

Underlying Statistical Models of Functional Genomics Data Analysis Tools

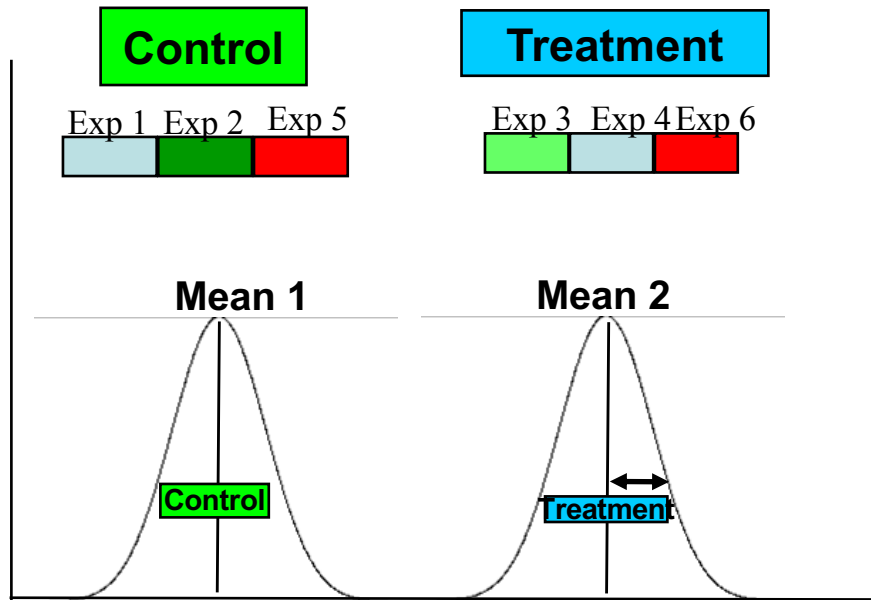
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BIOC 8145

Outline

- Classic two sample test:
 - t-test
- Multiple Hypothesis Testing:
 - Bonferroni p-value Correction
 - Benjamini and Hochberg (False Discovery Rate) p-value Correction
- Single Sample Data
 - Binomial Distribution
 - Poisson Distribution
- ChIP-seq Peak Calling: MACS2
 - Poisson Distribution
 - Negative Binomial Distribution
- RNA-seq Differential Expression Analysis: DESeq2
 - Normalization
 - Statistical Inference/Null Model

Two Sample Data: t-test

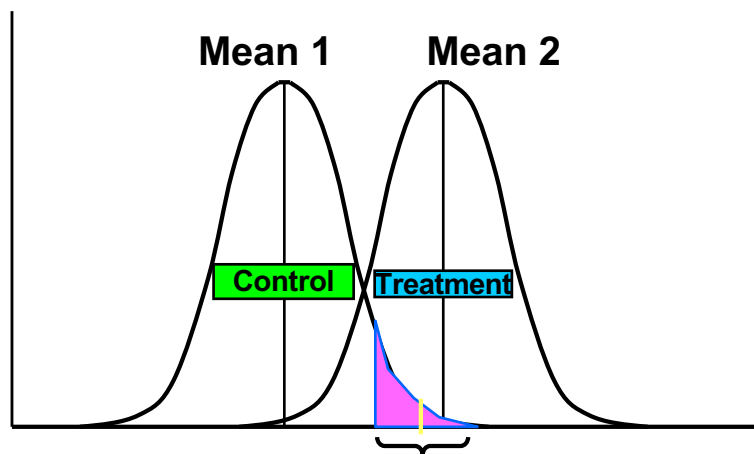


t-statistic

$$t = \frac{(m1 - m2)}{\text{stddev}(m1-m2)}$$

Calculating Significance of Differential Expression (p-value):

1. Randomly permute control and treatment data and compute t-statistic
2. Generate “null” t-distribution
3. p-value = fraction of random $t > \text{true } t$



In R: `t.test()`

Multiple Hypothesis Testing

- Assume we randomly split the same population of RNA into two samples and performed 10000 t-tests. Applying a cutoff of $p\text{-value} < 0.05$ would give 500 genes!!
- Fact: Applying t-tests to two populations with the same distributions generates a uniform distribution of p-values.
- Bonferroni correction: $p\text{-value}^* = \# \text{ tests} \times p\text{-value}$
 - Crudely, $p\text{-value}^*$ is the expected number of false positives
 - Apply $p\text{-value}^*$ as your new p-value cutoff
 - Very conservative for functional genomics but not statistical genetics

Multiple Hypothesis Testing (cont' d)

- Assume g genes.
- Two main approaches to defining false positive rates:
 - Family Wise Error Rate (FWER): *Probability* of having one (or more) false positives in the predicted set of genes
 - False Discovery Rate (FDR): *Expected* proportion of the predicted set which consists of false predictions
- If FWER or FDR $< \alpha$, a procedure controls the FDR or FWER error rate to level α
- Many set $\alpha = 0.05$
- FWER (5% chance of having ≥ 1 false positive)
 - Single Step Method: All p-values given the same correction
 - Bonferroni correction:
 - Select genes whose p-values $p_i < \alpha/g$
 - Overly stringent for functional genomics but not statistical genetics!
 - Step Down Method: p-values given different correction
 - Westfall and Young
 - Sort p-values in increasing order: $p_{(1)}, \dots, p_{(g)}$
 - If $p_{(1)} < \alpha/g$, then null hypothesis (1) is rejected, go to step 2
 - If $p_{(2)} < \alpha/(g-1)$, then null hypothesis (2) is rejected, go to step 3, continue until
 - If $p_{(j)} > \alpha/(g-j+1)$, then all null hypotheses $i=(j), \dots, (g)$ are accepted
 - Less stringent than Bonferroni correction
 - Still too conservative for gene expression studies

Multiple Hypothesis Testing (cont' d)

- FDR (5% of the predicted genes are false positives)
 - Benjamini and Hochberg Step-Up Methods
 - $Q = V/R$; $R = \#$ rejected null hypotheses; $V = \#$ rejected that are true nulls (false positives); assume g_0 out of g genes are true nulls
 - Theorem: $E(Q) = (g_0/g) * \alpha$
 - Assumes g different tests are independent
 - Sort p-values in increasing order: $p_{(1)}, \dots, p_{(i)}, \dots, p_{(g)}$
 - Let $q_i = i * \alpha / g$, $i=1, \dots, g$
 - α is the desired FDR
 - Let k be a maximum i such that $p_{(i)} \leq q_i$
 - If $k \geq 1$, reject null hypotheses $i=(1), \dots, (k)$ and accept all others
 - Note: There may be $i' < k$ such that $p_{(i')} > q_{i'}$
 - Appropriate for functional genomics studies which are followed by relatively easy experimental validation
 - Default multi-test correction in many Bioconductor packages.

Single Sample Data

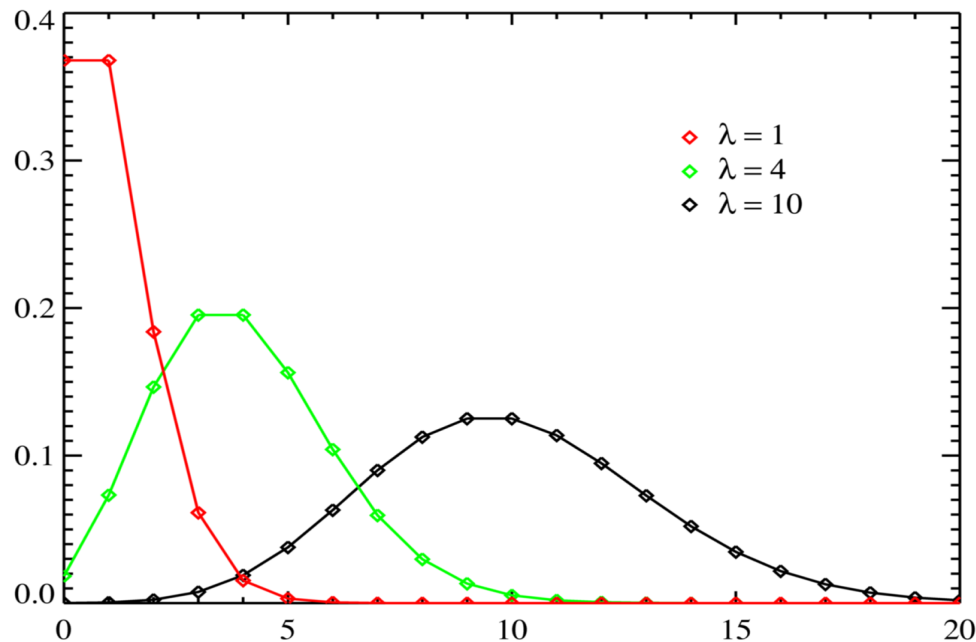
- The t-test described above was developed for treatment versus control (2 sample).
- Functional Genomics:
 - In a discovery/characterization phase.
 - Costly and labor/time intensive,
 - High throughput sequencing data (e.g., ChIP-Seq, RNA-Seq, MeDIP-Seq,...) sometimes comes in the form of single samples:
 - No replicates.
 - No comparisons between treatment and control or multiple groups.
- What are the analysis goals?
 - Identify significantly enriched sites above background/noise.
 - Compare sites to annotations.
- How do we identify the significantly enriched sites?
 - Generate a null distribution from the background/noise in the data.
 - Calculate p-values using this distribution.
 - FDR correct the p-values.
 - Apply 0.05 (or 5%) FDR cutoff.

Binomial Distribution

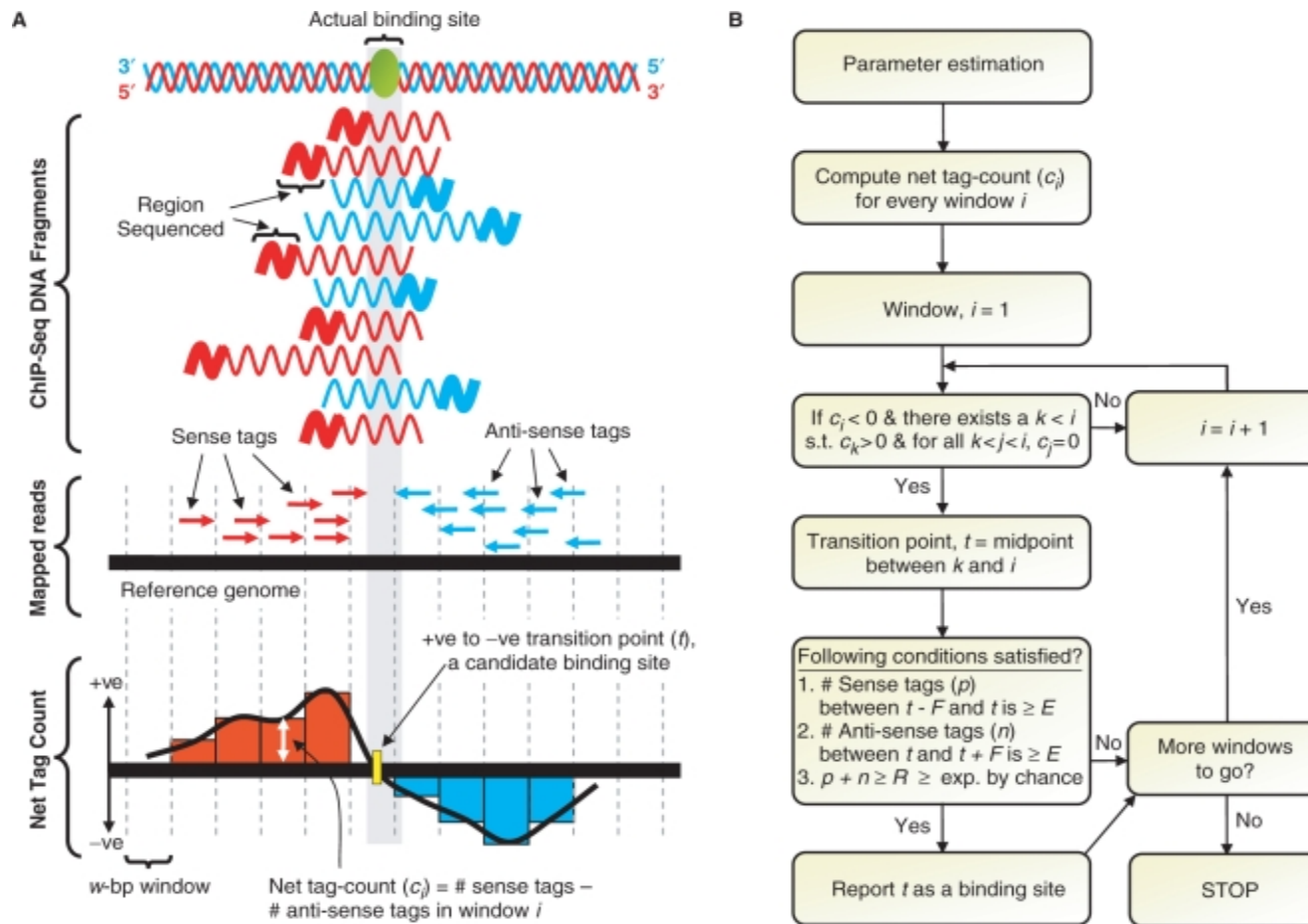
- Suppose that we divide the genome into non-overlapping windows of size w .
- Assume n reads could map to window w_i given our sample.
- Assume the probability of sequencing and mapping a read in w_i is p and not sequencing/mapping it within w_i is $1-p$.
- The total number of reads mapping within w_i , X , is a binomial random variable with parameters n and p .
- Note: $n! = n * (n-1) * (n-2) * (n-3) * \dots * 3 * 2 * 1$
- The probability that k reads map in w_i or $X = k$ can be found as follows:
 - Any *specific* set of k reads out of n mapping within w_i (one instance out of many!) is given by the probability $p^k(1-p)^{n-k}$ assuming read sequencing/mapping events are independent (multiplication principle of probabilities).
 - The *total number of ways* that k reads out of n (that could be sequenced and mapped to w_i) are sequenced and mapped to w_i is given by $n!/((n-k)!k!)$.
- Thus, the probability of $X = k$ reads being sequenced and mapped to w_i is
$$P(X = k) = \frac{n!}{((n-k)!k!)} p^k(1-p)^{n-k}.$$
- Expectation of X is $E(X) = n * p$
- Variance of X is $\text{Var}(X) = n * p * (1 - p)$
- For n large and p far from 0 or 1; $X \sim N(np, np(1-p))$ is a good approximation.

Poisson Distribution

- Derived from the binomial distribution in the limit where
 - n (the number of possible mapped reads) is large
 - p (the probability that a read maps in w) is small
 - $n \cdot p = \lambda$
- $P(X = k) = (\lambda^k / k!) e^{-\lambda}$;
- $E(X) = \lambda$;
- $\text{Var}(X) = \lambda$
- In R: `dpois()`; `ppois()`; `qpois()`; `rpois()`

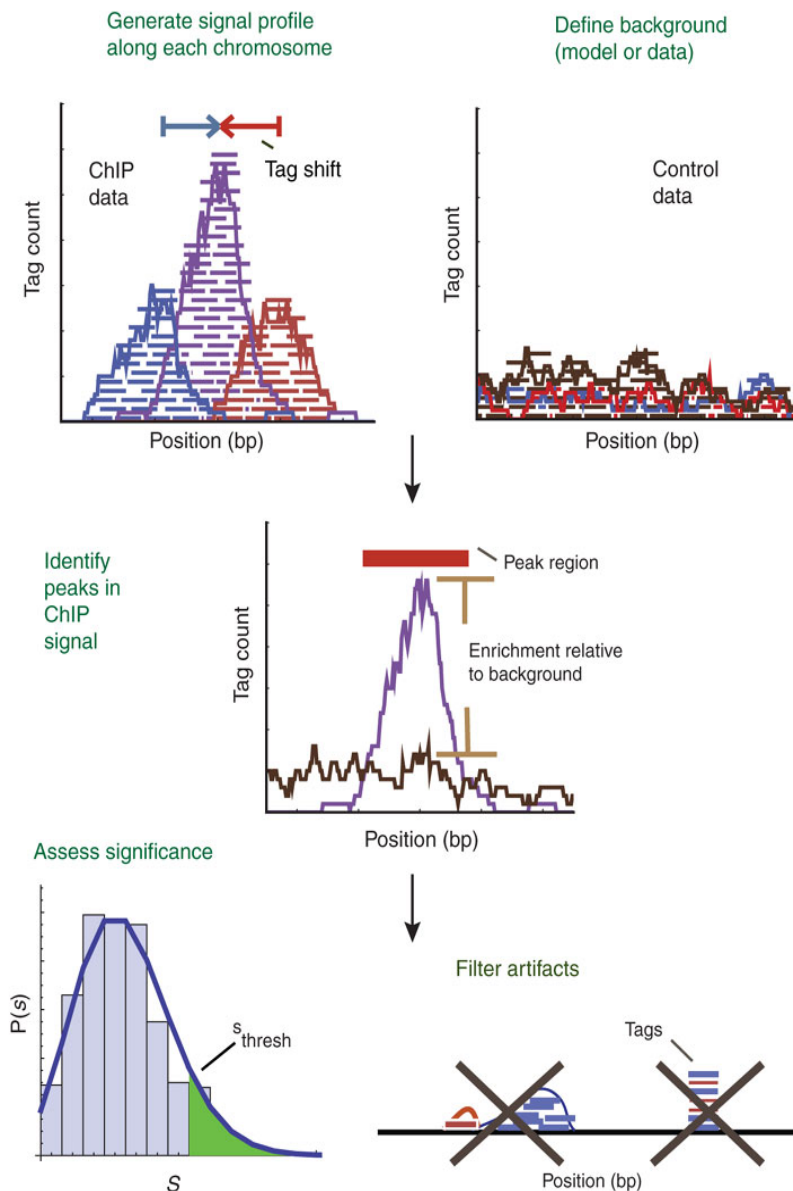


Identifying Sites from ChIP-seq Data (MACS2)



How do current algorithms identify sites?

Available Tools: Pepke, S. et al. Nat. Meth. Supp. 6, pp. S22-S32 (2009)

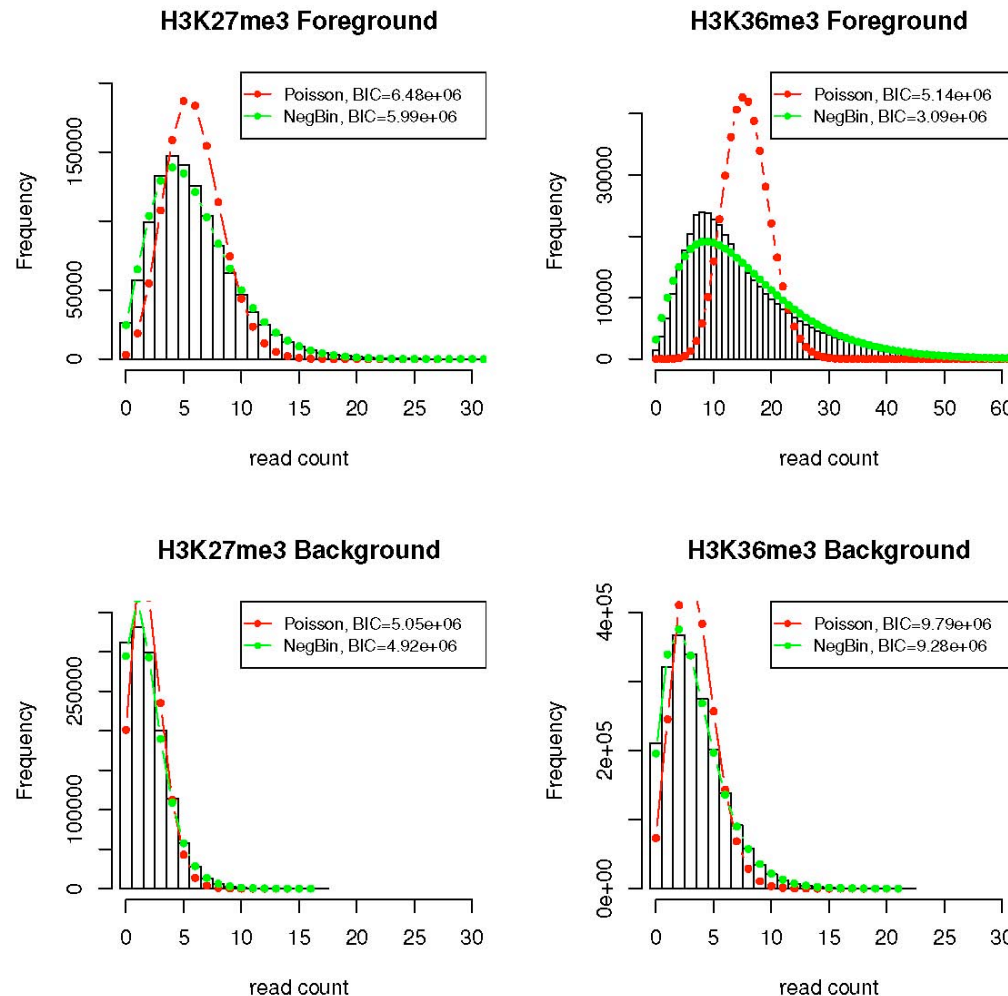


Poisson Background Model

- Bin the genome into windows of size w .
- λ = Expected (or average) number of reads in a window calculated from control or low read count data.
- Calculate p-value for each window.
 - $\text{p-value} = \sum_{k=R, \infty} e^{-\lambda} \lambda^k / k!$
 - R is the number of reads in a given window.
- FDR correct p-values and apply FDR cutoff.

Negative Binomial Distribution Background Model

The Negative Binomial Distribution is a mixture of a Poisson Distribution and a gamma distribution. It can be viewed as a Poisson distribution with a variable λ (or expected number of reads in a window) that itself varies as a gamma distribution.



Identify Differentially Expressed Genes from RNA-seq Data using DESeq2

- Assumes read count K_{ij} for gene i in sample j is described by a Generalized Linear Model where:
 - K_{ij} is distributed as a Negative Binomial with mean $= \mu_{ij}$ and variance $= \sigma_{ij}^2$; Accounts for shot/sampling noise plus additional experimental and biological variation
 - Normalized read counts $= q_{ij} = \mu_{ij}/s_j$
 - Assume no global changes in gene expression
 - $s_j = \text{median}_i(K_{ij}/K_i^R)$; $K_i^R = (\prod_{j=1..m} K_{ij})^{1/m}$
 - $\log(q_{ij}) = \sum_r x_{jr} \beta_{ir}$ (fit normalized read counts to line of user supplied covariates or contrasts/sample comparisons)
- Perform Wald test: compare $\beta_{ir}/\text{SE}(\beta_{ir})$ to standard normal distribution $N(0,1)$.
- Calculate p-values by summing/integrating tail of normal distribution: two tailed test.
- Filter low expressed genes whose average across samples is below a threshold which is optimized to maximize the number of genes found at a user specified false discover rate (FDR).
- Adjust for multiple hypothesis testing by calculating the FDR from the p-values using the procedure of Benjamini and Hochberg.