Will Landau, Eric Mittman

Iowa State University

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

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imulated data

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ROC (receiver operating characteristic) curves

The results

#### Outline

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#### The problem

#### Mock heterosis data

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

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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

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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

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edgeR

#### Simulation workflow

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  - ▶ 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

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### Apply edgeR to real data to get simulation parameters

Normalization factors



Main effects and dispersions

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

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

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

$$y_{f,i} \stackrel{\text{iid}}{\sim} NB \left( \exp \left( c_{\lceil 4i/N \rceil} + \mu_{f,\lceil i/N \rceil} \right), \ \psi_f \right)$$

- where:
  - ▶ Sample (library, column) i = 1, ..., 3N
  - $\triangleright$  N =samples per treatment group (4, 8, or 16)

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- where:
  - ▶ Sample (library, column) i = 1, ..., 3N
  - ightharpoonup N =samples per treatment group (4, 8, or 16)
- Remove extremely low-count features.

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- where:
  - ▶ Sample (library, column) i = 1, ..., 3N
  - ightharpoonup N =samples per treatment group (4, 8, or 16)
- ▶ 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

			Pare	nt (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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#### The contenders

- Fit a loglinear model to estimate main effects  $\mu_{f,t}$ 
  - Feature f = 1, ..., 25000
  - ▶ Treatment group t = 1 (parent), 2 (parent), 3 (hybrid)

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imulated data

edgeR baySeq

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

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

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

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

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The results

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

Final p-value	
$p_{f,1}/2$	
$p_{f,2}/2$	$ \widehat{\mu}_{f,3} < \widehat{\mu}_{f,2} \le \widehat{\mu}_{f,1} \text{ or } \widehat{\mu}_{f,3} > \widehat{\mu}_{f,2} \ge \widehat{\mu}_{f,1} $
1	$\widehat{\mu}_{f,1} \leq \widehat{\mu}_{f,3} \leq \widehat{\mu}_{f,2} \text{ or } \widehat{\mu}_{f,2} \leq \widehat{\mu}_{f,3} \leq \widehat{\mu}_{f,1}$

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#### baySeq

▶ Estimate main effects  $\mu_{f,t}$  using edgeR.

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Simulated data

edgeR
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

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$M_4$	$\mu_{f,2} = \mu_{f,3}$
$M_5$	All $\mu_{f,t}$ 's distinct

▶ Final posterior probabilities of heterosis:

Posterior probability	if
0 $P(M_3 \mid data) + P(M_5 \mid data)$	$\widehat{\mu}_{f,1} \leq \widehat{\mu}_{f,3} \leq \widehat{\mu}_{f,2}$ or
	$\widehat{\mu}_{f,2} \leq \widehat{\mu}_{f,3} \leq \widehat{\mu}_{f,1}$
$P(M_3 \mid data) + P(M_5 \mid data)$	otherwise

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#### ShrinkBayes

▶ Built on inla (integrated nested Laplace approximation).

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baySeq

#### ShrinkBayes

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

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edgeR baySeq

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

$$\begin{split} \phi_f &= \frac{\mu_{f,1} + \mu_{f,2}}{2} \qquad \text{(parental mean)} \\ \alpha_f &= \frac{\mu_{f,2} - \mu_{f,1}}{2} \qquad \text{(half parental difference)} \\ \delta_f &= \mu_{f,3} - \frac{\mu_{f,1} + \mu_{f,2}}{2} \qquad \text{(hybrid effect)} \end{split}$$

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#### ShrinkBayes

$\phi_{f}$	$\alpha_{f}$	$\delta_f$
parental mean	half parental difference	hybrid effect

Use contrasts to calculate final posterior probabilities of heterosis:

· cococ. p. cococy	if
0	$ \widehat{\delta}_f  <  \widehat{\alpha}_f $ , otherwise:
$P(\delta_f + lpha_f > 0 \mid data)$	$ \widehat{\delta}_f > -\widehat{\alpha}_f $
$P(\delta_f - lpha_f > 0 \mid data)$	$ \widehat{\delta}_f > \widehat{\alpha}_f $
$P(\delta_f - lpha_f < 0 \mid data)$	$\widehat{\delta}_f < \widehat{\alpha}_f$
$egin{aligned} 0 \ P(\delta_f + lpha_f > 0 \mid data) \ P(\delta_f - lpha_f > 0 \mid data) \ P(\delta_f - lpha_f < 0 \mid data) \ P(\delta_f + lpha_f < 0 \mid data) \end{aligned}$	$ \widehat{\delta}_f < -\widehat{\alpha}_f $

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

 $\triangleright$   $N_{\text{true}}$  heterosis features,  $N_{\text{false}}$  null features.

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Simulated data

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- $\triangleright$   $N_{\text{true}}$  heterosis features,  $N_{\text{false}}$  null features.
- ► Results of testing each feature for heterosis (25000 columns here):

pval	0.802	0.935	0.539	0.001		0.500	0.603
truth	0	0	1	1		1	0

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pval	0.802	0.935	0.539	0.001		0.500	0.603
truth	0	0	1	1		1	0

Sort table by p-value (or other binary classifier)

pval	0.000	0.001	0.005	0.006		0.901	1.000
truth	1	1	0	1		0	0

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

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

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pval	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,

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

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pval	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,

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

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

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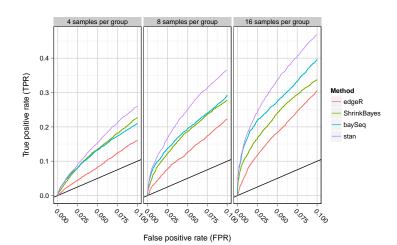
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#### Example ROC curves



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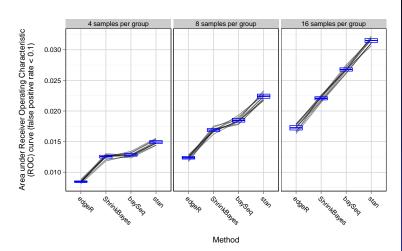
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#### Areas under ROC curves



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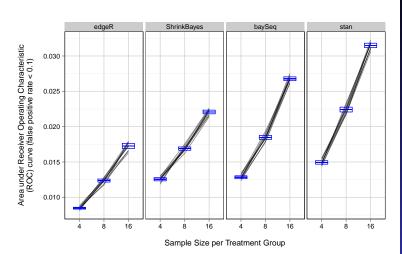
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