Statistical Methods for Quantitative MS-Based Proteomics:

1. Identification & False discovery rate

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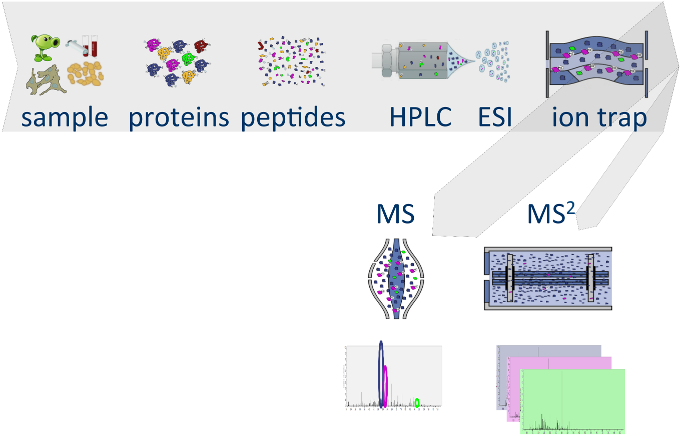
Challenges in Label Free MS-based Quantitative Proteomics

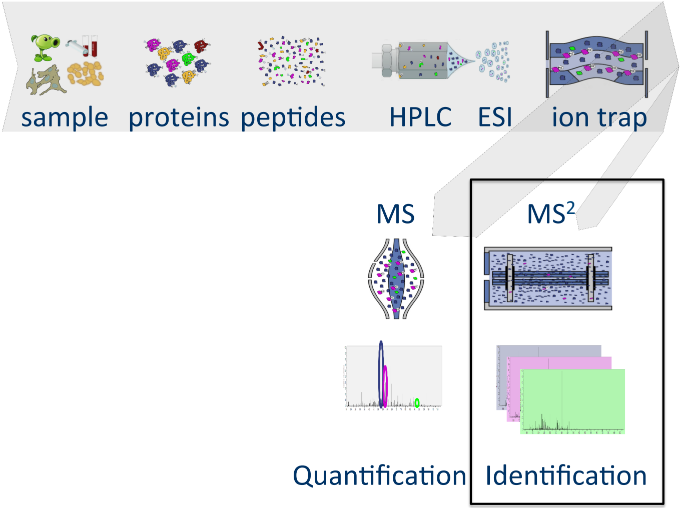


sample proteins peptides HPLC ESI ion trap

MS MS^2

Quantification Identification

Challenges in Label Free MS-based Quantitative Proteomics



sample proteins peptides HPLC ESI ion trap

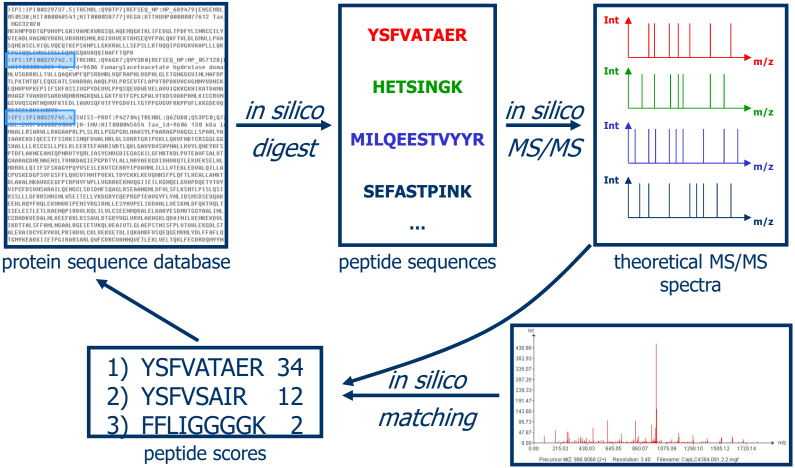
MS

MS^2

Quantification

Identification

Identification


in silico digest
YSFVATAER
HETSINGK
MILQEESTVYYR
SEFASTPINK
...
in silico MS/MS
Int
m/z
in silico matching
Int
430.80
382.93
335.07
287.20
239.33
191.47
143.60
95.73
47.87
0.00
0.00
215.02
430.03
645.05
860.07
1075.09
1290.10
1505.12
1720.14
m/z
Precursor M/Z: 986.9068 (2+)
Resolution: 3.40
Filename: CapLC4384.081.2.2.mgf

protein sequence database

peptide sequences

theoretical MS/MS spectra

1) YSFVATAER 34
2) YSFVSAIR 12
3) FFLIGGGGK 2

peptide scores

experimental MS/MS spectrum

(slide courtesy to Lennart Martens)

Table of Outcomes

	Called Bad	Called Correct	
Bad hit	TN	FP	m_0
Correct hit	FN	TP	m_1
Total	NR	R	m

- TN: number of true negatives
- FP: number of false positives
- FN: number of false negatives
- TP: number of true positives
- NR: number of non-rejections, R: number of rejections

Table of Outcomes

	Called Bad	Called Correct	
Bad hit	TN	FP	m_0
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Random Variables

Table of Outcomes

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Unobservable	Bad hit	TN	FP	m_0
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$FDP = \frac{FP}{FP+TP}$. But is unkown! (FDP: false discovery proportion)

Table of Outcomes

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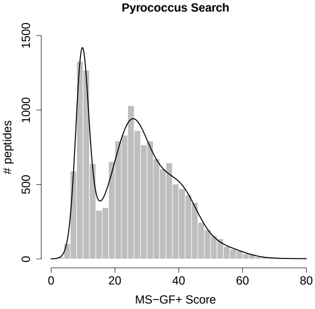
$FDR = E\left[\frac{FP}{FP+TP}\right]$. (FDR: false discovery rate)

Search engines return score that discriminates good from bad matches



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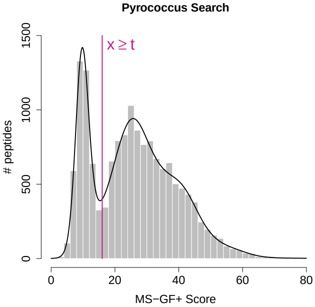

Score threshold t?

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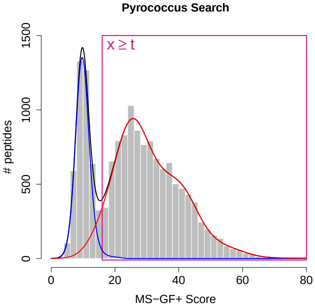
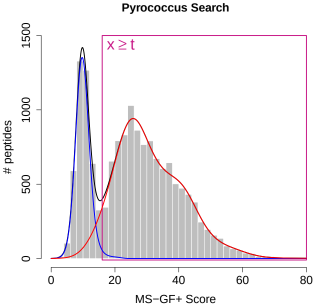


Score threshold t?

$$f(x) = \color{blue}{\pi_0 f_0(x)} + \color{red}{(1-\pi_0) f_1(x)}$$

Search engines return score that discriminates good from bad matches



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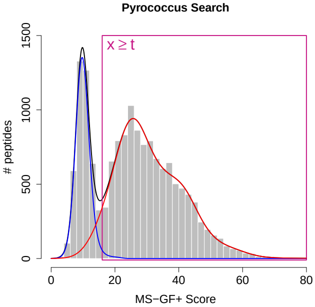

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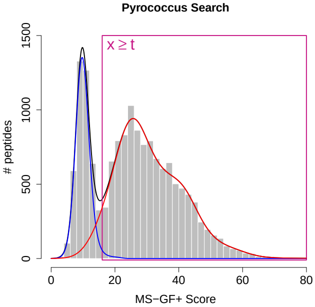

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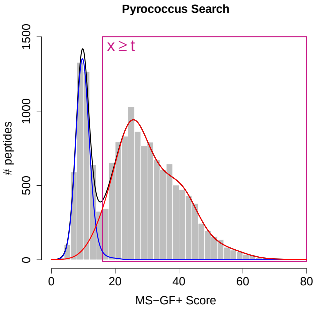

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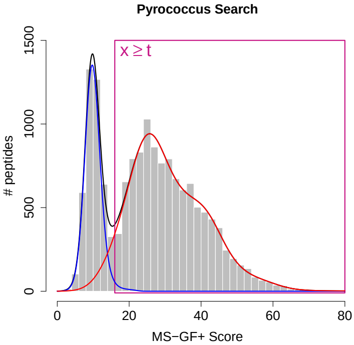
$$P_.[x \geq t] = \int_t^\infty f_.(x)$$

How to estimate FDR?



$$P_{\cdot}[x \geq t] = \int_t^{\infty} f_{\cdot}(x)$$

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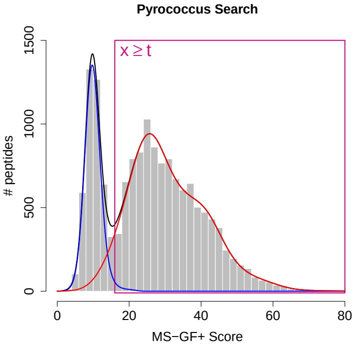


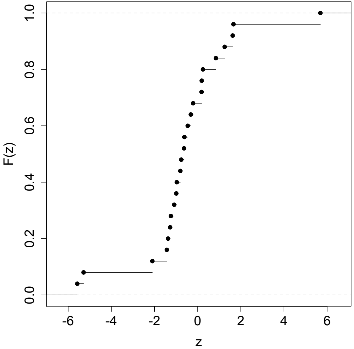
$$P_.[x \geq t] = \int_t^\infty f_.(x)$$

$$P_.[x \geq t] \approx \frac{\#x \geq t}{m}$$

$$\text{FDR}(t) = E\left[\frac{FP}{FP + TP}\right] = \frac{m\pi_0 P[x \geq t|FP]}{mP[x \geq t]}$$

$$\widehat{FDR}(t) = \frac{m\pi_0 P[x \geq t|FP]}{\#x \geq t}$$

- $F(t)=\int\limits_{-\infty}^{t} f(x)$ using the Empirical cumulative distribution function (ECDF): $\bar{F}(t)$



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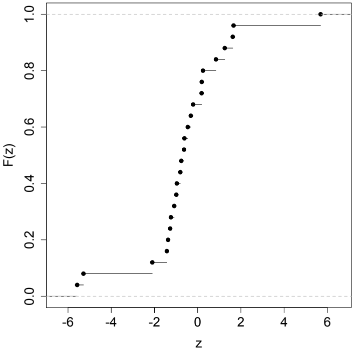

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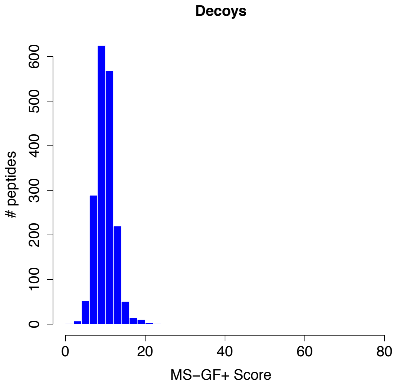
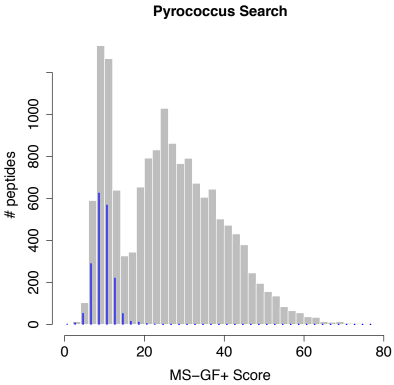
- How to characterize $F_0(t)$ and π_0 in proteomics?

Target-Decoy approach to establish null distribution



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

Target-Decoy approach to establish null distribution



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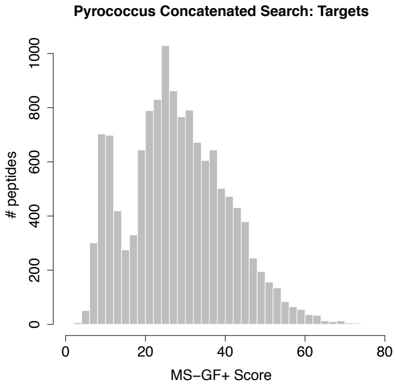

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- Assumption that bad hits have an equal probability to map on forward (target) and reverse database (decoy)

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

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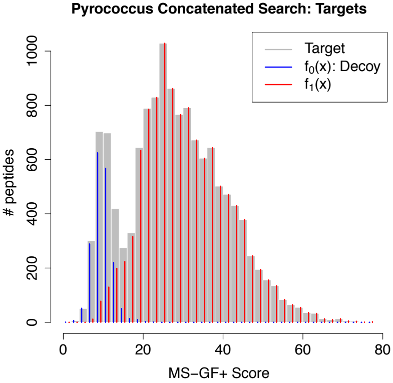

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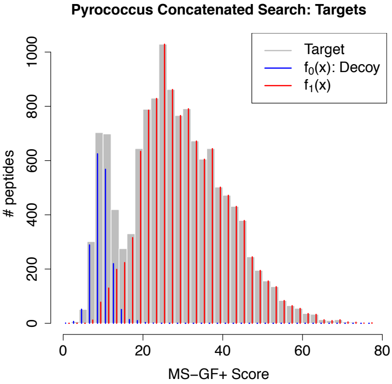

- Score cuttoff?

$$\text{FDR}(x)=E\left[\frac{FP}{FP+TP}\right]$$

- Competitive Target - decoy:

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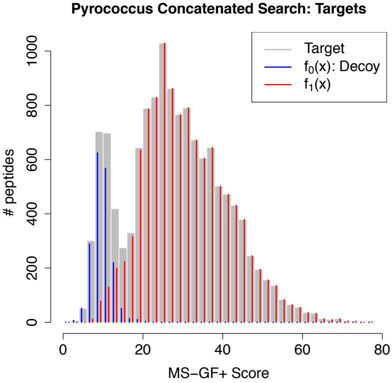

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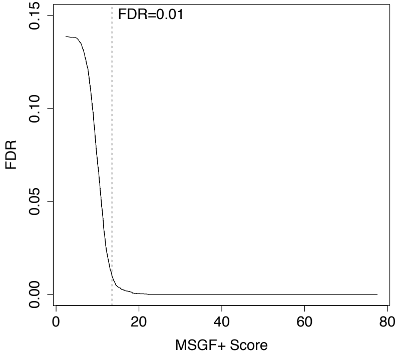

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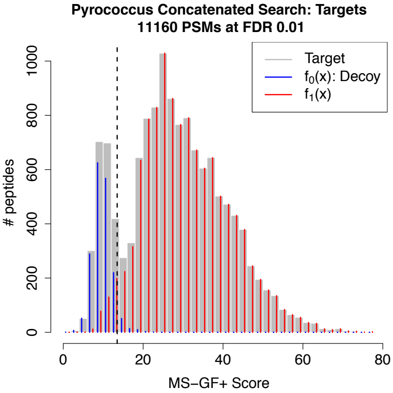

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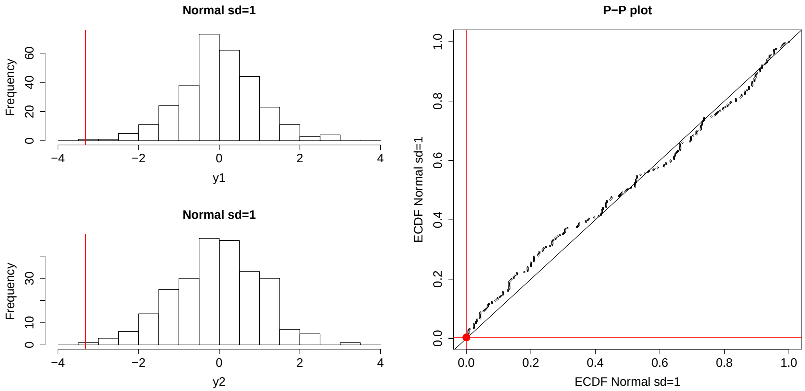
We have to evaluate that

- The decoys are good simulations of the targets: compare $\bar{F}_0(x)$ with $\bar{F}(x)$
- $\hat{\pi}_0 = \frac{\#decoys}{\#targets}$ is 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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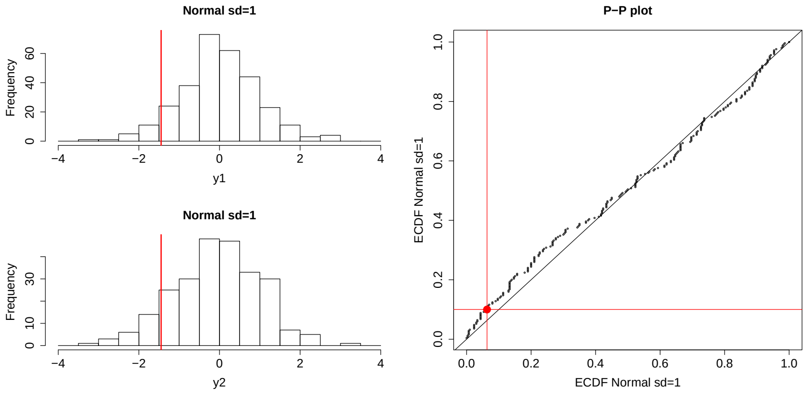

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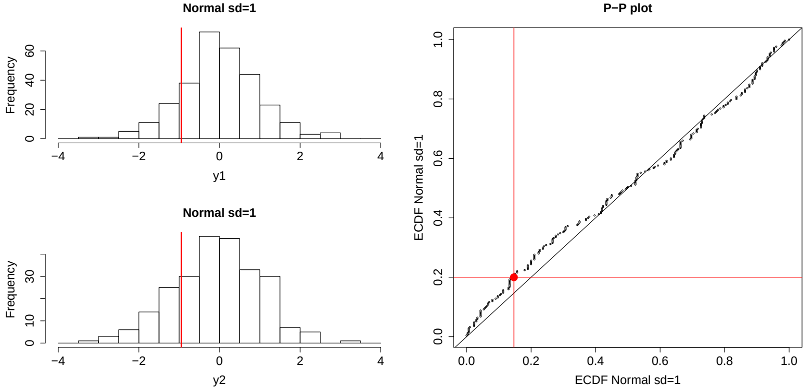

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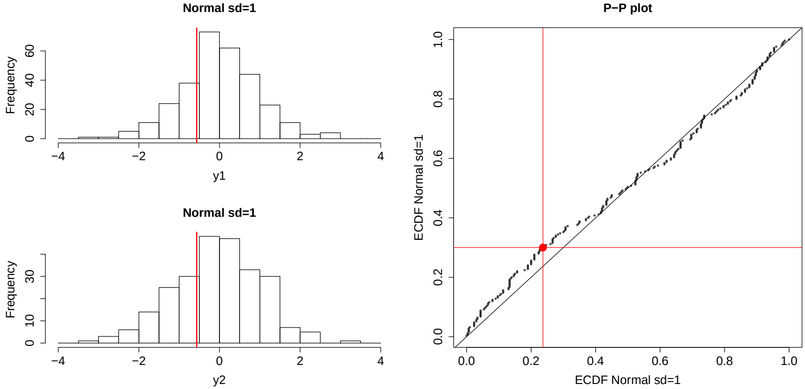

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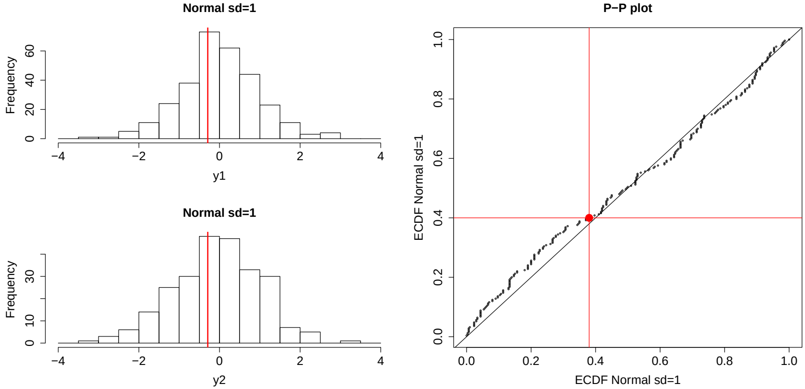

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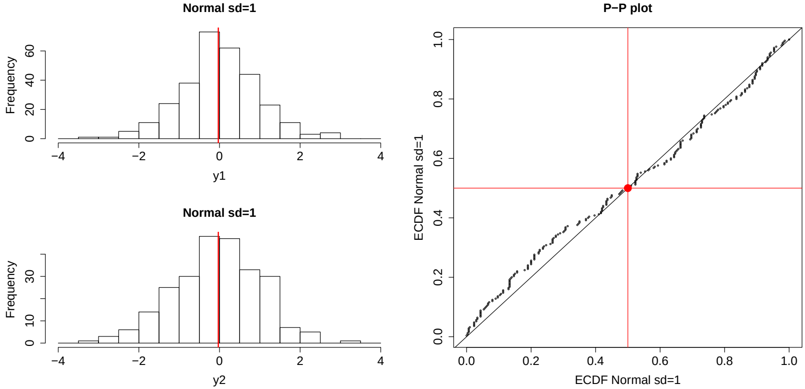

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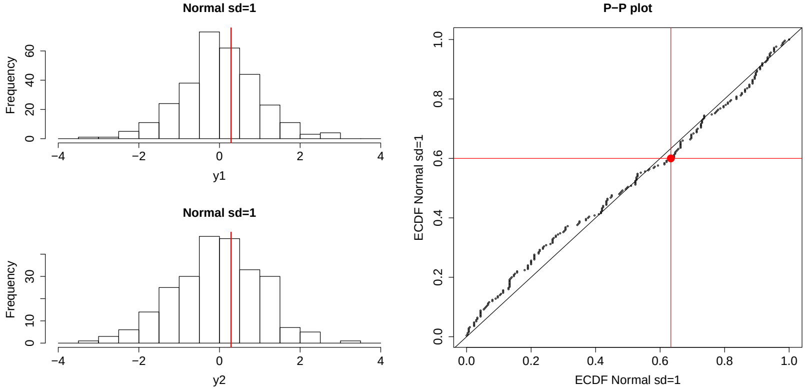

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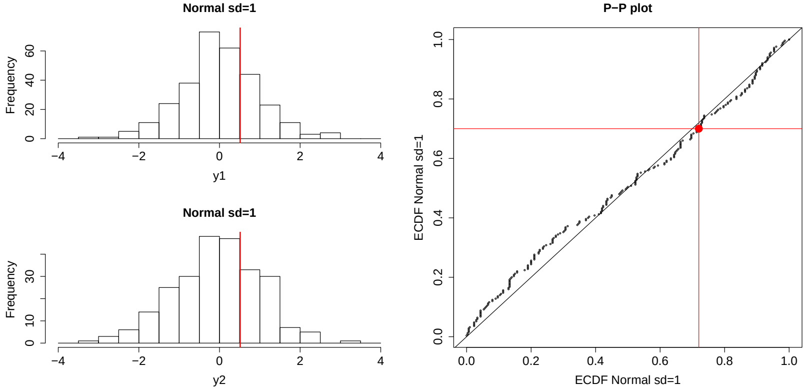

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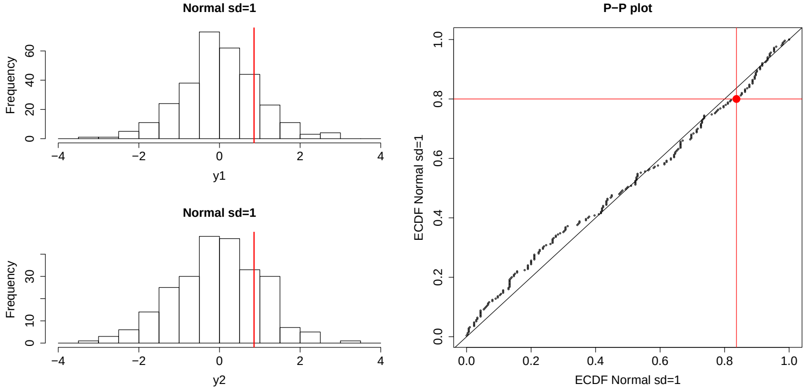

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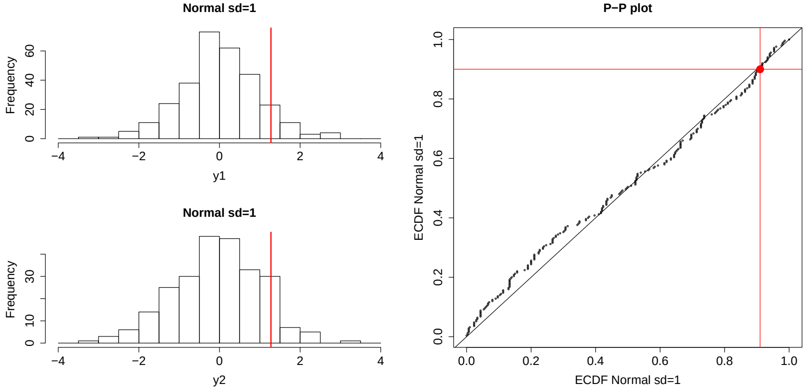

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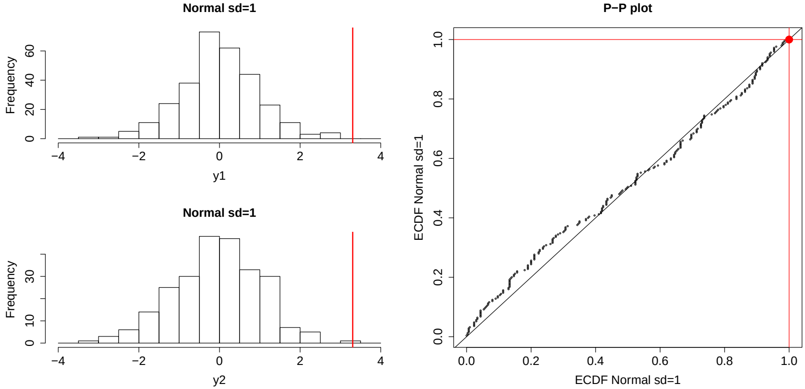

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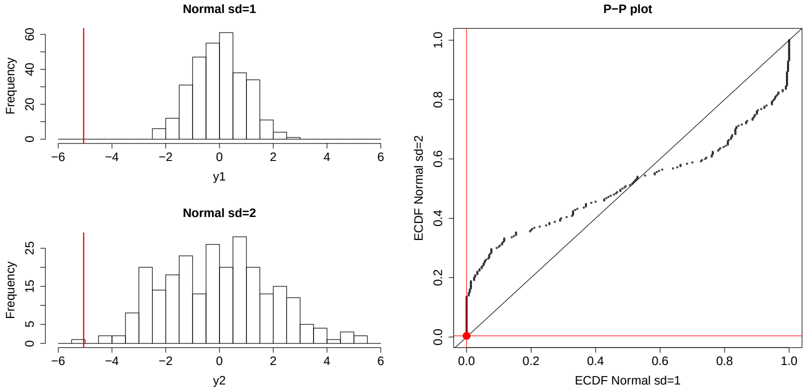
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Frequency
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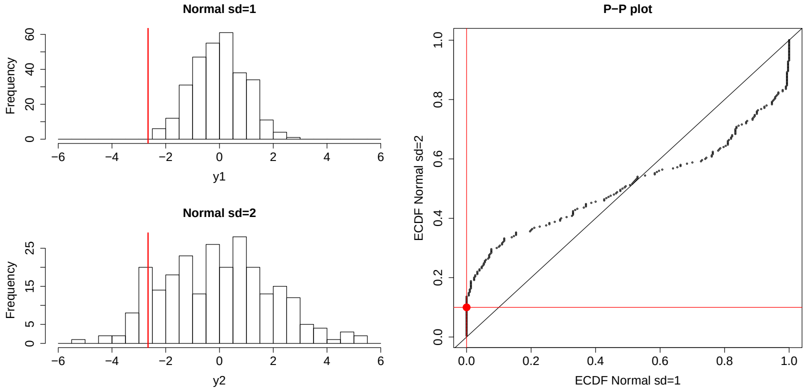
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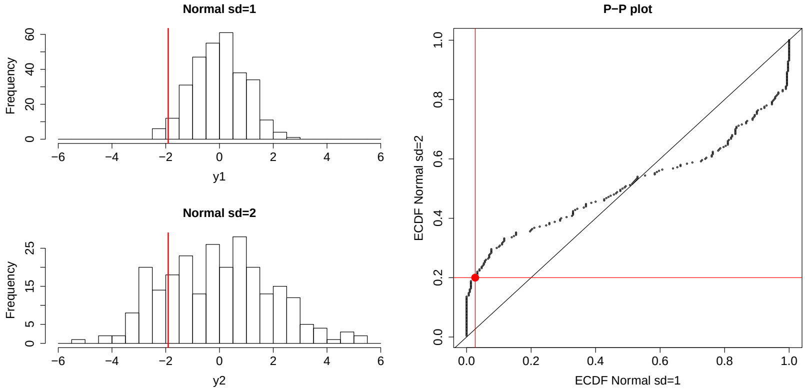
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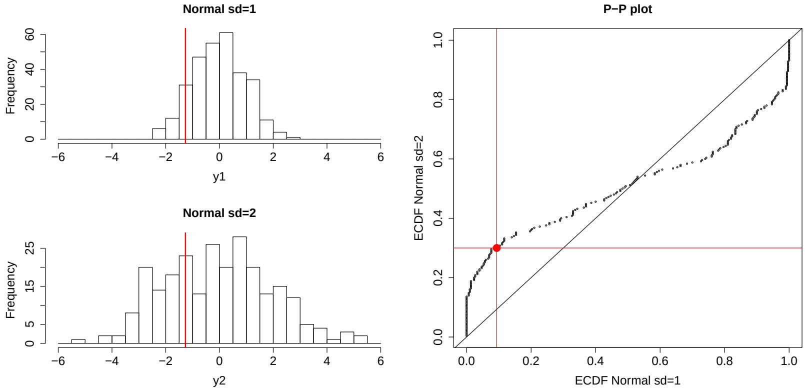
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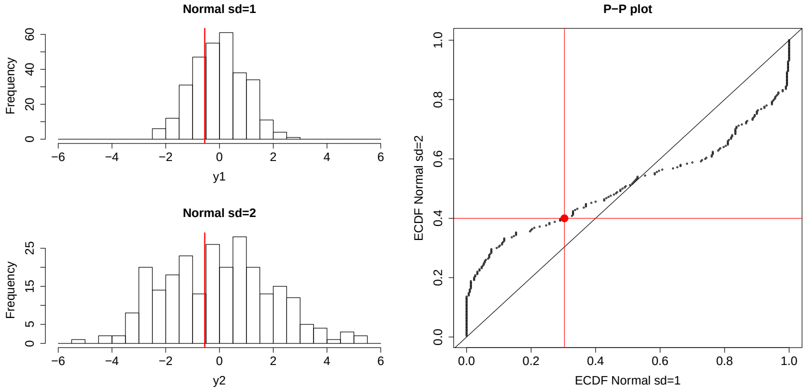
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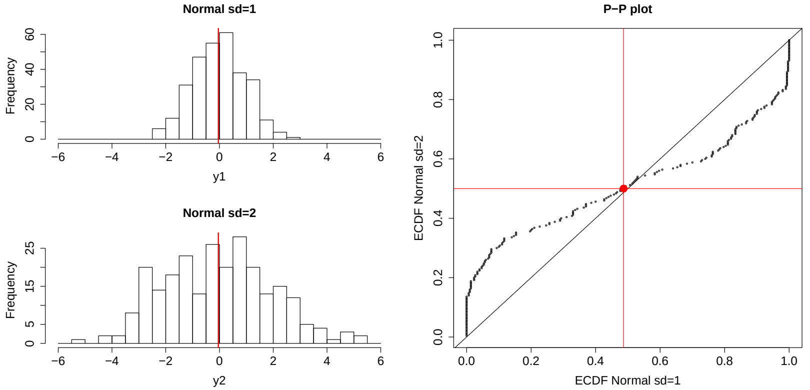
1.0

PP-plot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−4
−2
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2
4
6
y1
Normal sd=2
Frequency
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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
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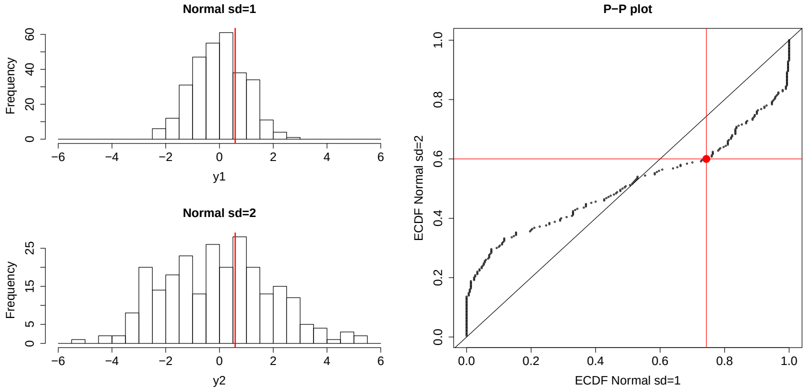
0.2
0.4
0.6
0.8
1.0

PP-plot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Frequency
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−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
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0.2
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ECDF Normal sd=1
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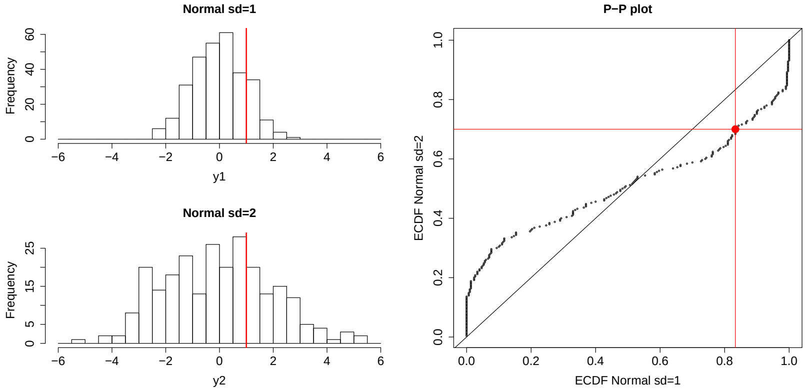
PP-plot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y2
P−P plot
ECDF Normal sd=2
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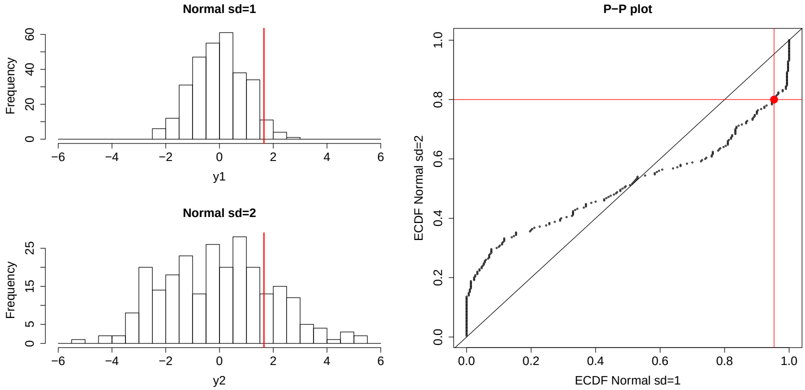
PP-plot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Frequency
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y2
P−P plot
ECDF Normal sd=2
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ECDF Normal sd=1
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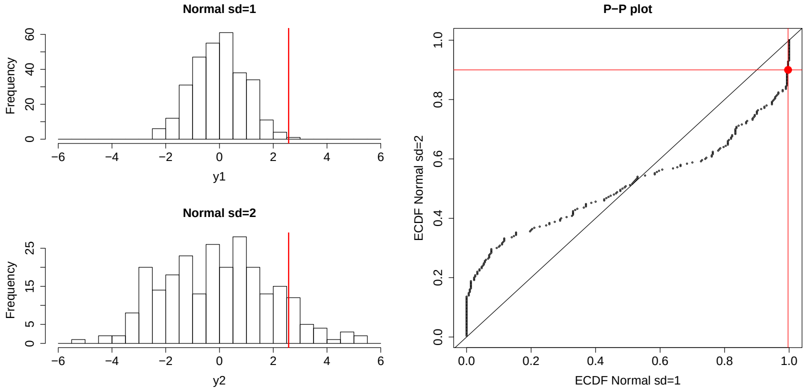
PP-plot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Frequency
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y2
P−P plot
ECDF Normal sd=2
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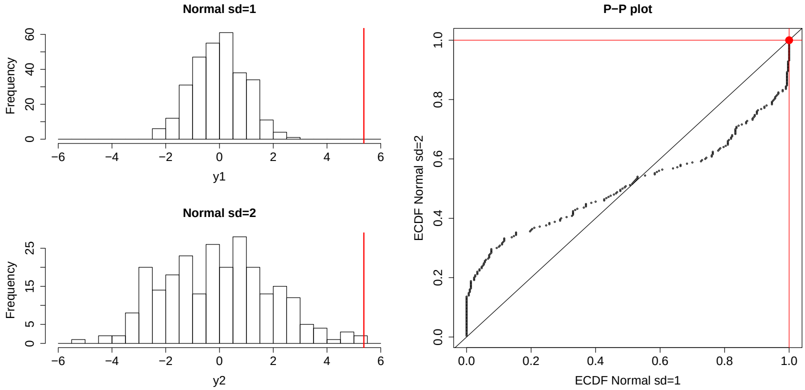
ECDF Normal sd=1

PP-plot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Frequency
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y2
P−P plot
ECDF Normal sd=2
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ECDF Normal sd=1
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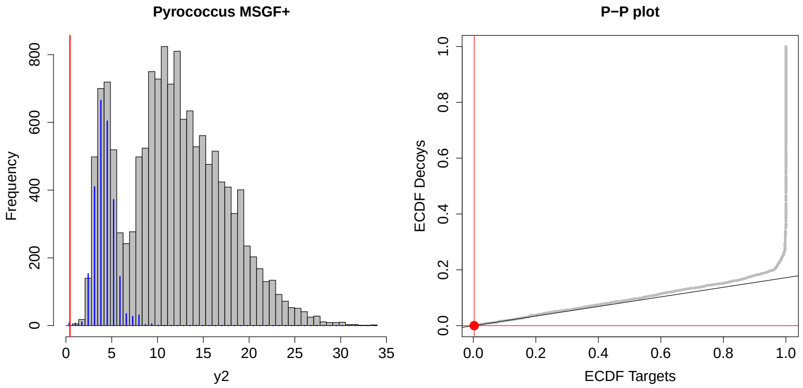
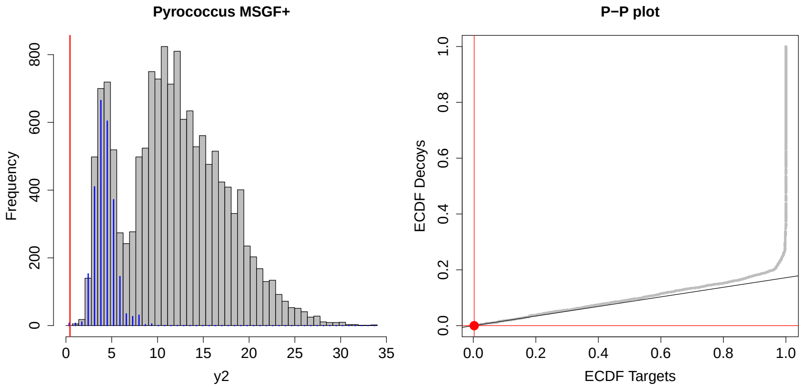
1.0

PP-plot: pyrococcus

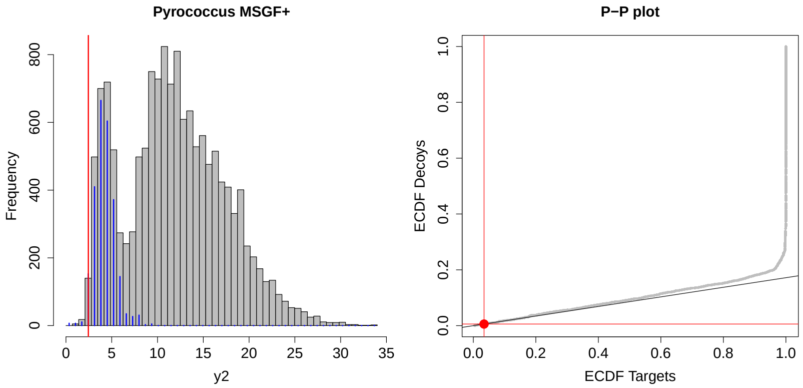


PP-plot: pyrococcus

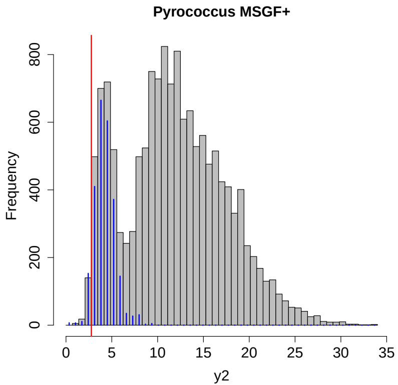


What about $\hat{\pi}_0$?

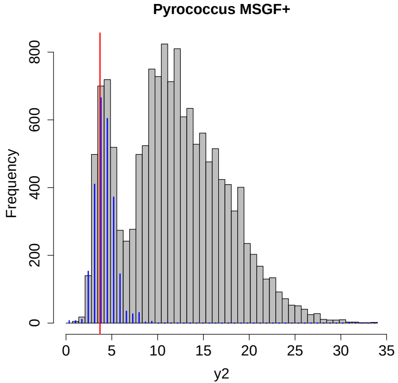
PP-plot: pyrococcus



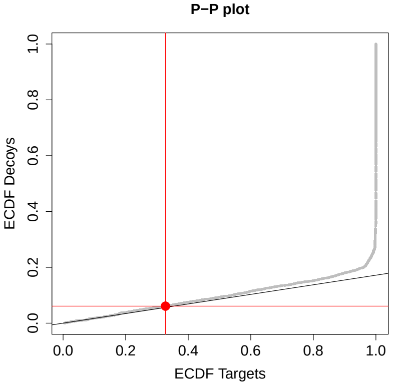
PP-plot: pyrococcus



PP-plot: pyrococcus

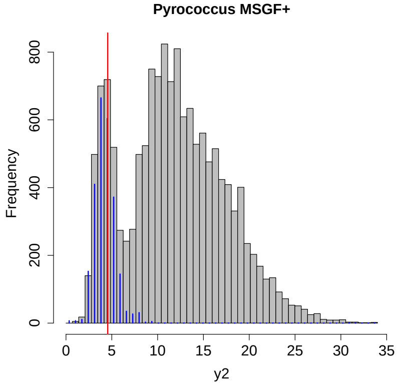


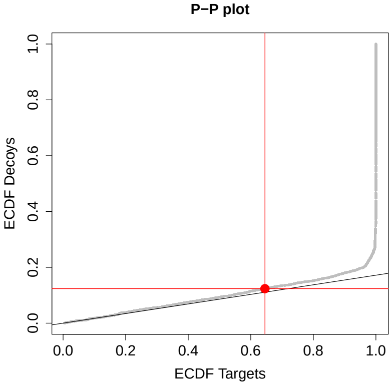
Pyrococcus MSGF+



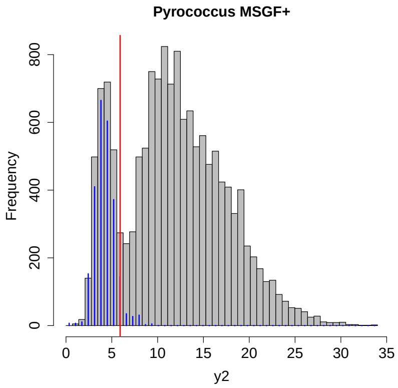
P−P plot

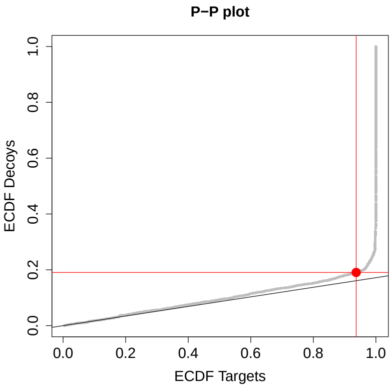
PP-plot: pyrococcus





PP-plot: pyrococcus





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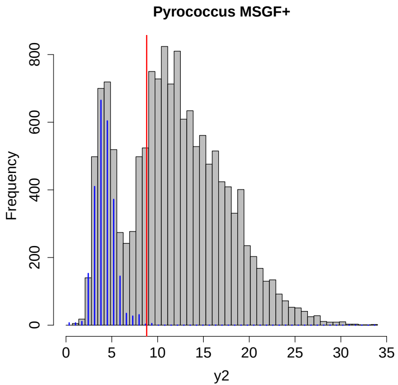
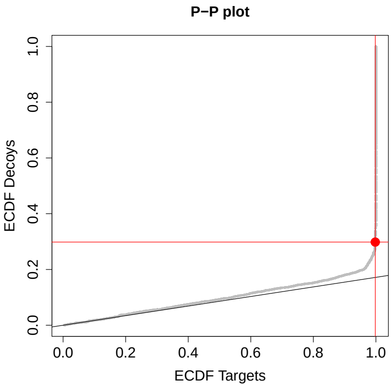
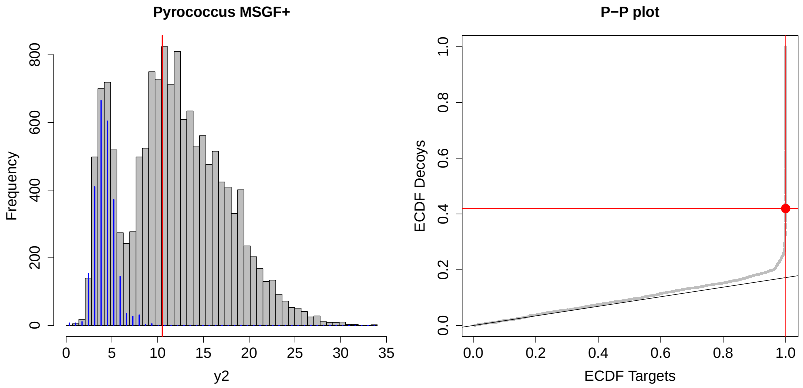
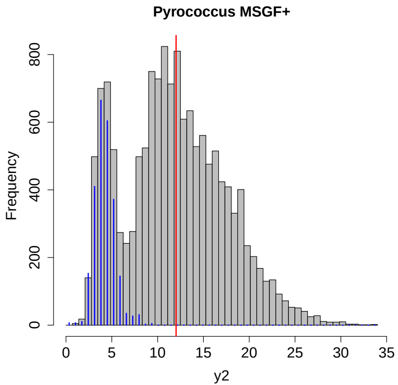


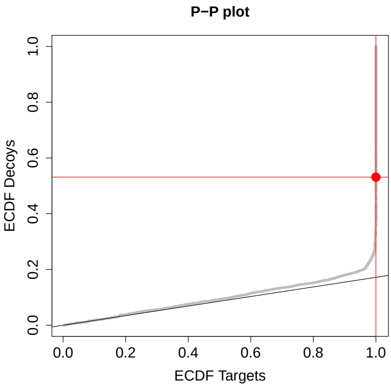


PP-plot: pyrococcus

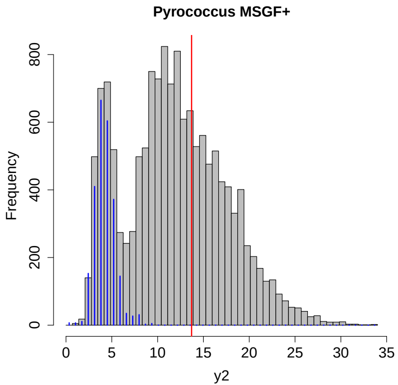


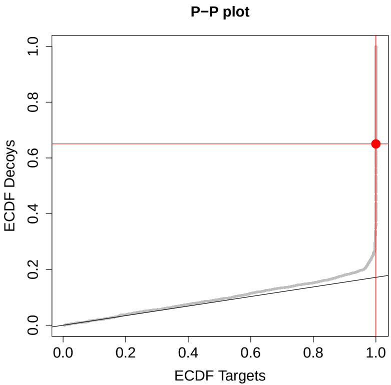
PP-plot: pyrococcus



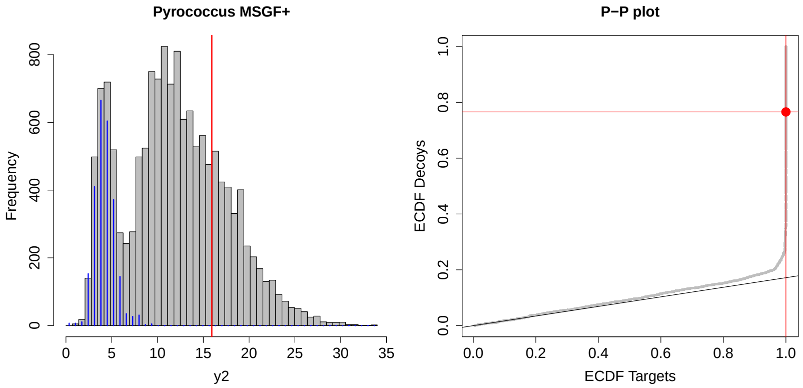


PP-plot: pyrococcus



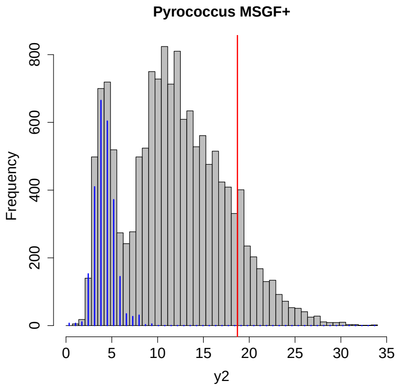


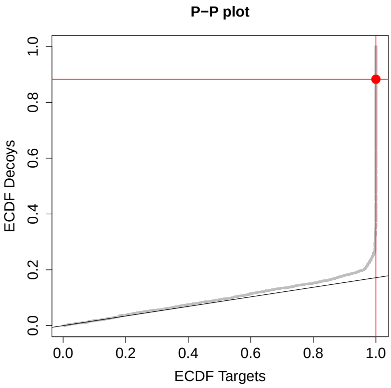
PP-plot: pyrococcus

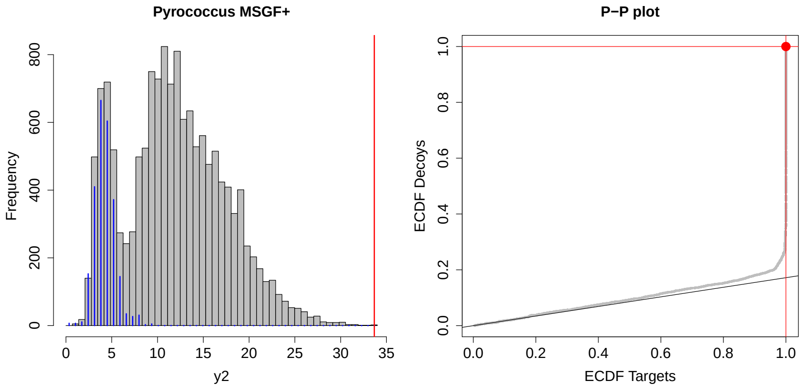


lieven.clement@ugent.be

PP-plot: pyrococcus





PP-plot: pyrococcus