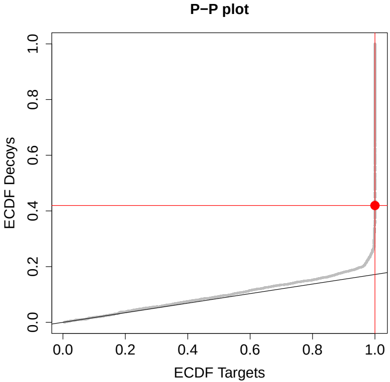

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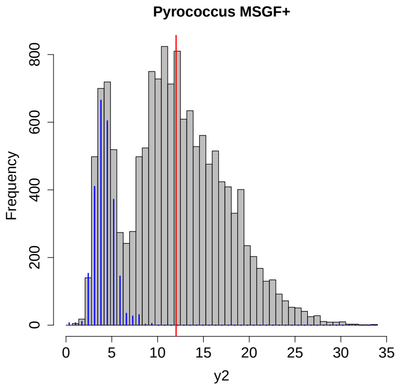

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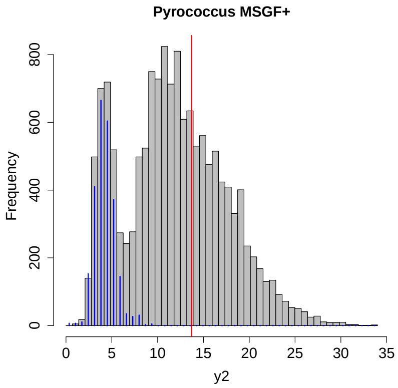

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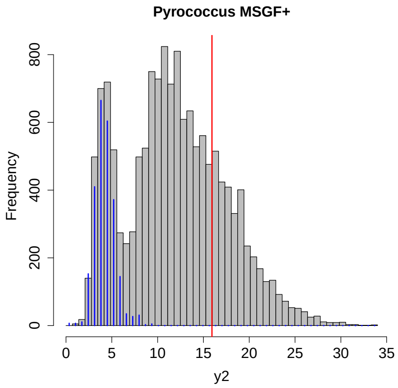

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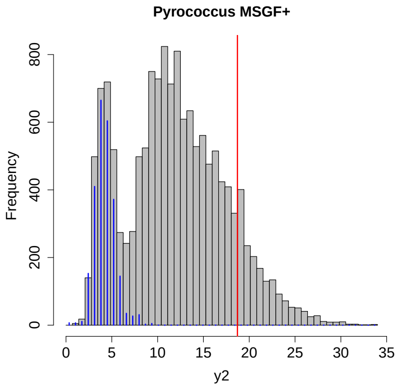

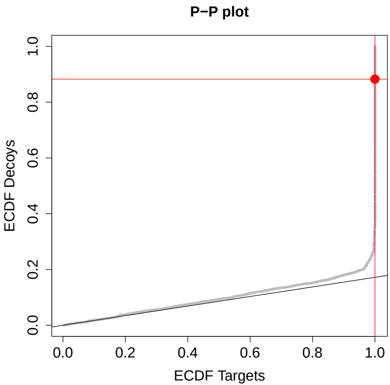

PP-plot: pyrococcus