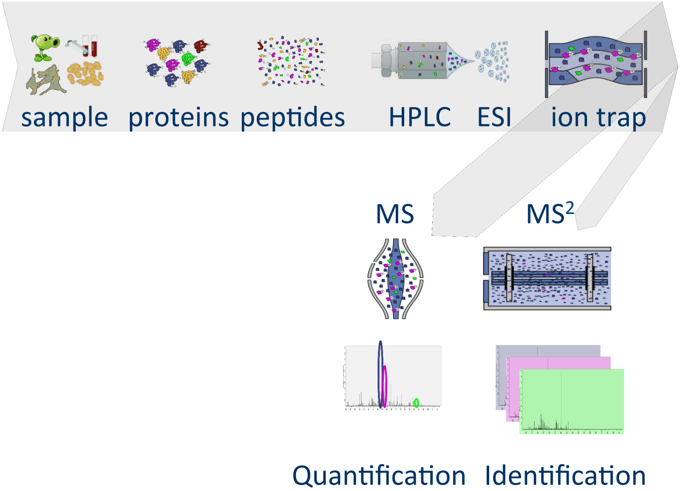
Statistical Methods for Quantitative MS-Based Proteomics:
1. Identification & False discovery rate

Lieven Clement

Statistics and Genomics Seminar, UCBerkeley, California

Challenges in Label Free MS-based Quantitative Proteomics



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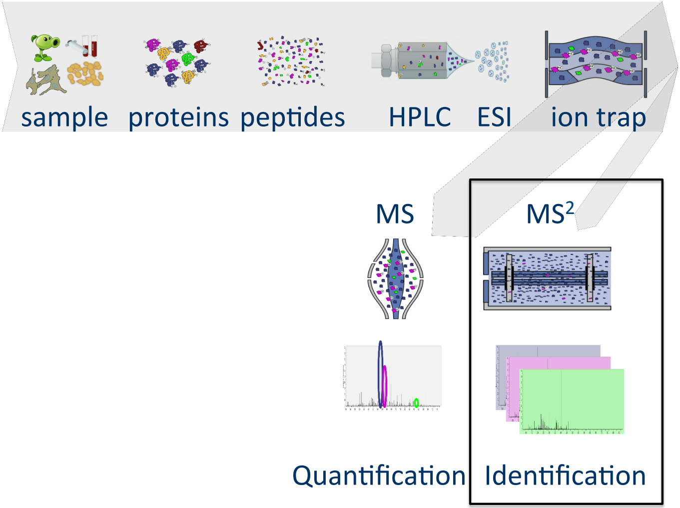
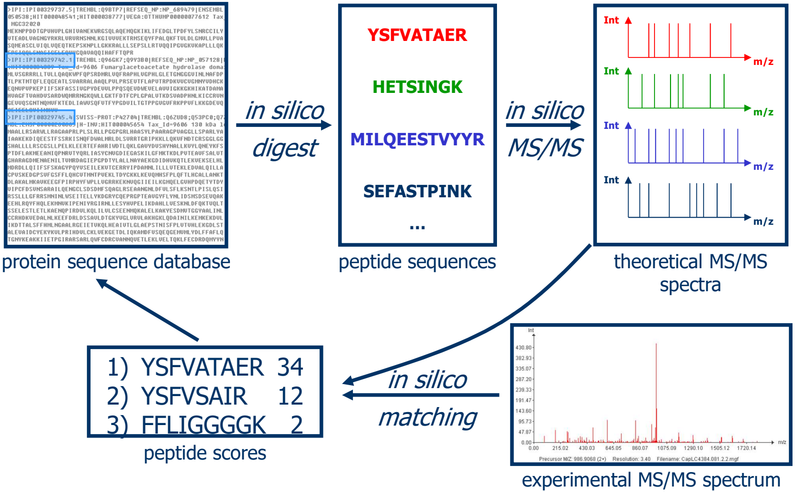


Identification



(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	
Bad hit	TN	FP	m_0
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Random Variables

Table of Outcomes

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

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

Table of Outcomes

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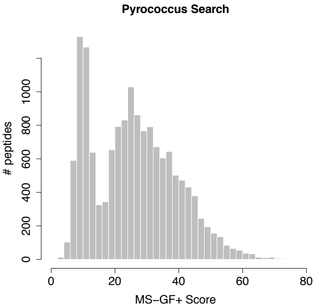
$FDR = E\left[\frac{FP}{FP+TP}\right]$. (FDR: false discovery rate)
What does it mean?

Search engines return score that discriminates good from bad matches



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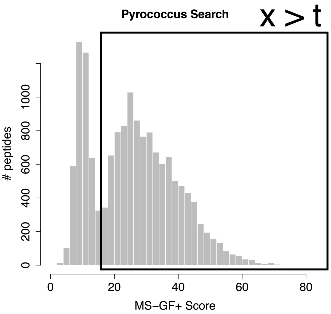

Score threshold t?

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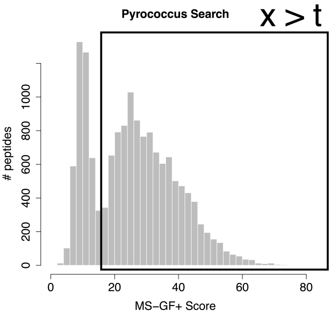

Score threshold t?

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

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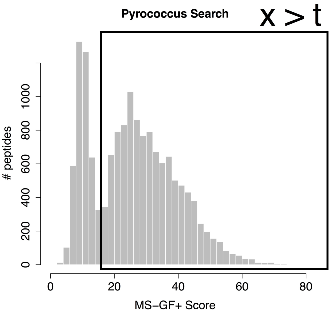

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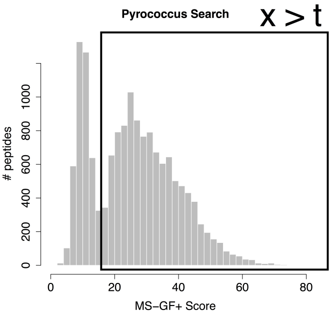

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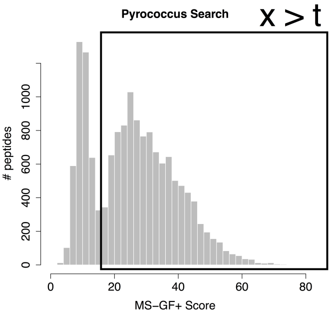

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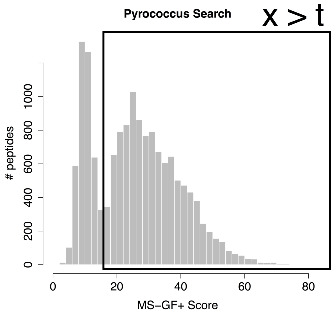

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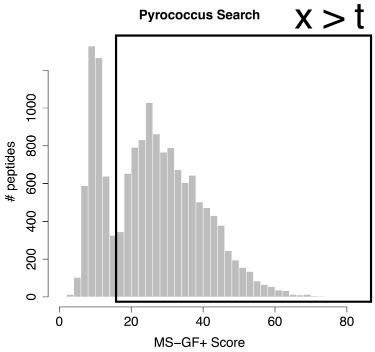
$$\text{FDR}(t) = Pr\left[FP|x>t\right]$$

$$\text{FDR}(t) = \frac{\pi_0[1-F_0(t)]}{1-F(t)} \text{ with } F.(t) = \int\limits_{-\infty}^{t} f.(x)dx$$

How to estimate FDR?



$$\mathrm{FDR}(t) = \frac{\pi_0\,[1 - F_0(t)]}{1 - F(t)} = \frac{\pi_0 Pr[x > t|FP]}{Pr[x > t]}$$

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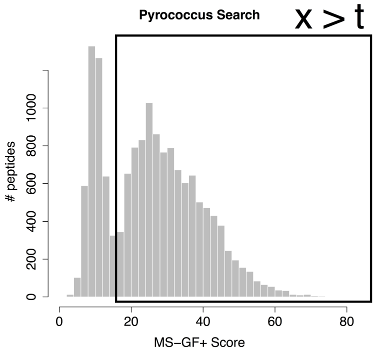

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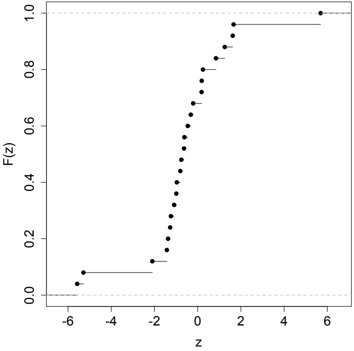
$$FDR(t) = \frac{\pi_0 \left[1 - F_0(t)\right]}{1 - \frac{\#x \leq t}{m}} = \frac{\pi_0 Pr[x > t|FP]}{\frac{\#x > t}{m}}$$

How to estimate FDR?

- $F(t)$ using the Empirical cumulative distribution function (ECDF)



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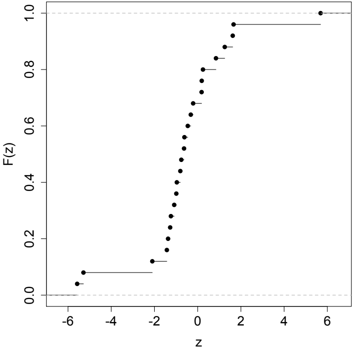
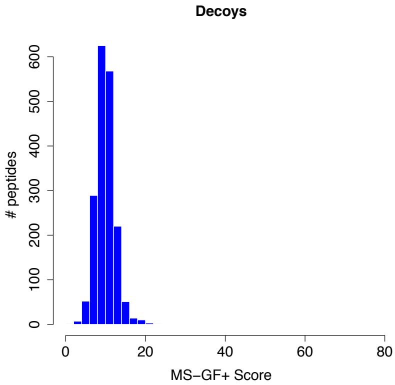


- How to characterize $F_0(t)$ and π_0 in proteomics?

Target-Decoy approach to establish null distribution



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

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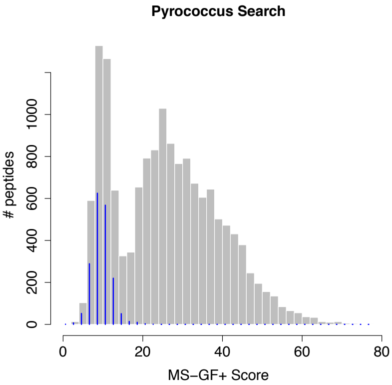

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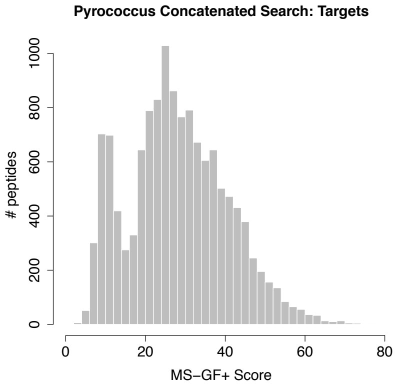

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- Assumption that bad hits have an equal probability to map on forward (target) and reverse database (decoy)

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

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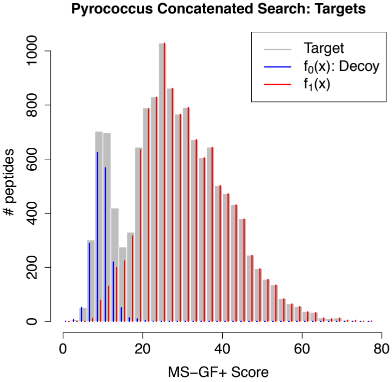

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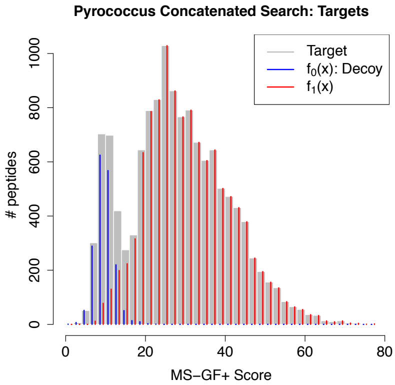

- Score cuttoff?

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

- Competitive Target - decoy:

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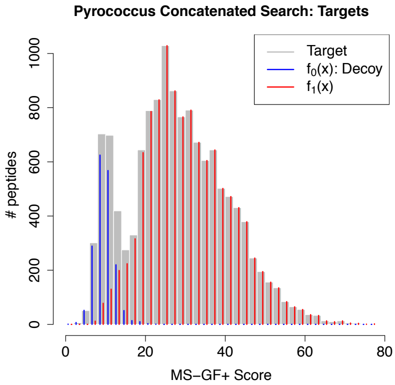

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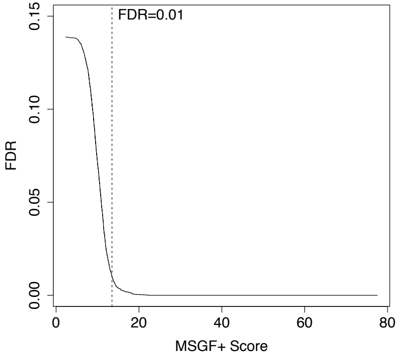

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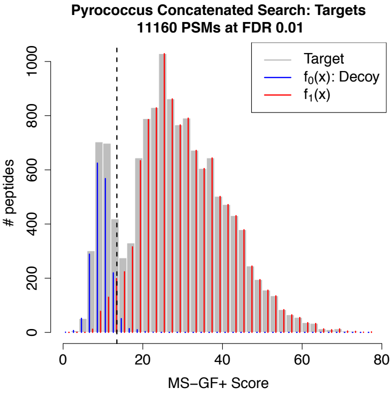

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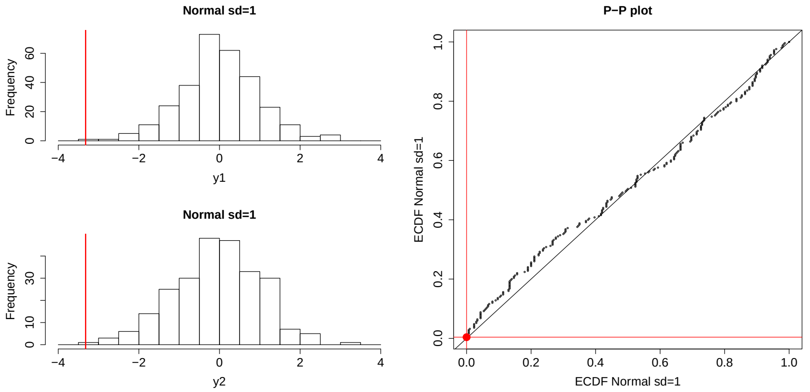
We have to evaluate that

- 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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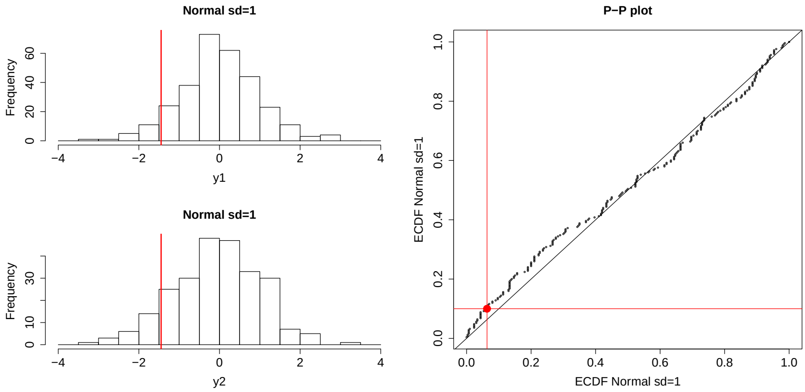

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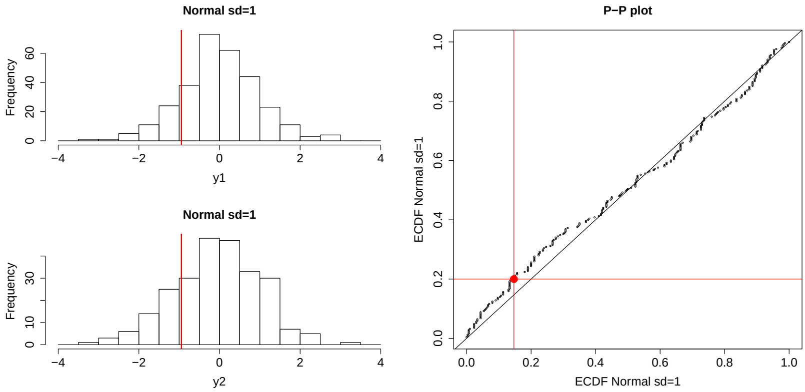

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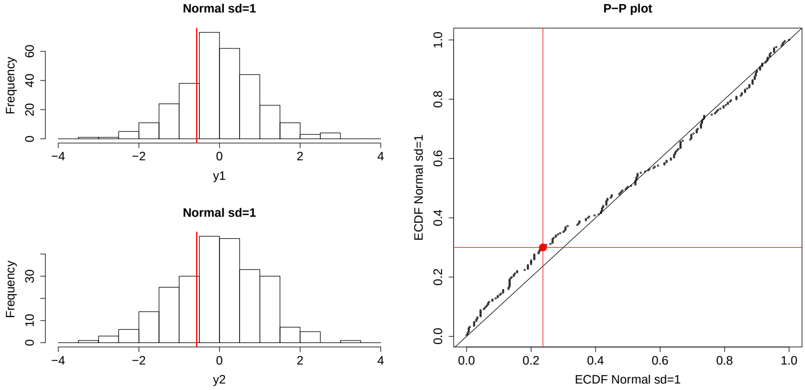

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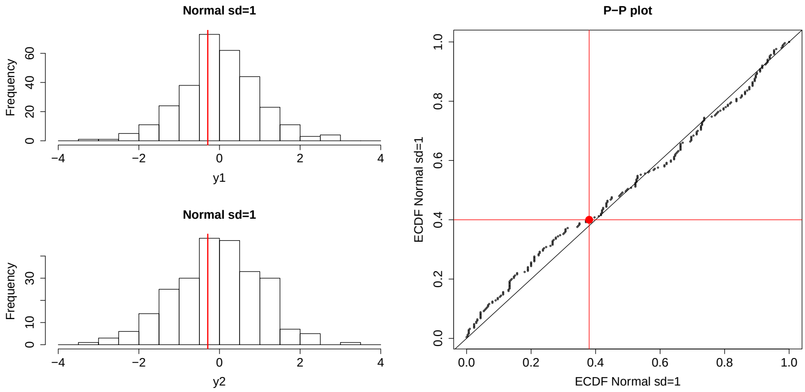

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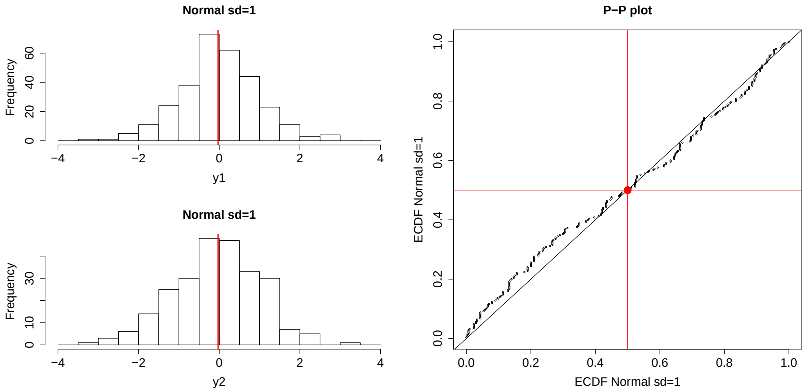

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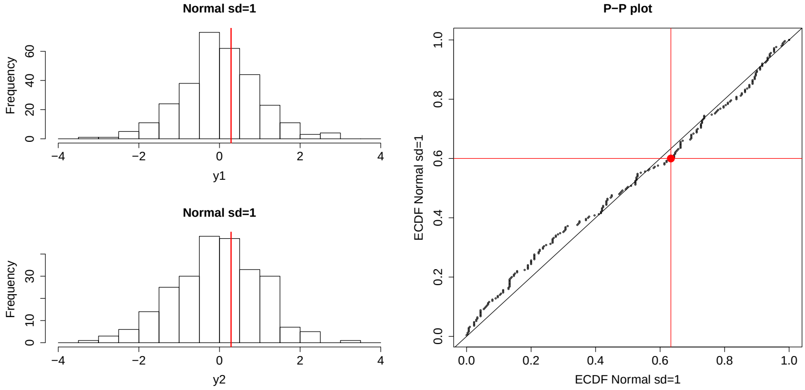

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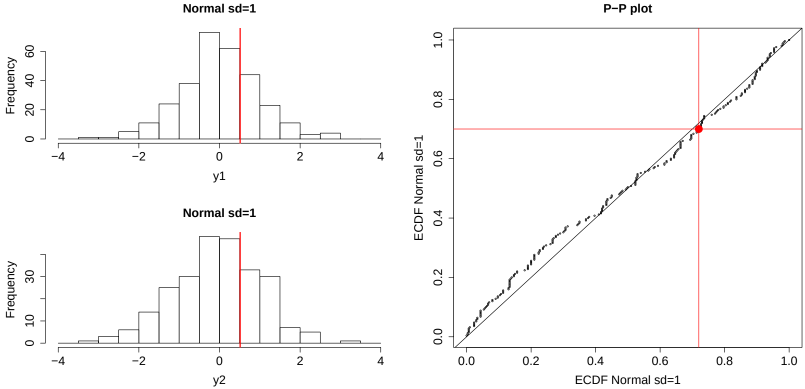

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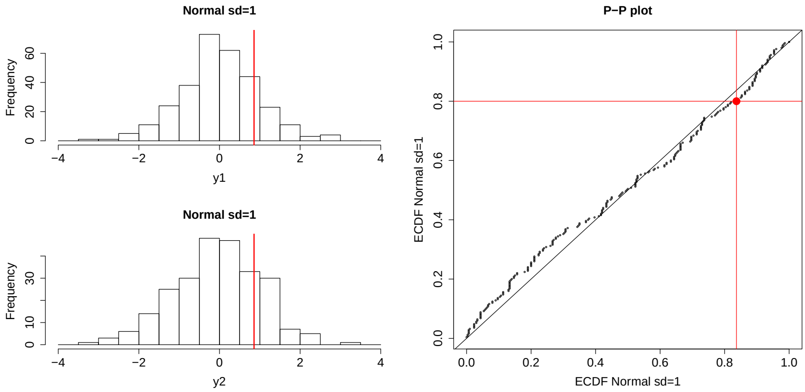

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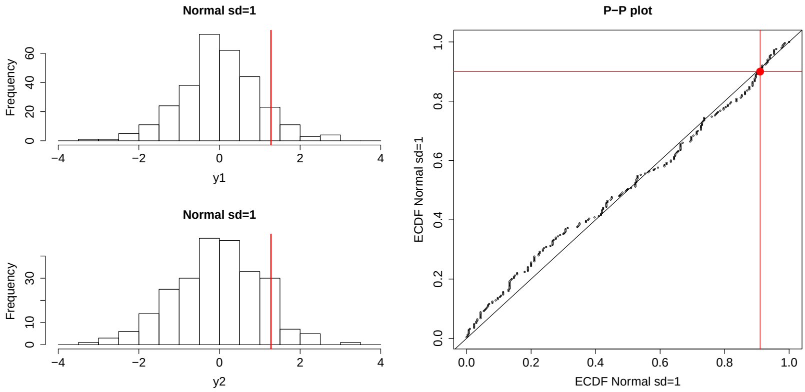

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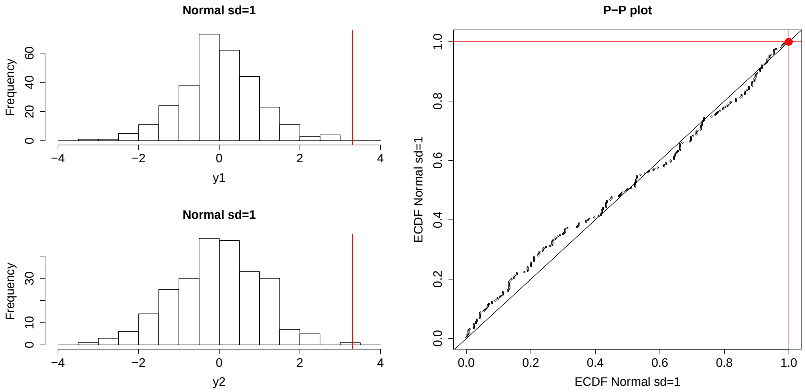

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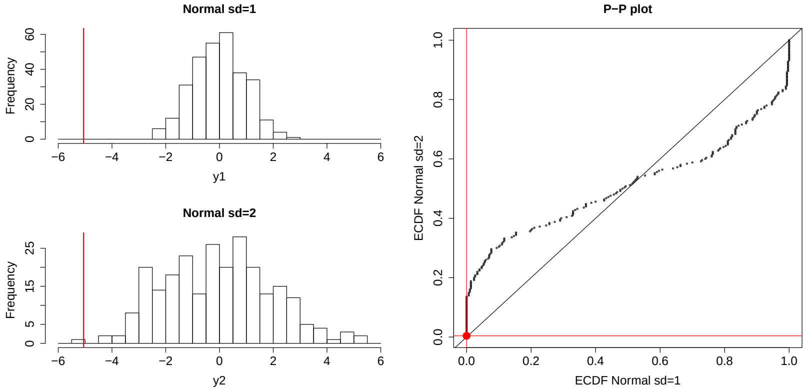

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P−P plot
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ECDF Normal sd=1

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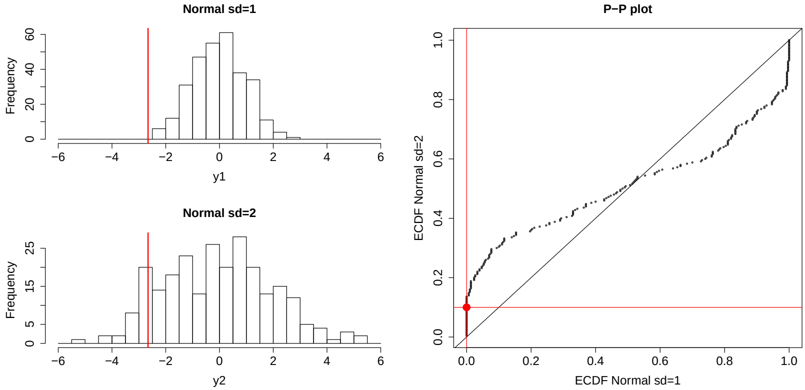
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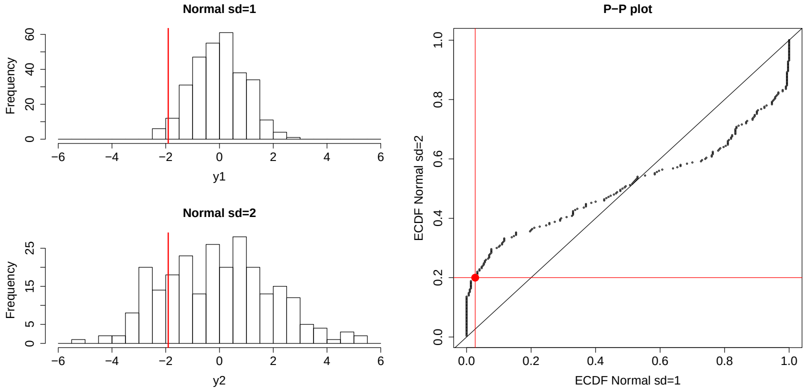
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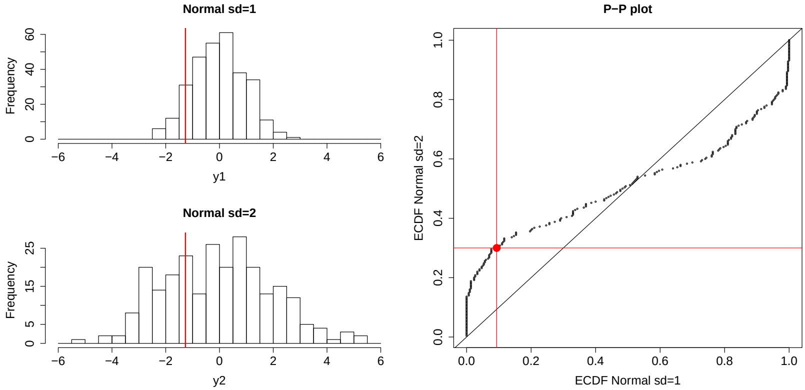
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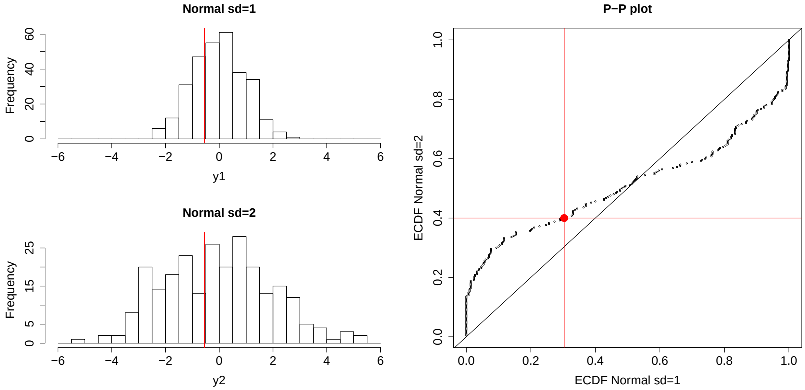

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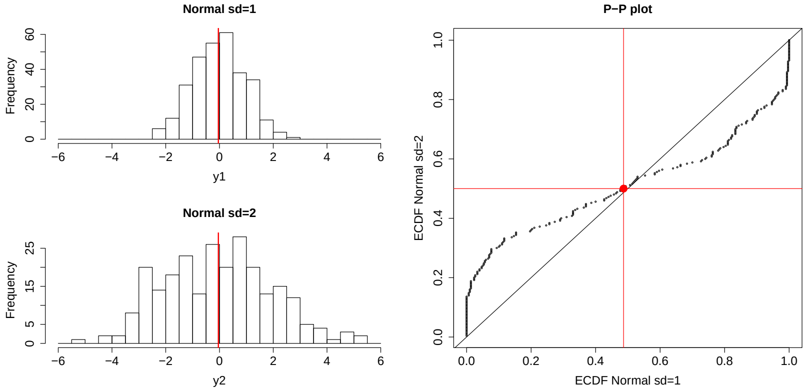
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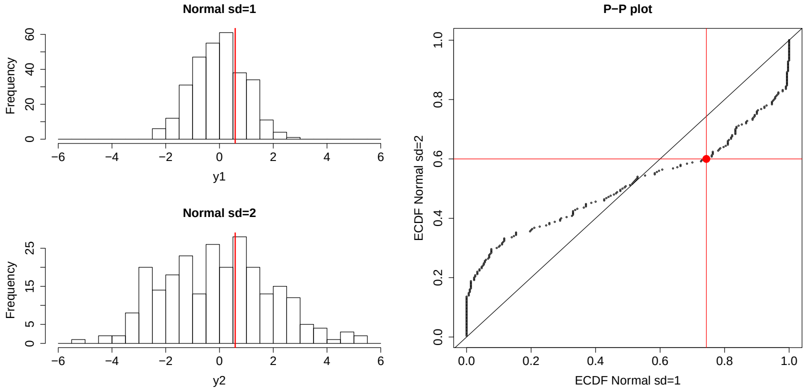
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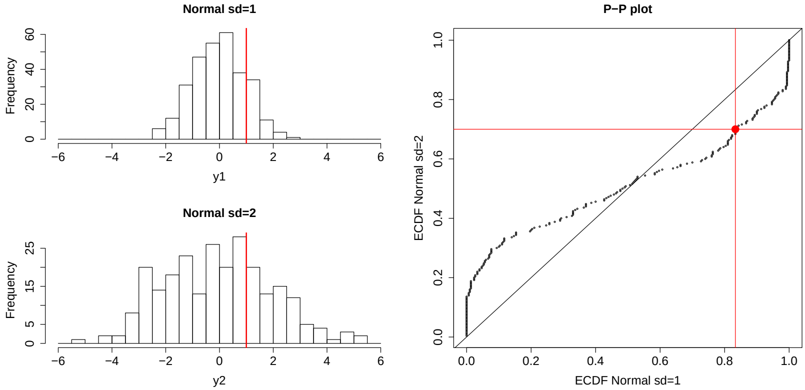
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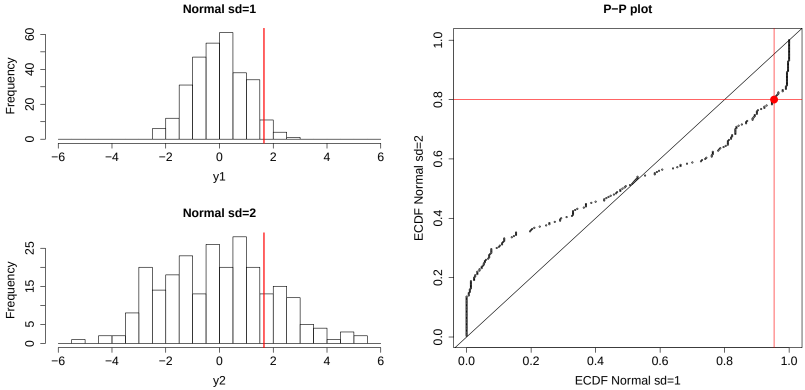
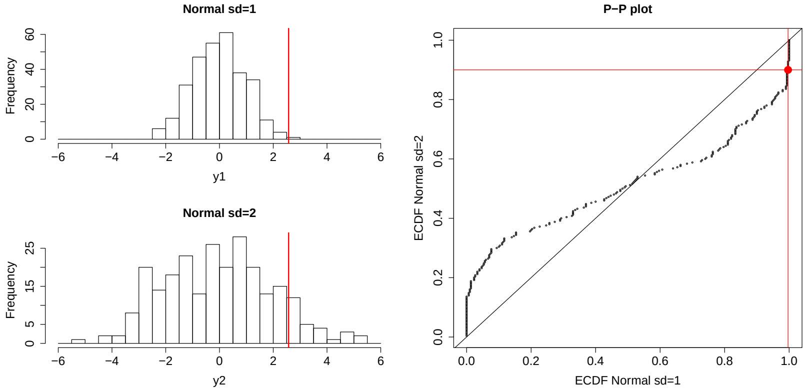
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

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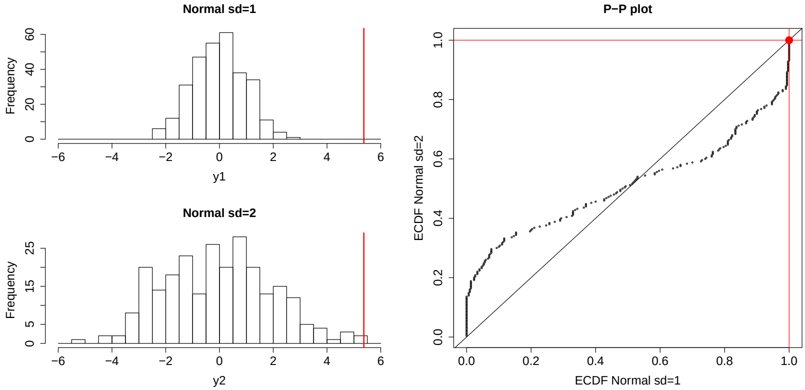
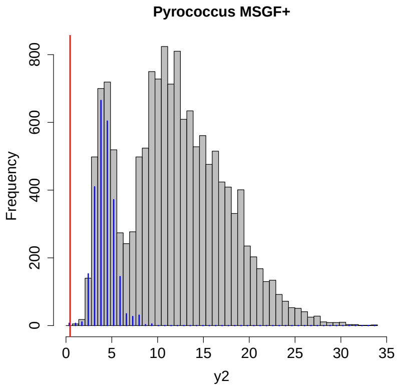
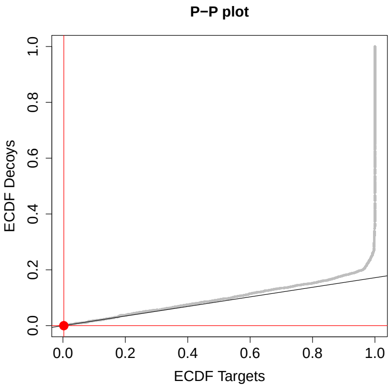
−4
−2
0
2
4
6
y1
Normal sd=2
Frequency
0
5
15
25
−6
−4
−2
0
2
4
6
y2
P−P plot
ECDF Normal sd=2
0.0
0.2
0.4
0.6
0.8
1.0
ECDF Normal sd=1

PP-plot

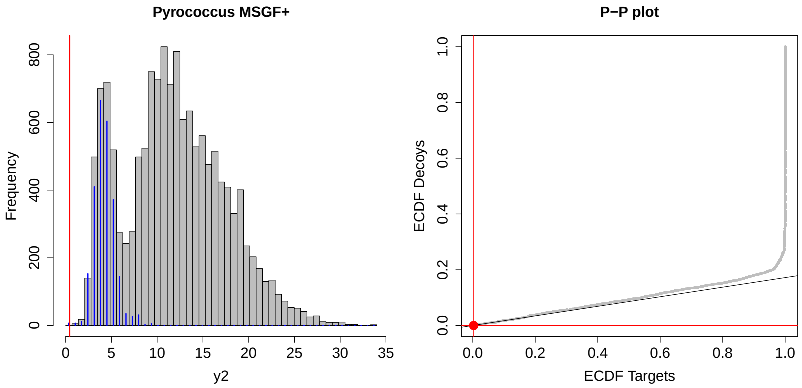

Normal sd=1
Frequency
y1
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

PP-plot: pyrococcus



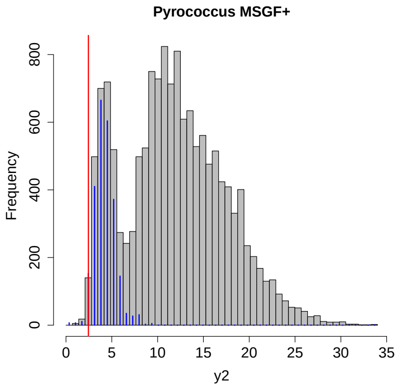


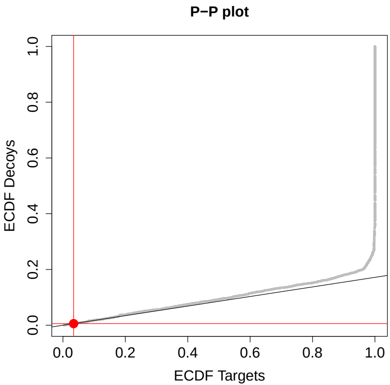
PP-plot: pyrococcus



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

PP-plot: pyrococcus





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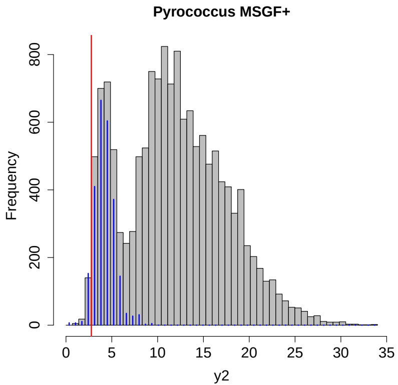

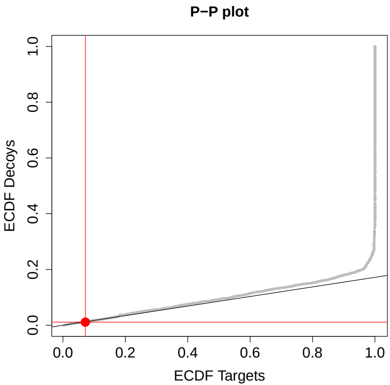

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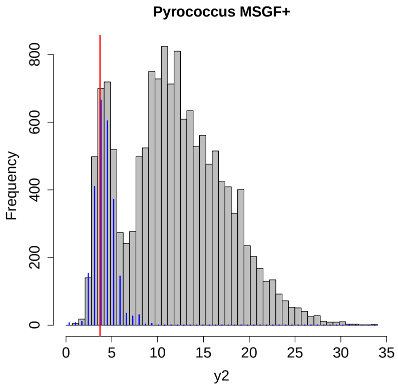

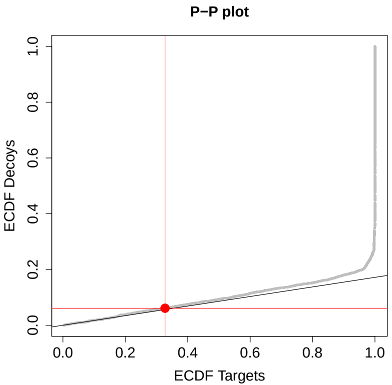

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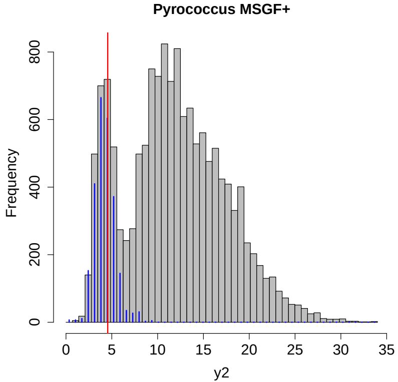

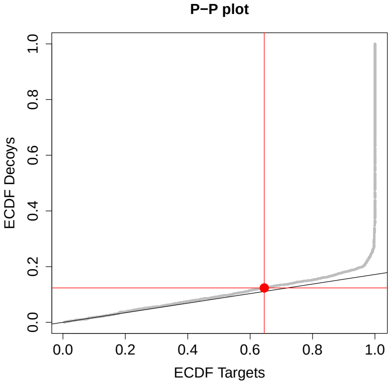

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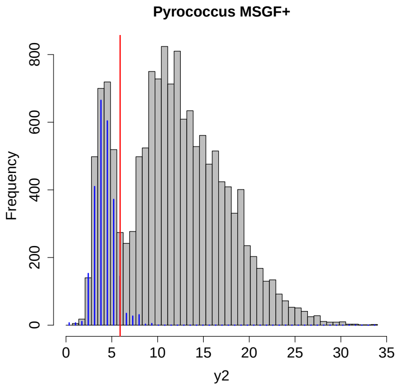

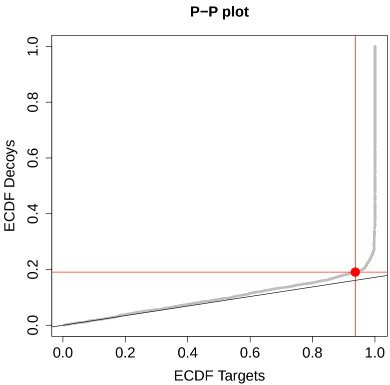

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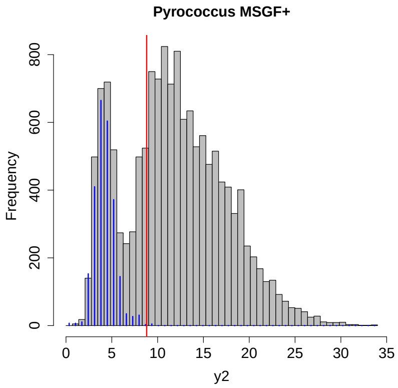

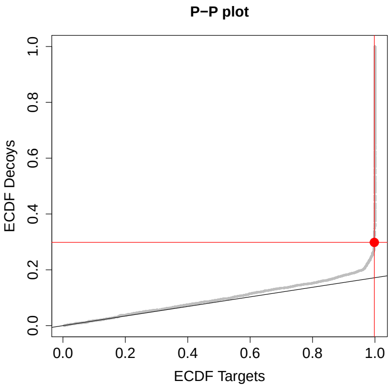

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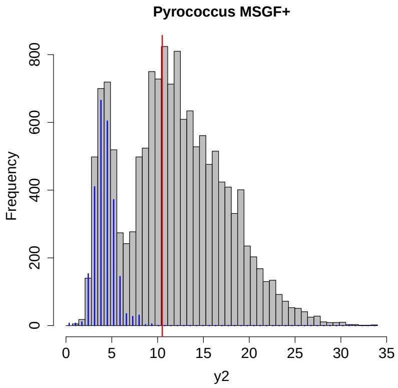

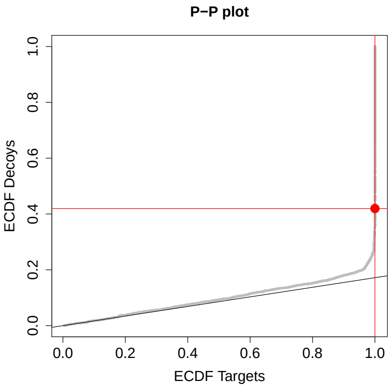

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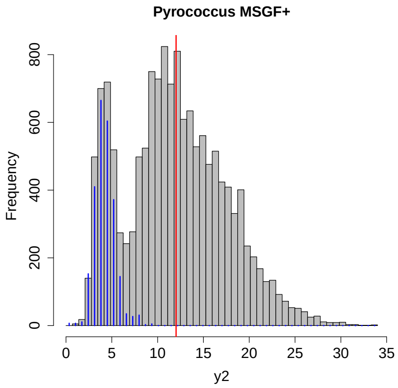
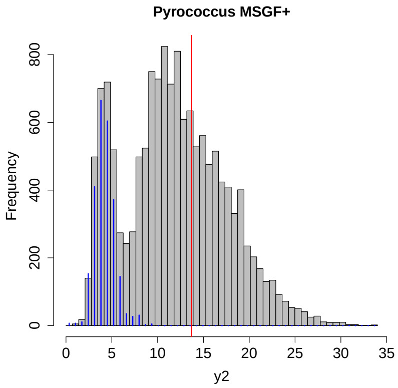


PP-plot: pyrococcus



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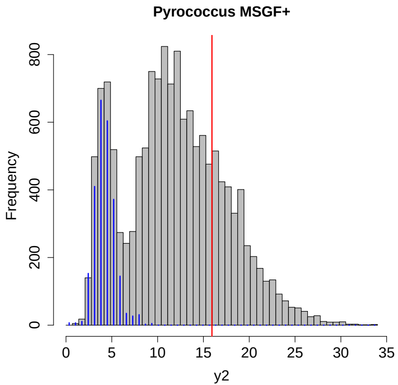

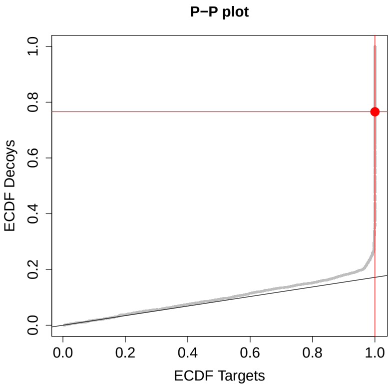

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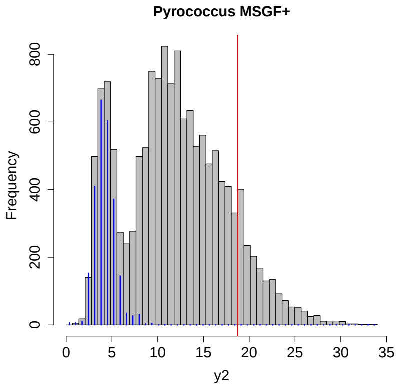

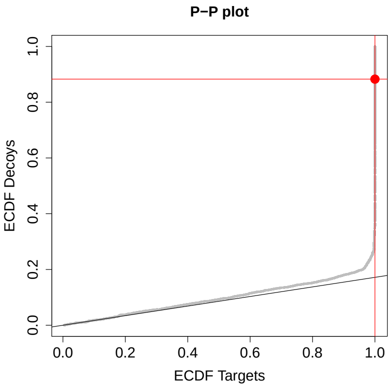

PP-plot: pyrococcus