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Gene expression

(Datta & Nettleton 2014, *Statistical Analysis of Next Generation Sequencing Data*)

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Gene expression is regulated by mRNA.

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Steps to producing RNA-seq data:

- isolate mRNA and fragment it
- match fragments back to genes/features (counts)

Motivating example

Heterosis

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- Low parent heterosis (LPH) $H < LP < HP$

RNA-seq data

	Population 1			Population 2		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Gene 1	7	2	0	14	18	41
Gene 2	55	42	40	32	22	37
Gene 3	41	40	32	61	61	60
Gene 4	40	43	35	15	24	39
raw library size	11569434	10079799	9028465	10028258	9010306	10283594

Data from Paschold et al. (2012)

Research goal: To use gene expression data to identify genes responsible for hybrid vigor (heterosis).

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- 2 recombinant inbred lines (homozygous): B73, Mo17
- 2 reciprocal hybrid crosses: B73×Mo17, Mo17×B73
- 4 replicates of each variety
- sequencing done on 2 flow cells, replicates balanced across flow cells

Normalization

Definitions

r_{gn} = count for gene g, sample n; $\quad g = 1, \dots, G, \quad n = 1, \dots, N$

R_n = library size for sample n

$$\log \tilde{R} = \frac{1}{N} \sum_{n=1}^{N} \log(R_n)$$

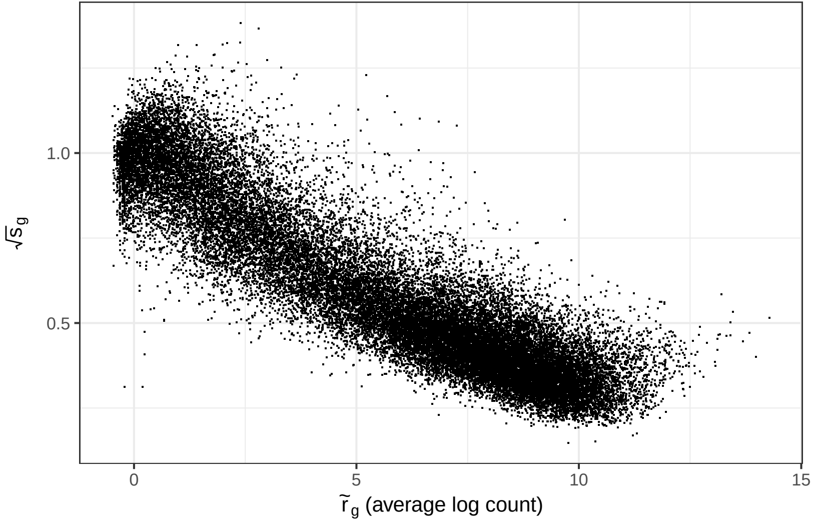
$$y_{gn} = \log_2 \left(\frac{r_{gn} + 0.5}{R_n + 1} \times 10^6 \right)$$

$$\tilde{r}_g = \frac{1}{N} \sum_{n=1}^{N} y_{gn} + \log_2(\tilde{R}) - \log_2(10^6)$$

X = model matrix

$s_g = \sqrt{MSE}$ from fitting a linear model, $y_g \sim N(X\beta_g, \sigma_g^2 I_n)$

Mean-variance in RNA-seq


$\sqrt{s_g}$
$\tilde{r}_g$ (average log count)