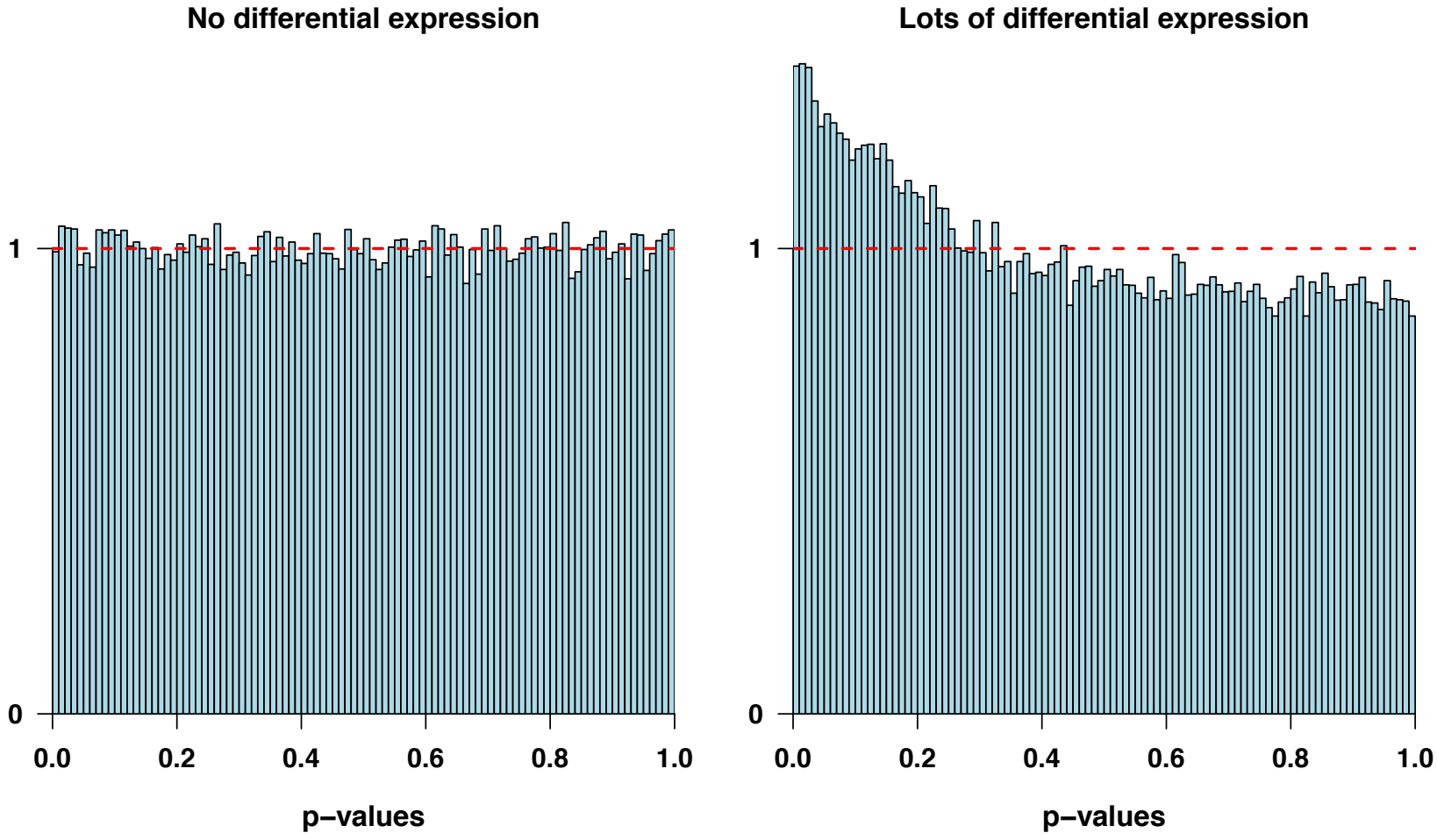

No differential expression
1
0
0.0
0.2
0.4
0.6
0.8
1.0
p−values
Lots of differential expression
1
0
0.0
0.2
0.4
0.6
0.8
1.0
p−values

Hypothetical example (no differential expression):

- Microarray with 10,000 genes.
- Calculate 10,000 p-values.
- Call genes “significant” if p-value $<$ 0.05.
- Expected Number of False Positives:

  10,000 $\times$ 0.05 = 500.

- Many procedures have been developed to control the Family Wise Error Rate (the probability of at least one type I error).

- Two general types of FWER corrections:
  - Single step: equivalent adjustments made to each p-value.
  - Sequential: adaptive adjustment made to each p-value.

Simple single step approach: Bonferroni.

- Very simple method for ensuring that the overall type I error rate of α is maintained when performing m hypothesis tests.
- Rejects any hypothesis with p-value $\leq \alpha$/m.
- The Bonferroni adjusted p-value is

$$p_j^{Bonf} = \min\left\{\mathrm{m} \times p_j, 1\right\}$$

- For example, if we want to have an experiment wide type I error rate of 0.05 when we perform 10,000 hypothesis tests, we needed a p-value of 0.05 / 10,000 = 5×10^{-6} to declare significance.

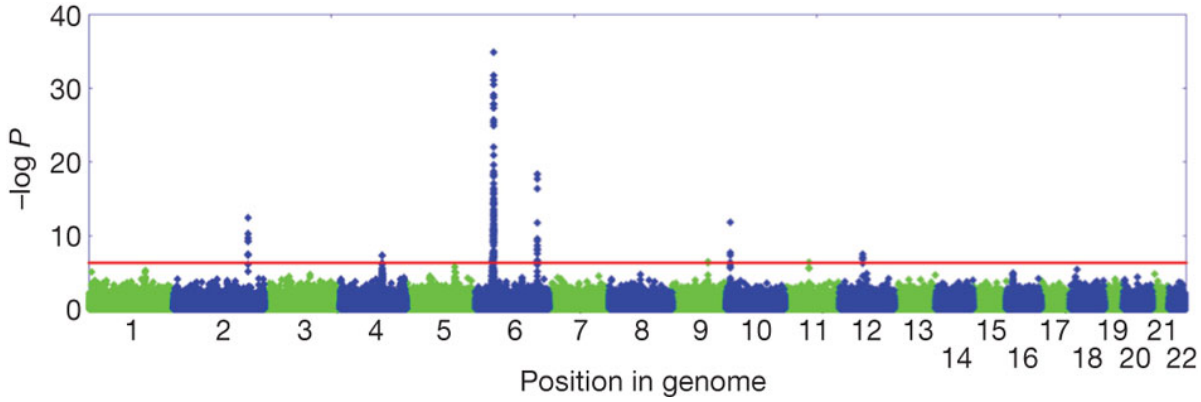
Simple sequential method: Holm-Bonferroni.

- Order the unadjusted p-values such that $p_1 \leq p_2 \leq \cdots \leq p_m$.
- Holm-Bonferroni uniformly delivers more power than the Bonferroni correction by testing only the most extreme p value against the strictest criterion, and the others against progressively less strict criteria.
- The Holm adjusted p-value is

$$p_j^{Holm} = \min\left\{\mathrm{m} - \mathrm{j} + 1 \times p_j, 1\right\}$$

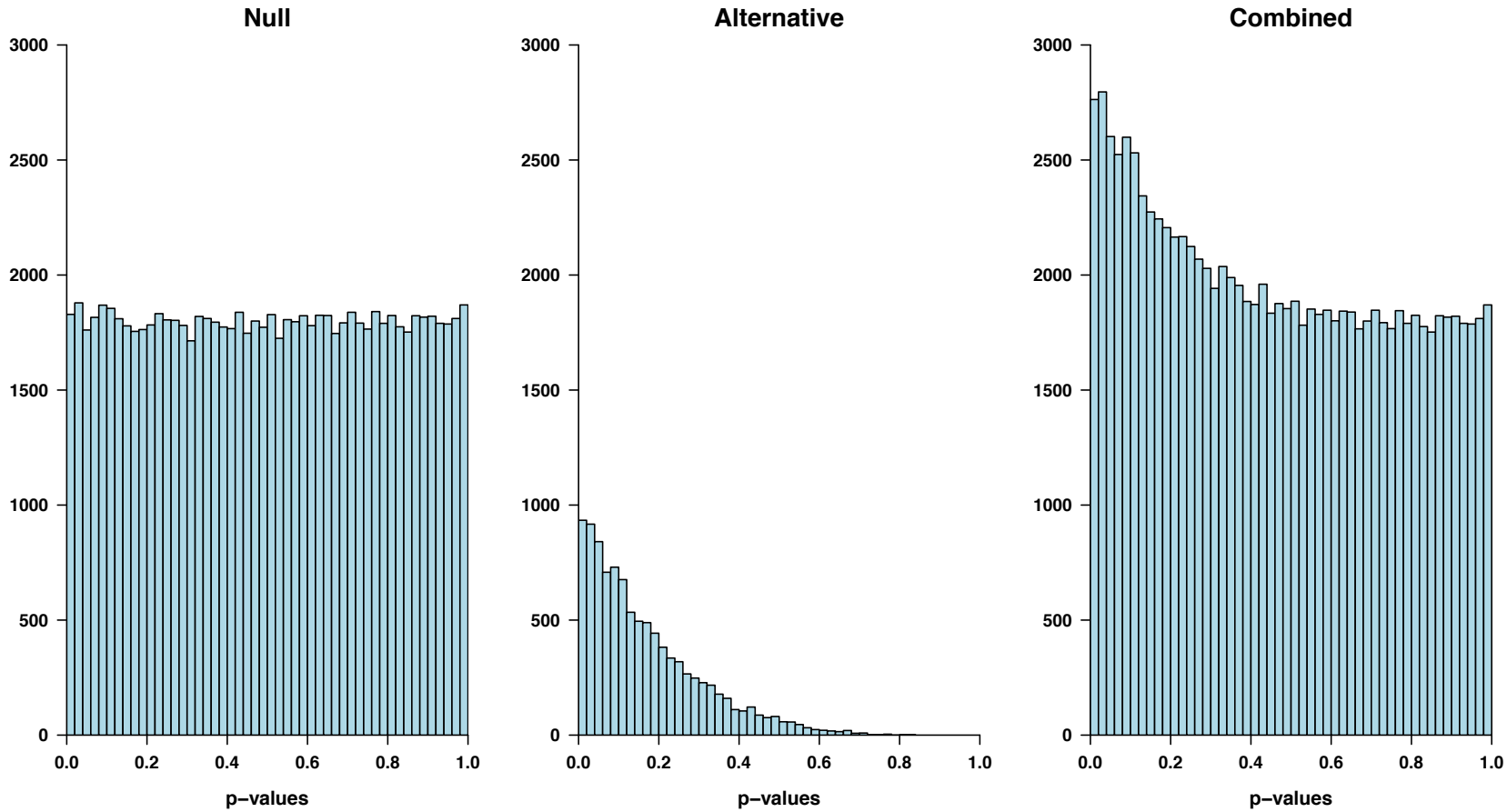
- The point here is that we do not multiply every p_j by the same factor m.

- The FWER is appropriate when you want to guard against **any** false positives.
- For example, this is usually done in genome-wide association studies.



- The general null hypothesis (that all the null hypotheses are true) is rarely of interest.
- There is a high probability of type 2 errors, i.e. of not rejecting the general null hypothesis when important effects exist.

- In many cases (particularly in genomics) we can live with a certain number of false positives.
- This is for example the case in gene expression studies, when we suspect a fair number of genes to be differentially expressed.
- In these cases, the more relevant quantity to control is the False Discovery Rate (FDR).
- The FDR is designed to control the proportion of false positives *among the set of rejected hypotheses.*


Null
Alternative
Combined
3000
2500
2000
1500
1000
500
0
0.0
0.2
0.4
0.6
0.8
1.0
p−values

Declared $\downarrow$	H_0 is true	H_a is true	Total
Significant	V	S	R
Non-significant	U	T	m – R
Total	m_0	$m - m_0$	m

- Bonferroni and such control the family-wise error rate.

  $\rightarrow$ V/(V+U).

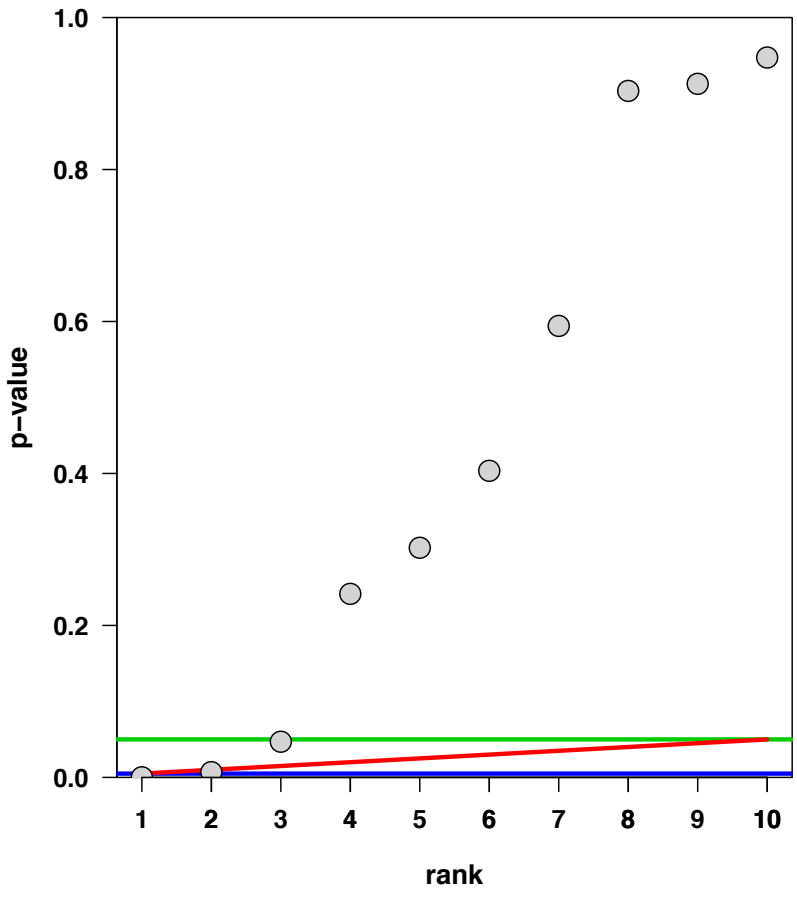
- The FDR controls the false positive rate.

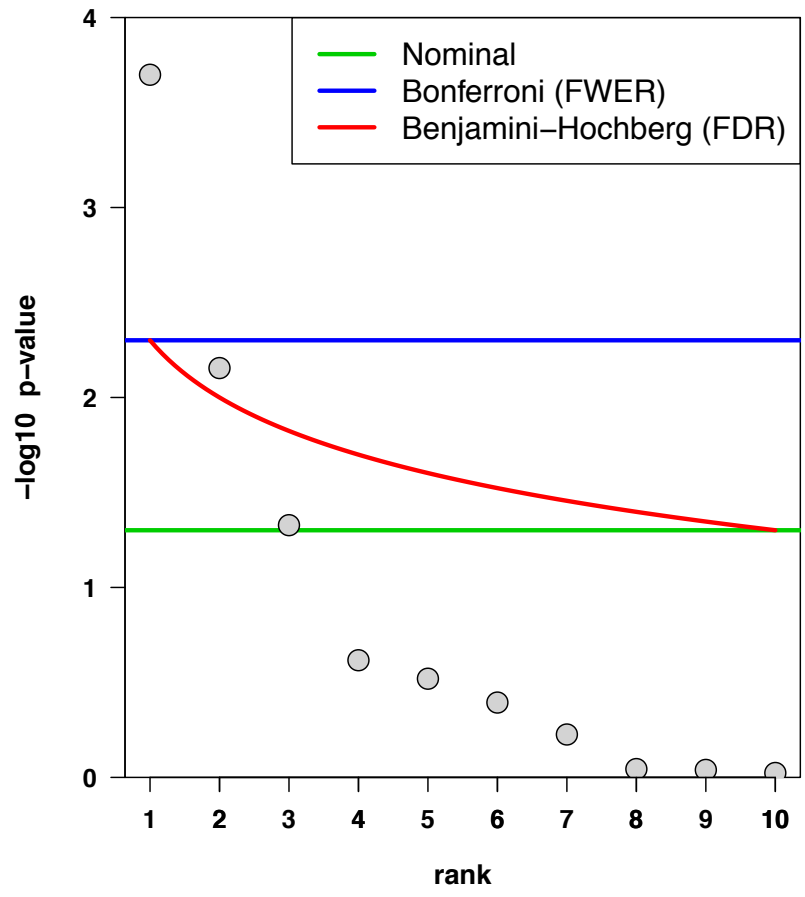
  $\rightarrow$ V/(V+S).

Benjamini and Hochberg FDR.

To control FDR at level δ:

- Order the unadjusted p-values: $p_1 \leq p_2 \leq \cdots \leq p_m$.
- Find the test with the highest rank j for which the p-value p_j is less than or equal to (j / m) $\times \delta$.
- Declare the tests of rank 1, 2, . . ., j as significant.


1.0
0.8
0.6
0.4
0.2
0.0
p−value
1
2
3
4
5
6
7
8
9
10
rank


Nominal
Bonferroni (FWER)
Benjamini–Hochberg (FDR)
4
3
2
1
0
−log10 p−value
1
2
3
4
5
6
7
8
9
10
rank

Difference in interpretation:

Suppose 550 out of 10,000 genes are significant at the 0.05 level.

- False Discovery Rate < 0.05:

  Expect $0.05 \times 550 = 27.5$ false positives.

- Family Wise Error Rate < 0.05:

  The probability of at least 1 false positive < 0.05.

- In most settings, the latter is extremely unlikely, unless the sample size is huge!

John Storey's positive FDR (pFDR):

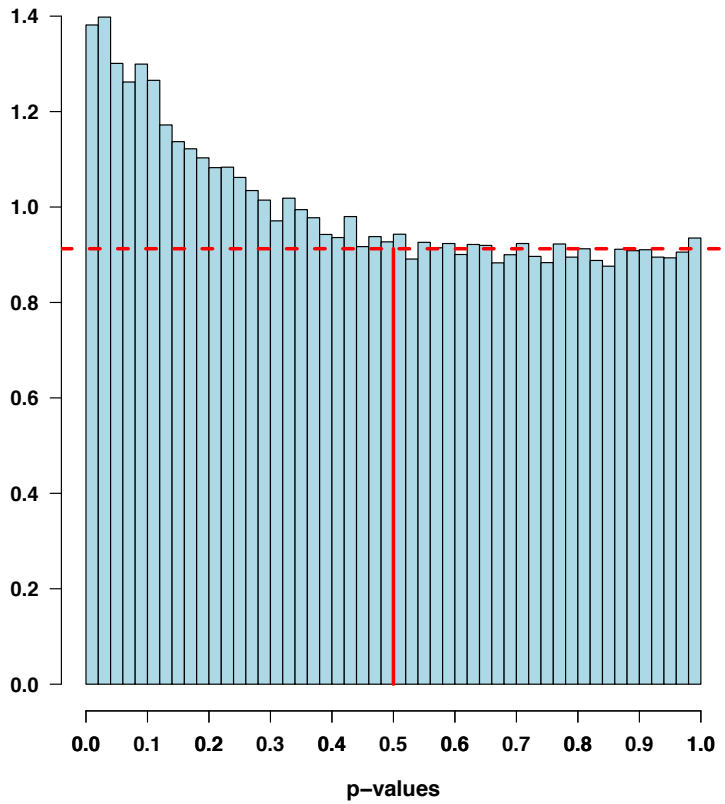
$$\text{FDR} = \text{E}\left[\frac{V}{R} \mid R > 0\right] \times P(R > 0)$$

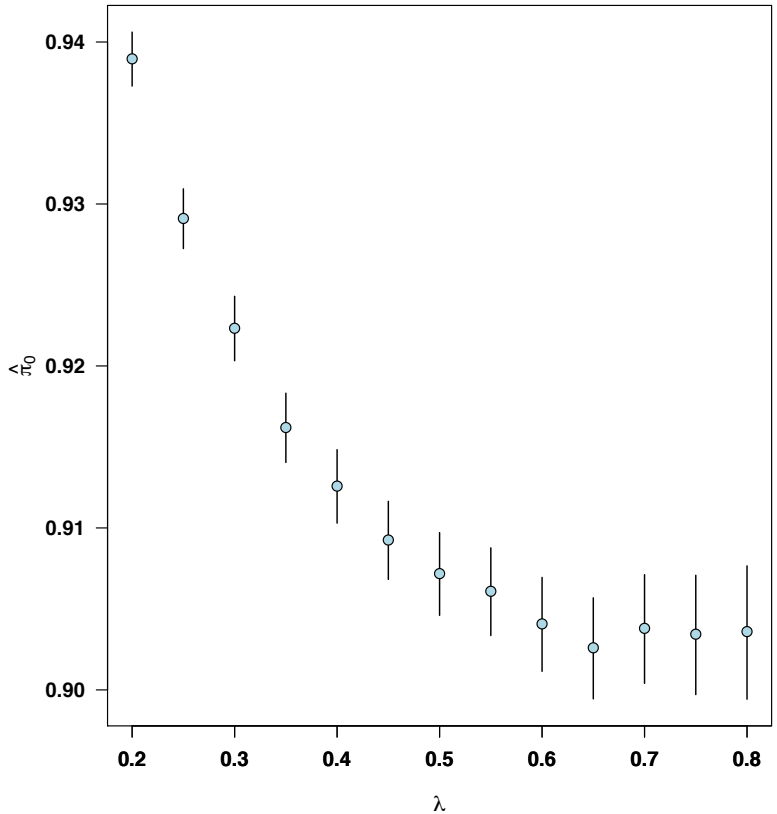
$$\text{pFDR} = \text{E}\left[\frac{V}{R} \mid R > 0\right]$$

- Since $P(R > 0)$ is ~ 1 in most genomics experiments, the FDR and the pFDR are very similar.
- Omitting $P(R > 0)$ facilitated the development of a measure of significance in terms of the FDR for each hypothesis.

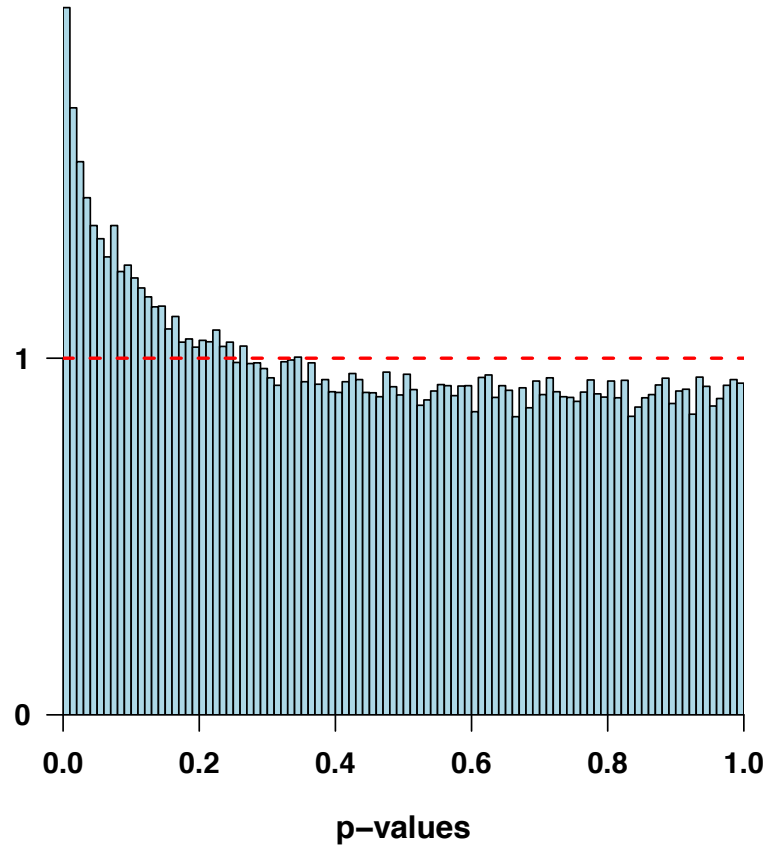
Q-values:

- The q-value is defined as the minimum FDR that can be attained when calling that gene significant (i.e., expected proportion of false positives incurred when calling that gene significant).
- The estimated q-value is a function of the p-value for that test and the distribution of the entire set of p-values from the family of tests being considered.
- In testing for differential expression, if a gene has a q-value of 0.10 it means that we can expect 10% of genes that show p-values at least as small as this gene to be false positives.


1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
p−values


0.94
0.93
0.92
0.91
0.90
$\hat{\pi}_0$
0.2 0.3 0.4 0.5 0.6 0.7 0.8
λ

	p	q	p.bonf
1	1.92e-06	0.11814	0.19188
2	3.90e-06	0.11814	0.38955
3	8.43e-06	0.11814	0.84317
4	9.35e-06	0.11814	0.93497
5	1.11e-05	0.11814	1.00000
6	1.14e-05	0.11814	1.00000
7	1.14e-05	0.11814	1.00000
8	1.22e-05	0.11814	1.00000
9	1.24e-05	0.11814	1.00000
10	1.44e-05	0.11814	1.00000
11	1.44e-05	0.11814	1.00000
12	1.64e-05	0.11997	1.00000
13	1.76e-05	0.11997	1.00000
14	1.97e-05	0.11997	1.00000
15	1.99e-05	0.11997	1.00000
16	2.53e-05	0.14035	1.00000
17	2.64e-05	0.14035	1.00000
18	3.03e-05	0.14795	1.00000
19	3.11e-05	0.14795	1.00000
20	3.67e-05	0.15919	1.00000



	p	q	p.bonf
1	2.27e-31	2.05e-26	2.27e-26
2	9.05e-13	4.09e-08	9.05e-08
3	3.37e-12	1.02e-07	3.37e-07
4	2.57e-11	5.80e-07	2.57e-06
5	2.20e-09	3.97e-05	2.20e-04
6	1.92e-06	2.89e-02	1.92e-01
7	3.90e-06	5.03e-02	3.90e-01
8	5.26e-06	5.94e-02	5.26e-01
9	8.43e-06	6.99e-02	8.43e-01
10	9.35e-06	6.99e-02	9.35e-01
11	9.55e-06	6.99e-02	9.55e-01
12	1.11e-05	6.99e-02	1.00e+00
13	1.14e-05	6.99e-02	1.00e+00
14	1.14e-05	6.99e-02	1.00e+00
15	1.22e-05	6.99e-02	1.00e+00
16	1.24e-05	6.99e-02	1.00e+00
17	1.44e-05	7.22e-02	1.00e+00
18	1.44e-05	7.22e-02	1.00e+00
19	1.64e-05	7.77e-02	1.00e+00
20	1.76e-05	7.95e-02	1.00e+00