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

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Outline

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

Outline

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

Outline

Apply edgeR to real data to get simulation parameters

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}

Generating the data

- Does feature f truly have heterosis?

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

- For a dataset with 4 libraries per group,

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

  - $t(i)$ 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.

Mock example data with 4 samples per treatment group

		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

Outline

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

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

-

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

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	$\widehat{\mu}_{f,1} \le \widehat{\mu}_{f,3} \le \widehat{\mu}_{f,2}$ or $\widehat{\mu}_{f,2} \le \widehat{\mu}_{f,3} \le \widehat{\mu}_{f,1}$
$P(M_3 \mid \text{data}) + P(M_5 \mid \text{data})$	otherwise

ShrinkBayes

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

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

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

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

ShrinkBayes

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	$\lvert\widehat{\delta}_f\rvert < \lvert\widehat{\alpha}_f\rvert$, otherwise:
$P(\delta_f + \alpha_f > 0 \mid \text{data})$	$\widehat{\delta}_f > -\widehat{\alpha}_f$
$P(\delta_f - \alpha_f > 0 \mid \text{data})$	$\widehat{\delta}_f > \widehat{\alpha}_f$
$P(\delta_f - \alpha_f < 0 \mid \text{data})$	$\widehat{\delta}_f < \widehat{\alpha}_f$
$P(\delta_f + \alpha_f < 0 \mid \text{data})$	$\widehat{\delta}_f < -\widehat{\alpha}_f$

Outline

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	$\cdots$	0.500	0.603
truth	0	0	1	1	$\cdots$	1	0

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

pval	0.000	0.001	0.005	0.006	$\cdots$	0.901	1.000
truth	1	1	0	1	$\cdots$	0	0

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

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

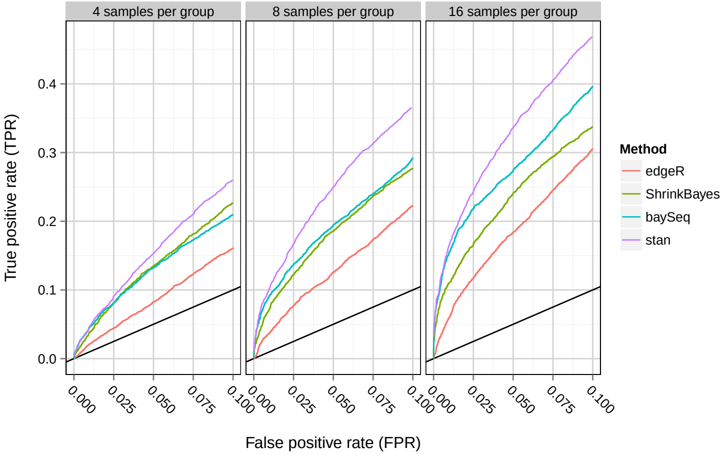
pval	0.000	0.001	0.005	0.006	⋯	0.901	1.000
truth	1	1	0	1	⋯	0	0

- With 2 heterosis genes and 1 null gene,

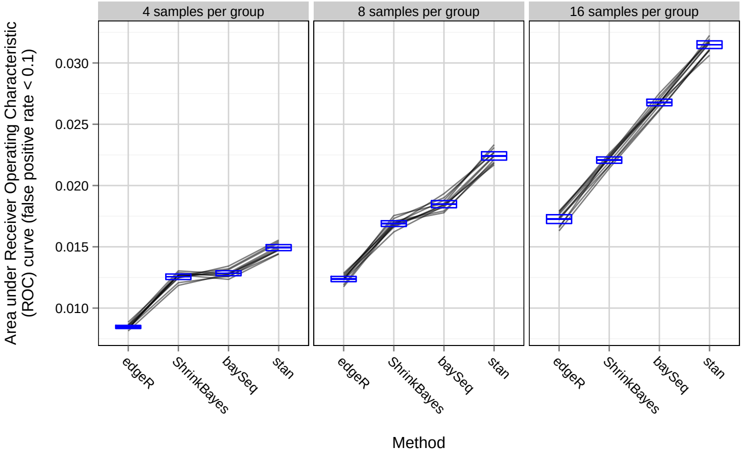
$$FPR = \frac{1}{N_{false}} \qquad TPR = \frac{2}{N_{true}}$$

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

Example ROC curves



Areas under ROC curves



Areas under ROC curves