Whole transcriptome sequencing data analysis workshop

P-value correction methods and plots: ROC, FDR, FPs and precision-recall

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All models are wrong

George Box:

"Essentially, all models are wrong, but some are useful."

"Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful."

David Cox:

"The very word model implies simplification and idealization. The idea that complex physical, biological or sociological systems can be exactly described by a few formulae is patently absurd. The construction of idealized representations that capture important stable aspects of such systems is, however, a vital part of general scientific analysis and statistical models."

Andrew Gelman:

"The saying, "all models are wrong", is helpful because it is not completely obvious...This is a simple point...But, the trouble is, many people don't realize that all models are wrong."

1. p-value correction

2. Plots

The p-value

- First of all, for a test we need a system of hypothesis, H_0 vs H_1, and a test statistic.
- A p-value is the probability of generating, under H_0, a value of the statistic as extreme (or more) as the one observed in our sample.
- A p-value of 0.05 means that, under H_0, there is a 5% probability of generating a value as extreme (or more) than the observed one: so, under H_0, every 100 tests, on average, 5 will be false positives (FPs) at the 5% significance threshold.
- If we perform 20,000 tests (one per gene) and H_0 is true for all of them, on average 1,000 will be FPs.
- Given a p-value, we can separate our features (genes, transcripts, exons, etc...) in "significant" (p-value $< \alpha$) and "non-significant" (p-value $> \alpha$), where typically $\alpha = 0.05$ or 0.01.
- Features can also be separated according to other statistics, e.g., the fold-change (FC) between conditions.

Definitions

Test result	Real state: Positive	Negative	
Significant	TP	FP	S
Non-significant	FN	TN	NS
	P	N	

- True positive (TPs) are positive cases that are (correctly) detected by a test (i.e., significant p-value).
- True negatives (TNs) are negative cases that are (correctly) not detected by a test (i.e., non-significant p-value).
- False positive (FPs) are negative cases that are (mistakenly) detected by a test (i.e., significant p-value).
- False negatives (FNs) are positive cases that are (mistakenly) not detected by a test (i.e., non-significant p-value).

Sensitivity and Specificity

Test result	Real state: Positive	Negative	
Significant	TP	FP	S
Non-significant	FN	TN	NS
	P	N	

- The true positive rate (TPR), also called Sensitivity, power or recall, measures the proportion of actual positives that are correctly identified as such: $TPR = TP/P = TP/(TP + FN)$.
- The true negative rate (TNR), also called Specificity, measures the proportion of actual negatives that are correctly identified as such: $TNR = TN/N = TP/(TN + FP)$.
- The type *I* error, false positive rate (FPR) or 1 - Specificity, is the proportion of negatives wrongly classified as significant.
- The type *II* error, false negative rate (FNR) or 1 - Sensitivity, is the proportion of positives wrongly classified as non-significant.

FWER & FDR

- Alternative ways of selecting the significant genes (w.r.t. the p-value):
  - family wise error rate (**FWER**);
  - false discovery rate (**FDR**).
- The family wise error rate (FWER) is the probability of making at least 1 type *I* error: $\text{FWER} = \Pr(\text{FP} > 0)$.
- FWER is very strict when testing thousands of genes.
- Define the proportion of FPs among significant results as: $Q = \text{FP} / S = \text{FP} / \text{TP} + \text{FP}$.
- The FDR is the expected proportion of FPs in the set of significant results: $\text{FDR} = E(Q)$
- Unlike the measures introduces so far, the FDR represents a property of the set of significant features.

Controlling the FDR

- Instead of selecting the significant genes according to the p-value, we can correct the p-value such that:
  - the ordering of genes is preserved;
  - the FDR is controlled at the α threshold.
- The Benjamini-Hochberg (BH) correction is the most popular method for controlling the FDR.
- We select the genes whose corrected p-value (often called q.value) is $< \alpha$: therefore for $\alpha = 0.05$, we expect that 5% of the selected genes will be FPs and 95% will be TPs.
- This is typically performed in both differential expression and differential splicing methods.

Independent filtering

- We often filter out genes *a priori*, i.e. before testing.
- Typically we filter genes with very low expression, below a specified threshold.

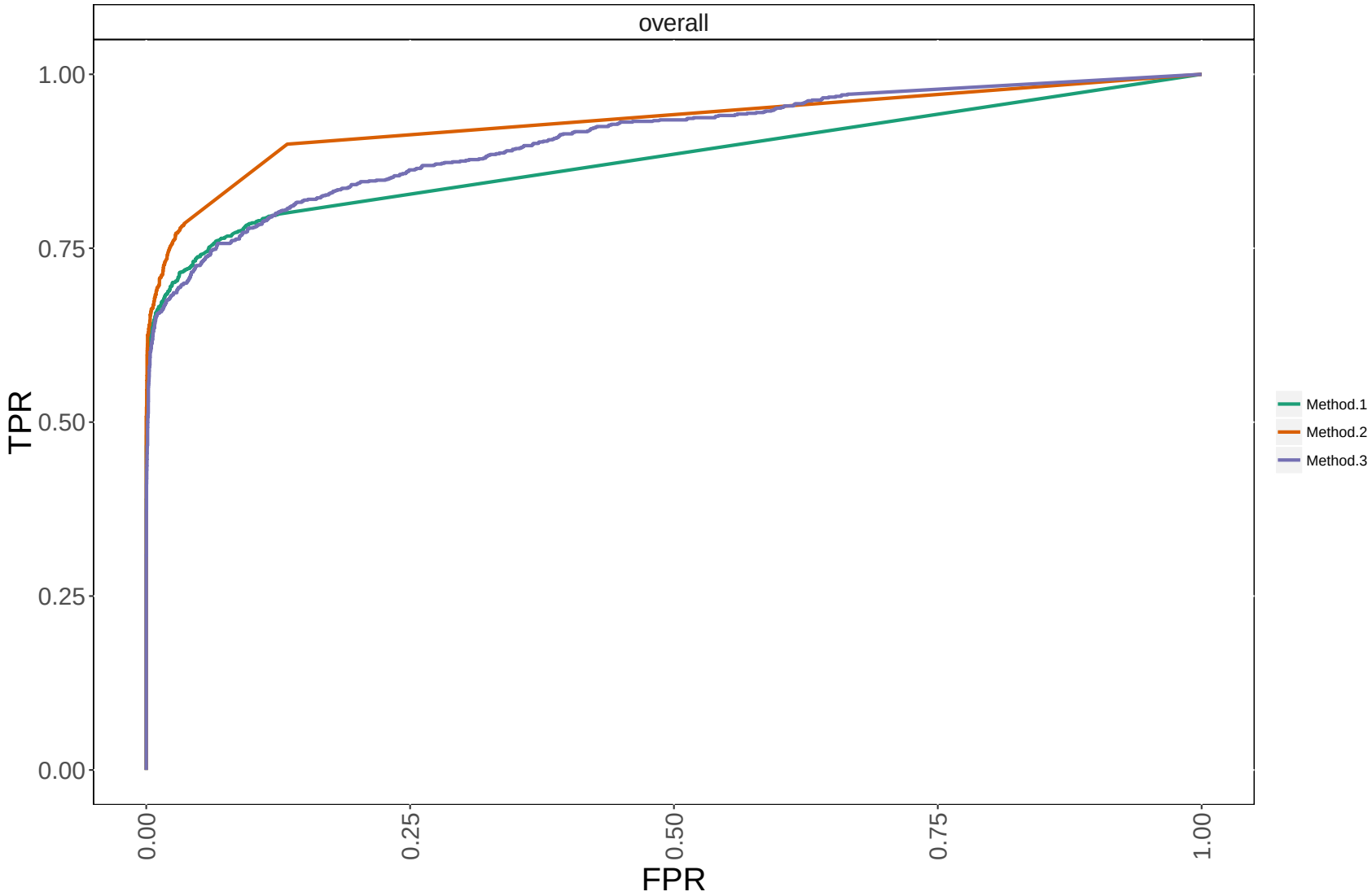
1. p-value correction

2. Plots

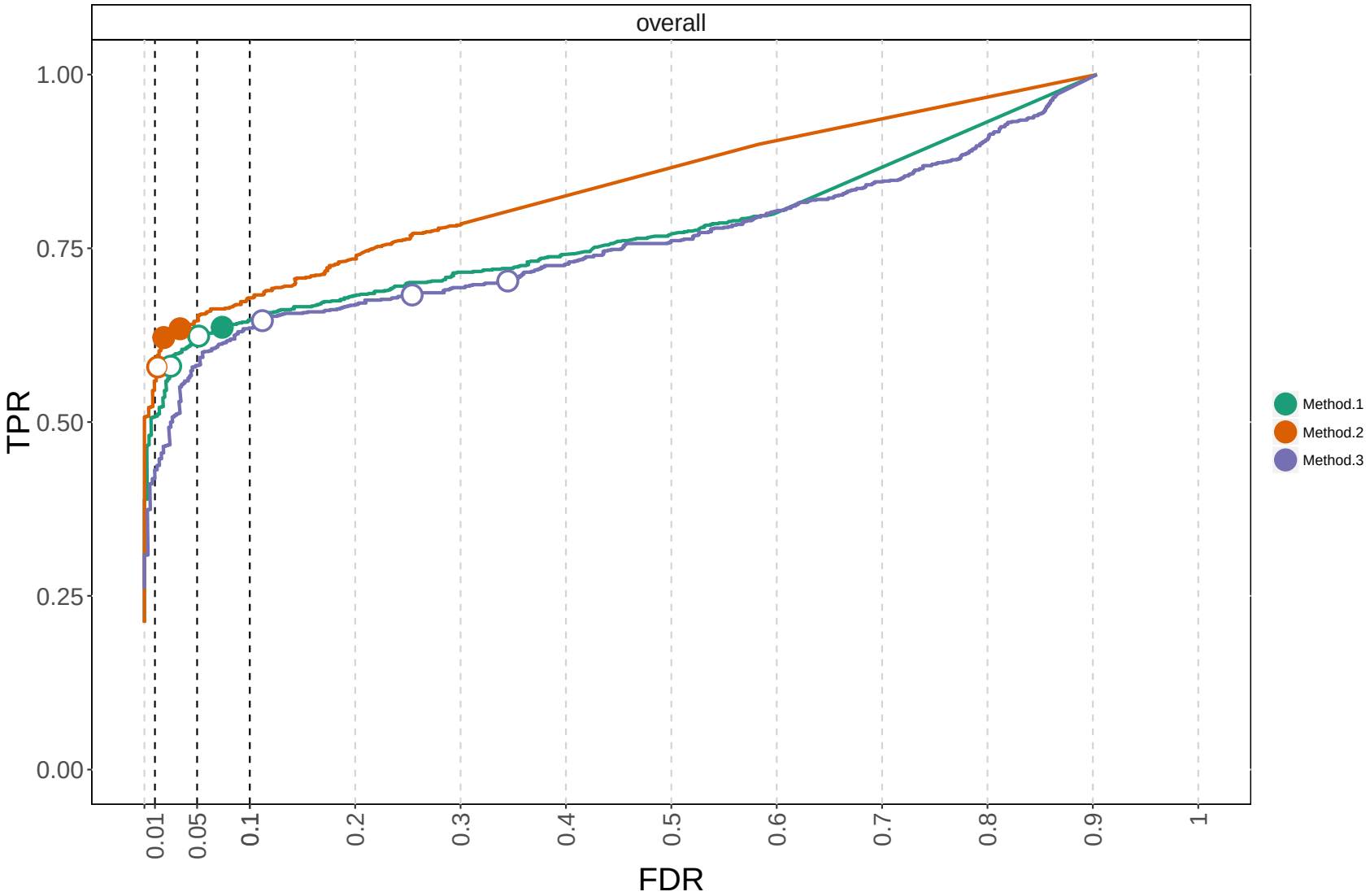
Assessing the performance of a method

- When assessing the performance of methods (DGE, eQTL, DTU, etc...) and comparing them, we mostly investigate several plots.
- This is always done in simulations where we know the **truth**.
- The main plots are:
  - **ROC**: true positive rate (TPR) vs false positive rate (FPR); The diagonal line, from (0,0) to (1,1), represents the blind model, which chooses at random.
  - **FDR**: TPR vs FDR (more interesting than the ROC in this field): important to check if the FDR is controlled at a chosen threshold;
  - **FPs** vs top significant genes: it tells us how well a model does in the very top genes, which are those we are mostly interested in;
  - Precision vs recall plot, where:
    - precision = TP/(TP + FP), i.e. TP over all significant results,
    - recall = TP/(TP + FN), i.e. TP over (actual) positive results.
- **iCOBRA** is a very useful R package for plotting these curves.

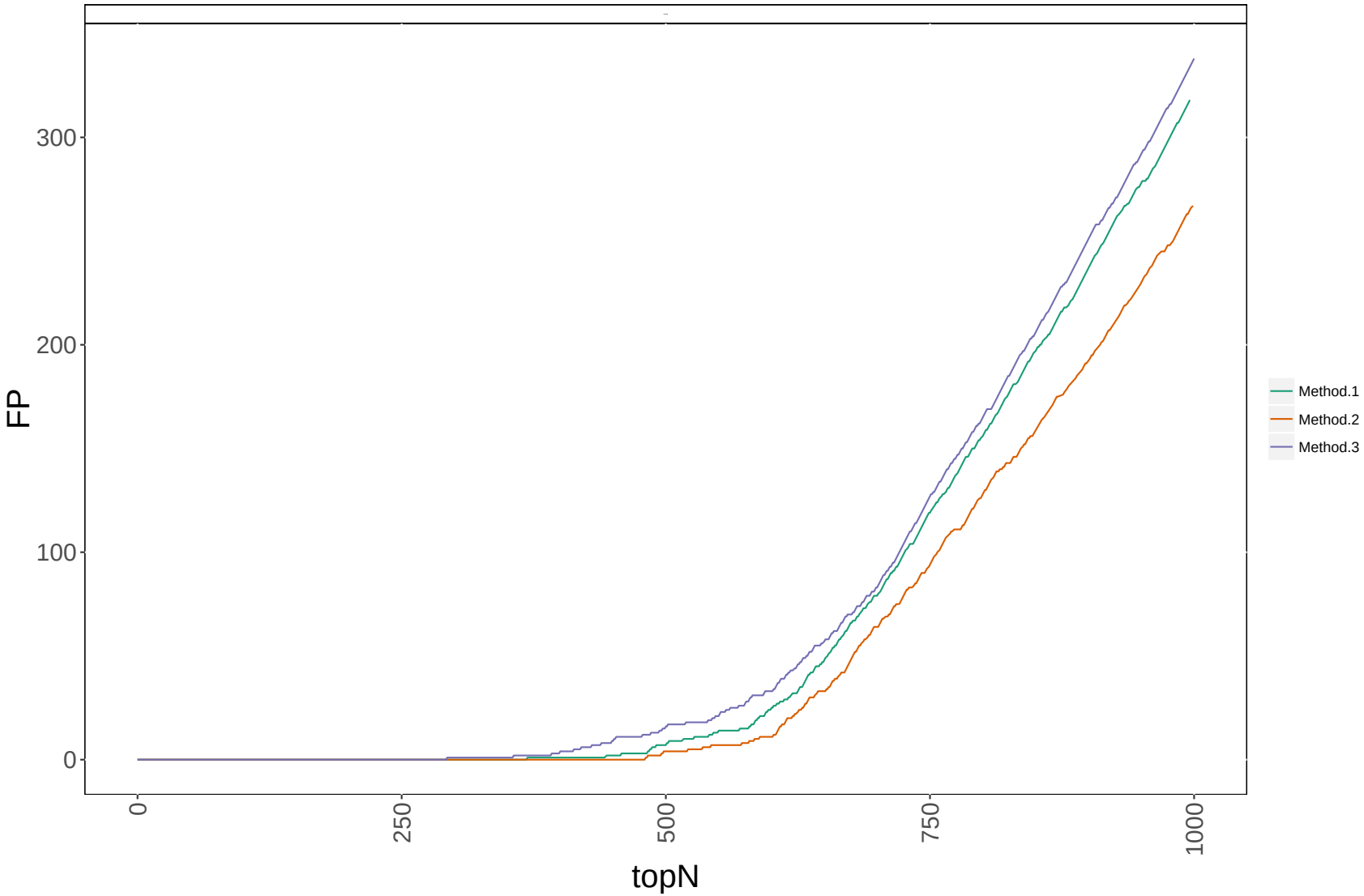
ROC curve


overall
1.00
0.75
0.50
0.25
0.00
TPR
0.00
0.25
0.50
0.75
1.00
FPR
Method.1
Method.2
Method.3

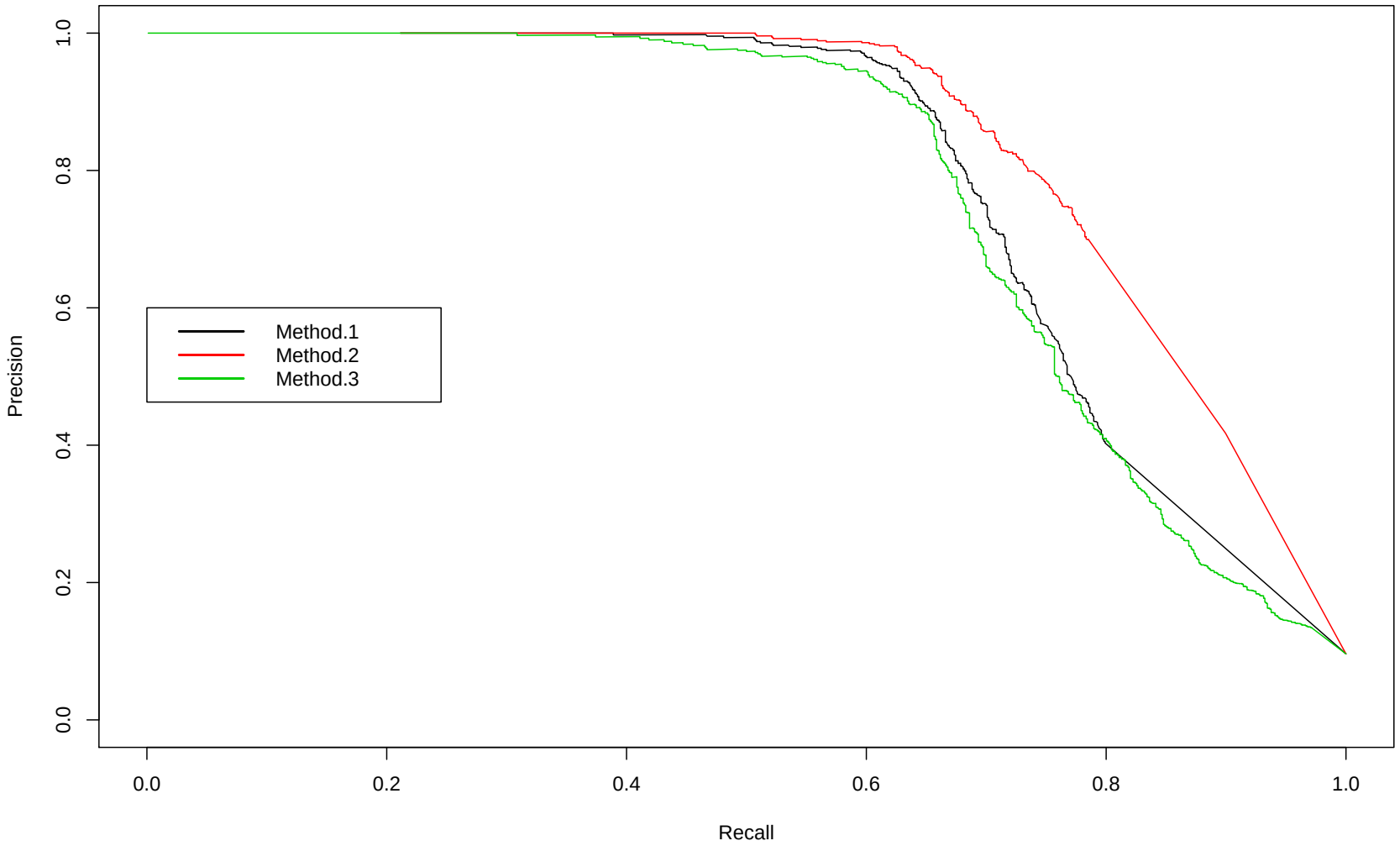
FDR plot


overall
1.00
0.75
0.50
0.25
0.00
TPR
0.01
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
FDR
Method.1
Method.2
Method.3

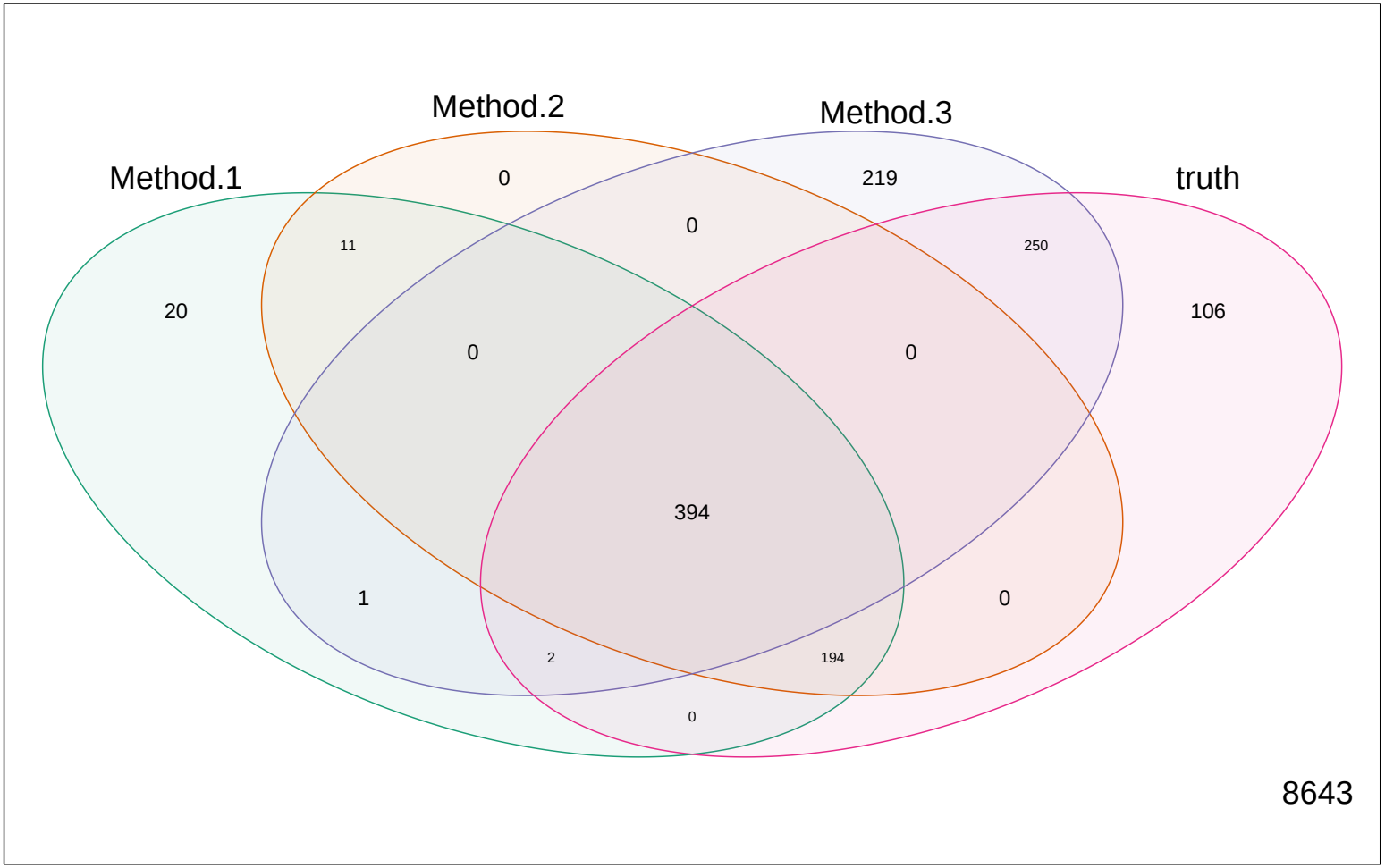
FPs vs top selected genes



Precision recall plot



Venn diagram



What comes after RNA-seq studies?

- The main aim of RNA-seq studies is to identify genes (but also transcripts or pathways) which show interesting characteristics (DGE, differential splicing, etc...): these genes will typically be studied more in depth in further biological analyses.
- This is why we are mostly interested in selecting correctly the top genes: we often want to select a small amount of (very) significant genes, with very few FPs, that might undergo further studies.

Questions?