

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

Will Landau, Eric Mittman

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The problem

The workflow

Simulated data

The contenders

edgeR
baySeq
ShrinkBayes

The contest

ROC (receiver operating characteristic) curves
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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

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- ▶ 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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 - ▶ 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

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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}
-------	-------	-------	-------	-------	-------	-------	-------	-------	----------	----------	----------

Main effects and dispersions

Parent (1)	Parent (2)	Hybrid (3)	Dispersion
$\mu_{1,1}$	$\mu_{1,2}$	$\mu_{1,3}$	ψ_1
$\mu_{2,1}$	$\mu_{2,2}$	$\mu_{2,3}$	ψ_2
...
$\mu_{27888,1}$	$\mu_{27888,2}$	$\mu_{27888,3}$	ψ_{27888}

A single simulation (of 30)

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

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A single simulation (of 30)

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

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

► where:

- Sample (library, column) $i = 1, \dots, 3N$
- N = samples per treatment group (4, 8, or 16)

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

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

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

- ▶ where:
 - ▶ Sample (library, column) $i = 1, \dots, 3N$
 - ▶ 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

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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
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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)

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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 $p_{f,1}$, $p_{f,2}$

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

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

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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 $p_{f,1}, p_{f,2}$

$$\begin{array}{ll} H_{0,1} : \mu_{f,3} = \mu_{f,1} & H_{a,1} : \mu_{f,3} \neq \mu_{f,1} \\ H_{0,2} : \mu_{f,3} = \mu_{f,2} & H_{a,2} : \mu_{f,3} \neq \mu_{f,2} \end{array}$$

Final p-value	if...
$p_{f,1}/2$	$\hat{\mu}_{f,3} < \hat{\mu}_{f,1} \leq \hat{\mu}_{f,2}$ or $\hat{\mu}_{f,3} > \hat{\mu}_{f,1} \geq \hat{\mu}_{f,2}$
$p_{f,2}/2$	$\hat{\mu}_{f,3} < \hat{\mu}_{f,2} \leq \hat{\mu}_{f,1}$ or $\hat{\mu}_{f,3} > \hat{\mu}_{f,2} \geq \hat{\mu}_{f,1}$
1	$\hat{\mu}_{f,1} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,2}$ or $\hat{\mu}_{f,2} \leq \hat{\mu}_{f,3} \leq \hat{\mu}_{f,1}$

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baySeq

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

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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

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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:

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

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ShrinkBayes

- Built on `inla` (integrated nested Laplace approximation).

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ShrinkBayes

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

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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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ϕ_f	α_f	δ_f
parental mean	half parental difference	hybrid effect

- Use contrasts to calculate final posterior probabilities of heterosis:

Posterior probability	if...
0	$ \hat{\delta}_f < \hat{\alpha}_f $, otherwise:
$P(\delta_f + \alpha_f > 0 \mid \text{data})$	$\hat{\delta}_f > -\hat{\alpha}_f$
$P(\delta_f - \alpha_f > 0 \mid \text{data})$	$\hat{\delta}_f > \hat{\alpha}_f$
$P(\delta_f - \alpha_f < 0 \mid \text{data})$	$\hat{\delta}_f < \hat{\alpha}_f$
$P(\delta_f + \alpha_f < 0 \mid \text{data})$	$\hat{\delta}_f < -\hat{\alpha}_f$

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

- ▶ N_{true} heterosis features, N_{false} null features.

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

- ▶ N_{true} heterosis features, N_{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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- ▶ 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

- ▶ 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

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

- ▶ 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,

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

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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,

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

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

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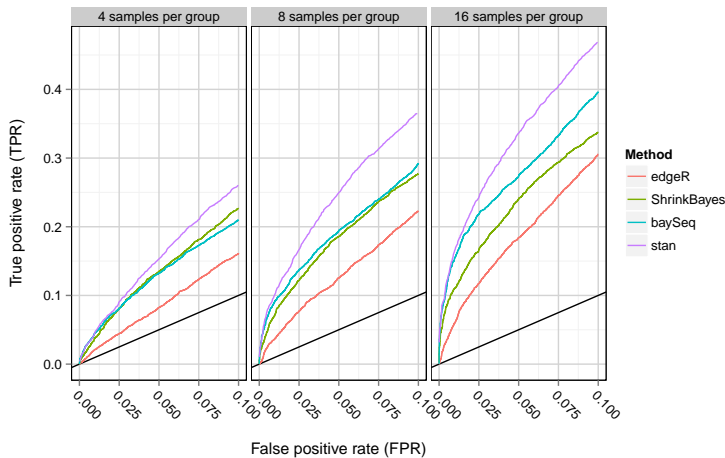
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Example ROC curves



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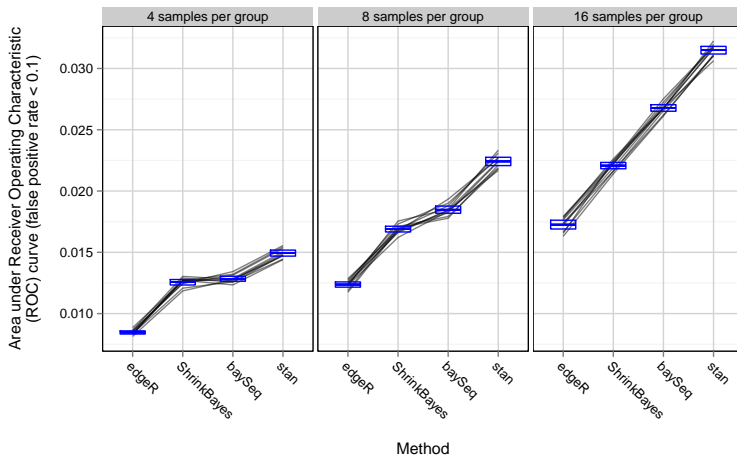
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Areas under ROC curves



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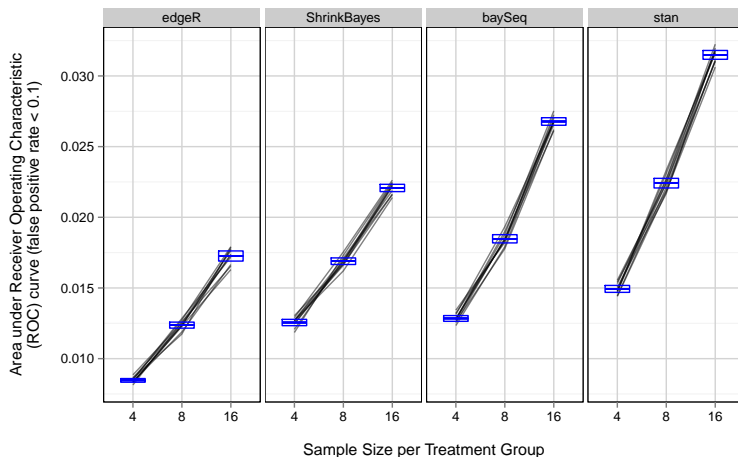
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