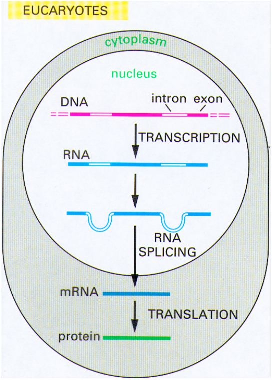
UNIVERSITEIT GENT

BIG N2N
Bioinformatics Institute Ghent
from nucleotides to networks

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

Lieven Clement

Proteomics Data Analysis Shortcourse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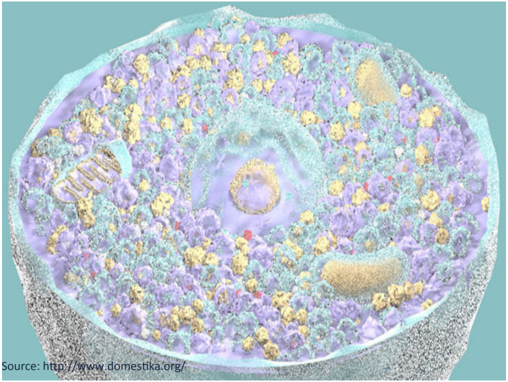
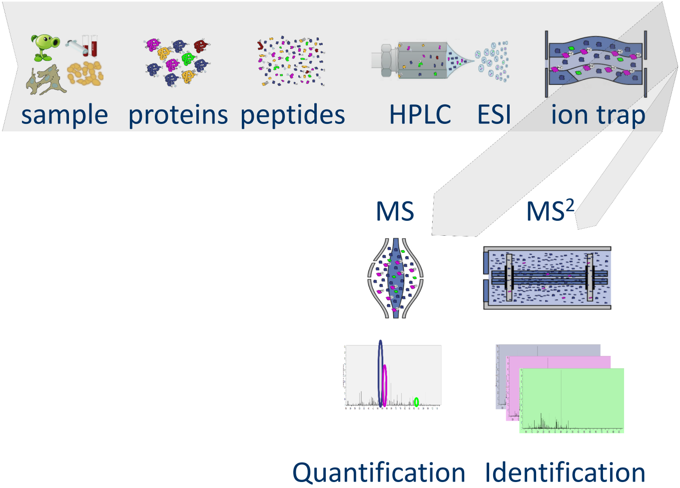

Source: http://www.domestika.org/

# Challenges in Label Free MS-based Quantitative Proteomics



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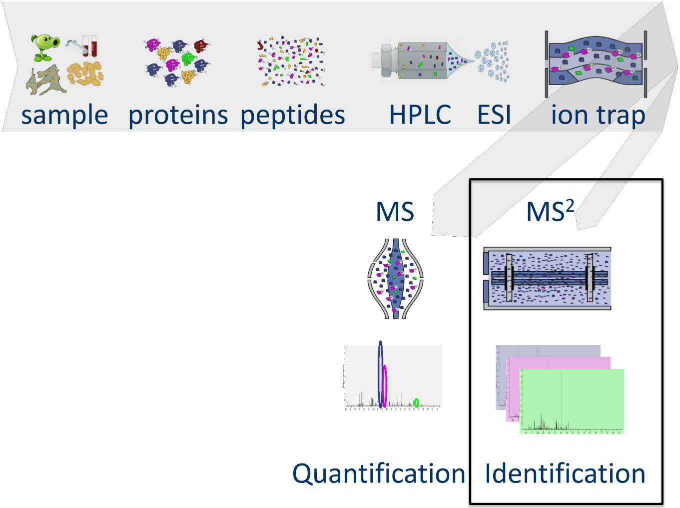
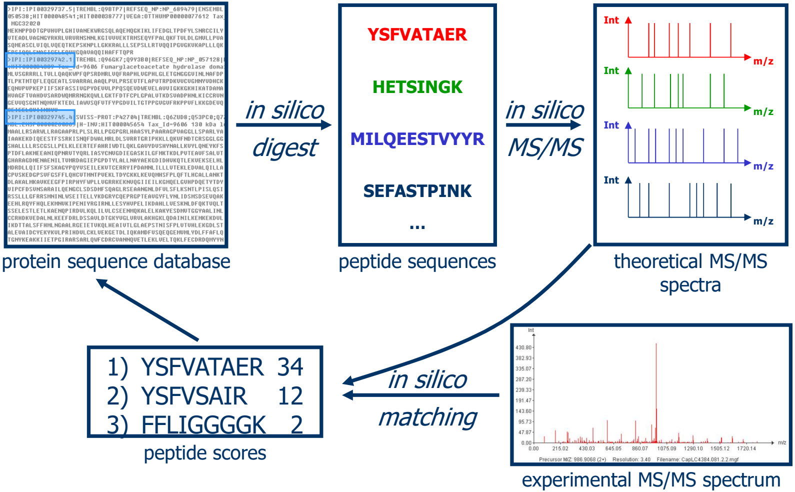


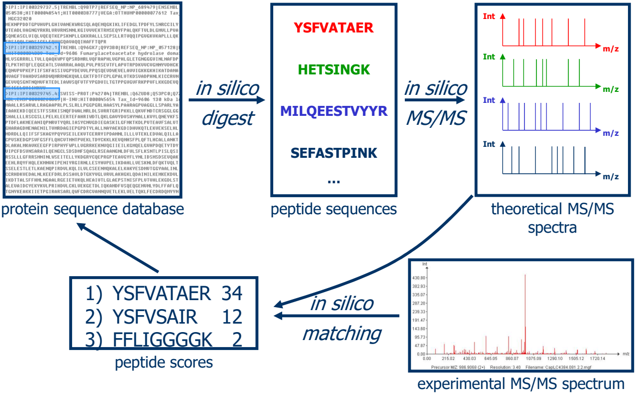
# Identification




(slide courtesy to Lennart Martens)

# E-values

Probability that a random candidate peptide produces a higher score that the observed PSM score.



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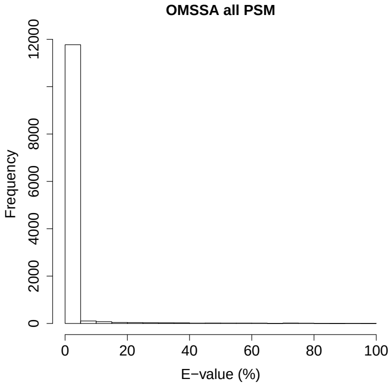

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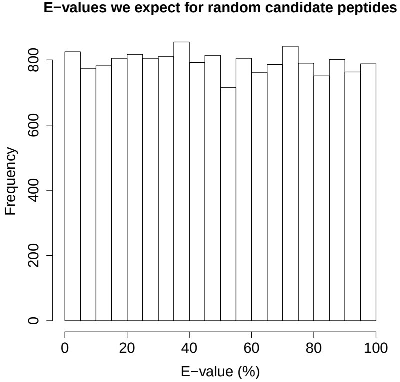

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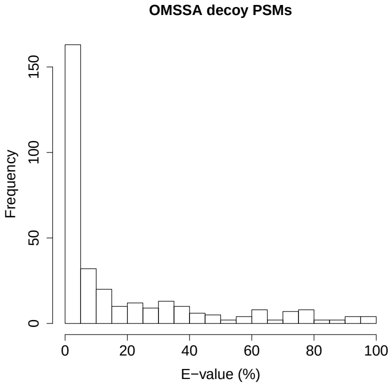

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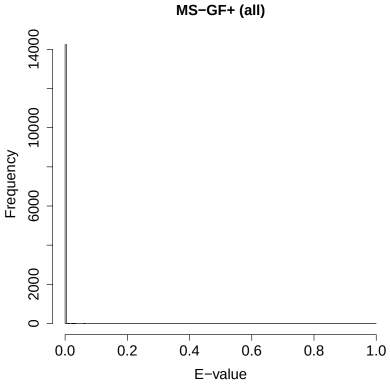

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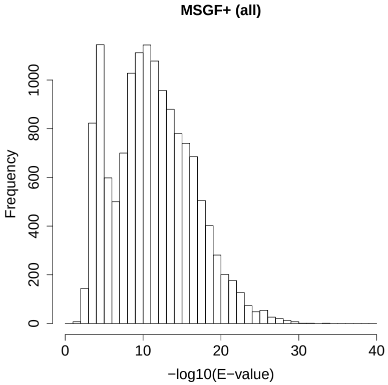

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Probability that a random candidate peptide produces a higher score that the observed PSM score.

- A bad hit is the random hit with the best score so it is also bound to have a low E-value.
- If we look at E-values for all PSMs they are only useful as a score.
- We should know the distribution of the maximum score of random candidate peptides when we want to do the statistics.

# 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

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

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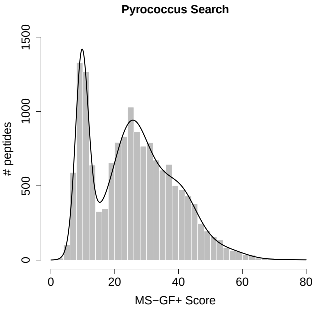
$FDR = E\left[\frac{FP}{FP+TP}\right]$. (FDR: false discovery rate)

# Search engines return score that discriminates good from bad matches



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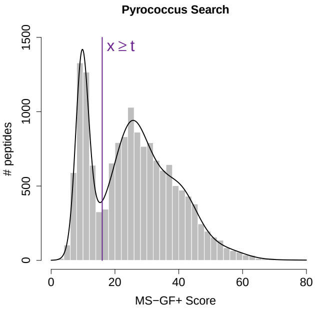
Score threshold $t$?



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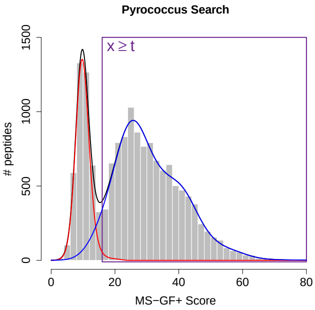


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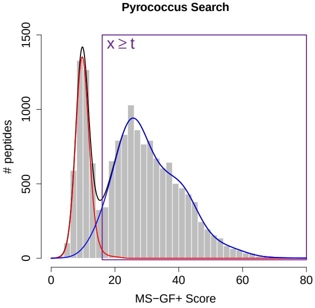


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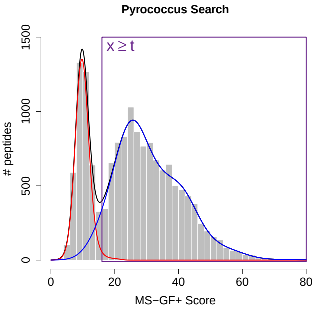


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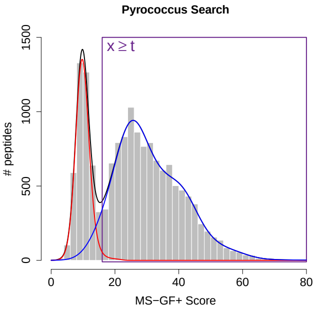


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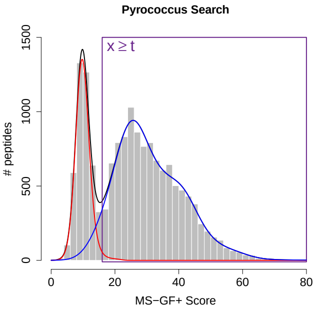


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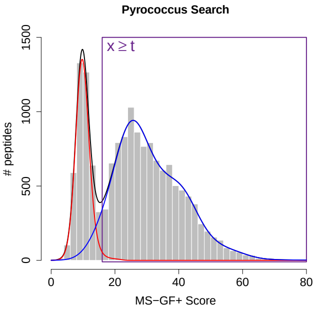


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FDR is a set property: $FDR(t) = \dfrac{\pi_0 \int_{x=t}^{+\infty} f_0(x)dx}{\int_{x=t}^{+\infty} f(x)dx}$

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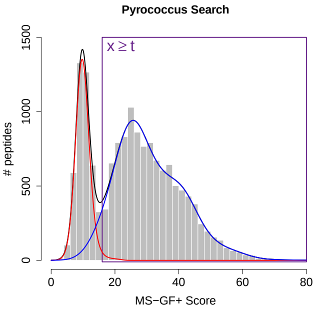


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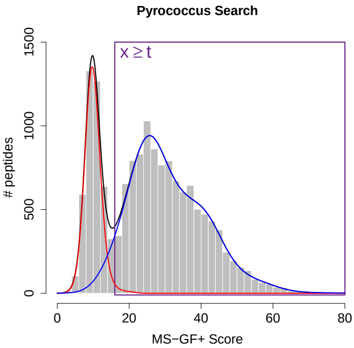
local fdr (posterior error probability, PEP): $fdr(x) = \frac{\pi_0 f_0(x)}{f(x)}$

Probability that an individual PSM is a bad hit.

# How to estimate FDR?

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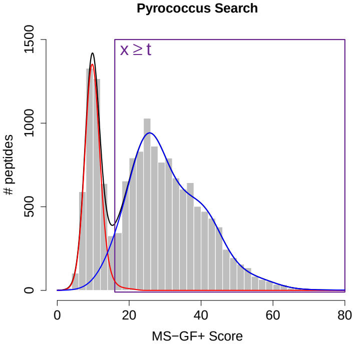


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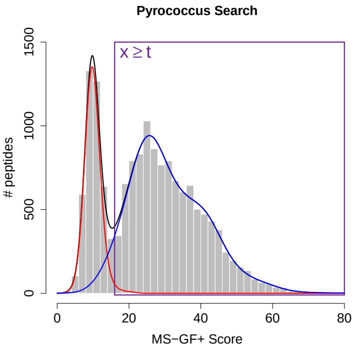


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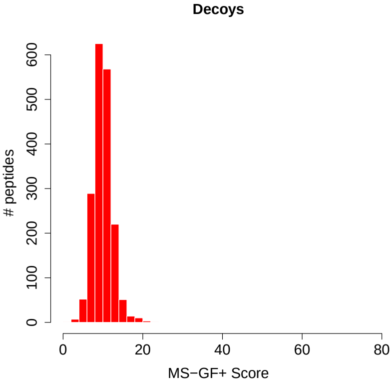
How to characterize $f_0(t)$ and $\pi_0$ in proteomics?

# Target-Decoy approach to establish null distribution

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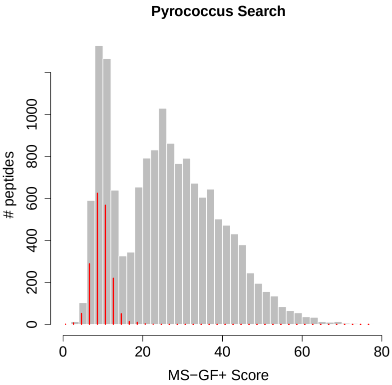


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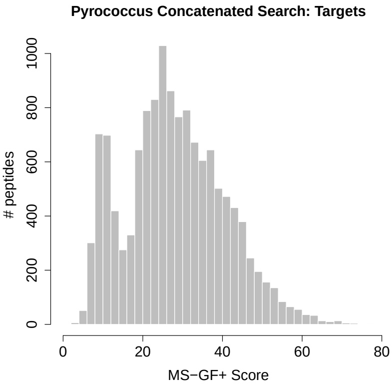


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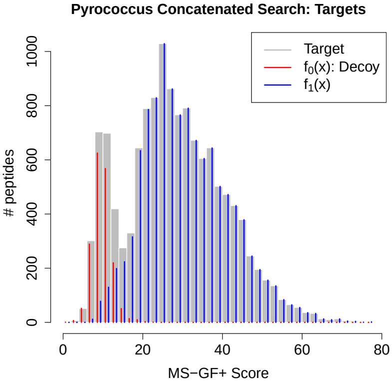


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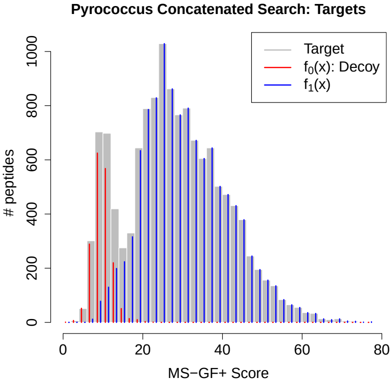


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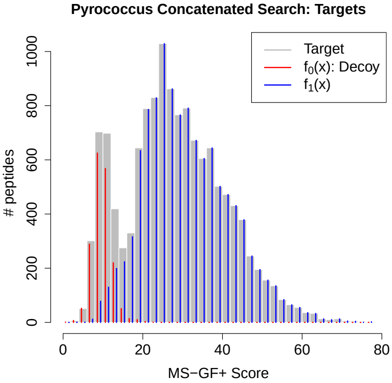


- Competitive Target - decoy:

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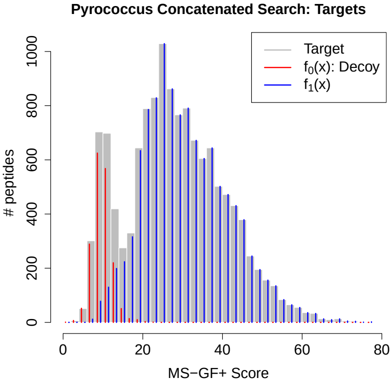


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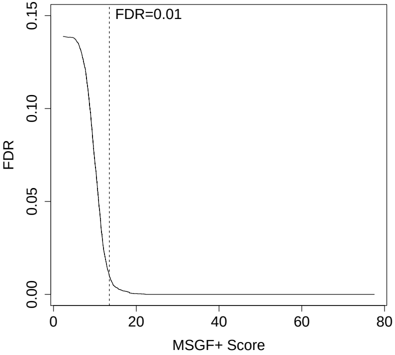


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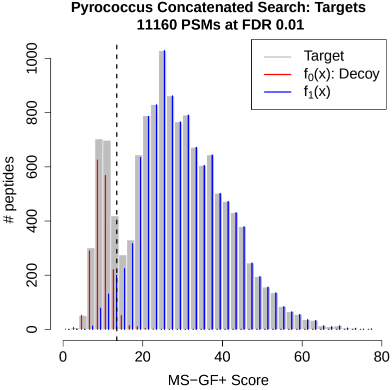


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# Assess TDA assumptions

We have to evaluate that

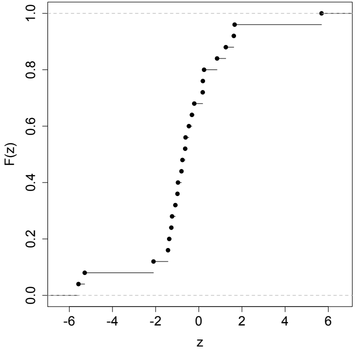
- The decoys are good simulations of the bad target hits: compare distributions $F_D(x)$ with $F(x)$

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

- $\hat{\pi}_0 = \frac{\#decoys}{\#targets}$ is a good estimator for $\pi_0$.

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

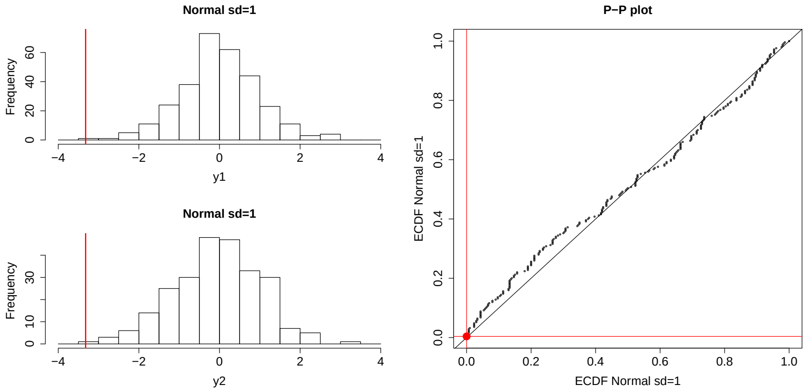
- To make PP-plots we need estimates for $F_D(x)$ and $F(x)$.
- The empirical cumulative distribution (ECDF) is used for that purpose




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

# 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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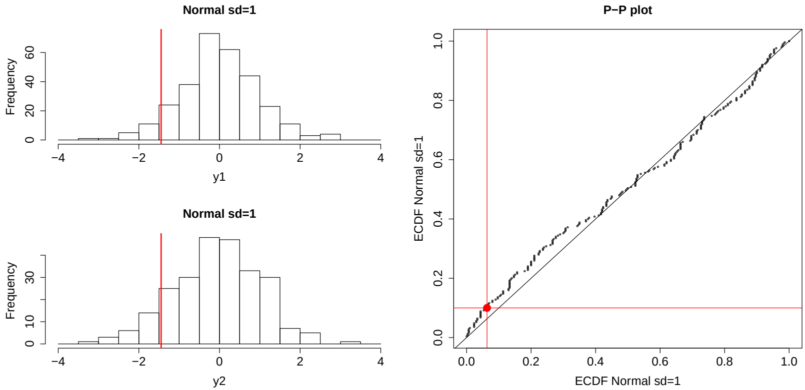

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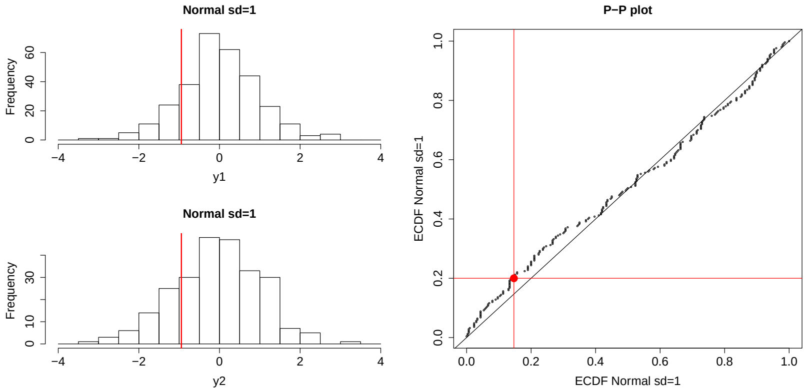

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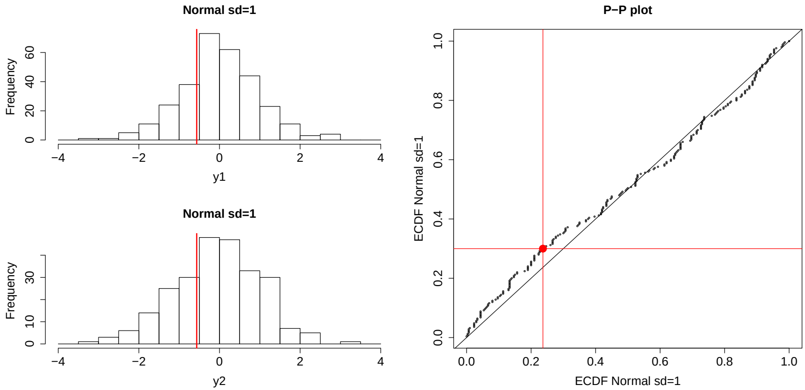

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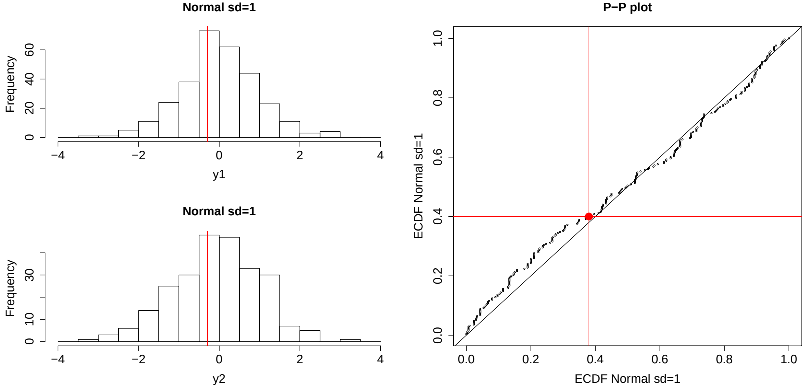

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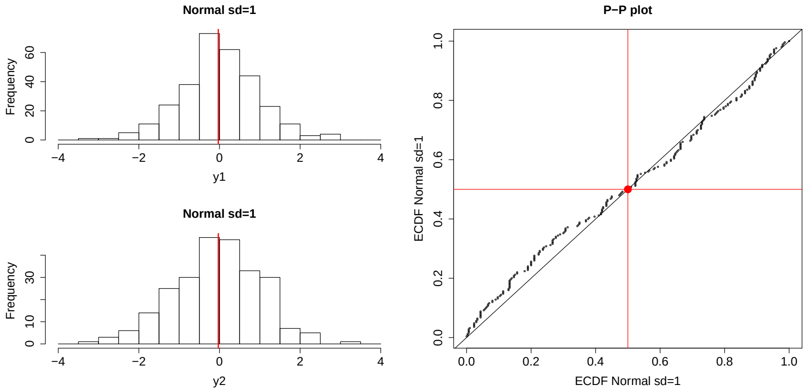

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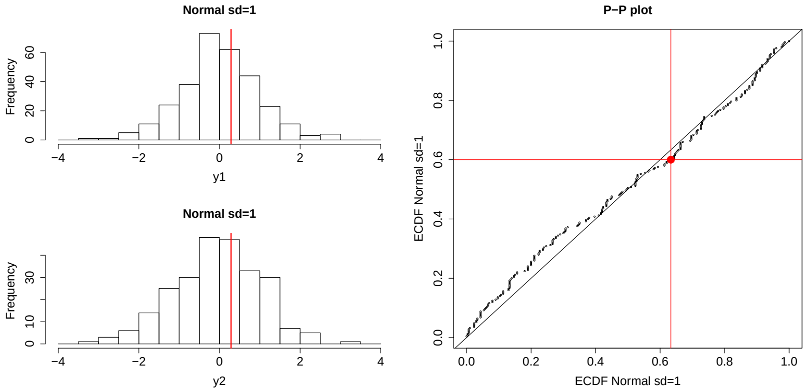

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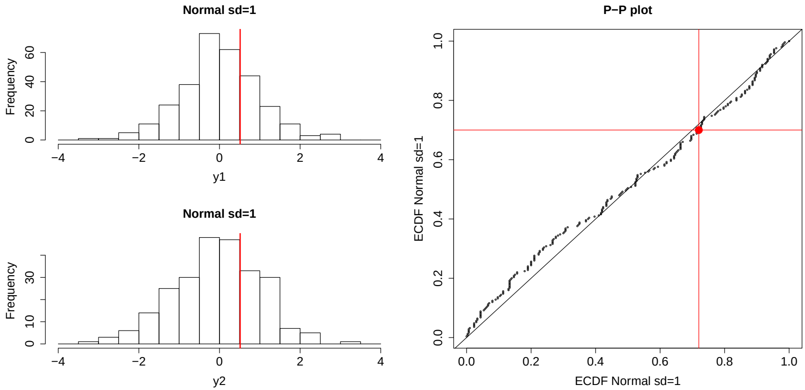

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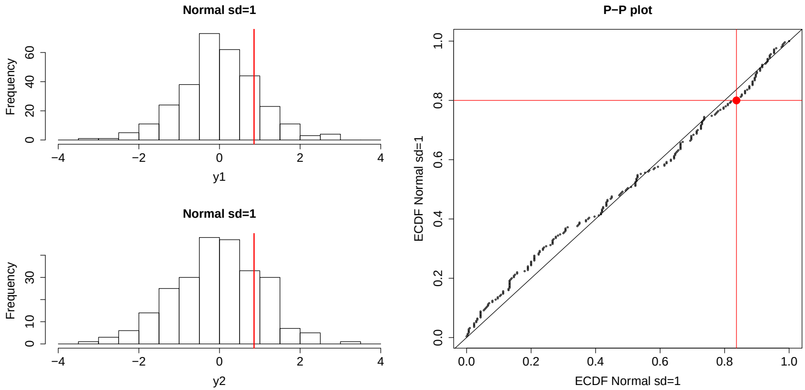

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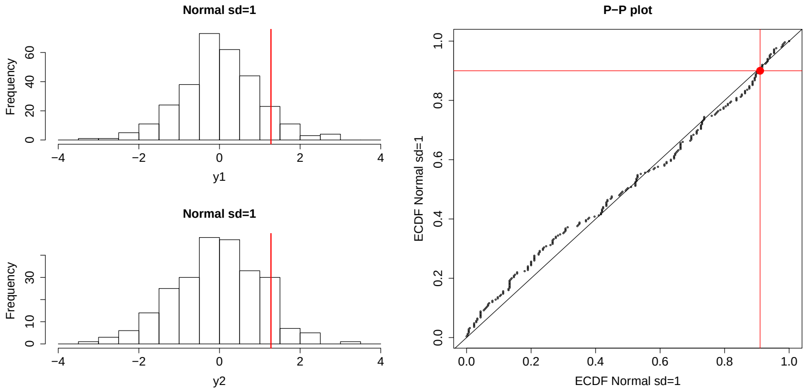
PP-plots have the property that they show a straight 45 degree line through the origin if and only if both distributions are equivalent.



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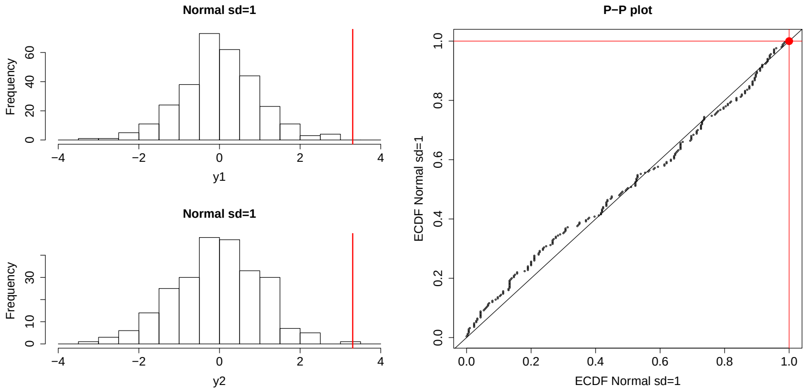
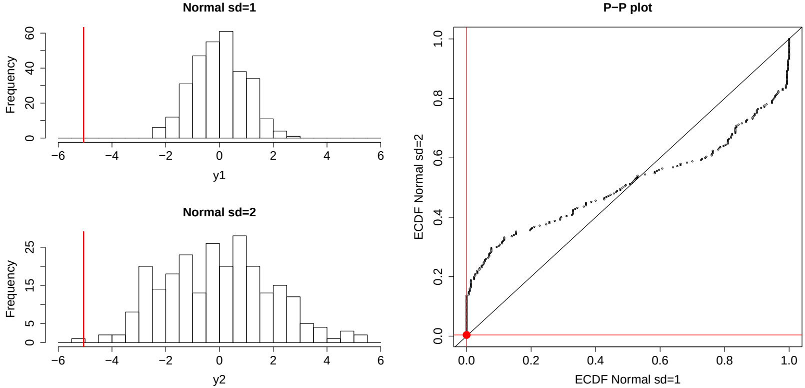


# PP-plot



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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
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0.0
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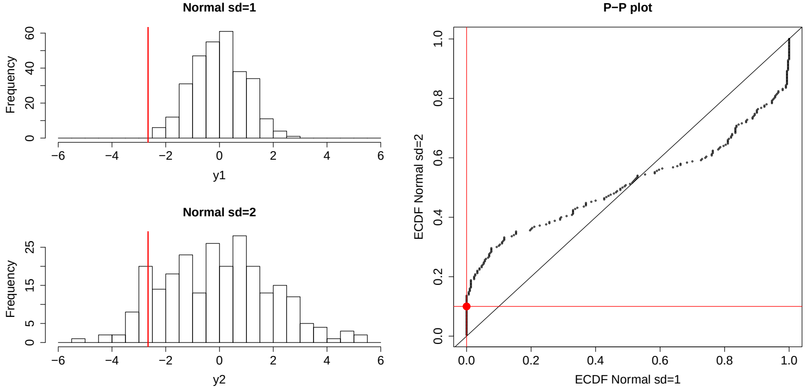
0.8
1.0
ECDF Normal sd=1

# PP-plot



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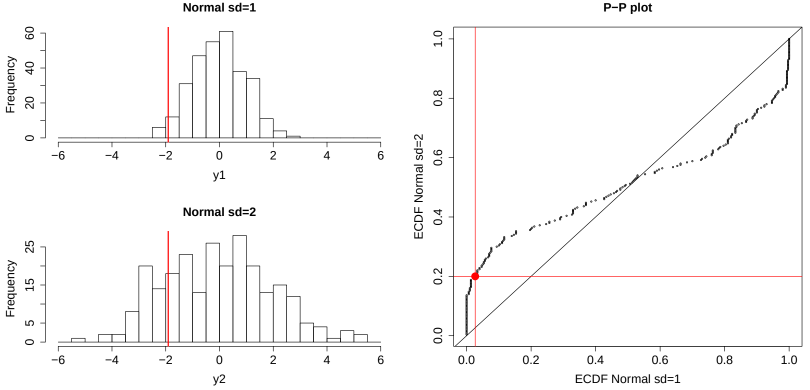
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

# PP-plot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Frequency
y2
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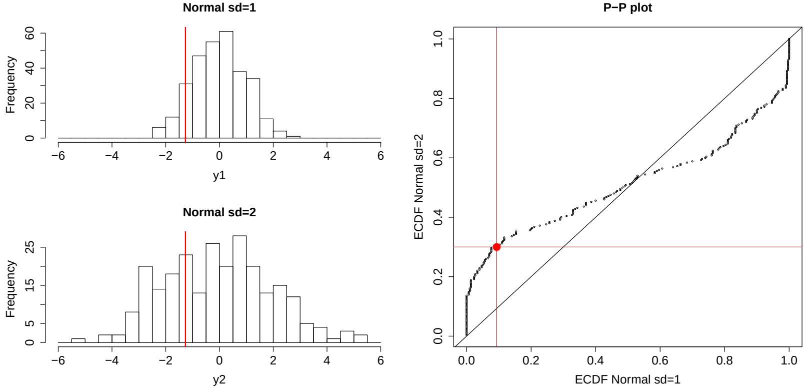
ECDF Normal sd=2
ECDF Normal sd=1

# PP-plot



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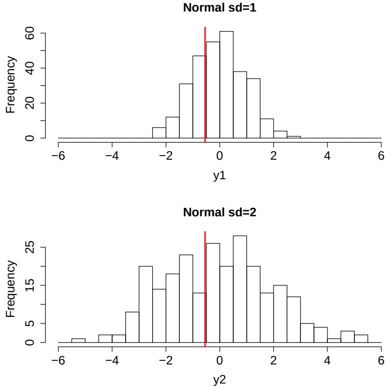
ECDF Normal sd=1

# PP-plot



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Normal sd=2
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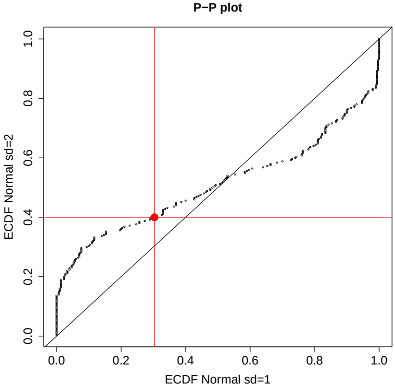
6
y2

P−P plot
ECDF Normal sd=2
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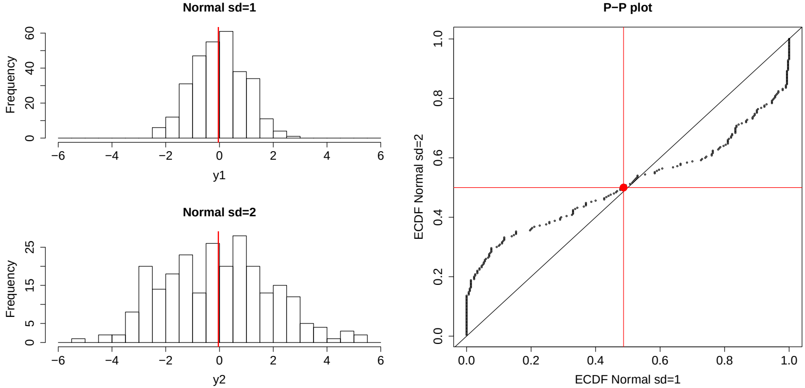
ECDF Normal sd=1

# PP-plot



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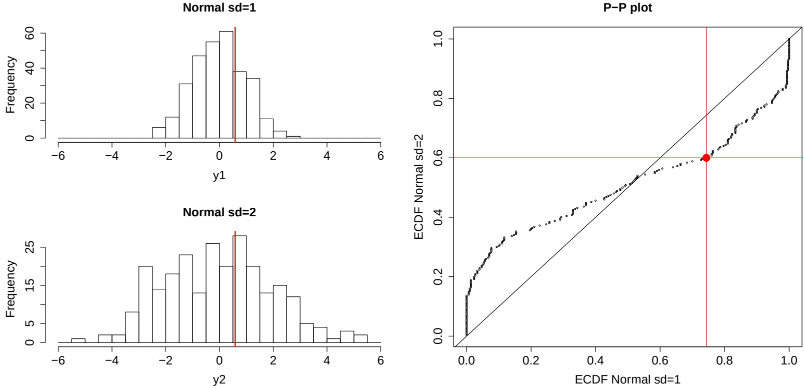
Normal sd=2
Frequency
y2
P−P plot
ECDF Normal sd=2
ECDF Normal sd=1

# PP-plot



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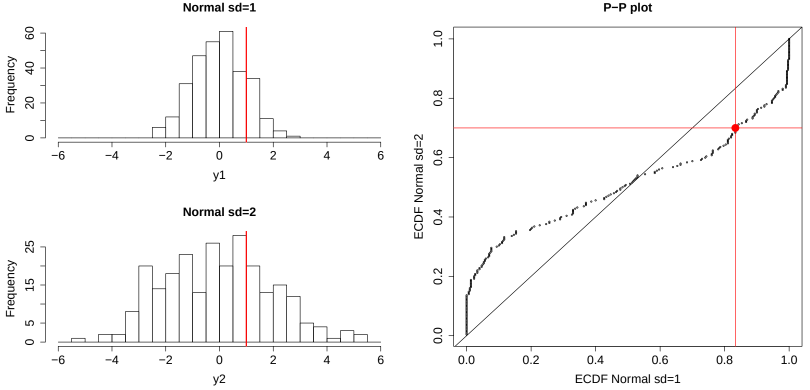
ECDF Normal sd=1

# PP-plot



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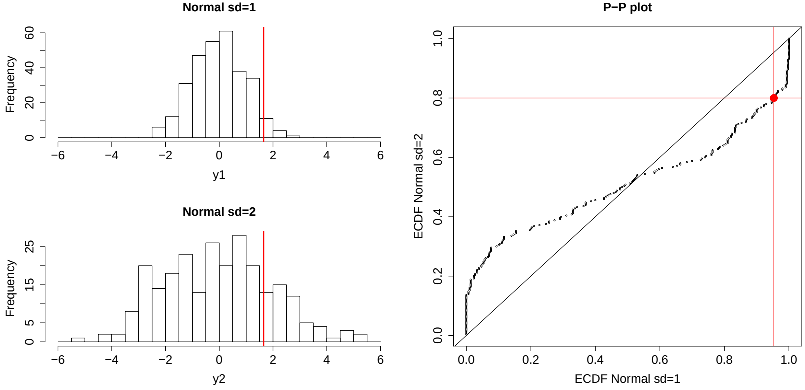
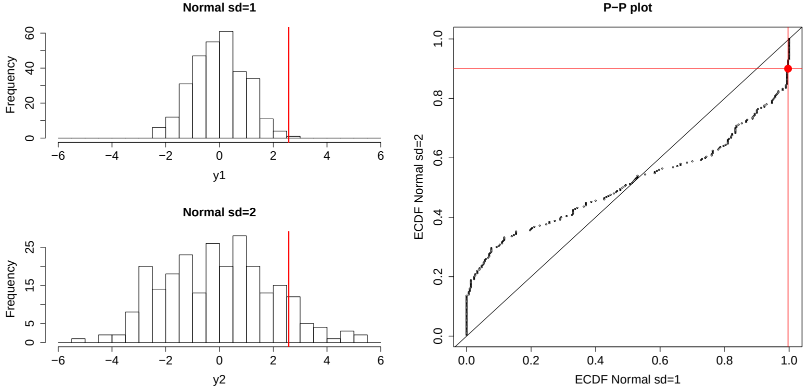
ECDF Normal sd=1

# 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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Normal sd=2
Frequency
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P−P plot
ECDF Normal sd=2
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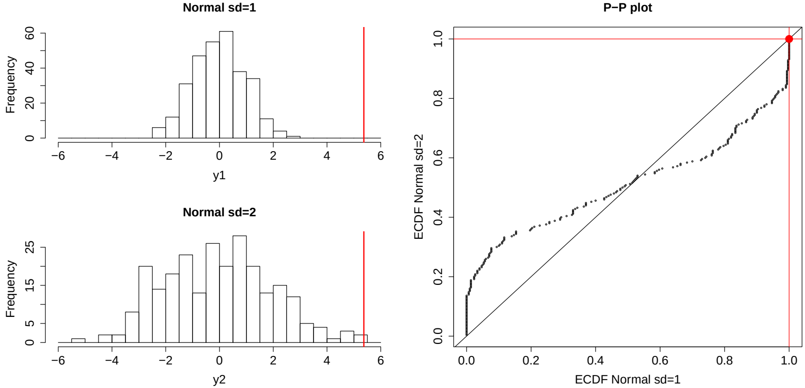
1.0
ECDF Normal sd=1

# PP-plot


Normal sd=1
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y1
Normal sd=2
Frequency
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−2
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2
4
6
y2
P−P plot
ECDF Normal sd=2
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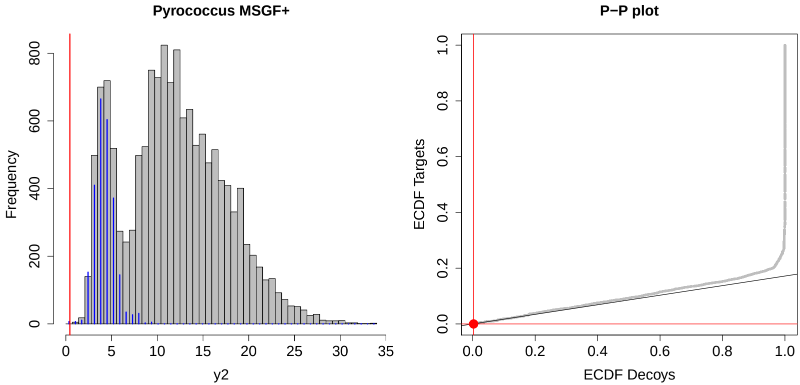
0.8
1.0
ECDF Normal sd=1

# PP-plot: pyrococcus



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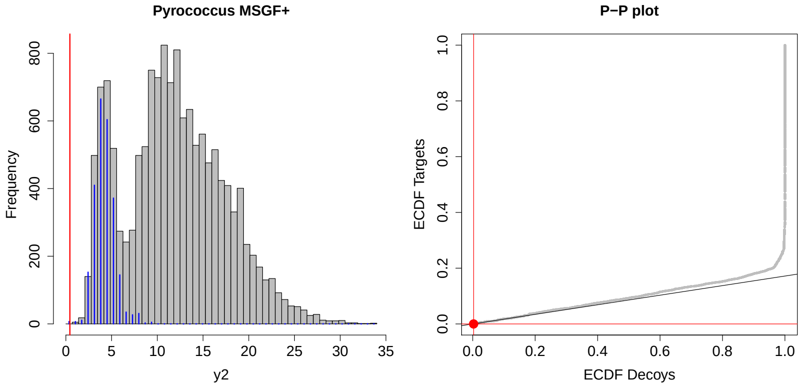
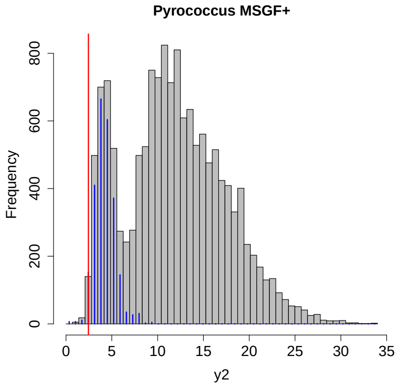
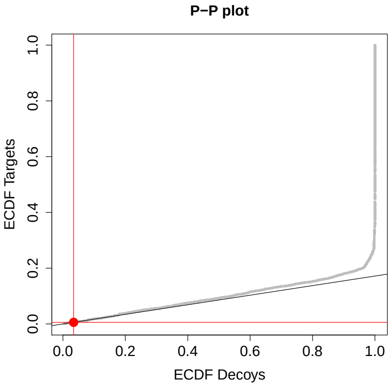



What about $\hat{\pi}_0$?

# PP-plot: pyrococcus



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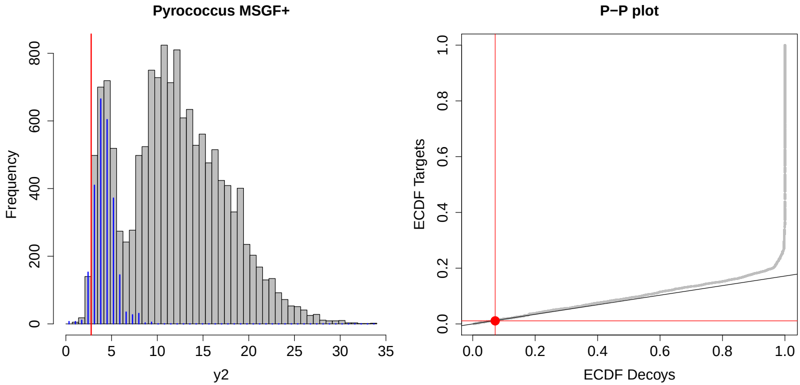

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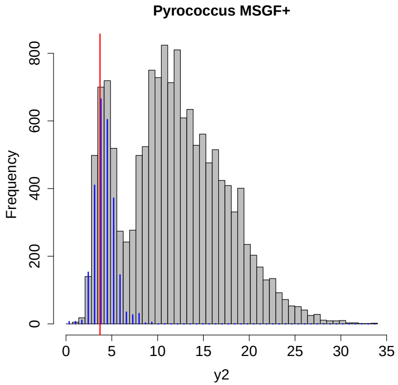

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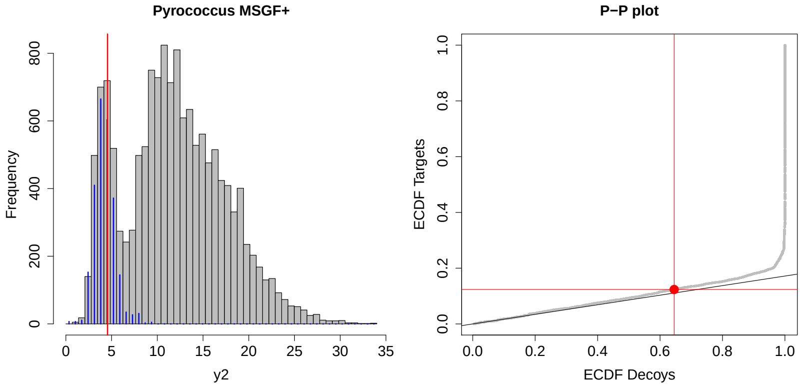
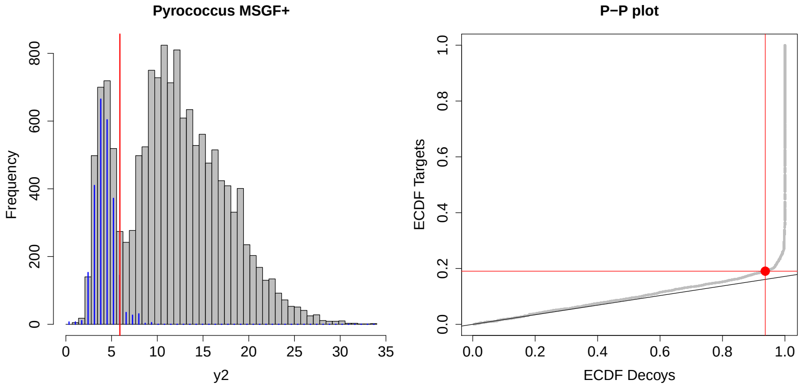


# PP-plot: pyrococcus



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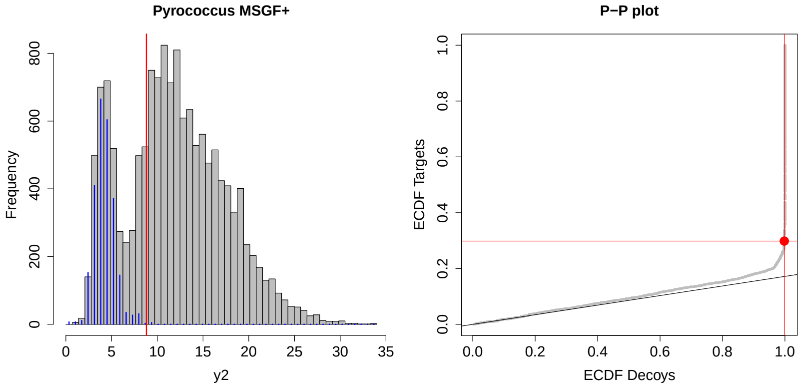

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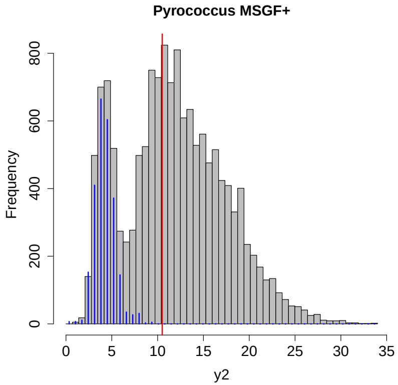


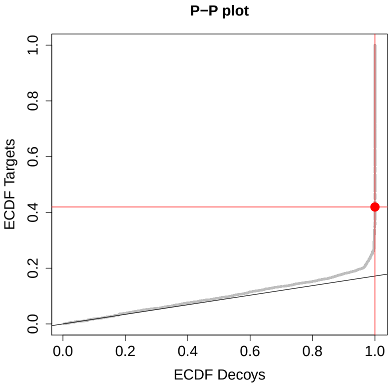

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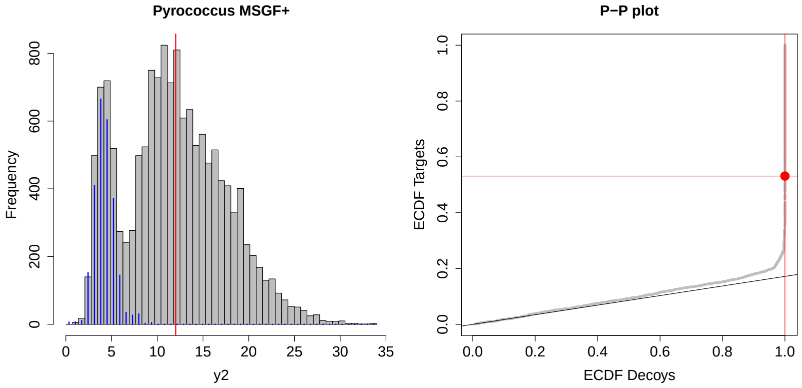

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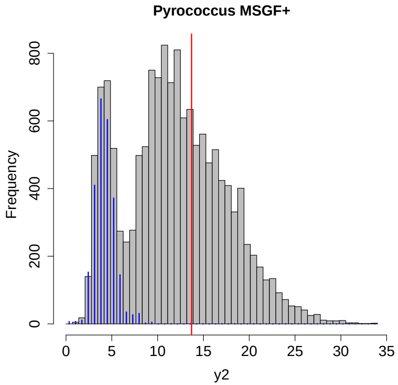


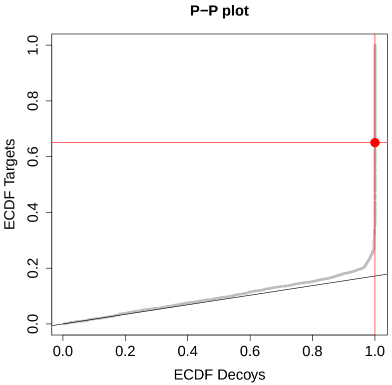

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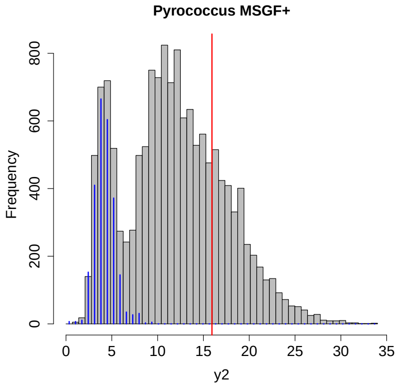


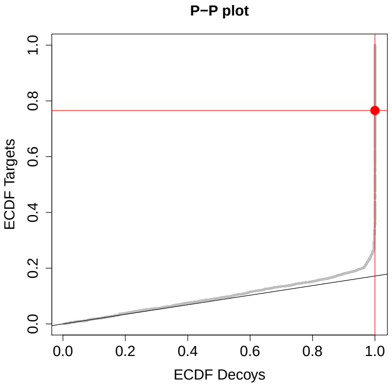

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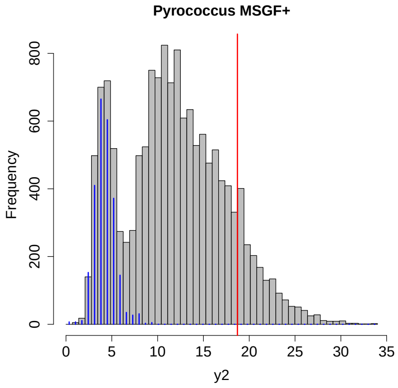

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