High-Throughput Sequencing Course

DESeq Model for RNA-Seq

Biostatistics and Bioinformatics

Summer 2018

Outline

- Review: Standard linear regression model (e.g., to model gene expression as function of an experimental condition or continuous covariate)
- Review: Logistic model: To model probability of abinary event as a function of a covariate
- Parameter interpretation: Linear and logistic regression
- Introduction: Negative binomial regression model for RNA-Seq
- Overview: Maximum likelihood estimation

Linear Regression Example: Gene Expression

- Consider the simple linear regression model

  $$Y = \beta_0 + \beta_1 x + \epsilon,$$

  where
  - $x = 0$ (untreated)
  - or $x = 1$ (treated)
- Y is the observed "expression" of the gene
- ϵ is the measurement noise term
- We assume that it follows a normal distribution with mean 0 and variance σ^2

Reminder: Important Fact about Normal Distribution

- Consider a normal distribution with mean 0 and standard deviation σ
- If the data are shifted by a constant μ, then
  1. resulting distribution remains normal
  2. The mean of the new distribution is $\mu + 0 = \mu$
  3. Its standard deviation remains unchanged
- The last two (but not first) property are true for any distribution
- Recall $Y = \beta_0 + \beta_1 x + \epsilon$
- Y follows a normal distribution with mean $\mu = \beta_0 + \beta_1 x$ and variance σ^2
- IMPORTANT: μ depends on x (unless of course $\beta_1 = 0$)

Linear Regression Example: Interpretation

- Model
$$Y = \beta_0 + \beta_1 x + \epsilon,$$
- The goal of (mean) regression is to estimate the expected value of Y given treatment status
- Conditional on $x = 0$ (i.e., not receiving treatment), the expected value of Y is
$$\beta_0 + \beta_1 \times 0 = \beta_0$$
- Conditional on $z = 1$ (i.e., receiving treatment), the expected value of Y is
$$\beta_0 + \beta_1 \times 1 = \beta_0 + \beta_1$$

General Conditional Expectation

- Expectation is another word for average
- We can write the conditional expectation of Y given that $X = x$ as $E[Y|X = x]$
- English: This is the average value of the outcome Y if the value of X is equal to x
- The unconditional expectation of Y is denoted by $E[Y]$
- If Y does not depend on X, then $E[Y|X = x] = E[Y]$ for every x
- The goal of linear regression is to model $E[Y|X = x]$ as "Linear" function
- Our Example: $E[Y|X = x] = \beta_0 + \beta_1 x$

Linear Regression Example: Interpretation

- Model
$$Y = \beta_0 + \beta_1 x + \epsilon,$$
- β_0 (the intercept) is the expected value of Y if no treatment is administered (average baseline value)
- β_1 is the treatment effect
- If treatment is administered, the expected value of expression is
  - increased by β_1 units if $\beta_1 > 0$
  - decreased by β_1 units if $\beta_1 < 0$
  - unchanged if $\beta_1 = 0$

Linear Regression Example: Continuous covariate

- Model
$$Y = \beta_0 + \beta_1 x + \epsilon,$$
where x is continuous (quantitative)
-
  - If $\beta_1 > 0$, then increasing x by one unit, increases Y on average by β_1 units
  - If $\beta_1 < 0$, then increasing x by one unit, decreases Y on average by β_1 units
  - If $\beta_1 = 0$, then changes in x do not affect the expected value of Y

Regression for Binary Outcomes

- Suppose that Y is a binary outcome
- It assumes values 0 or 1
- This is a count outcome
- Consider the previous model
$$Y = \beta_0 + \beta_1 x + \epsilon,$$
- Is it appropriate? Why or why not?

Logistic Regression

- Relate the probability of the outcome of the event $Y = 1$ to treatment
- More specifically, relate the log-odds to the treatment
- The log-odds will be modeled as a linear function of x

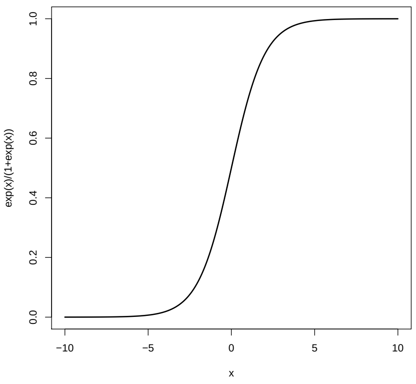
$$\beta_0 + \beta_1 x + \epsilon$$

- This is an example of a generalized linear model (GLM)
- Note: The model used by DESeq is a GLM on the basis of the NB (instead of binomial distribution)
- The expected outcome of Y is not modeled directly as a linear function
- A transformation of the expected outcome of Y is modeled as a linear function

Expected value of a binary event

- Suppose that Y assumes 1 with probability π or 0 with probability $1 - \pi$
- $P(Y = 1) = \pi$ and $P(Y = 0) = 1 - \pi$
- IMPORTANT: $P(Y = 1) = E(Y)$
- The expected value of Y is the probability that it assumes the value 1
- Why?

Relationship between x and $\frac{\exp(x)}{1+\exp(x)}$



Odds vs Probability

- Suppose that $\pi = P(Y = 1)$
- The odds of the event $Y = 1$ (to occur) is defined as

$$\text{Odds}[Y = 1] = \frac{\text{Probability that } Y = 1 \text{ occurs}}{\text{Probability that } Y = 1 \text{ does not occur}} = \frac{\pi}{1 - \pi}$$

Odds Ratio Versus Relative Risk

- $\pi_0 = P[Y = 1|X = 0]$: Probability that the event occurs if sample is not treated
- $\pi_1 = P[Y = 1|X = 1]$: Probability that the event occurs if $X = 1$sample is treated
- The odds-ratio is

$$\text{OR} = \frac{\frac{\pi_1}{1-\pi_1}}{\frac{\pi_0}{1-\pi_0}}$$

- The relative risk is

$$\text{RR} = \frac{\pi_1}{\pi_0}$$

The Logistic Model

- The log-odds of the event $Y = 1$

$$\log \frac{P(Y = 1|X = x)}{1 - P(Y = 1|X = x)} = \beta_0 + \beta_1 x$$

- or equivalently

$$\log \frac{E(Y|X = x)}{1 - E(Y|X = x)} = \beta_0 + \beta_1 x$$

- or equivalently

$$P(Y = 1|X = x) = E(Y|X = x) = \frac{\exp(\beta_0 + \beta_1 x)}{1 + \exp(\beta_0 + \beta_1 x)}$$

Parameter Interpretation

- If $\beta_1 > 0$, a unit increase in x, results in an expected increase of $\exp(\beta_1)$ in the odds of the event
- If $\beta_1 < 0$, a unit increase in x, results in an expected decrease of $\exp(\beta_1)$ in the odds of the event
- If $\beta_1 = 0$, then changes in x do not affect the odds of realization of the event

Link Function

- For a probability π, define the "logit" transformation as

$$\log \frac{\pi}{1-\pi}$$

- This is the log-odds of an event with probability π
- Note that in the logistic model, the probability of the event is linear in the parameter through this logit transformation

$$\log \frac{E(Y|X=x)}{1-E(Y|X=x)} = \beta_0 + \beta_1 x$$

- In the GLM literature, this is called the link function

Overdispersion

- Recall that if K follows a binomial distribution with parameters n and π, then
  - mean $\mu = n\pi$
  - variance $\sigma^2 = n\pi(1-\pi)$
- Clustering in the data results in the actual variance to be different than the nominal variance $(n\pi(1-\pi))$
  - Overdispersion: Actual variance is larger than nominal variance
  - Underdispersion: Actual variance is smaller than nominal variance
- The choice of a GLM and evaluation of its performance *should* start and end with considering/addressing the overdispersion issue
- The use of Poisson (actually a variation thereof) and Negative Binomial models are two common choices for GLM for overdispersed data

Generalized Linear Models (GLM)

Define $\mu_x = E(Y|X = x)$ as the expected value of the outcome given treatment status ($x = 0$ or $x = 1$)

Distribution	Link	Mean	
Binomial	$0, 1, \ldots, n$	$\beta_0 + \beta_1 x = \log \frac{\mu_x}{1-\mu_x}$	$\mu_x = \frac{\exp(\beta_0+\beta_1 x)}{1+\exp(\beta_0+\beta_1 x)}$
Poisson	$0, 1, 2, \ldots$	$\beta_0 + \beta_1 x = \log(\mu_x)$	$\mu_x = \exp(\beta_0 + \beta_1 x)$
Negative Binomial	$0, 1, 2, \ldots$	$\beta_0 + \beta_1 x = \log(\mu_x)$	$\mu_x = \exp(\beta_0 + \beta_1 x)$

General Note

- Recall the simple linear regression model for expression

  $$Y = \beta_0 + \beta_1 x + \epsilon,$$

  where
  - $x = 0$ (untreated)
  - or $x = 1$ (treated)
- Y is the observed "expression" of the gene
- ϵ is the measurement noise term
- The parameter of interest is β_1 (the treatment effect)
- There are two other unknown parameters, β_0 and σ^2 the estimation procedure has to deal with in a *principled* manner
- β_0 and σ^2 are *nuisance* parameters
- They are not of primary (or any) interest. But you have to deal with them!

General Hypothesis

- Is the RNA abundance level for any of the m genes affected by treatment
- Let H_j denote the null hypothesis for gene j
- H_j: The RNA abundance level for gene j is not affected by treatment
- $\bar{H}_j$: The RNA abundance level for gene j is affected by treatment
- The global null hypothesis: H_1 and H_2 and .... and H_m are all true
- The global alternative: $\bar{H}_1$ or $\bar{H}_2$ or .... or $\bar{H}_m$ is true
- In other words, under the alternative at least one of the marginal null hypotheses is false

Observed Data

- Some notation
  - n denotes the number of samples
  - m denotes the number of genes
  - K_{ij} denotes the *observed* number of reads mapped to gene i for sample j
  - $x_j = 0$ or 1 denotes the treatment status for sample j
- What is observed for sample j is the vector

$$K_{1j}, \ldots, K_{mj}, x_j$$

- In other words m counts (one per gene) and the experimental factor
- Note that the K_{ij} form a table of counts of dimension $n \times m$ (n samples and m genes)

DESeq: Notation for Negative Binomial Distribution

- The count K is assumed to follow a negative binomial distribution with parameters $p \in (0,1)$ and $r > 1$
- The distribution is PMF is

$$P(K = k) = \binom{k + r - 1}{r - 1} p^r (1-p)^k,$$

  for $k = r, r+1, \ldots$
- Rather than considering the model as NB$[p, r]$ we will consider it as NB$[\mu, \alpha]$, where

$$P[K = k] = \frac{\Gamma[k + \alpha^{-1}]}{\Gamma[\alpha^{-1}]\Gamma[k+1]} \left(\frac{1}{1 + \mu\alpha}\right)^{\alpha^{-1}} \left(\frac{\mu}{\alpha^{-1} + \mu}\right)^k,$$

  where $k = 0, 1, \ldots$

DESeq: Notation

- K_{ij} denotes the *observed* number of reads mapped to gene i for sample j
- K_{ij} follows a negative binomial distribution with
  - Mean μ_{ij} (indexed by gene i *and* sample j)
  - Dispersion parameter α_i (indexed by the gene i)
- The mean is assumed to be $\mu_{ij} = s_j q_{ij}$ where
  - $\log q_{ij} = \beta_{i0} + \beta_{i1} x_j$
  - s_j is a gene j specific normalization constant

DESeq: Reformulate Hypotheses

- Hypotheses of interest
  - The global null hypothesis: H_1 and H_2 and .... and H_m are all true
  - The global alternative: $\bar{H}_1$ or $\bar{H}_2$ or .... or $\bar{H}_m$ is true
- Reformulation
  - The global null hypothesis: $\beta_{11} = 0$ and $\beta_{21} = 0$ and .... and $\beta_{m1} = 0$
  - In other words, all of the β_{j1} are equal to zero
  - The global alternative: $\beta_{11} \neq 0$ or $\beta_{21} = 0$ or .... or $\beta_{m1} = 0$
  - In other words, at least one of the β_{j1} is not equal to zero

DESeq: Assumption on Distribution

K_{ij} follows a negative binomial distribution with mean μ and dispersion parameter α

DESeq: Assumption on Mean of Distribution

- Conditional on the treatment status of sample j ($x_j = 0$ or 1), the expected value of K_{ij} is

$$\mu_{ij} = s_j \times q_{ij}$$

  where

$$\log q_{ij} = \beta_{i0} + \beta_{i1} x_j$$

- Note that two regression parameters are indexed by i
- Why? Because these are gene i specific parameters
- Why is x_j not indexed by i?
- Final Assumption: $s_{ij} = s_j$
- In other words: Within sample j, the normalization parameter is constant across the genes
- How many assumptions so far?

DESeq: Main parameters and Nuisance Parameters

- The m main parameters of interest

$$\beta_{11}, \dots, \beta_{m1}$$

- The unknown nuisance parameters are
  - The m gene specific intercepts

$$\beta_{10}, \dots, \beta_{m0}$$

  - the n sample specific normalization constants

$$s_1, \dots, s_n$$

  - The m gene specific nuisance parameters

$$\alpha_1, \dots, \alpha_m$$

DESeq: Main parameters and Nuisance Parameters

- Assuming the model assumptions are correct, the estimation of the regression parameters β_{i0}, β_{i1} is fairly straightforward
- The DESeq authors propose to estimate the normalization constant for sample j as

$$s_j = \text{median} \frac{K_{ij}}{K_i^R},$$

where

$$K_i^R = \left(\prod_{j=1}^{m} K_{ij} \right)^{\frac{1}{m}}$$

- Here K_i^R is the geometric mean of $K_{i1}, \dots, K_{in}$ (the n counts for gene i)
- The median is taken over all m genes for which K_i^R is positive

DESeq: Dispersion parameter

- A key issue in using the NB model is proper handling of the gene specific dispersion parameters

$$\alpha_1, \dots, \alpha_m$$

- The estimation of the dispersion parameter is a challenging task
- DESeq2 assumes that α_i is random following a normal distribution
- The results are sensitive to the estimates
- One of the key differences between DESeq2 and DESeq is the approach taken to estimate these nuisance parameters

DESeq Software Overview

- The analysis of RNA-Seq data using the `DESeq2` package will be reviewed in detail in the upcoming weeks
- The estimation and inference for the model is done through the `DESeq` function
- It performs the following steps in the order give
  1. estimation of size factors $s_1, \dots, s_n$
  2. estimation of dispersion parameters $\alpha_1, \dots, alpha_m$
  3. Fit NB GLM model

DESeq: Model Exercise

- K_{ij} denotes the *observed* number of reads mapped to gene i for sample j
- $x_j = 0$ or 1 denotes the treatment status for sample j
- Say we want to account for another covariate z_j (e.g., temperature)
- What is observed for sample j is the vector

$$K_{1j}, \dots, K_{mj}, x_j, z_j$$

- Questions
  - State the hypotheses
  - Propose a model (that incorporates the additional covariate)
  - List any assumptions that you have made

DESeq: Model Exercise

- The null hypothesis
  $H_0 : \beta_{11} = 0$ and $\beta_{21} = 0$ and $\dots \beta_{m1} = 0$
- Conditional on x_j and z_j, the observed number of reads mapped to gene i for sample j, K_{ij}, follows a negative binomial distribution with
  - Mean μ_{ij}
  - Dispersion parameter α_i (gene specific)
- Conditional on the treatment status of sample j ($x_j = 0$ or 1) and the temperature z_j, the expected value of K_{ij} is

$$\mu_{ij} = s_j \times q_{ij}$$

  where

$$\log q_{ij} = \beta_{i0} + \beta_{i1} x_j + \beta_{i2} z_j$$

- The normalization parameters are assumed to be sample (not gene) specific ($s_{ij} = s_j$)

DESeq: Model Nuisance Parameter

- The m main parameters of interest

$$\beta_{11}, \ldots, \beta_{m1}$$

- The unknown nuisance parameters are
  - The m gene specific intercepts

$$\beta_{10}, \ldots, \beta_{m0}$$

  - The m gene specific coefficients for the new covariate

$$\beta_{12}, \ldots, \beta_{m2}$$

  - the n sample specific normalization constants

$$s_1, \ldots, s_n$$

  - The m gene specific nuisance parameters

$$\alpha_1, \ldots, \alpha_m$$

edgeR: Another NB Model for RNA-Seq Counts

- Assume that the K_{ij} follows a NB distribution with mean μ_{ij} and dispersion paramater α_i
- The mean (conditional on treatment status x) is

$$\mu_i j = M_j p_{xi}$$

  where
  - M_j is the library size (total number of reads for sample j
  - p_{xi} is the relative abudance of the gene i given treatment status x
    - p_{0i} is the relative abudance of the gene i given no treatment
    - p_{1i} is the relative abudance of the gene i given treatment
- Treatment changes the abudance of RNA in gene i if $p_{0i} \neq p_{1i}$
- This is same distributional assumption as in DESeq

MLE Illustration

- In a GLM, the parameters β_{i0} and β_{i1} are estimated using the method of Maximum likelihood (MLE)
- We illustrate the method using this coin tossing example:
- We toss a coin once and record the number of heads
- Suppose that you conduct two independent replicates of this experiment
- K_1 the number of events (among $n = 1$ trial) in experiment 1
- K_2 the number of events (among $n = 1$ trial) in experiment 2
- The PMF of K_1 is

$$P(K_1 = k) = \pi^k (1 - \pi)^{1-k}$$

- The PMF of K_1 is

$$P(K_2 = k) = \pi^k (1 - \pi)^{1-k}$$

- Here $k = 0$ or 1

Joint Distribution

- $P(K_1 = k_1)$ denotes the probability of the event that $K_1 = k_1$
- $P(K_2 = k_2)$ denotes the probability of the event that $K_2 = k_2$
- These are called marginal probabilties
- What is $P(K_1 = k_1, K_2 = k_2)$
- This is probability of the event that $K_1 = k_1$ *and* $K_2 = k_2$
- If you assume that these are independent tosses then
- $P(K_1 = k_1, K_2 = k_2) = P(K_1 = k_1) \times P(K_2 = k_2)$
- In other words, the probability of the *joint* event is equal to the probability of the marginal events.

Likelihood

- Suppose that the realized value of K_1 is k_1
- Unlike K_1, k_1 is a fixed non-random number
- The likelihood of π given the observed data k_1, k_2 is

$$L(\pi) = \pi^{k_1}(1-\pi)^{1-k_1}\pi^{k_2}(1-\pi)^{1-k_2}$$

- Note that this is the joint probability of the events evaluated at the realized values

Joint Distribution

- Repeat the experiment B times
- The joint PMF is

$$P(K_1 = k_1, \ldots, K_B = k_B) = \pi^{k_1}(1-\pi)^{1-k_1} \times \ldots \times \pi^{k_B}(1-\pi)^{1-k_B}$$

- Note that the implicit assumption is that the experiments are mutually independent
- Under this assumption, the joint PMF is the product of the marginal PMFs
- Plugging in the *observed* counts into the joint PMF yields the likelihood function

Binomial Example: Observed data

```
set.seed(2131)
x = rbinom(5, 1, 0.5)
x


## [1] 1 0 0 0 1
```

- Observed data $x_1 = 1$, $x_1 = 0$, $x_3 = 0$, $x_4 = 0$ and $x_5 = 1$
- What is the likelihood?

Binomial Example: Likelihood

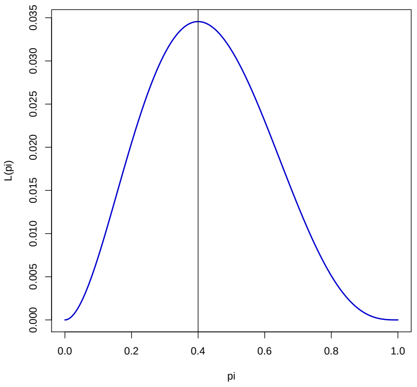
- Observed data $x_1 = 1$, $x_1 = 0$, $x_3 = 0$, $x_4 = 0$ and $x_5 = 1$
- The likelihood

$$\begin{aligned} L[\pi] &= \pi^{x_1}(1-\pi)^{x_1} \times \pi^{x_2}(1-\pi)^{x_2} \times \pi^{x_3}(1-\pi)^{x_3} \times \\ &\quad \pi^{x_4}(1-\pi)^{x_4} \times \pi^{x_5}(1-\pi)^{x_5} \times \\ &= \pi^{1}(1-\pi)^{1-1} \times \pi^{0}(1-\pi)^{1-0} \times \pi^{0}(1-\pi)^{1-0} \times \\ &\quad \pi^{0}(1-\pi)^{1-0} \times \pi^{1}(1-\pi)^{1-1} \\ &= \pi^2(1-\pi)^3 \end{aligned}$$

- Given the observed data find the value of π that maximizes this probability

Binomial Example: Maximum Likelihood

The maximum value of the function $L[\pi] = \pi^2(1-\pi)^3$ occurs at $\pi = 0.4$.



Maximum Likelihood Calculation for NB

- For gene i, let $k_{11}, \ldots, k_{1n}$ the n observed counts
- For patient j plug the observed count k_{ij} into the PMF of the NB distribution $f[k_{ij}; \mu_{ij}; \alpha_i]$
- Write the likelihood function as a product of these n terms

$$L = \prod_{j=1}^{n} f[k_{ij}; \mu_{ij}; \alpha_i] = f[k_{ij}; \beta_{0i}, \beta_{1i}, s_j, \alpha_i]$$

- The function depends on $\beta_{0i}, \beta_{1i}, s_j$ and α_i
- One approach: Come up with some estimates of s_j and α_i and plug them into the likelihood
- Pretend that these are the *true* values
- Now the likelihood is only a function of β_{0i} and β_{1i}