# Lecture 10: Multiple Testing

# Goals

- Define the multiple testing problem and related concepts
- Methods for addressing multiple testing (FWER and FDR)
- Correcting for multiple testing in R

# Type I and II Errors

| Decision | **Actual Situation "Truth"** $H_0$ True | $H_0$ False |
|---|---|---|
| Do Not Reject $H_0$ | Correct Decision $1 - \alpha$ | Incorrect Decision Type II Error $\beta$ |
| Rejct $H_0$ | Incorrect Decision Type I Error $\alpha$ | Correct Decision $1 - \beta$ |

$$\alpha = P(Type\ I\ Error) \quad \beta = P(Type\ II\ Error)$$

# Why Multiple Testing Matters

**Genomics = Lots of Data = Lots of Hypothesis Tests**

A typical microarray experiment might result in performing 10000 separate hypothesis tests. If we use a standard p-value cut-off of 0.05, we'd expect ***500*** genes to be deemed "significant" by chance.

# Why Multiple Testing Matters

- In general, if we perform m hypothesis tests, what is the probability of at least 1 false positive?

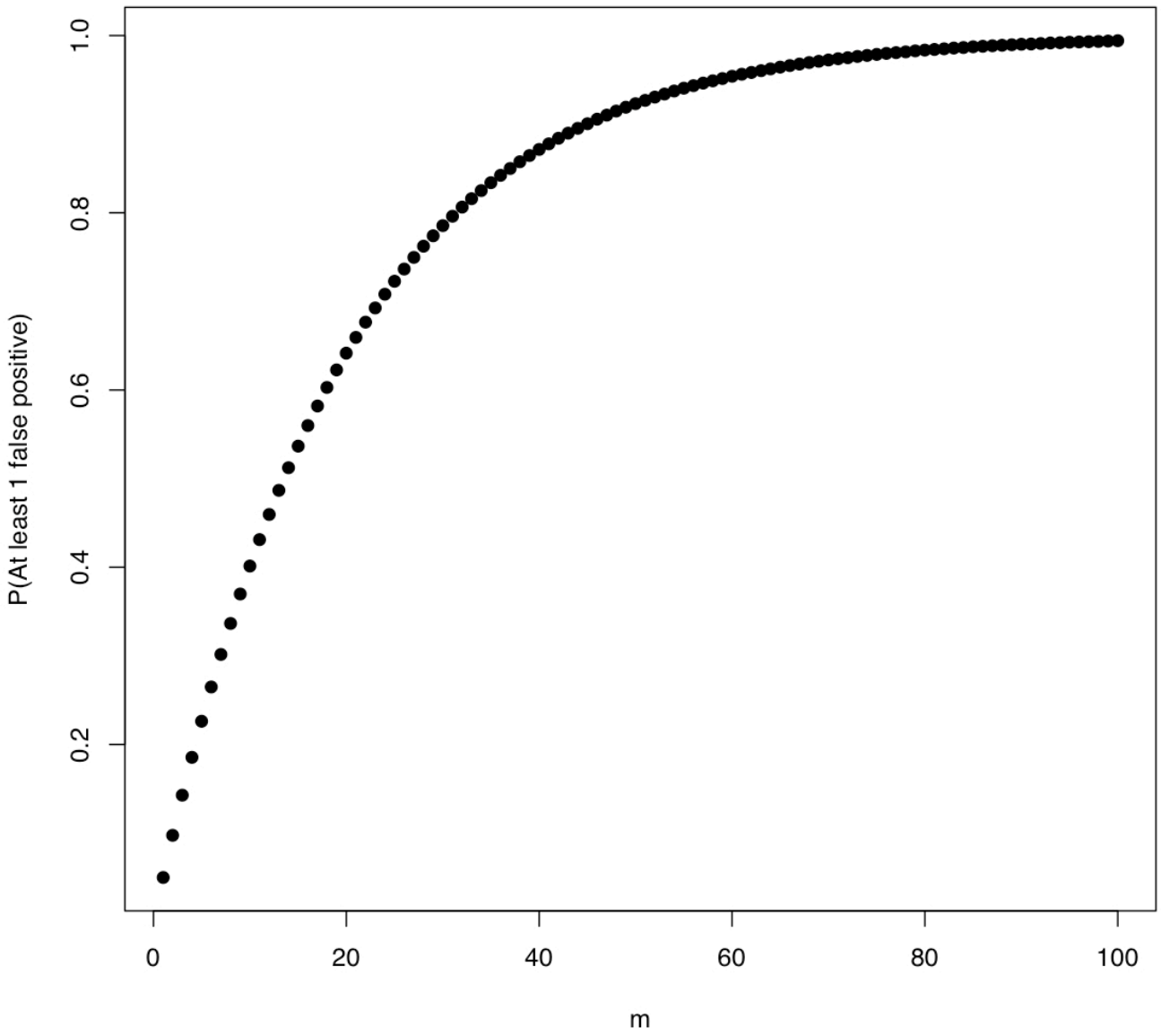
$$P(\text{Making an error}) = \alpha$$

$$P(\text{Not making an error}) = 1 - \alpha$$

$$P(\text{Not making an error in m tests}) = (1 - \alpha)^m$$

$$P(\text{Making at least 1 error in m tests}) = 1 - (1 - \alpha)^m$$

# Probability of At Least 1 False Positive



# Counting Errors

Assume we are testing $H^1, H^2, \ldots, H^m$

$m_0$ = # of true hypotheses  $R$ = # of rejected hypotheses

| | **Null True** | **Alternative True** | **Total** |
|---|---|---|---|
| **Not Called Significant** | ***U*** | ***T*** | ***m - R*** |
| **Called Significant** | ***V*** | ***S*** | ***R*** |
| | $m_0$ | $m-m_0$ | ***m*** |

$V$ = # Type I errors [false positives]

# What Does Correcting for Multiple Testing Mean?

- When people say "adjusting p-values for the number of hypothesis tests performed" what they mean is ***controlling the Type I error rate***

- Very active area of statistics - many different methods have been described

- Although these varied approaches have the same goal, they go about it in fundamentally different ways

# Different Approaches To Control Type I Errors

- **Per comparison error rate** (PCER): the expected value of the number of Type I errors over the number of hypotheses,

  $$PCER = E(V)/m$$

- **Per-family error rate** (PFER): the expected number of Type I errors,

  $$PFE = E(V).$$

- **Family-wise error rate**: the probability of at least one type I error

  $$FEWR = P(V \geq 1)$$

- **False discovery rate** (FDR) is the expected proportion of Type I errors among the rejected hypotheses

  $$FDR = E(V/R \mid R>0)P(R>0)$$

- **Positive false discovery** rate (pFDR): the rate that discoveries are false

  $$pFDR = E(V/R \mid R > 0)$$

# Digression: p-values

- Implicit in all multiple testing procedures is the assumption that the distribution of p-values is "correct"

- This assumption often is not valid for genomics data where p-values are obtained by asymptotic theory

- Thus, resampling methods are often used to calculate calculate p-values

# Permutations

1. Analyze the problem: think carefully about the null and alternative hypotheses
2. Choose a test statistic
3. Calculate the test statistic for the original labeling of the observations
4. Permute the labels and recalculate the test statistic
   - Do all permutations: Exact Test
   - Randomly selected subset: Monte Carlo Test
5. Calculate p-value by comparing where the observed test statistic value lies in the permuted distributed of test statistics

# Example: What to Permute?

- Gene expression matrix of m genes measured in 4 cases and 4 controls

| Gene | Case 1 | Case 2 | Case 3 | Case 4 | Control 1 | Control 2 | Control 3 | Control 4 |
|---|---|---|---|---|---|---|---|---|
| 1 | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ | $X_{15}$ | $X_{16}$ | $X_{17}$ | $X_{18}$ |
| 2 | $X_{21}$ | $X_{22}$ | $X_{23}$ | $X_{24}$ | $X_{25}$ | $X_{26}$ | $X_{27}$ | $X_{28}$ |
| 3 | $X_{31}$ | $X_{32}$ | $X_{33}$ | $X_{34}$ | $X_{35}$ | $X_{36}$ | $X_{37}$ | $X_{38}$ |
| 4 | $X_{41}$ | $X_{42}$ | $X_{43}$ | $X_{44}$ | $X_{45}$ | $X_{46}$ | $X_{47}$ | $X_{48}$ |
| ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ |
| m | $X_{m1}$ | $X_{m2}$ | $X_{m3}$ | $X_{m4}$ | $X_{m5}$ | $X_{m6}$ | $X_{m7}$ | $X_{m8}$ |

# Back To Multiple Testing: FWER

- Many procedures have been developed to control the Family Wise Error Rate (the probability of at least one type I error):

$$P(V \geq 1)$$

- Two general types of FWER corrections:
  1. **Single step**: equivalent adjustments made to each p-value
  2. **Sequential**: adaptive adjustment made to each p-value

# Single Step Approach: Bonferroni

- Very simple method for ensuring that the overall Type I error rate of $\alpha$ is maintained when performing m independent hypothesis tests

- Rejects any hypothesis with p-value ≤ $\alpha$/m:

$$\tilde{p}_j = \min[mp_j, 1]$$

- For example, if we want to have an experiment wide Type I error rate of 0.05 when we perform 10,000 hypothesis tests, we'd need a p-value of 0.05/10000 = 5 x $10^{-6}$ to declare significance

# Philosophical Objections to Bonferroni Corrections

**"Bonferroni adjustments are, at best, unnecessary and, at worst, deleterious to sound statistical inference" Perneger (1998)**

- Counter-intuitive: interpretation of finding depends on the number of other tests performed
- The general null hypothesis (that all the null hypotheses are true) is rarely of interest
- High probability of type 2 errors, i.e. of not rejecting the general null hypothesis when important effects exist

# FWER: Sequential Adjustments

- Simplest sequential method is Holm's Method
  - Order the unadjusted *p*-values such that $p_1 \le p_2 \le \ldots \le p_m$
  - For control of the FWER at level $\alpha$, the step-down Holm adjusted p-values are

$$\tilde{p}_j = \min[(m - j + 1) \bullet p_j, 1]$$

  - The point here is that we don't multiply every $p_i$ by the same factor m

- For example, when m = 10000:

$$\tilde{p}_1 = 10000 \bullet p_1, \tilde{p}_2 = 9999 \bullet p_2, \ldots, \tilde{p}_m = 1 \bullet p_m$$

# Who Cares About Not Making ANY Type I Errors?

- FWER is appropriate when you want to guard against ANY false positives
- However, in many cases (particularly in genomics) we can live with a certain number of false positives
- In these cases, the more relevant quantity to control is the false discovery rate (FDR)

# False Discovery Rate

| | Null True | Alternative True | Total |
|---|---|---|---|
| **Not Called Significant** | ***U*** | ***T*** | ***m - R*** |
| **Called Significant** | ***V*** | ***S*** | ***R*** |
| | $m_0$ | $m-m_0$ | ***m*** |

*V* = # Type I errors [false positives]

- False discovery rate (FDR) is designed to control the proportion of false positives ***among the set of rejected hypotheses*** (R)

# FDR vs FPR

| | Null True | Alternative True | Total |
|---|---|---|---|
| **Not Called Significant** | ***U*** | ***T*** | ***m - R*** |
| **Called Significant** | ***V*** | ***S*** | ***R*** |
| | $m_0$ | $m-m_0$ | ***m*** |

$$FDR = \frac{V}{R} \qquad FPR = \frac{V}{m_0}$$

# Benjamini and Hochberg FDR

- To control FDR at level $\delta$:

  1. Order the unadjusted p-values: $p_1 \leq p_2 \leq \ldots \leq p_m$

  2. Then find the test with the highest rank, j, for which the p value, $p_j$, is less than or equal to (j/m) x $\delta$

  3. Declare the tests of rank 1, 2, …, j as significant

$$p(j) \leq \delta \frac{j}{m}$$

# B&H FDR Example

Controlling the FDR at $\delta = 0.05$

| Rank (j) | P-value | (j/m)× $\delta$ | Reject $H_0$ ? |
|---|---|---|---|
| 1 | 0.0008 | 0.005 | 1 |
| 2 | 0.009 | 0.010 | 1 |
| 3 | 0.165 | 0.015 | 0 |
| 4 | 0.205 | 0.020 | 0 |
| 5 | 0.396 | 0.025 | 0 |
| 6 | 0.450 | 0.030 | 0 |
| 7 | 0.641 | 0.035 | 0 |
| 8 | 0.781 | 0.040 | 0 |
| 9 | 0.900 | 0.045 | 0 |
| 10 | 0.993 | 0.050 | 0 |

# Storey's positive FDR (pFDR)

$$\text{BH}: FDR = E\left[\frac{V}{R} \mid R > 0\right] P(R > 0)$$

$$\text{Storey}: \text{p}FDR = E\left[\frac{V}{R} \mid R > 0\right]$$

- Since P(R > 0) is ~ 1 in most genomics experiments FDR and pFDR are very similar

- Omitting P(R > 0) facilitated development of a measure of significance in terms of the FDR for each hypothesis

# What's a q-value?

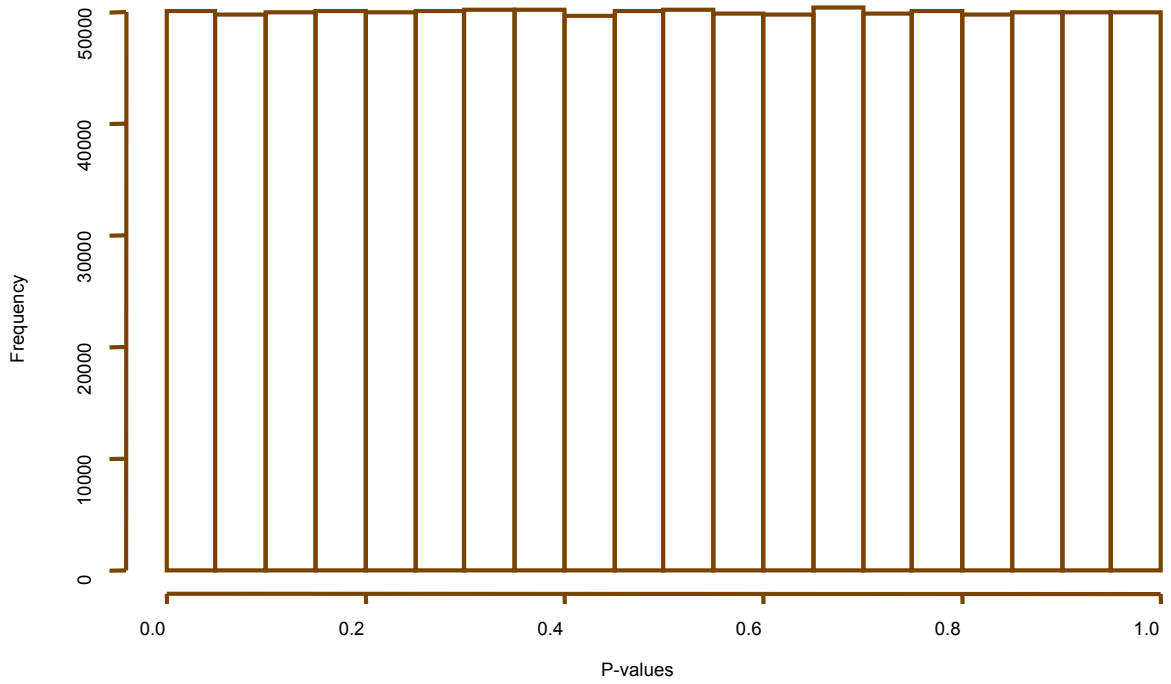
- q-value is defined as the minimum FDR that can be attained when calling that "feature" significant (i.e., expected proportion of false positives incurred when calling that feature significant)

- The estimated q-value is a function of the p-value for that test and the distribution of the entire set of p-values from the family of tests being considered (Storey and Tibshiriani 2003)

- Thus, in an array study testing for differential expression, if gene X has a q-value of 0.013 it means that 1.3% of genes that show p-values at least as small as gene X are false positives

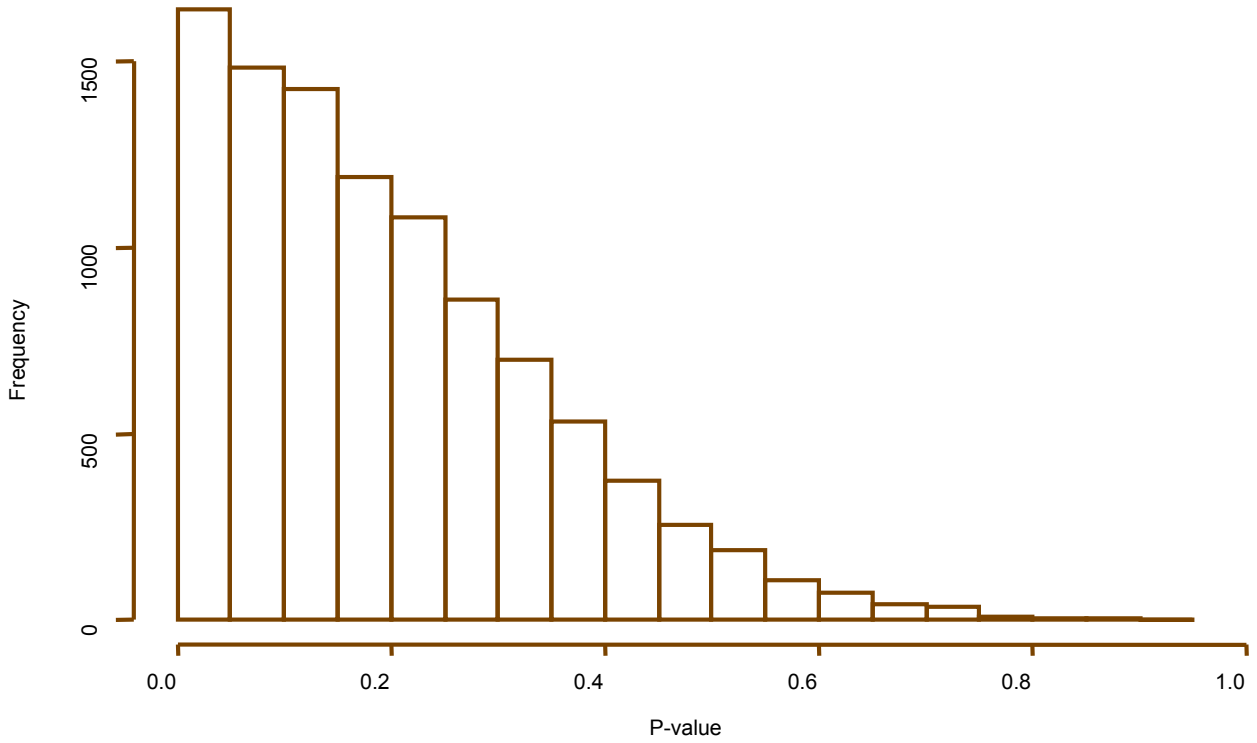
# Estimating The Proportion of Truly Null Tests

- Under the null hypothesis p-values are expected to be uniformly distributed between 0 and 1



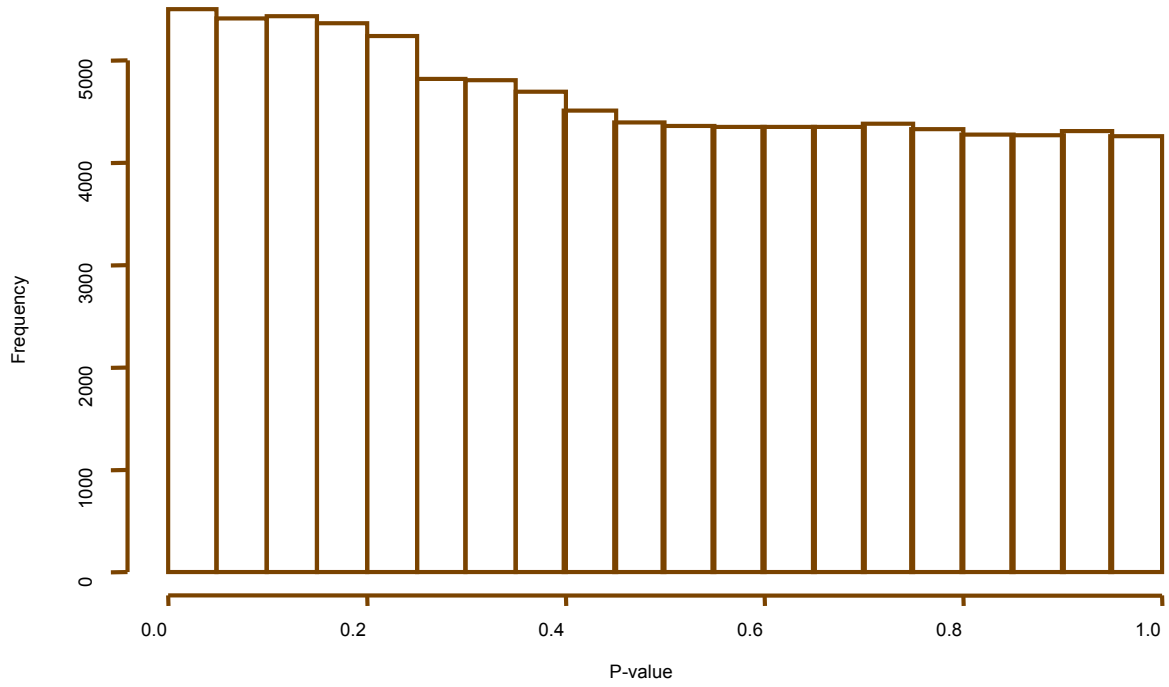
# Estimating The Proportion of Truly Null Tests

- Under the alternative hypothesis p-values are skewed towards 0



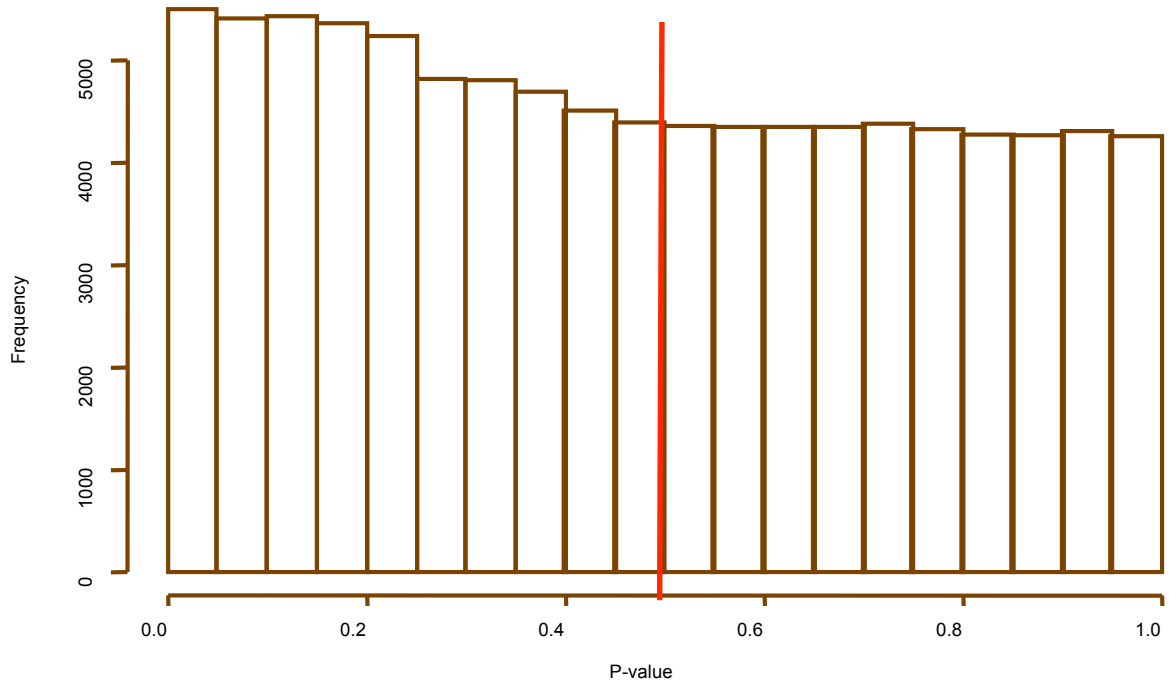
# Estimating The Proportion of Truly Null Tests

- Combined distribution is a mixture of p-values from the null and alternative hypotheses



# Estimating The Proportion of Truly Null Tests

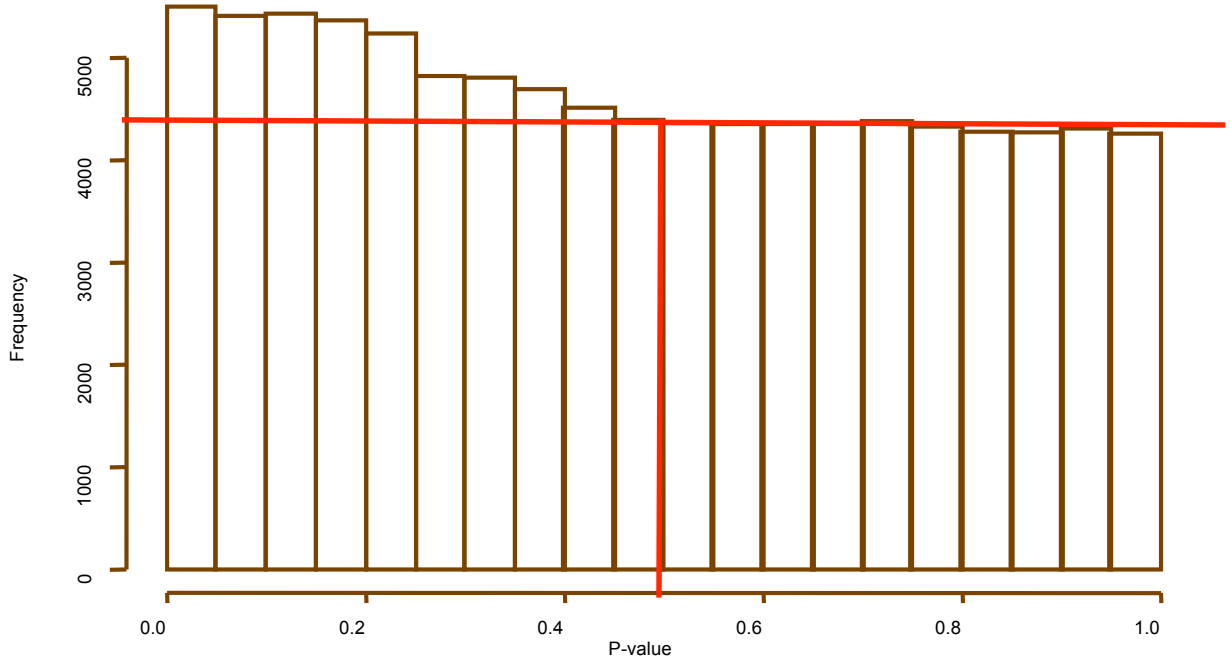
- For p-values greater than say 0.5, we can assume they mostly represent observations from the null hypothesis



# Definition of $\pi_0$

- $\hat{\pi}_0$ is the proportion of truly null tests:

$$\hat{\pi}_0(\lambda) = \frac{\#\{p_i > \lambda; i = 1,2,\ldots,m\}}{m(1-\lambda)}$$




- 1 - $\hat{\pi}_0$ is the proportion of truly alternative tests (very useful!)