

# The heterosis problem: a comparison of Eric's method with edgeR, baySeq, and ShrinkBayes

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# Mock heterosis data

|     | Parent (1)    |     |     |     | Parent (2) |      |     |     | Hybrid (3) |     |     |     | Truth |     |
|-----|---------------|-----|-----|-----|------------|------|-----|-----|------------|-----|-----|-----|-------|-----|
| HPH | Feature 1     | 3   | 4   | 2   | 1          | 0    | 0   | 1   | 0          | 700 | 900 | 825 | 860   | 1   |
| HPH | Feature 2     | 0   | 1   | 1   | 0          | 2    | 7   | 5   | 18         | 50  | 501 | 400 | 90    | 1   |
|     | Feature 3     | 100 | 225 | 0   | 15         | 300  | 106 | 200 | 400        | 70  | 279 | 100 | 123   | 0   |
| LPH | Feature 4     | 893 | 400 | 760 | 901        | 1000 | 513 | 760 | 580        | 5   | 5   | 6   | 7     | 1   |
|     | ...           | ... | ... | ... | ...        | ...  | ... | ... | ...        | ... | ... | ... | ...   | ... |
|     | Feature 25000 | 10  | 13  | 6   | 4          | 902  | 912 | 999 | 825        | 819 | 761 | 800 | 465   | 0   |

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# Simulation workflow

- ▶ Simulate 30 datasets:
  - ▶ 10 datasets with 4 samples (libraries, columns, etc.) per group
  - ▶ 10 with 8 per group
  - ▶ 10 with 16 per group
- ▶ For each simulated dataset, test for heterosis with
  - ▶ empirical Bayes with STAN (Eric's method)
  - ▶ edgeR
  - ▶ baySeq
  - ▶ ShrinkBayes
- ▶ Compare methods with ROC curves
- ▶ Check FDR control

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# Apply edgeR to real data to get simulation parameters

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Normalization factors

|       |       |       |       |       |       |       |       |       |          |          |          |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|----------|
| $c_1$ | $c_2$ | $c_3$ | $c_4$ | $c_5$ | $c_6$ | $c_7$ | $c_8$ | $c_9$ | $c_{10}$ | $c_{11}$ | $c_{12}$ |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|----------|----------|----------|

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Main effects and dispersions

| Parent (1)      | Parent (2)      | Hybrid (3)      | Dispersion     |
|-----------------|-----------------|-----------------|----------------|
| $\mu_{1,1}$     | $\mu_{1,2}$     | $\mu_{1,3}$     | $\psi_1$       |
| $\mu_{2,1}$     | $\mu_{2,2}$     | $\mu_{2,3}$     | $\psi_2$       |
| ...             | ...             | ...             | ...            |
| $\mu_{27888,1}$ | $\mu_{27888,2}$ | $\mu_{27888,3}$ | $\psi_{27888}$ |

# Generating the data

- ▶ Does feature  $f$  truly have heterosis?

$$\text{truth}_f = I(\mu_{f,3} > \max(\mu_{f,1}, \mu_{f,2}) \text{ or } \mu_{f,3} < \min(\mu_{f,1}, \mu_{f,2}))$$

- ▶ For a dataset with 4 libraries per group,

$$y_{f,i} \stackrel{\text{iid}}{\sim} \text{NB}(\exp(c_i + \mu_{f,t(i)}), \psi_f)$$

- ▶  $t(i)$  is the group of library  $i$ .
  - ▶ Resimulate to increase the number of libraries per group.
- ▶ Remove extremely low-count features.
- ▶ Take a random subset of 25000 features from the remaining ones.

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# Mock example data with 4 samples per treatment group

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|-----|---------------|-----|-----|-----|------------|------|-----|-----|------------|-----|-----|-----|-------|-----|
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|     | ...           | ... | ... | ... | ...        | ...  | ... | ... | ...        | ... | ... | ... | ...   | ... |
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## edgeR

- ▶ Fit a loglinear model to estimate main effects  $\mu_{f,t}$ 
  - ▶ Feature  $f = 1, \dots, 25000$
  - ▶ Treatment group  $t = 1$  (parent),  $2$  (parent),  $3$  (hybrid)
- ▶ Likelihood ratio tests to get p-values  $p_{f,1}$ ,  $p_{f,2}$

$$H_{0,1} : \mu_{f,3} = \mu_{f,1} \quad H_{a,1} : \mu_{f,3} \neq \mu_{f,1}$$

$$H_{0,2} : \mu_{f,3} = \mu_{f,2} \quad H_{a,2} : \mu_{f,3} \neq \mu_{f,2}$$

- ▶ Final p-value:

$$p_{f,\text{edgeR}} = \begin{cases} p_{f,1}/2 & \hat{\mu}_{f,3} < \hat{\mu}_{f,1} \leq \hat{\mu}_{f,2} \text{ or } \hat{\mu}_{f,3} > \hat{\mu}_{f,1} \geq \hat{\mu}_{f,2} \\ p_{f,2}/2 & \hat{\mu}_{f,3} < \hat{\mu}_{f,2} \leq \hat{\mu}_{f,1} \text{ or } \hat{\mu}_{f,3} > \hat{\mu}_{f,2} \geq \hat{\mu}_{f,1} \\ 1 & \hat{\mu}_{f,1} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,2} \text{ or } \hat{\mu}_{f,2} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,1} \end{cases}$$

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# ShrinkBayes

- ▶ Built on `inla` (integrated nested Laplace approximation).
- ▶ empirical Bayes with a zero-inflated NB likelihood and normal priors.
- ▶ I reparameterize

$$\phi_f = \frac{\mu_{f,1} + \mu_{f,2}}{2} \quad (\text{parental mean})$$

$$\alpha_f = \frac{\mu_{f,2} - \mu_{f,1}}{2} \quad (\text{half parental difference})$$

$$\delta_f = \mu_{f,3} - \frac{\mu_{f,1} + \mu_{f,2}}{2} \quad (\text{hybrid effect})$$

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# ShrinkBayes

$\alpha_f$  = half parental difference

$\delta_f$  = hybrid effect

- Use contrasts to calculate final posterior probabilities of heterosis:

$$p_{f, \text{ShrinkBayes}} = \begin{cases} 0 & \left| \hat{\delta}_f \right| < \left| \hat{\alpha}_f \right|, \text{ o.w.:} \\ P(\delta_f + \alpha_f > 0 \mid \text{data}) & \hat{\delta}_f > -\hat{\alpha}_f \\ P(\delta_f - \alpha_f > 0 \mid \text{data}) & \hat{\delta}_f > \hat{\alpha}_f \\ P(\delta_f - \alpha_f < 0 \mid \text{data}) & \hat{\delta}_f < \hat{\alpha}_f \\ P(\delta_f + \alpha_f < 0 \mid \text{data}) & \hat{\delta}_f < -\hat{\alpha}_f \end{cases}$$

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# baySeq

- ▶ Estimate main effects  $\mu_{f,t}$  using edgeR.
- ▶ Calculate the posterior probability that each feature satisfies:

| Model | Constraint                  |
|-------|-----------------------------|
| $M_1$ | All $\mu_{f,t}$ 's equal    |
| $M_2$ | $\mu_{f,1} = \mu_{f,2}$     |
| $M_3$ | $\mu_{f,1} = \mu_{f,3}$     |
| $M_4$ | $\mu_{f,2} = \mu_{f,3}$     |
| $M_5$ | All $\mu_{f,t}$ 's distinct |

- ▶ Final posterior probabilities of heterosis:

$$p_{f,\text{baySeq}} = \begin{cases} 0 & \hat{\mu}_{f,1} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,2} \text{ or} \\ & \hat{\mu}_{f,2} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,1} \\ P(M_2 \mid \text{data}) + & \\ P(M_5 \mid \text{data}) & \text{otherwise} \end{cases}$$

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# Calculating false positive rate (FPR) and true positive rate (TPR)

- ▶ ROC = Receiver Operating Characteristic
- ▶  $N_{\text{true}}$  heterosis features,  $N_{\text{false}}$  null features.
- ▶ Results of testing each feature for heterosis
  - ▶ For edgeR, the ranks are p-values.
  - ▶ For the other methods, take  $p(\text{no heterosis} \mid \text{data})$  for each feature.

|       |       |       |       |       |     |       |       |
|-------|-------|-------|-------|-------|-----|-------|-------|
| rank  | 0.802 | 0.935 | 0.539 | 0.001 | ... | 0.500 | 0.603 |
| truth | 0     | 0     | 1     | 1     | ... | 1     | 0     |

- ▶ Sort by rank.

|       |       |       |       |       |     |       |       |
|-------|-------|-------|-------|-------|-----|-------|-------|
| rank  | 0.000 | 0.001 | 0.005 | 0.006 | ... | 0.901 | 1.000 |
| truth | 1     | 1     | 0     | 1     | ... | 0     | 0     |

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# Calculating false positive rate (FPR) and true positive rate (TPR)

- ▶ In practice, we would declare the lowest-rank features to have heterosis.

|       |       |       |       |       |     |       |       |
|-------|-------|-------|-------|-------|-----|-------|-------|
| rank  | 0.000 | 0.001 | 0.005 | 0.006 | ... | 0.901 | 1.000 |
| truth | 1     | 1     | 0     | 1     | ... | 0     | 0     |

- ▶ With 2 heterosis genes and 1 null gene, say,

$$FPR = \frac{1}{N_{false}} \quad TPR = \frac{2}{N_{true}}$$

- ▶ Repeat for multiple cutoffs to get multiple (FPR, TPR) pairs.

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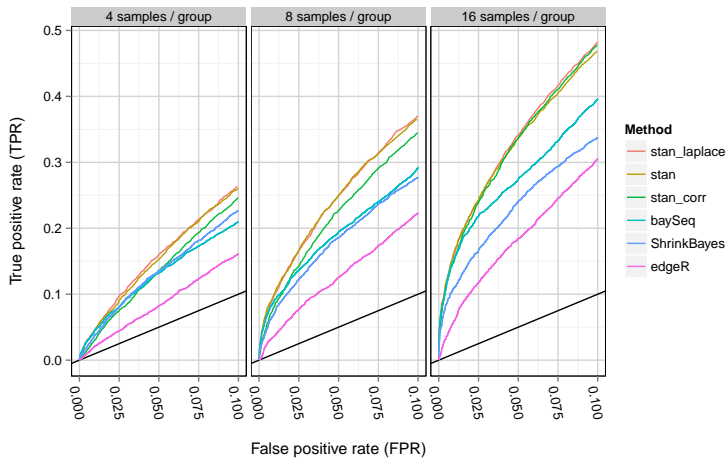
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# Example ROC curves



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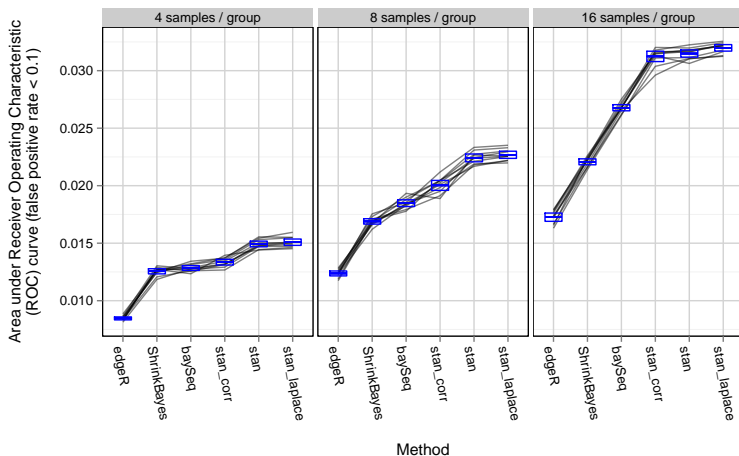
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# Areas under ROC curves



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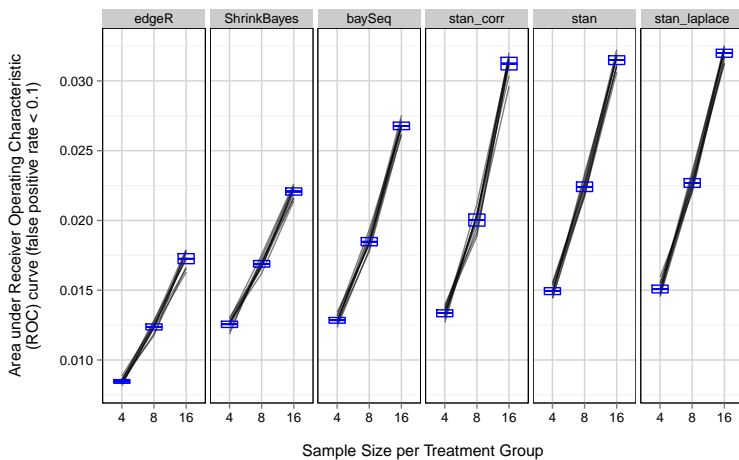
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# FDR control procedures

- ▶ Let  $p_{(f)}$  ( $f = 1, \dots, 25000$ ) be the ordered ranks
  - ▶ For edgeR, the ranks are now q-values.
  - ▶ For the other methods, take  $p(\text{no heterosis} \mid \text{data})$  for each feature.

- ▶ Let  $I_{(f)} = I(\text{feature of } p_{(f)} \text{ is not heterosis})$

- ▶ Method 1:

$$\bar{p}_m = \frac{1}{m} \sum_{f=1}^m p_{(f)} \quad \bar{I}_m = \frac{1}{m} \sum_{f=1}^m I_{(f)}$$

- ▶ To control FDR, we need  $\bar{I}_m \leq \bar{p}_m$ .
  - ▶ Plot  $\bar{I}_m - \bar{p}_m$  vs  $\bar{p}_m$  for multiple  $m$ .
- ▶ Method 2:

$$\bar{p}_m = \frac{1}{100} \sum_{f=m}^{m+100} p_{(f)} \quad \bar{I}_m = \frac{1}{100} \sum_{f=m}^{m+100} I_{(f)}$$

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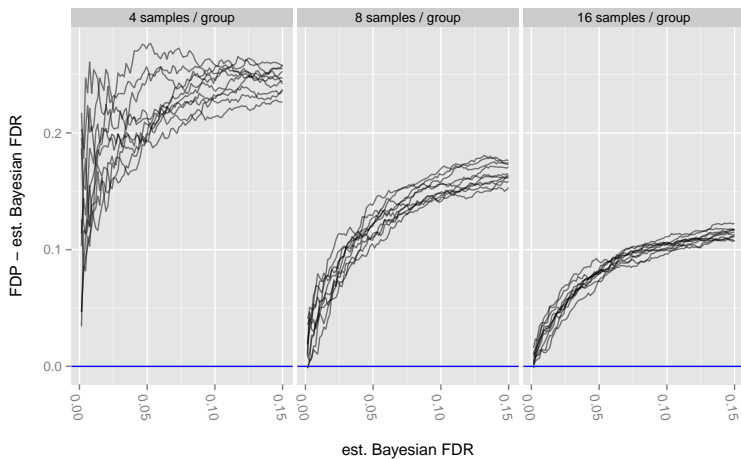
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# Method 1: stan\_laplace



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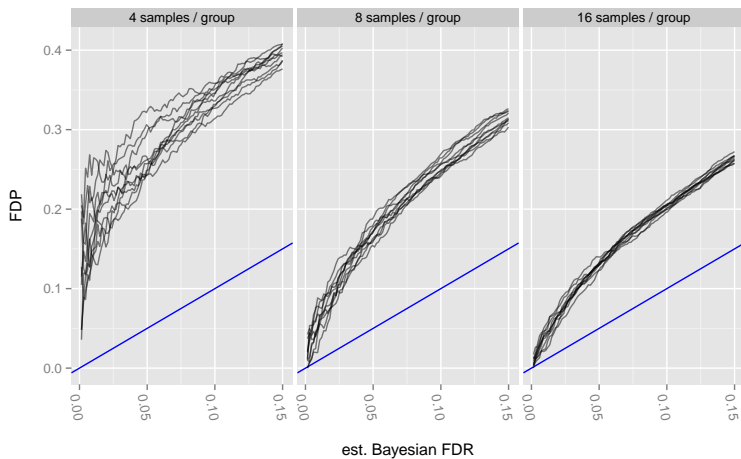
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# Method 1: stan\_laplace



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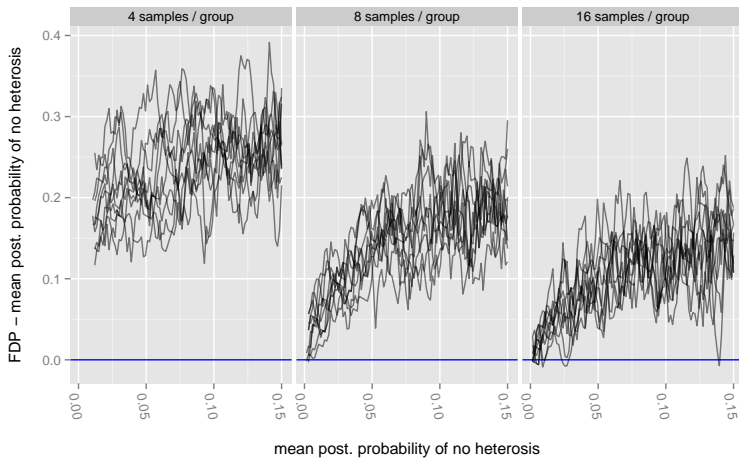
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## Method 2: stan\_laplace



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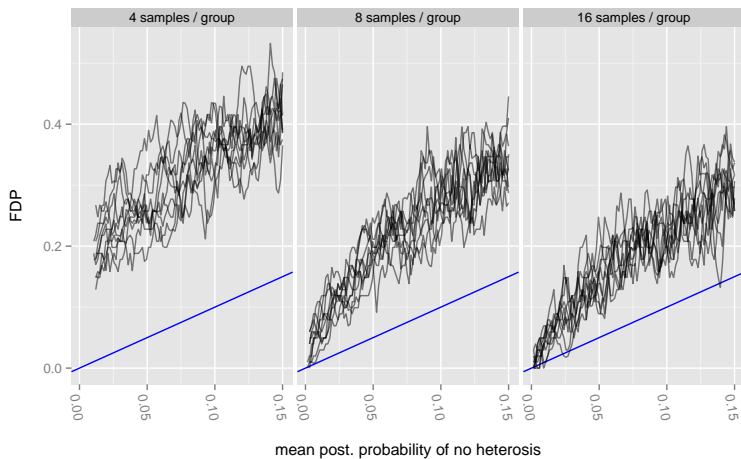
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## Method 2: stan\_laplace



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