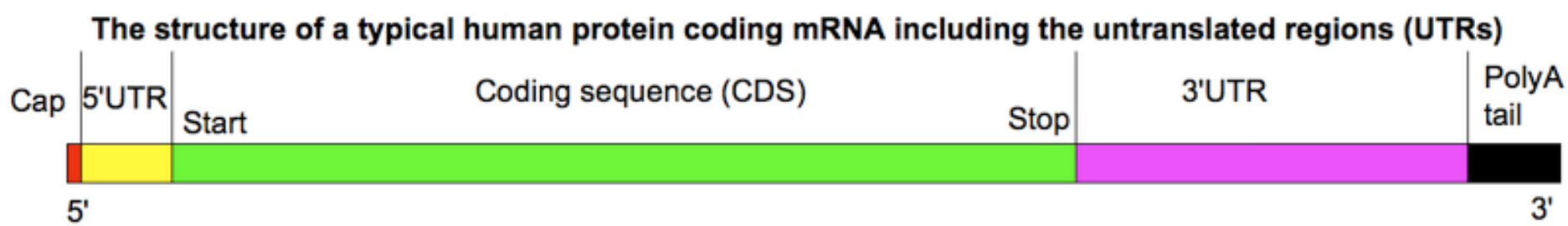
RNASeq Introduction

Malay (malay@uab.edu)

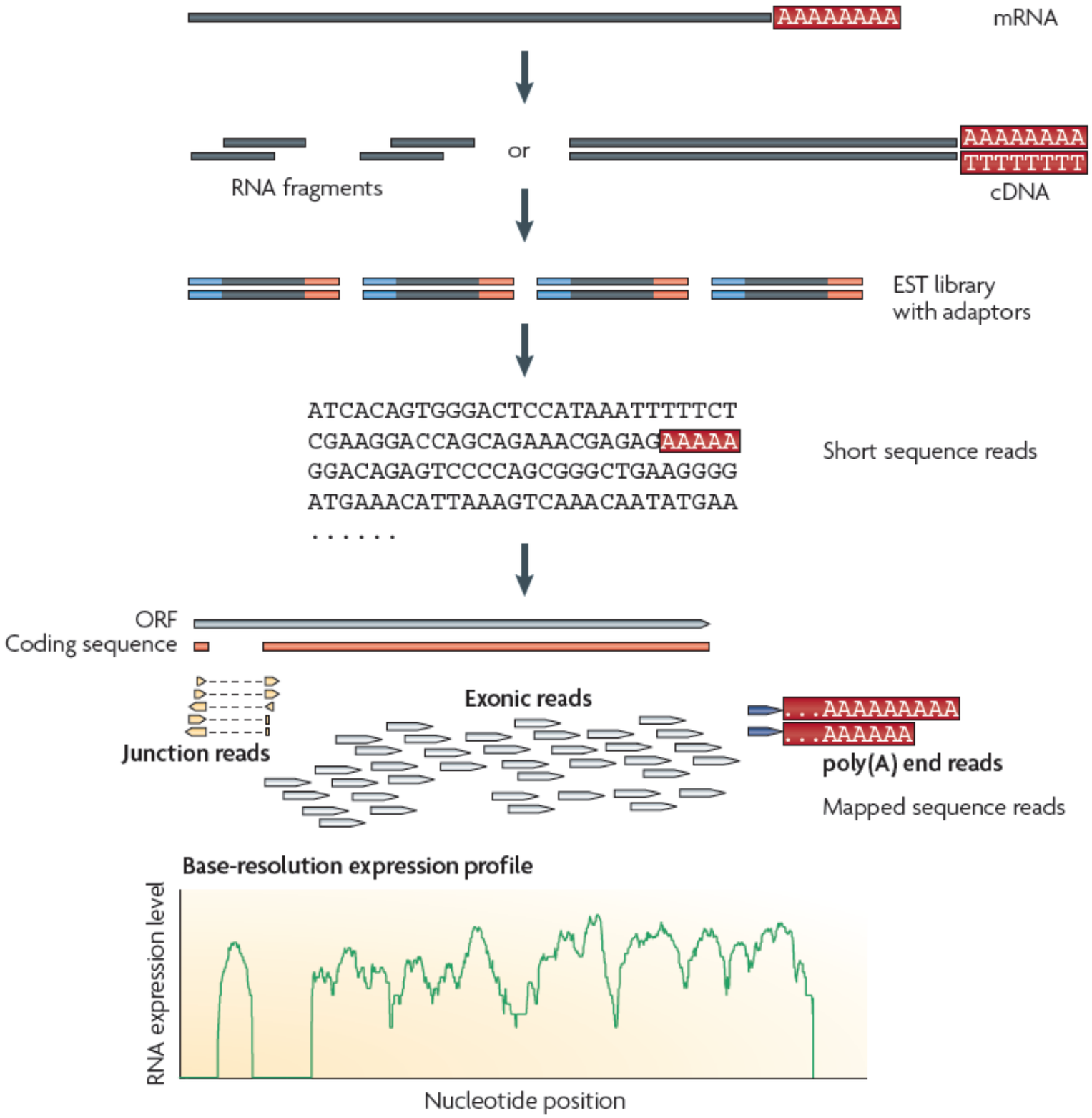
Structure of mRNA



Other RNAs:

Type	Size	Function
▪ microRNA (miRNA)	21-23 nt	regulation of gene expression
▪ small interfering RNA (siRNA)	19-23 nt	antiviral mechanisms
▪ piwi-interacting RNA (piRNA)	26-31 nt	interaction with piwi proteins/spermatogenesis
▪ small nuclear RNA (snRNA)	100-300 nt	RNA splicing
▪ small nucleolar RNA (snoRNA)	-	modification of other RNAs

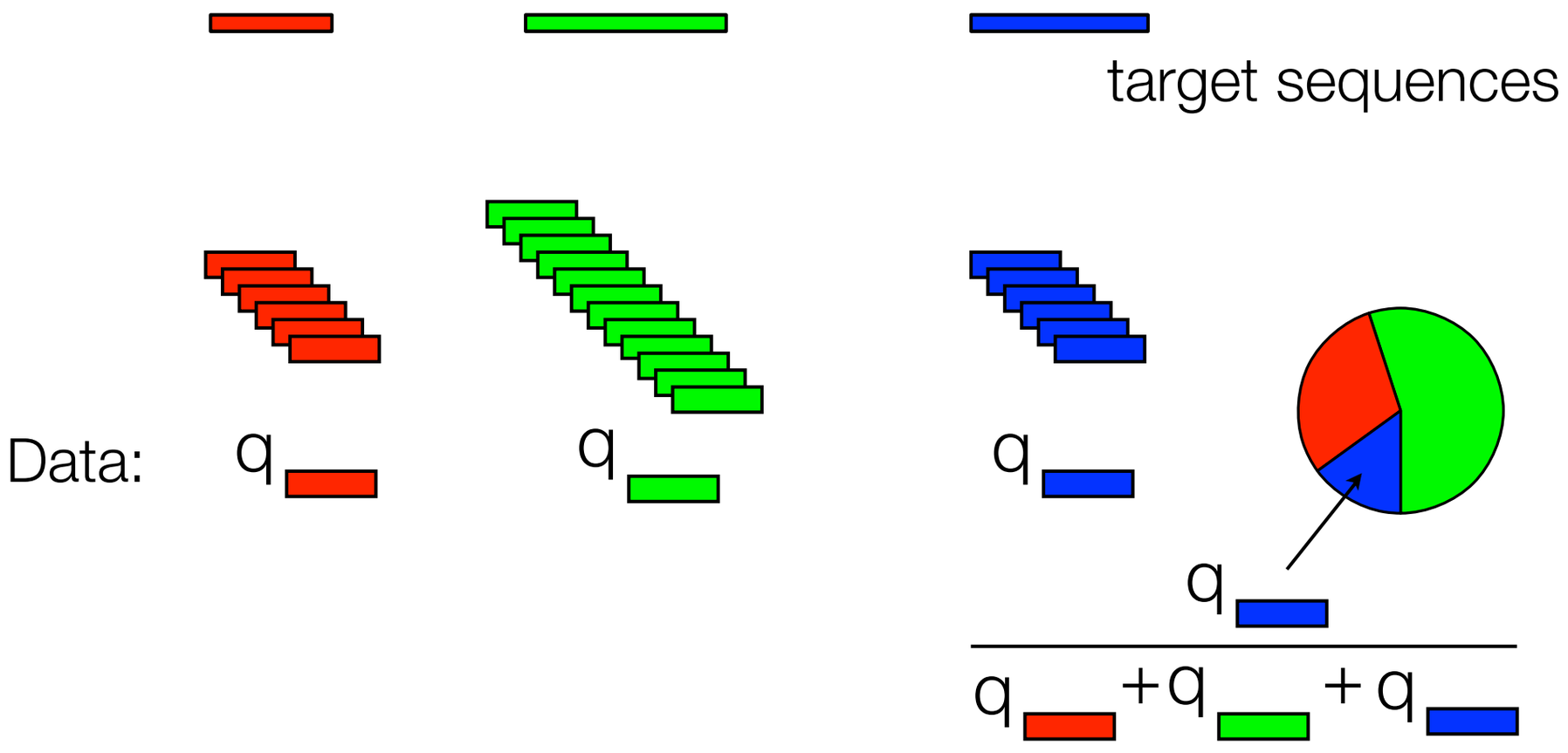
RNASeq overview



Various applications of RNASeq

Differential expression
Novel transcript detection
Fusion transcript detection
Mutation detection (not common)

The fragment assignment problem



Example count tables:

http://bowtie-bio.sourceforge.net/recount/

Normalization methods in RNASeq

Upper Quartile (UQ): Very similar in principle to TC, the total counts are replaced by the upper quartile of counts different from 0 in the computation of the normalization factors.

DESeq: This normalization method is included in the DESeq

Trimmed Mean of M-values (TMM): This normalization method is implemented in the edgeR

Reads Per Kilobase per Million mapped reads (RPKM): This approach was initially introduced to facilitate comparisons between genes within a sample and combines between- and within-sample normalization.

FPKM: Same as RPKM, but for fragments.

TPM: comes from RSEM

$$RPKM = \frac{Count}{\frac{N}{10^6} \times \frac{length}{10^3}}$$

$$\mathrm{TPM}_i = \frac{X_i}{\widetilde{l}_i} \cdot \left(\frac{1}{\sum_j \frac{X_j}{\widetilde{l}_j}} \right) \cdot 10^6$$

$$\mathrm{TPM}_i = \left(\frac{\mathrm{FPKM}_i}{\sum_j \mathrm{FPKM}_j} \right) \cdot 10^6$$

Upper quartile normalization

Sample_ID gene raw_1Kb_read_count
1 Act7 1000
1 GapDH 2000
1 Sec4 500
...
1 Bglob1 10000000
TOTAL WITH Bglob1: 12,000,000
TOTAL WITHOUT: 2,000,000

2 Act7 500
2 GapDH 1000
2 Sec4 250
...
2 Bglob1 10
TOTAL WITH Bglob1: 1,000,010
TOTAL WITHOUT: 1,000,000

$$FPKMAct7 = \frac{1000}{\frac{1000}{10^3} \times \frac{12^6}{10^6}} = 83.3$$

$$FPKMAct7 = \frac{500}{\frac{1000}{10^3} \times \frac{1{,}000{,}010}{10^6}} = 499.995$$

$$FPKMAct7 = \frac{1000}{\frac{1000}{10^3} \times \frac{2^6}{10^6}} = 500$$

$$FPKMAct7 = \frac{500}{\frac{1000}{10^3} \times \frac{1^6}{10^6}} = 500$$

DESEQ normalization

size factor

count of gene in one sample

$$\hat{s}_j = \underset{i}{\text{median}} \frac{k_{ij}}{\left(\prod_{v=1}^{m} k_{iv} \right)^{1/m}}.$$

samples

Geometric mean across the samples

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-10-r106

TMM normalization (EDGER)

log fold change of a gene →

$$M_g = \log_2 \frac{Y_{gk}/N_k}{Y_{gk'}/N_{k'}}$$

and absolute expression levels:

$$A_g = \frac{1}{2}\log_2\left(Y_{gk}/N_k \bullet Y_{gk'}/N_{k'}\right) \text{ for } Y_{g\bullet} \neq 0$$

TMM of the sample k with reference sample r →

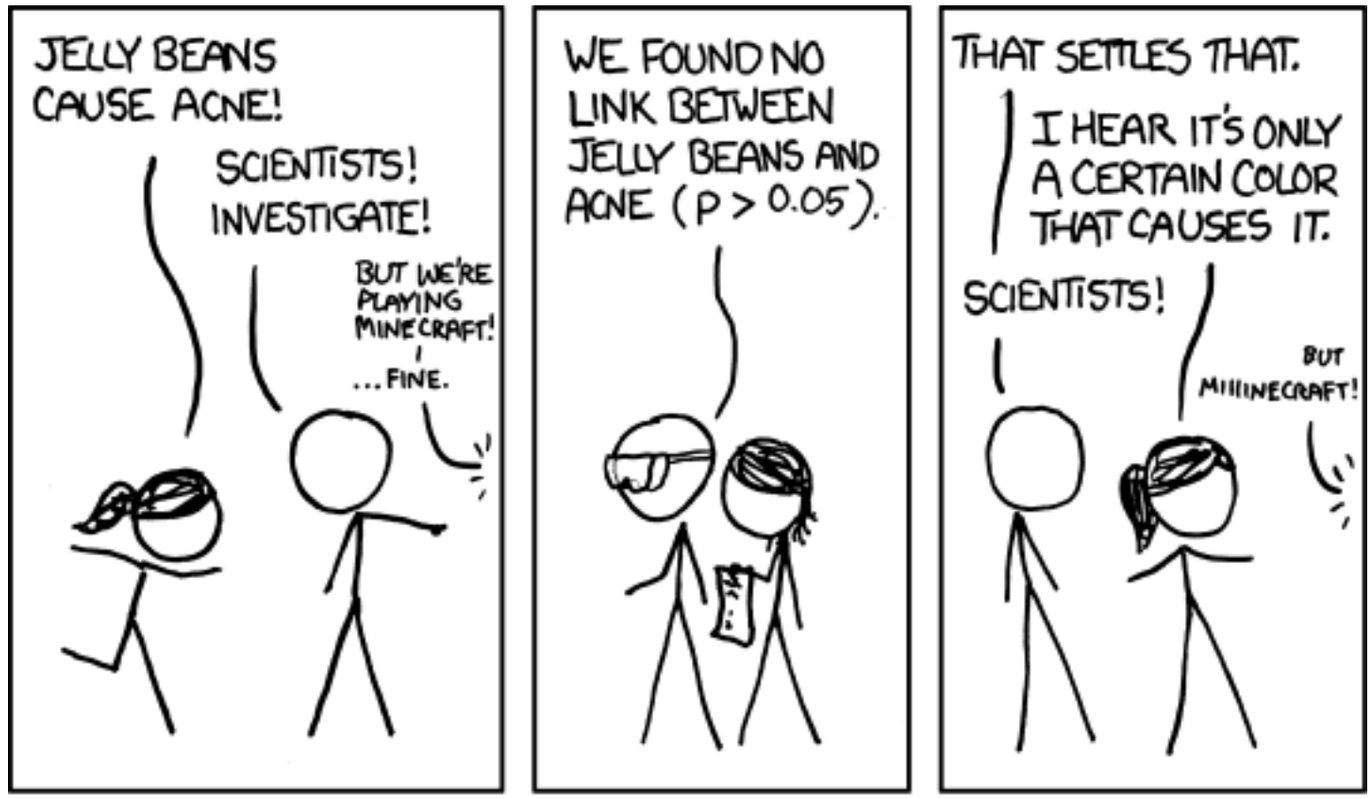
$$\log_2(TMM_k^{(r)}) = \frac{\sum_{g \in G^*} w_{gk}^r M_{gk}^r}{\sum_{g \in G^*} w_{gk}^r} \text{ where } M_{gk}^r = \frac{\log_2\left(Y_{gk}/N_k\right)}{\log_2\left(Y_{gr}/N_r\right)} \text{ and } w_{gk}^r = \frac{N_k - Y_{gk}}{N_k Y_{gk}} + \frac{N_r - Y_{gr}}{N_r Y_{gr}};$$

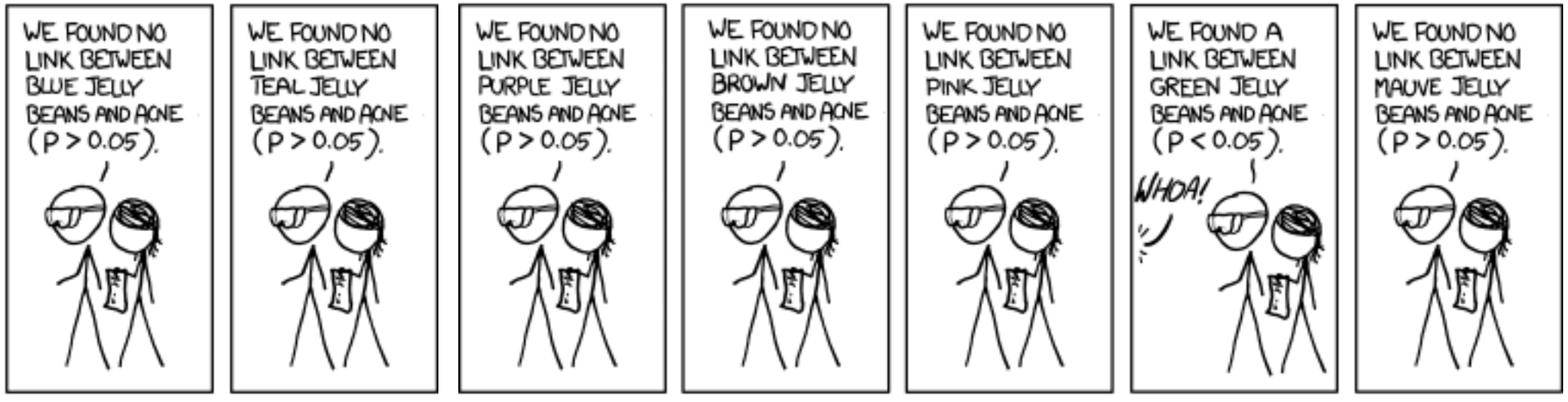
$Y_{gk}, Y_{gr} > 0.$

Remove top and bottom 30%

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2010-11-3-r25

False Discovery Rate and q-value


JELLY BEANS CAUSE ACNE!
SCIENTISTS! INVESTIGATE!
BUT WE'RE PLAYING MINECRAFT!
... FINE.
WE FOUND NO LINK BETWEEN JELLY BEANS AND ACNE (P > 0.05).
THAT SETTLES THAT.
I HEAR IT'S ONLY A CERTAIN COLOR THAT CAUSES IT.
SCIENTISTS!
BUT MILLINECRAFT!


WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE (P > 0.05).
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WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND A LINK BETWEEN GREEN JELLY BEANS AND ACNE (P < 0.05).
WHOA!
WE FOUND NO LINK BETWEEN MAUVE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN MAGENTA JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN YELLOW JELLY BEANS AND ACNE (P > 0.05).
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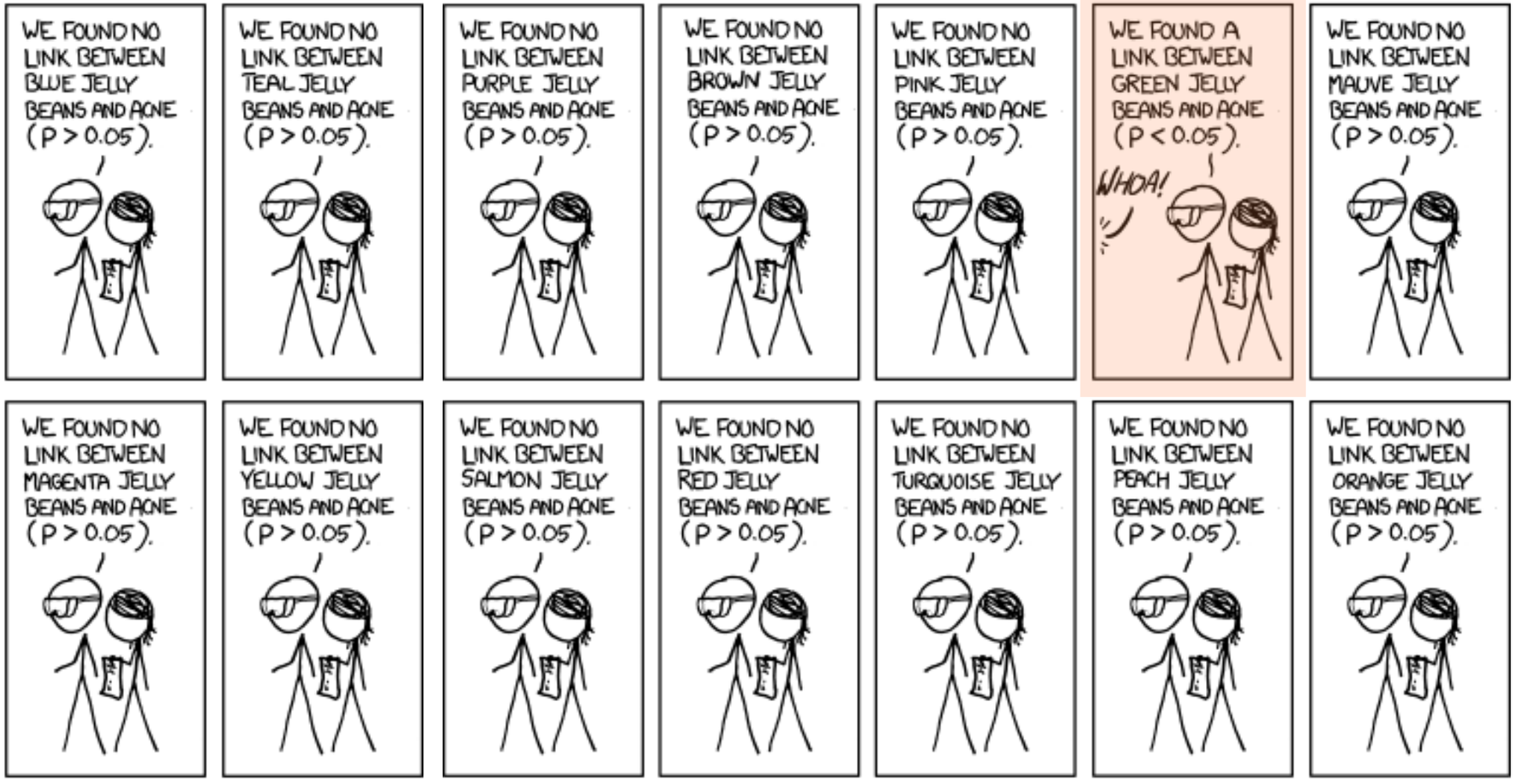
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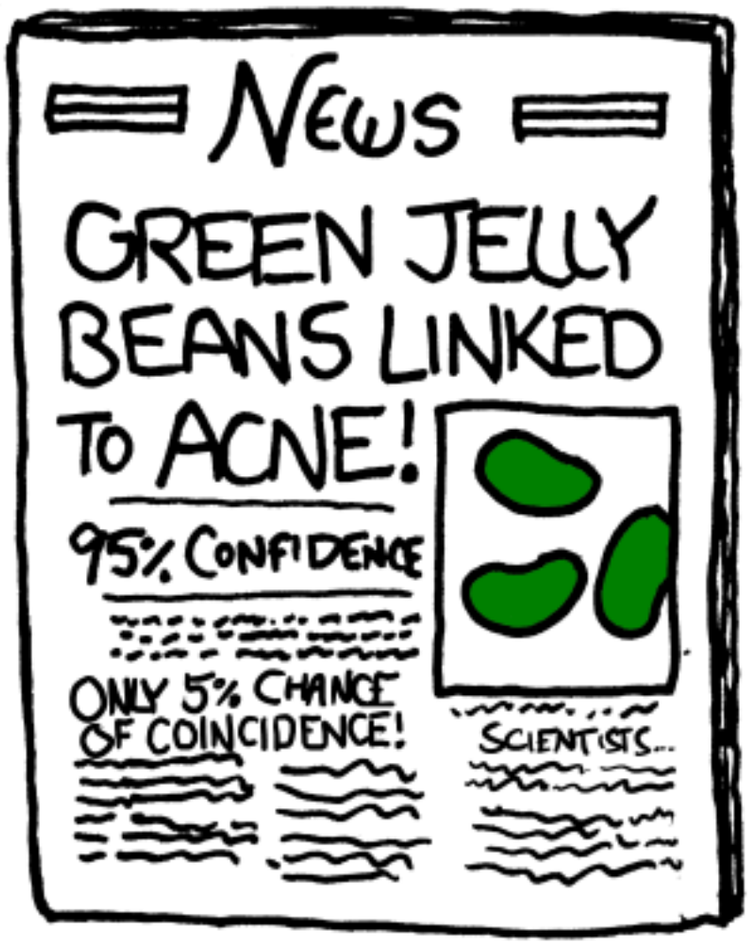
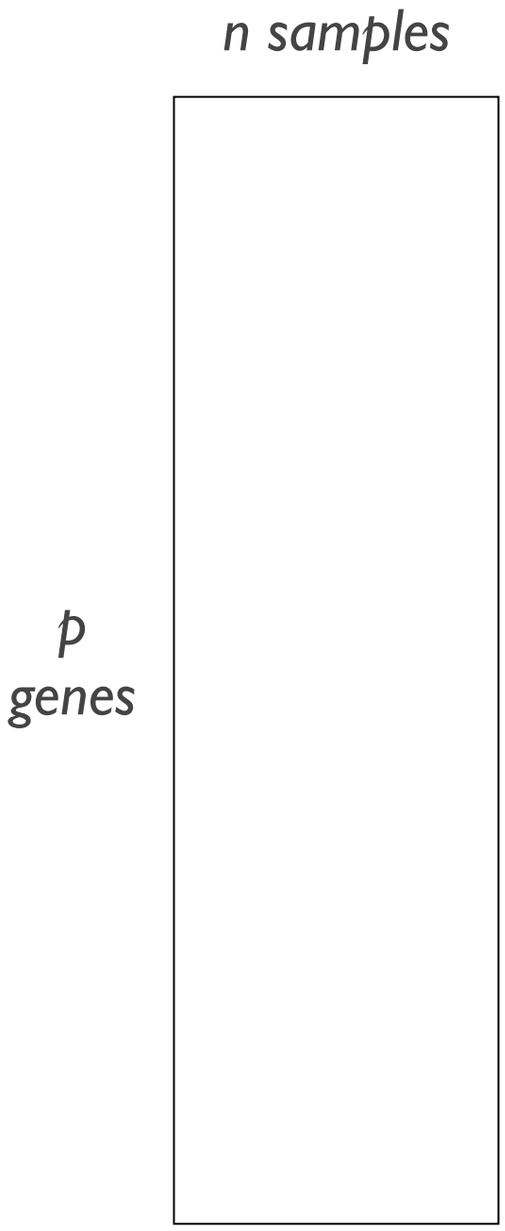

News
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...



We're doing p simultaneous tests!

$H_1, H_2, H_3, \ldots, H_p$

Bonferonni Correction

Storey's q-value