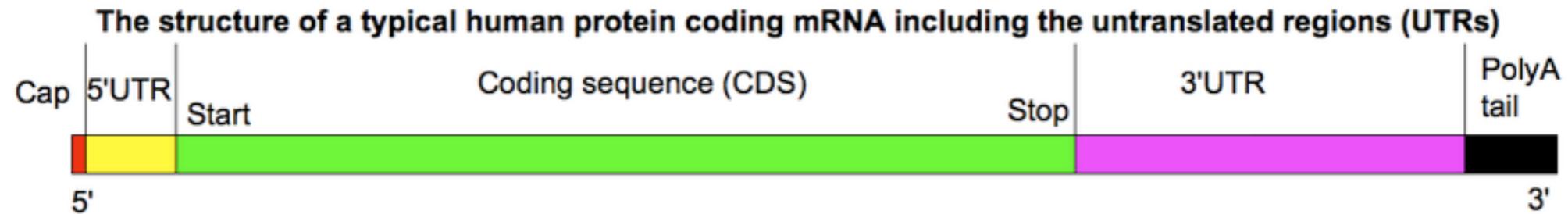


RNASeq Introduction

Malay (malay@uab.edu)

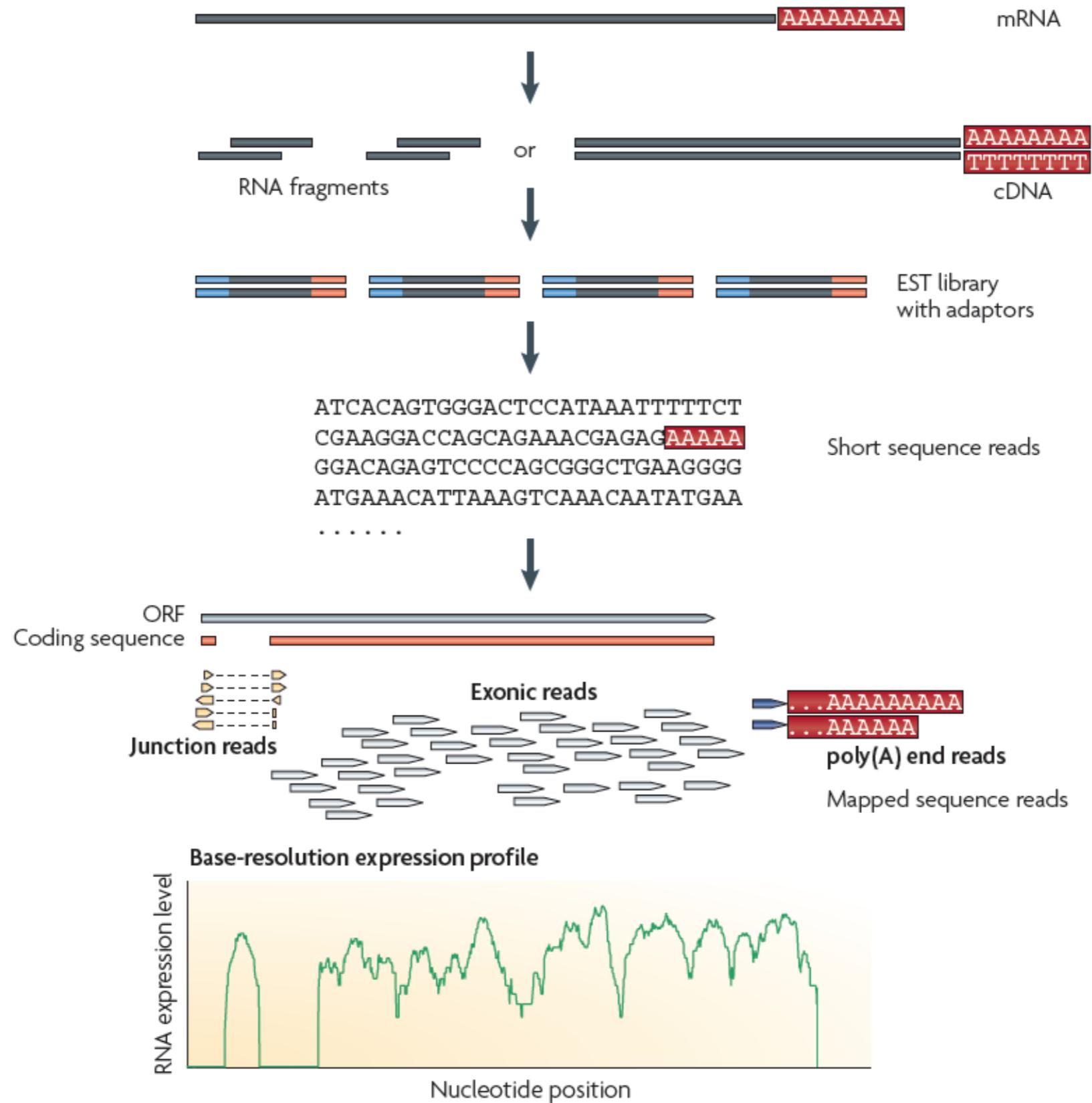
Structure of mRNA



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

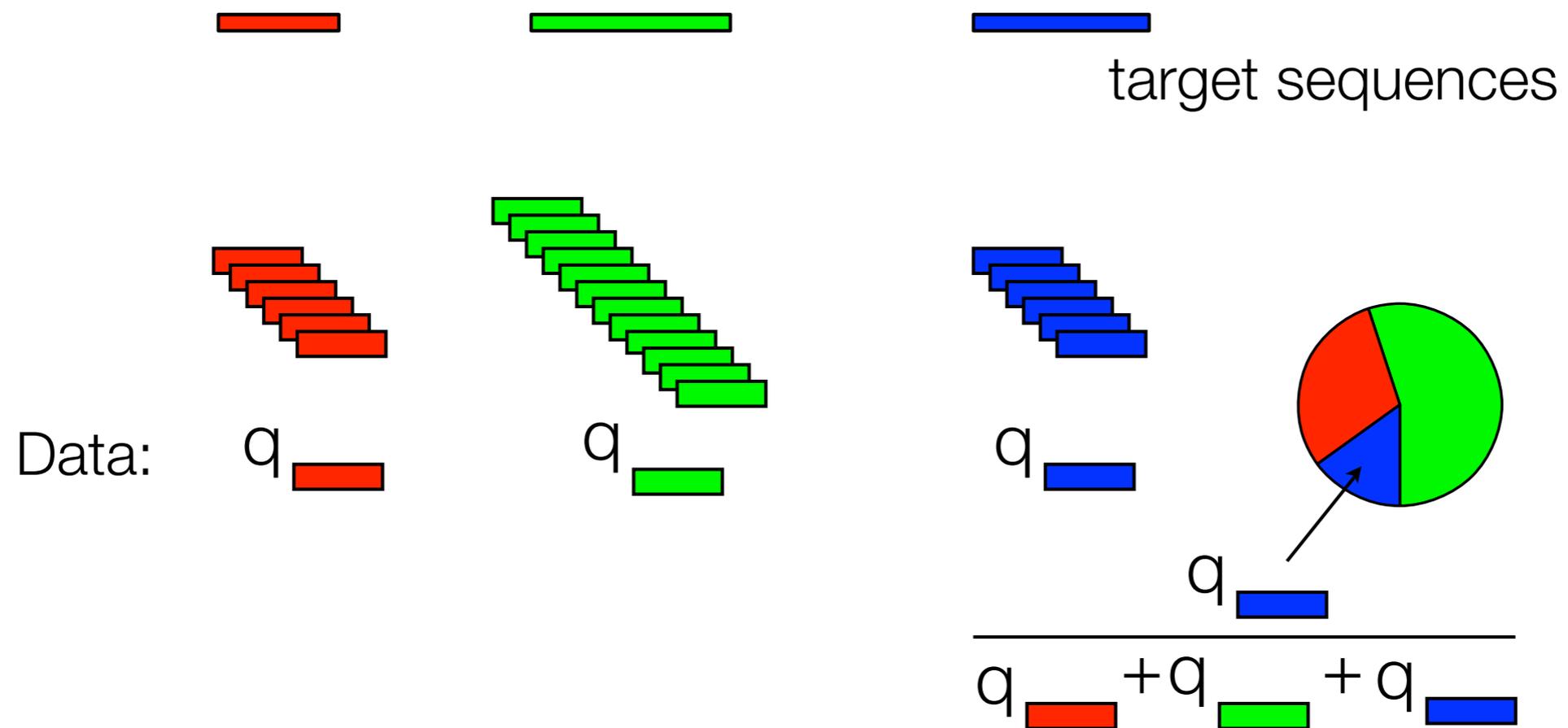
Fusion transcript detection

Mutation detection (not common)

Example count tables:

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

The fragment assignment problem



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.

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

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

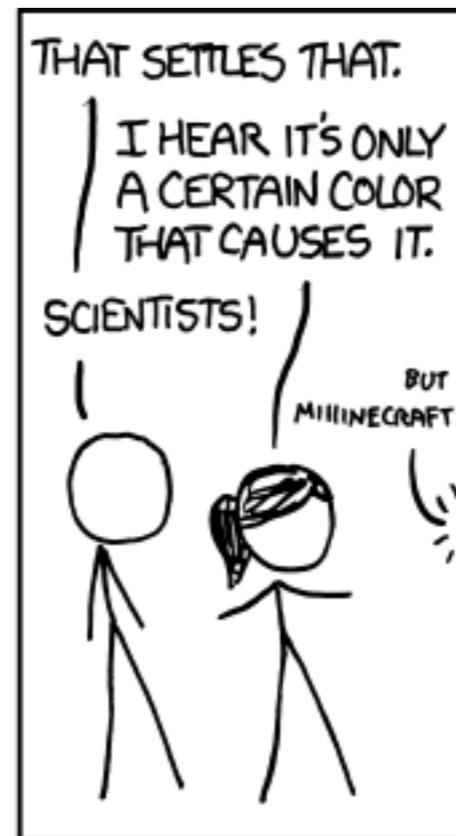
$$FPKM_{Act7} = \frac{1000}{\frac{1000}{10^3} \times \frac{12^6}{10^6}} = 83.3$$

$$FPKM_{Act7} = \frac{500}{\frac{1000}{10^3} \times \frac{1,000,010}{10^6}} = 499.995$$

$$FPKM_{Act7} = \frac{1000}{\frac{1000}{10^3} \times \frac{2^6}{10^6}} = 500$$

$$FPKM_{Act7} = \frac{500}{\frac{1000}{10^3} \times \frac{1^6}{10^6}} = 500$$

False Discovery Rate and q-value



WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE ($P > 0.05$).



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 ($P > 0.05$).



WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN TURQUOISE JELLY BEANS AND ACNE ($P > 0.05$).

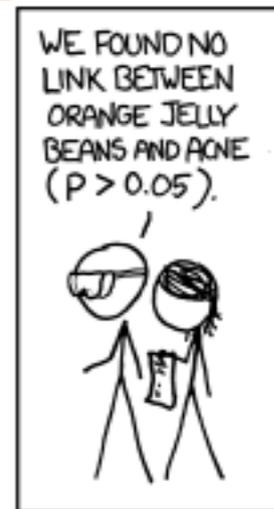
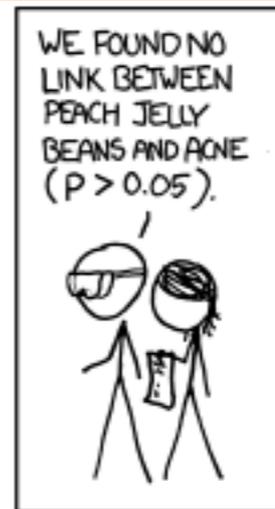
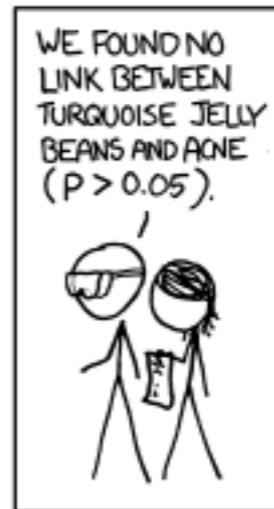
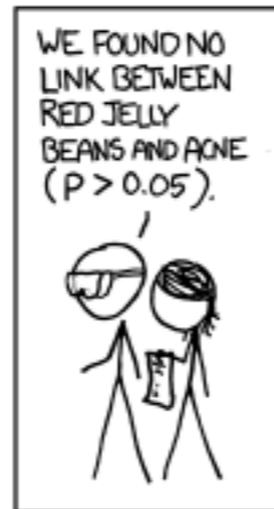
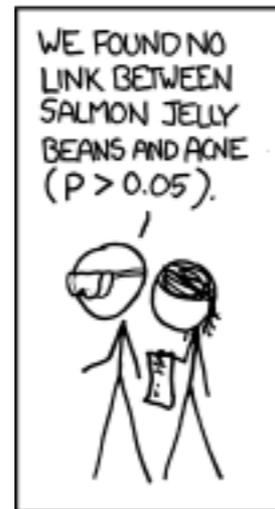
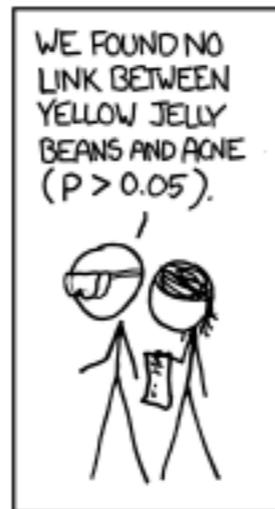
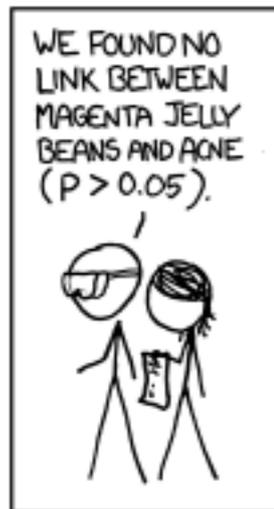
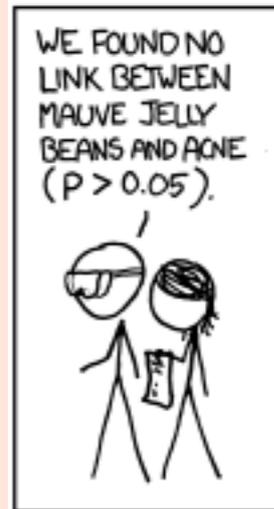
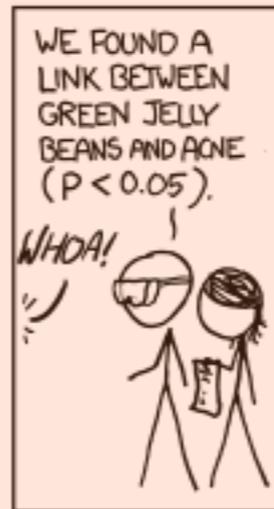
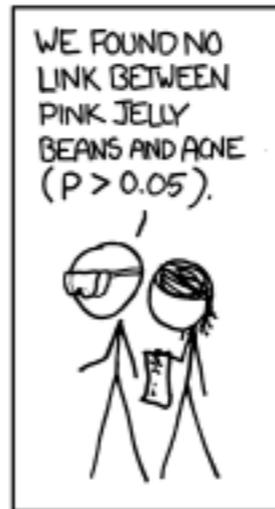
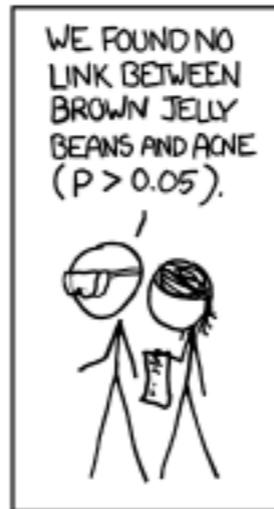
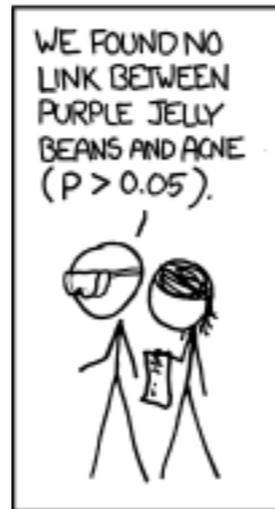
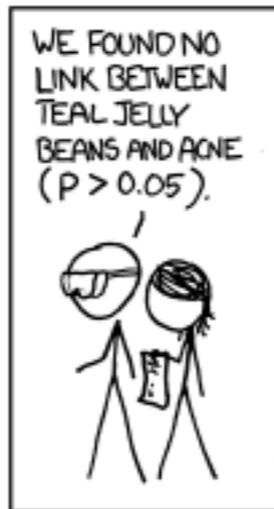
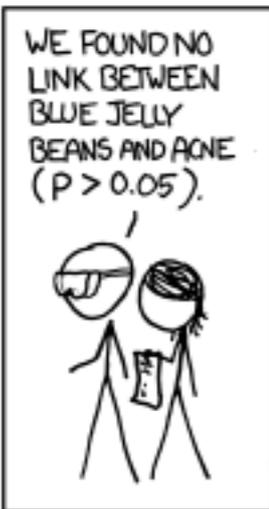


WE FOUND NO LINK BETWEEN PEACH JELLY BEANS AND ACNE ($P > 0.05$).



WE FOUND NO LINK BETWEEN ORANGE JELLY BEANS AND ACNE ($P > 0.05$).





≡ News ≡

GREEN JELLY BEANS LINKED TO ACNE!

95% CONFIDENCE



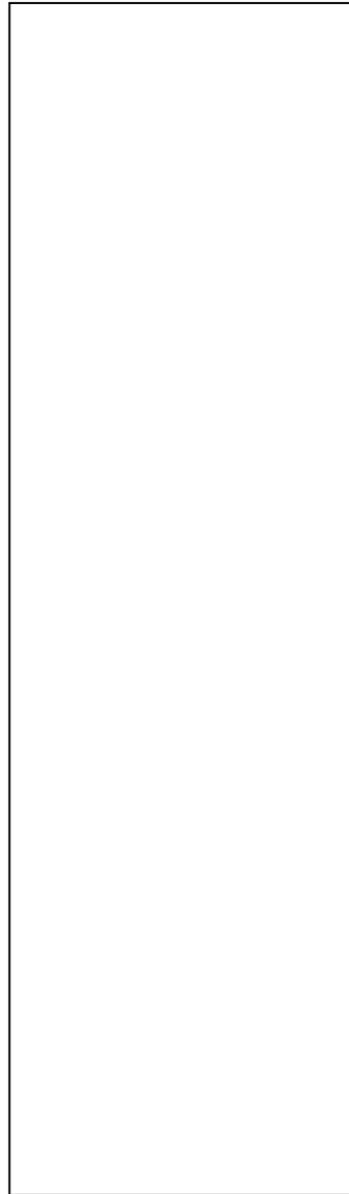
ONLY 5% CHANCE OF COINCIDENCE!

SCIENTISTS...

Wavy lines representing filler text in the article.

n samples

p
genes



We're doing p
simultaneous tests!

$H_1, H_2, H_3, \dots, H_p$

Bonferroni Correction

Storey's q-value