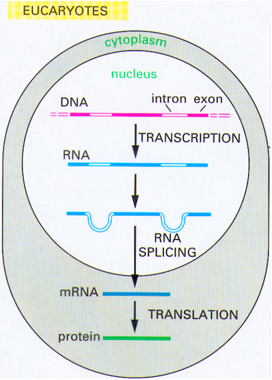
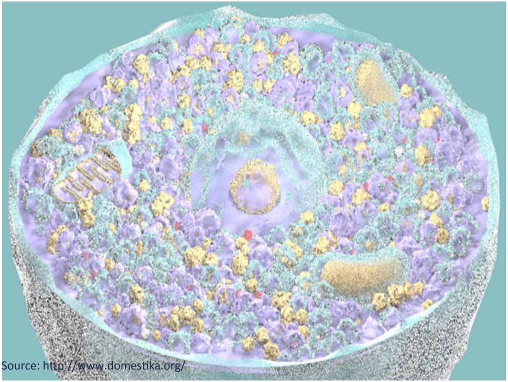
Statistical Methods for Quantitative MS-Based Proteomics: 1. Identification & False discovery rate

Lieven Clement

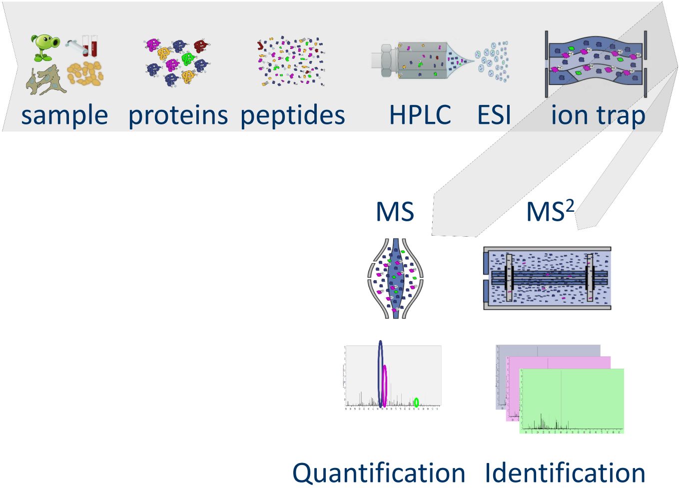
Proteomics Data Analysis Shortcourse



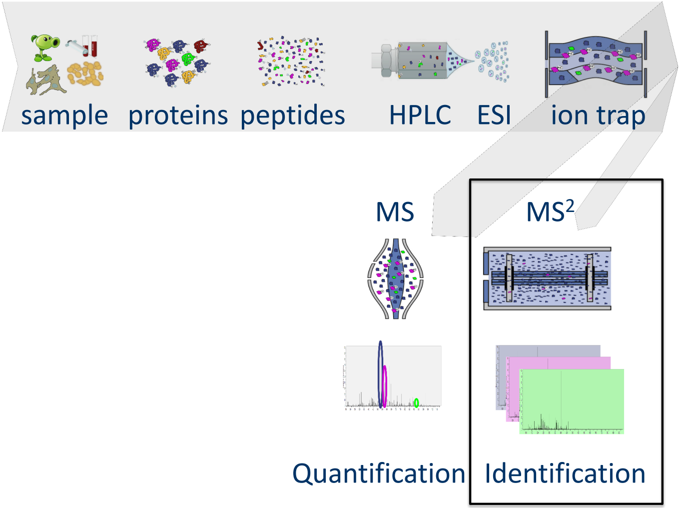


Source: http://www.domestika.org/

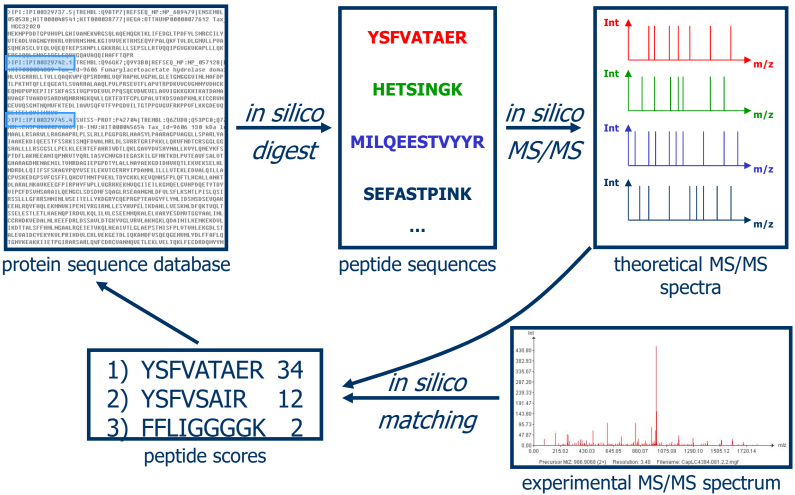
Challenges in Label Free MS-based Quantitative Proteomics



Challenges in Label Free MS-based Quantitative Proteomics



Identification



(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	
Unobservable	Bad hit	TN	FP	m_0
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Observable	Total	NR	R	m

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

Table of Outcomes

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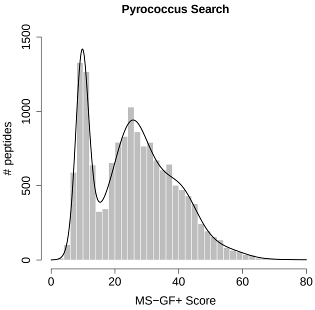
$FDR = E\left[\frac{FP}{FP+TP}\right]$. (FDR: false discovery rate)

Search engines return score that discriminates good from bad matches



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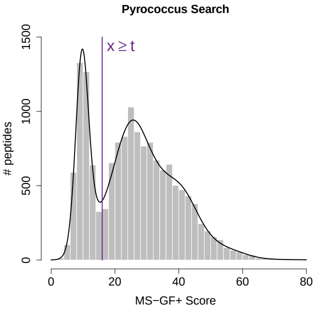

Score threshold t?

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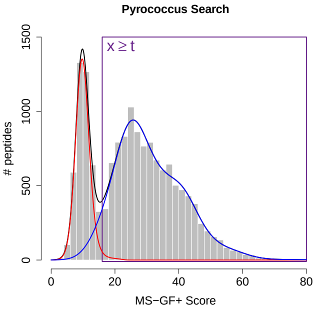

Score threshold t?

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

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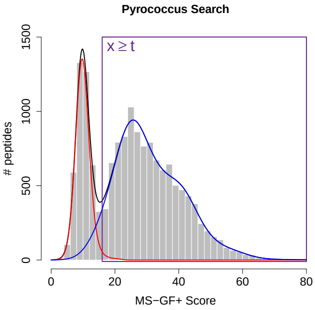

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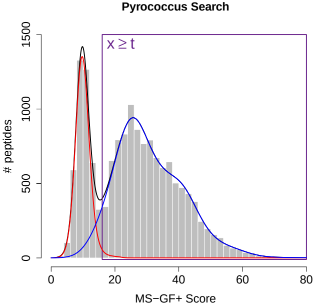

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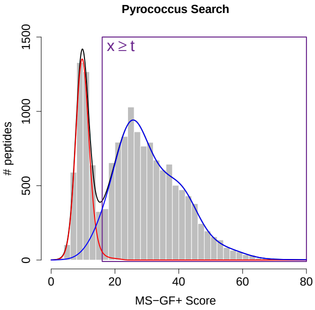

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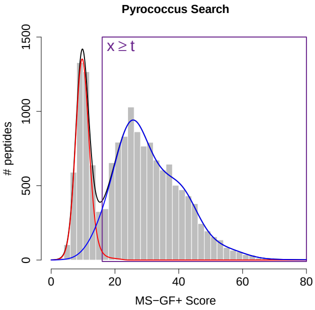

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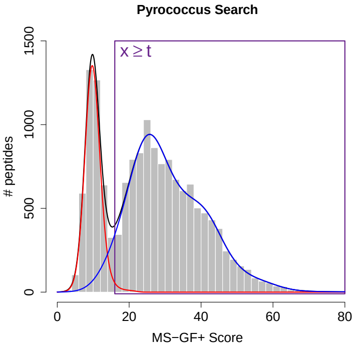
$$P_.[x \geq t] = \int_t^\infty f_.(x)dx$$

How to estimate FDR?



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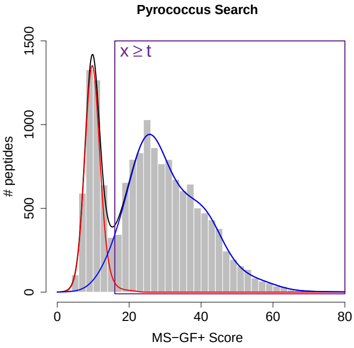

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$$\hat{P}[x \geq t] = \frac{\#x \geq t}{m} \Rightarrow \widehat{\mathrm{FDR}}(t) = \frac{\pi_0 P_0[x \geq t]}{\frac{\#x \geq t}{m}}$$

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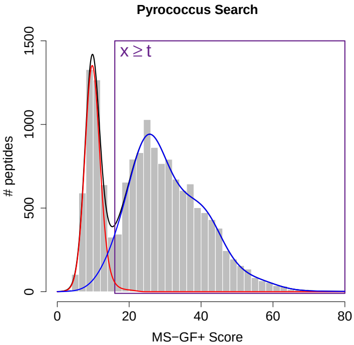

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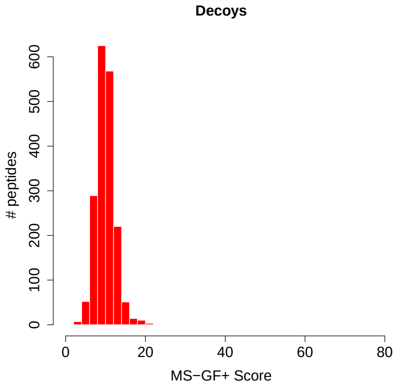
How to characterize $f_0(t)$ and π_0 in proteomics?

Target-Decoy approach to establish null distribution



- Search against decoy database to generate representative bad hits
- Reversed databases are popular

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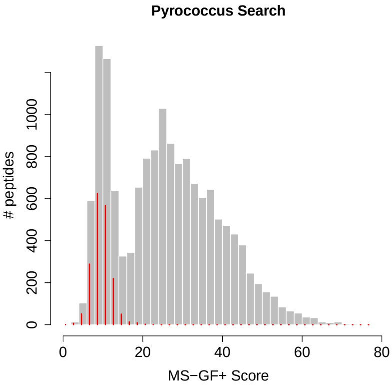

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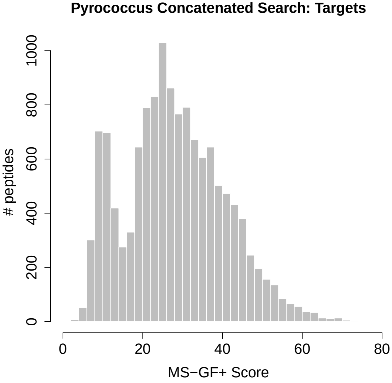

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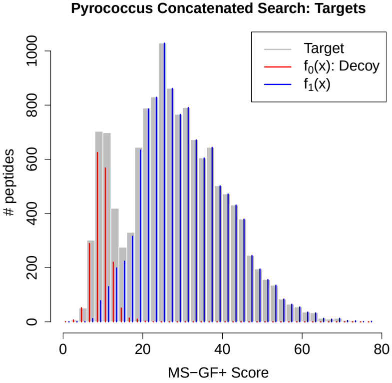

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- Assumption: bad hits has equal probability to map on target and decoy

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

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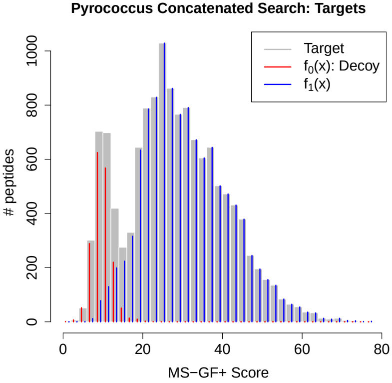

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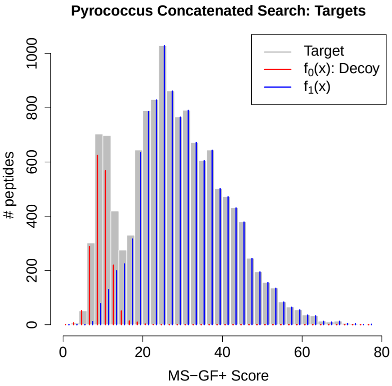

- Competitive Target - decoy:

$$\widehat{\text{FDR}}(x) = \frac{\#decoys|X \geq x}{\#targets|X \geq x}$$

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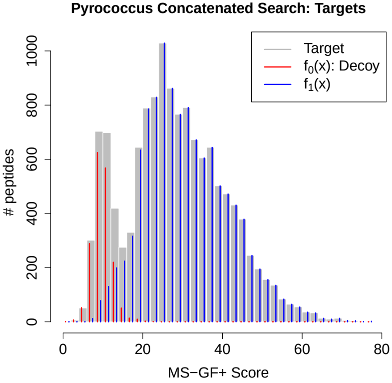

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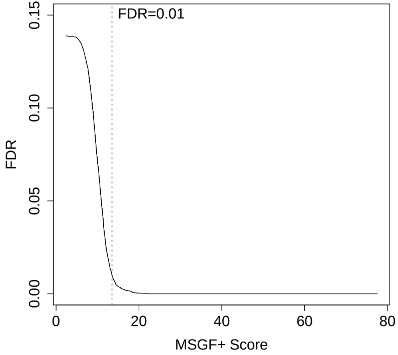

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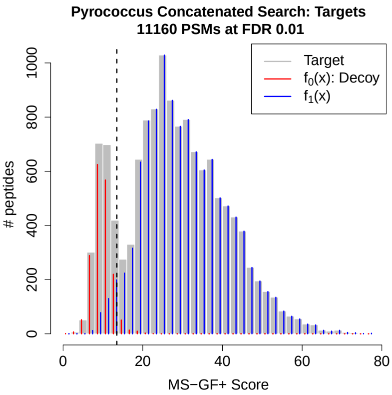

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Assess TDA assumptions

We have to evaluate that

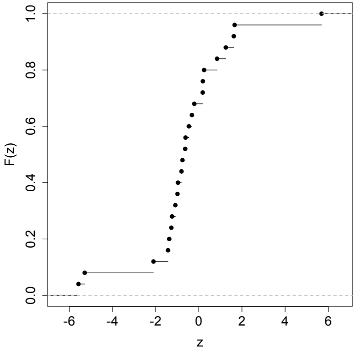
- The decoys are good simulations of the bad target hits: compare distributions $F_0(x)$ with $F(x)$

$$F_0(x) = \int_{-\infty}^{t} f_0(x)dx \quad \leftrightarrow \quad F(x) = \int_{-\infty}^{t} f(x)dx$$

- $\hat{\pi}_0 = \frac{\#decoys}{\#targets}$ is a good estimator for π_0.

- We will use Probability-Probability-plots (PP-plot) for this purpose.

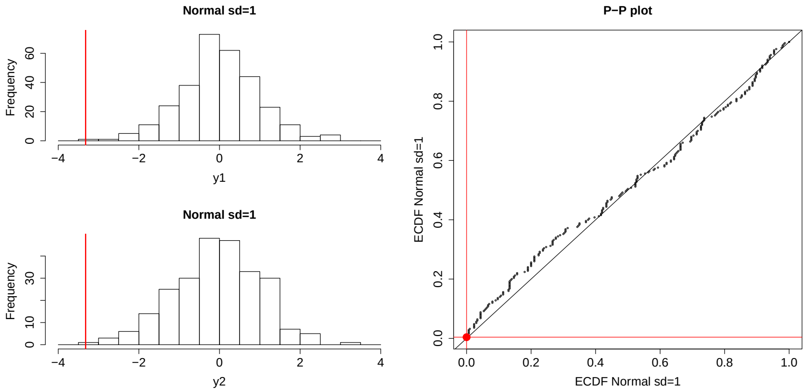
- To make PP-plots we need estimates for $F_0(x)$ and $F(x)$.
- The empirical cumulative distribution (ECDF) is used for that purpose



$$\hat{F}_0(x) = \frac{\#decoys|X \leq x}{\#decoys}, \quad \hat{F}(x) = \frac{\#targets|X \leq x}{\#targets}$$

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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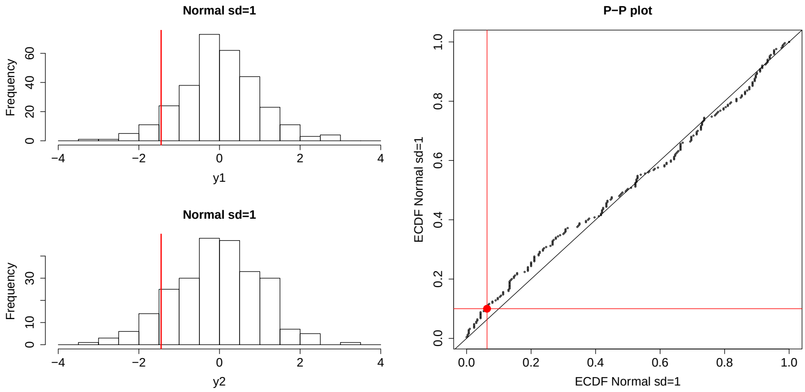

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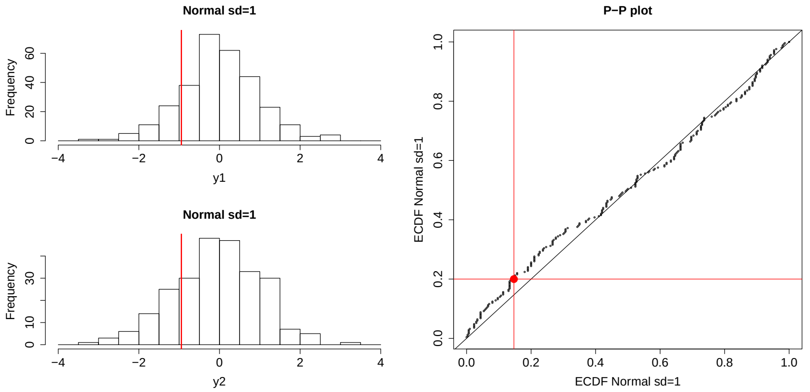

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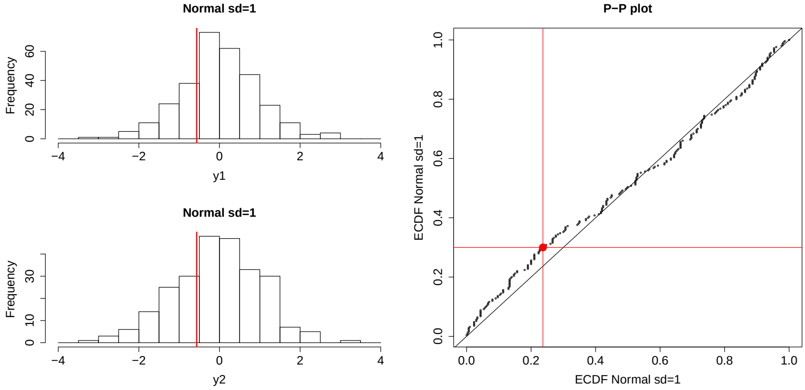

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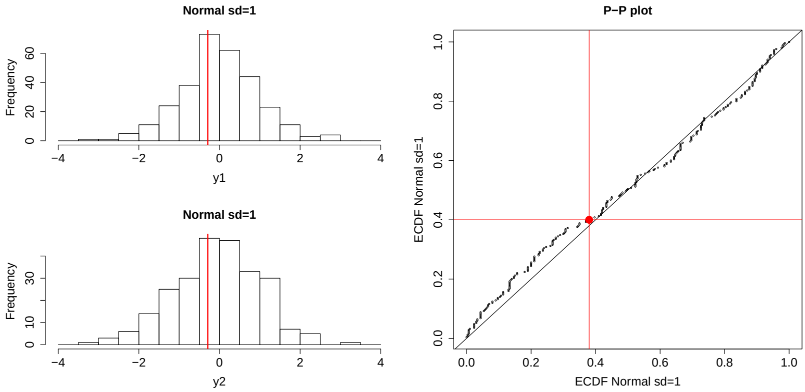

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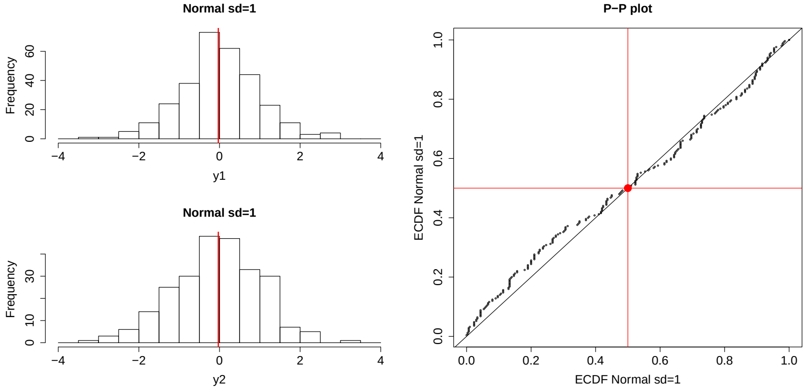

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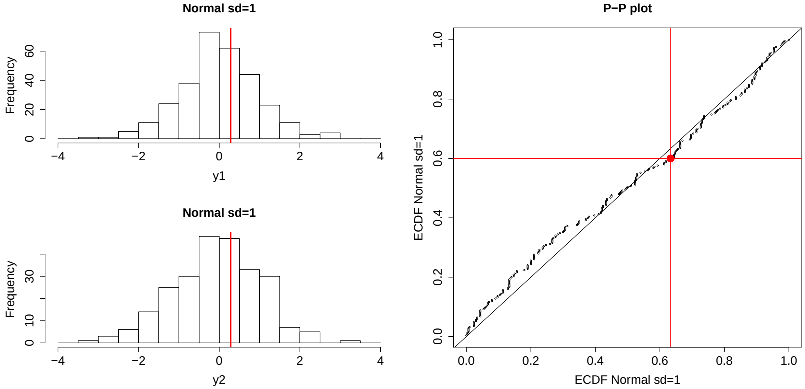

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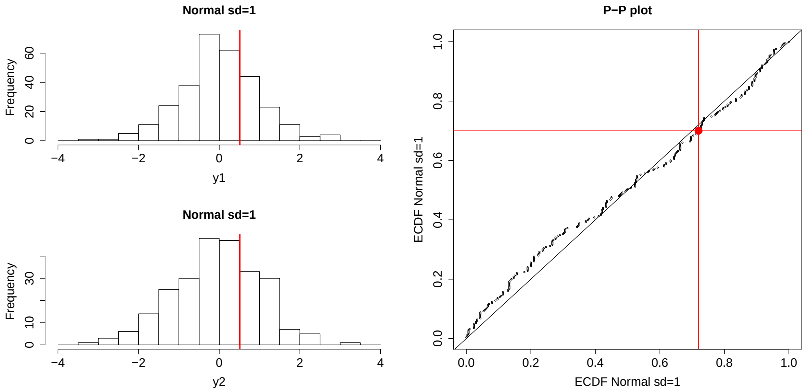

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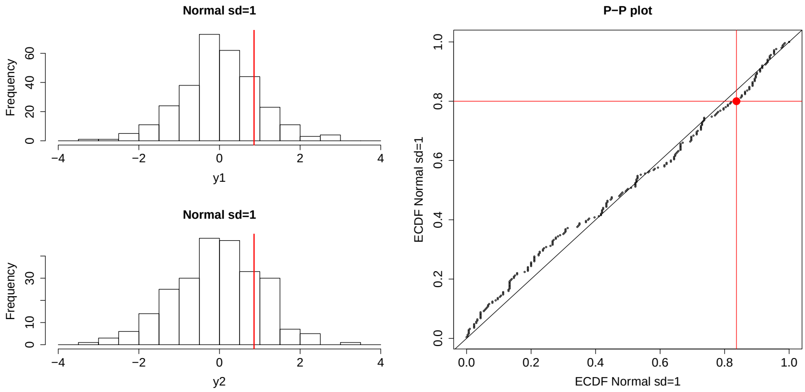

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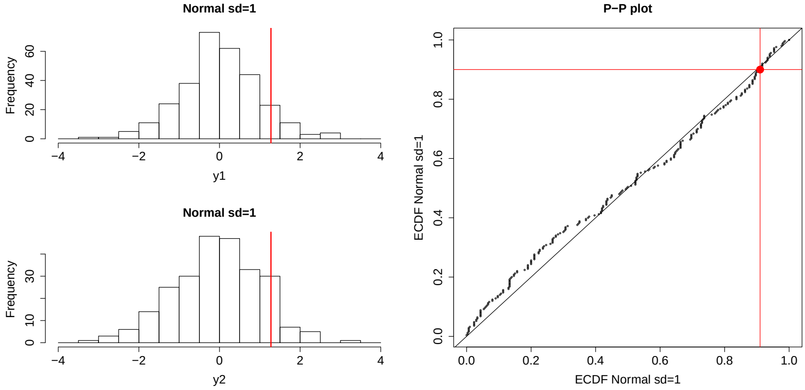

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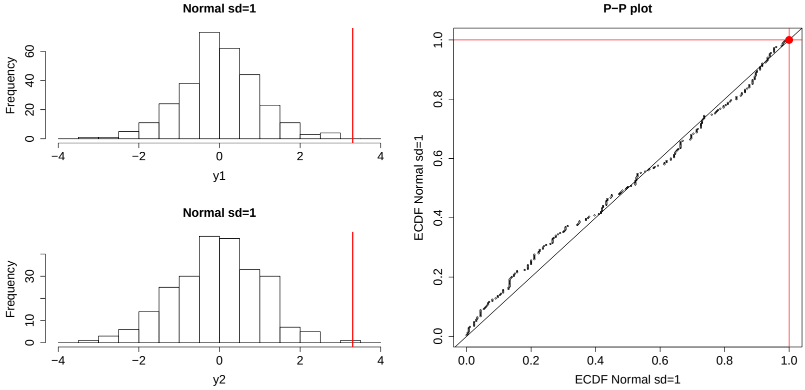

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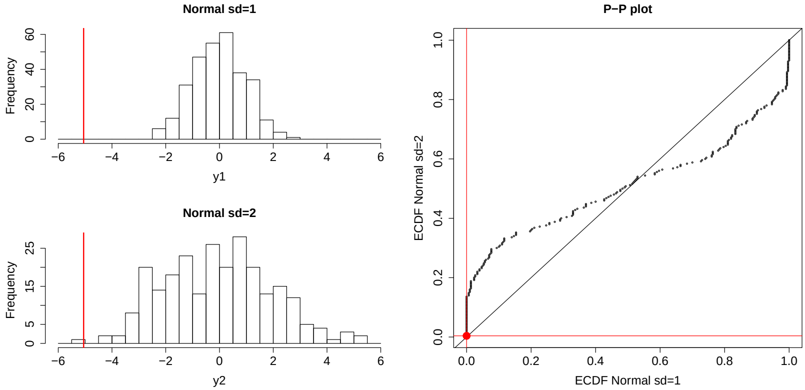

Normal sd=1
Frequency
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y1
Normal sd=2
Frequency
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y2
P−P plot
ECDF Normal sd=2
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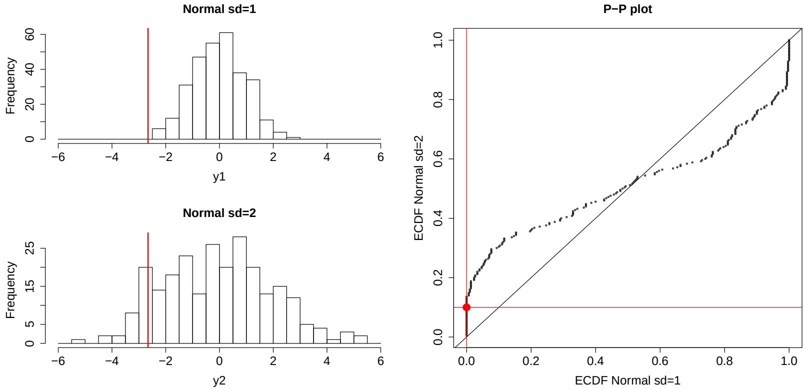
ECDF Normal sd=1

PP-plot



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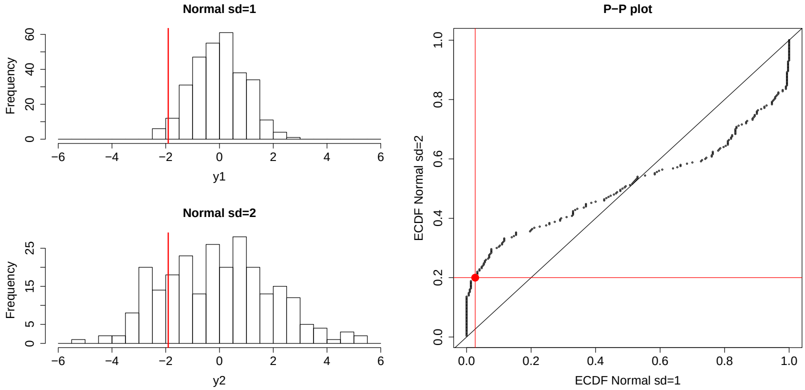
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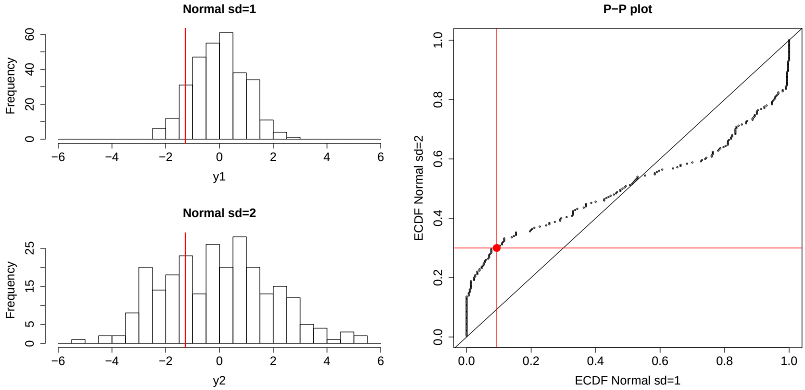
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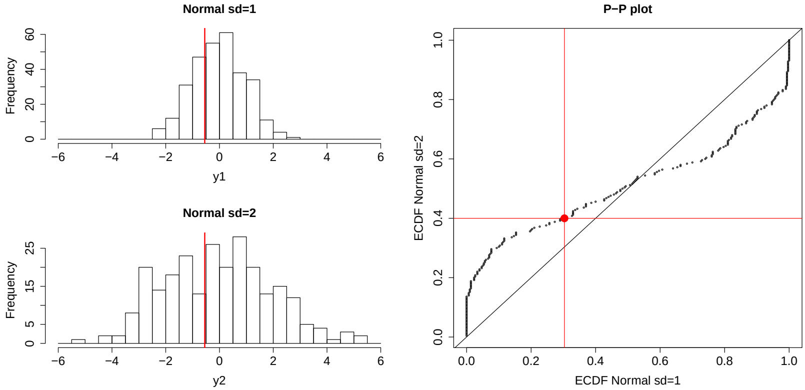
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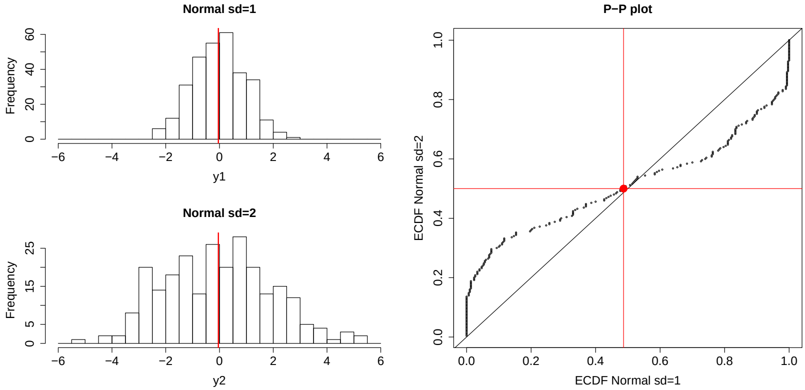

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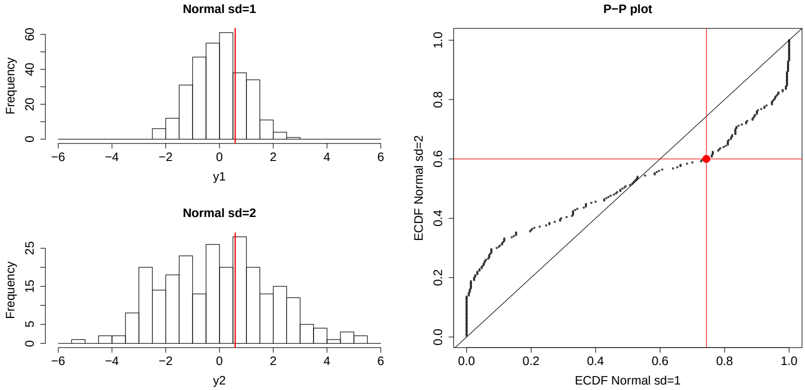
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1
0.0
0.2
0.4
0.6
0.8
1.0

PP-plot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Frequency
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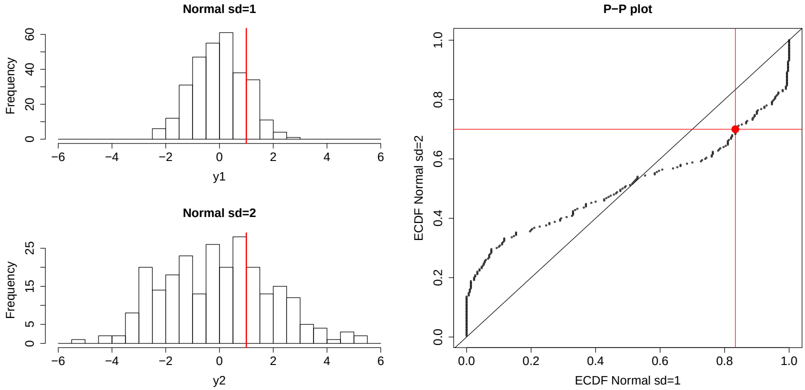
60
−6
−4
−2
0
2
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1
0.0
0.2
0.4
0.6
0.8
1.0

PP-plot



Normal sd=1
Frequency
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20
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60
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−2
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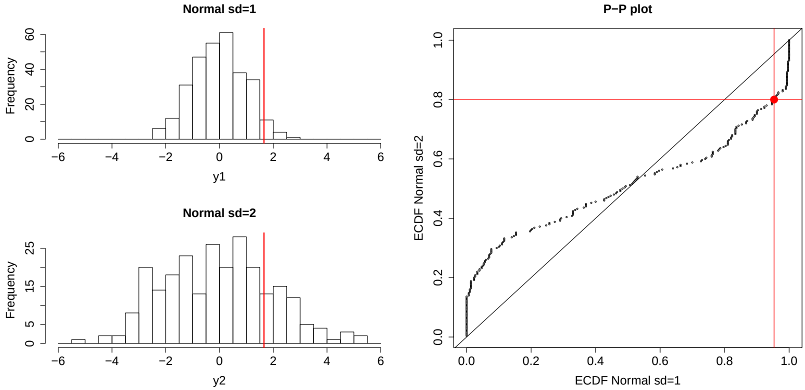
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
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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
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0.2
0.4
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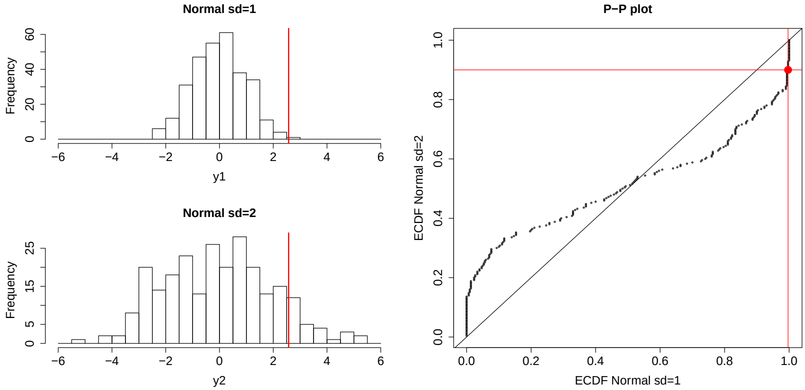
0.8
1.0
ECDF Normal sd=1

PP-plot


Normal sd=1
Frequency
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60
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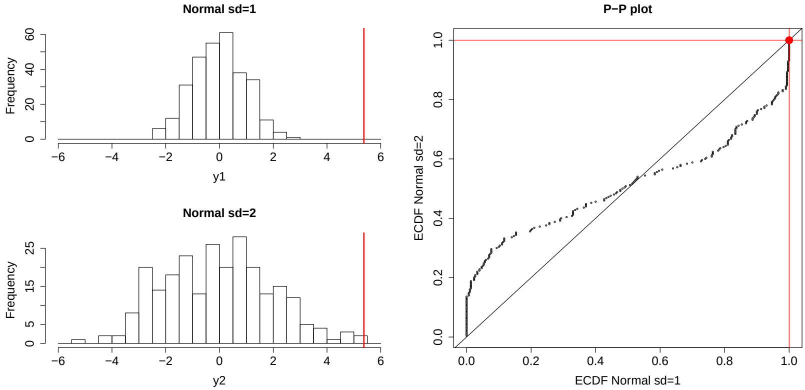
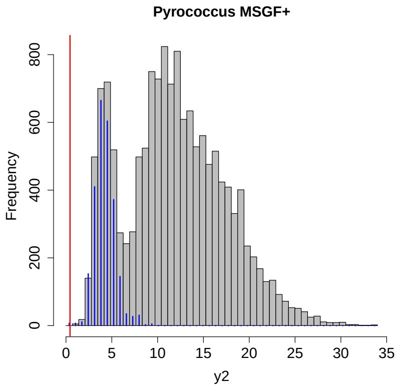
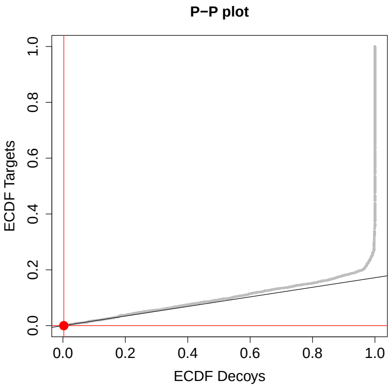
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

PP-plot

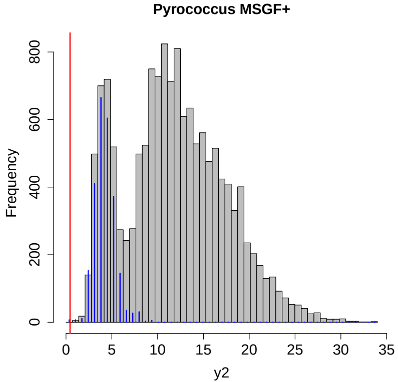

Normal sd=1
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

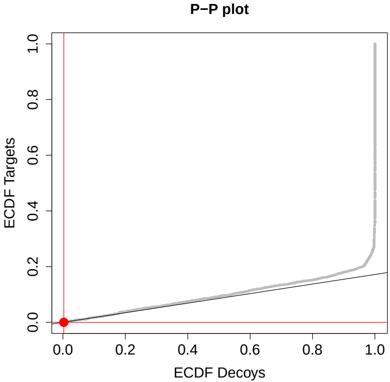
PP-plot: pyrococcus





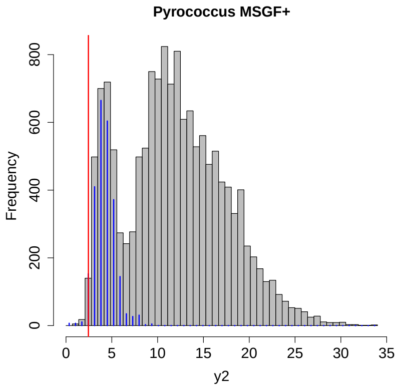
PP-plot: pyrococcus

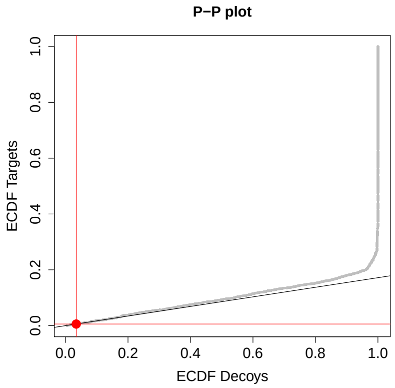




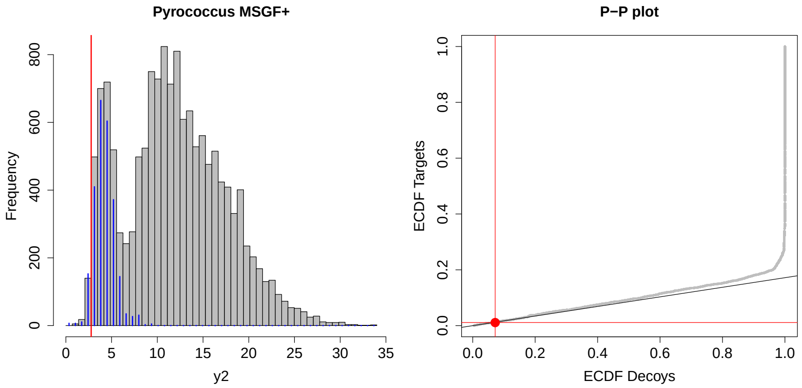
What about $\hat{\pi}_0$?

PP-plot: pyrococcus





PP-plot: pyrococcus



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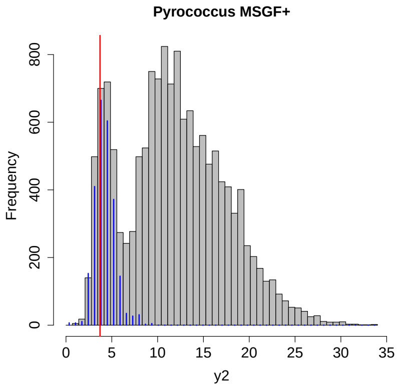
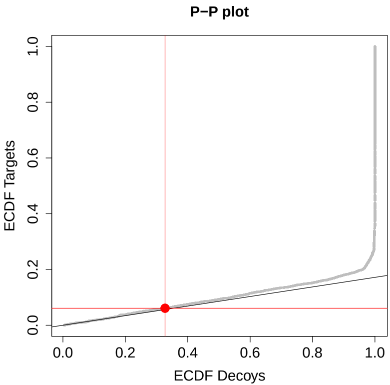
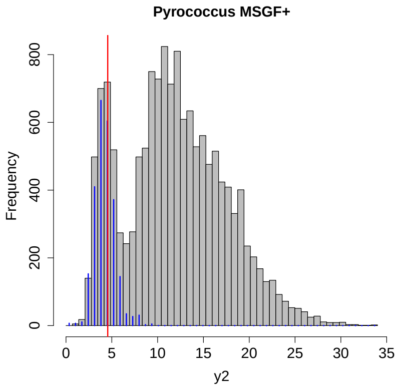
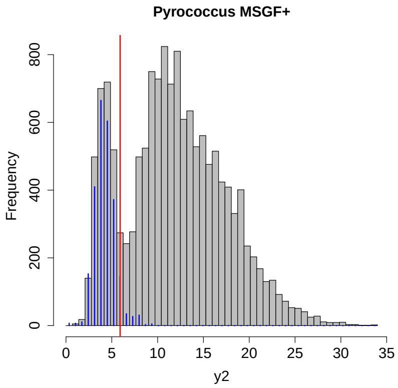




PP-plot: pyrococcus



PP-plot: pyrococcus



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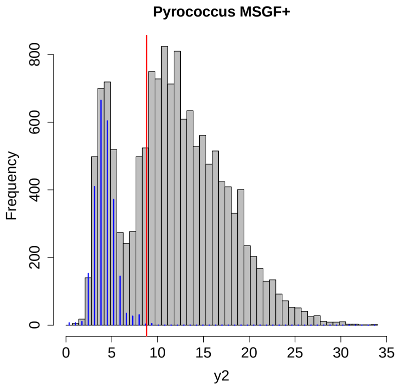

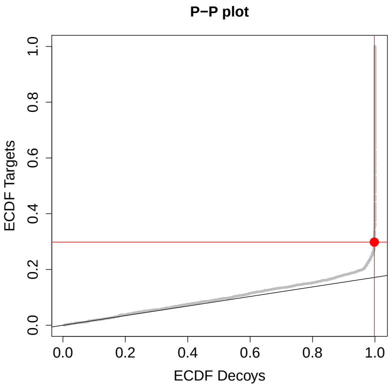

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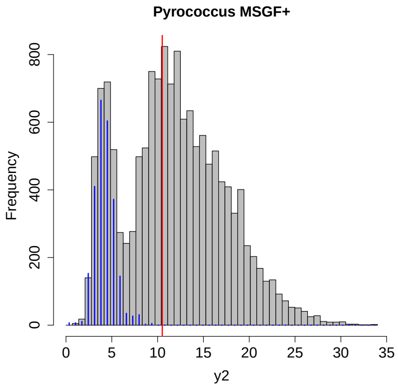

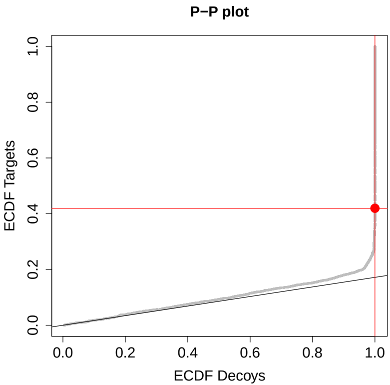

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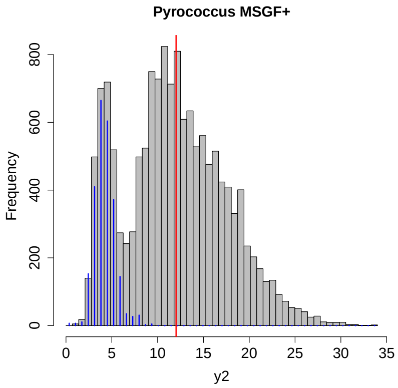
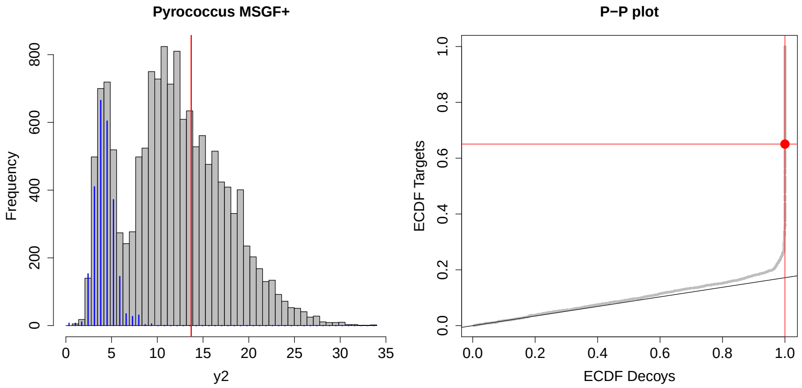


PP-plot: pyrococcus



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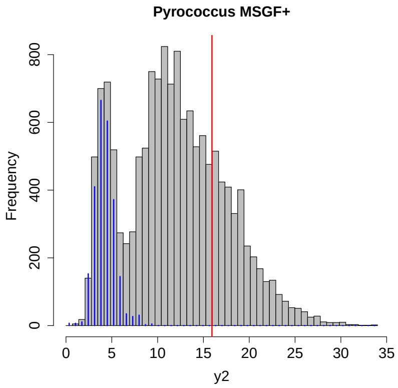

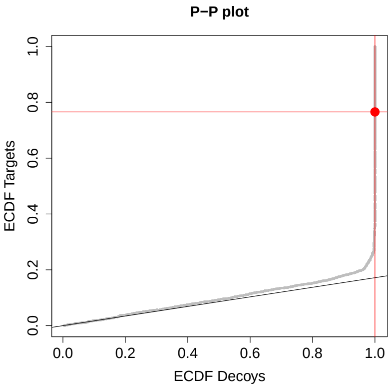

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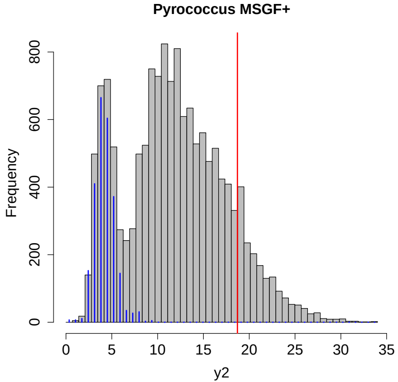

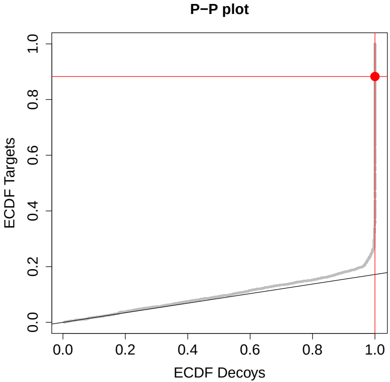

PP-plot: pyrococcus