3000788 Intro to Comp Molec Biol

Lecture 2: Probability and statistics

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Probability is the basis of statistics

- P-value = probability of observing the same or more extreme result, given that the null hypothesis is true
  - Probability of seeing >2-fold up-regulation of gene A in drug treated patient by chance, given that the drug does not affect gene A

- Likelihood ratio = $\frac{\mathrm{P(observed\ data\ |\ model\ 1)}}{\mathrm{P(observed\ data\ |\ model\ 2)}}$
  - If LR is high, reject model 2. If LR is low, reject model 1
  - $\frac{\mathrm{P(observed\ monkey\ pox\ genome\ diversity\ |\ mutation\ rate{=}0.3)}}{\mathrm{P(observed\ monkey\ pox\ genome\ diversity\ |\ mutation\ rate{=}0.01)}}$

Probability is about what can happen

- In a human genome with 20,000 genes, there are 518 kinases. What is the probability that a randomly selected gene is a kinase?

- Gene A has two alleles, *A* and *a*. The frequency of *A* in Thai population is 0.8. What is the probability that your genotype is *AA*? What about *Aa*?

- If the rate of death due to pancreatic cancer is 15% per year, what is the probability that a pancreatic cancer patient survives for at least 5 years?

Probability is about what can happen

- In the human genome with 3 billion base pairs, what is the probability of observing the pattern TAATTA by chance?

- Given a 50-bp DNA sequencing read, what is the expected number of locations on a 3 billion base pairs genome that this read will match to by chance?

More advanced examples

- In a human genome with 20,000 genes, there are 518 kinases. From a comparison of gene expression between control and drug-treated cells, there are 300 differentially expressed genes (DEGs). What is the expected number of kinases among these DEGs?

- Gene A has two alleles, *A* and *a*. The frequency of *A* in Thai population is 0.8, and the allele *aa* is embryonic lethal. What is the probability that your genotype is *AA*? What about *Aa*?

Permutation and combination

- There are *N*! = *N*(*N*-1)(*N*-2)…x1 ways to permute *N* objects (order them)
  - O_1 O_2 … O_N
  - There are *N* choices for the first position
  - There are *N*-1 choices for the second position, anything but the first object
  - And so on

- There are $\binom{N}{k} = \frac{N!}{k!(N-k)!}$ ways to select *k* objects from a pool of *N* objects
  - Do you know how we get this expression?

Permutation and combination

- There are $\binom{N}{k} = \frac{N!}{k!(N-k)!}$ ways to select *k* objects from a pool of *N* objects
  - Order all *N* objects and choose the first *k* objects
  - There are *N*! ways. But some of them result in the same selection
    - $[O_1\ O_2 \ldots O_k]\ O_{k+1}\ O_{k+2} \ldots O_N$
    - $[O_2\ O_1 \ldots O_k]\ O_{k+2}\ O_{k+1} \ldots O_N$
    - $[O_k\ O_2 \ldots O_1]\ O_N\ O_{k+2} \ldots O_{k+1}$
  - There are *k*! ways to order the first *k* objects and get the same selection
  - There are (*N*-*k*)! ways to order the last *N*-*k* objects and get the same selection

- In probability, division usually means you overcount and then compensate by dividing by the number of duplicates

Discrete probability distributions

- Binomial distribution
  - Independent trial with two mutually exclusive outcomes: Win and Lose
  - P(Win) = p, P(Lose) = 1 – p
  - Probability of getting k Wins out of N trials = $\binom{N}{k} p^k (1-p)^{N-k}$
  - Expected number of Win = pN

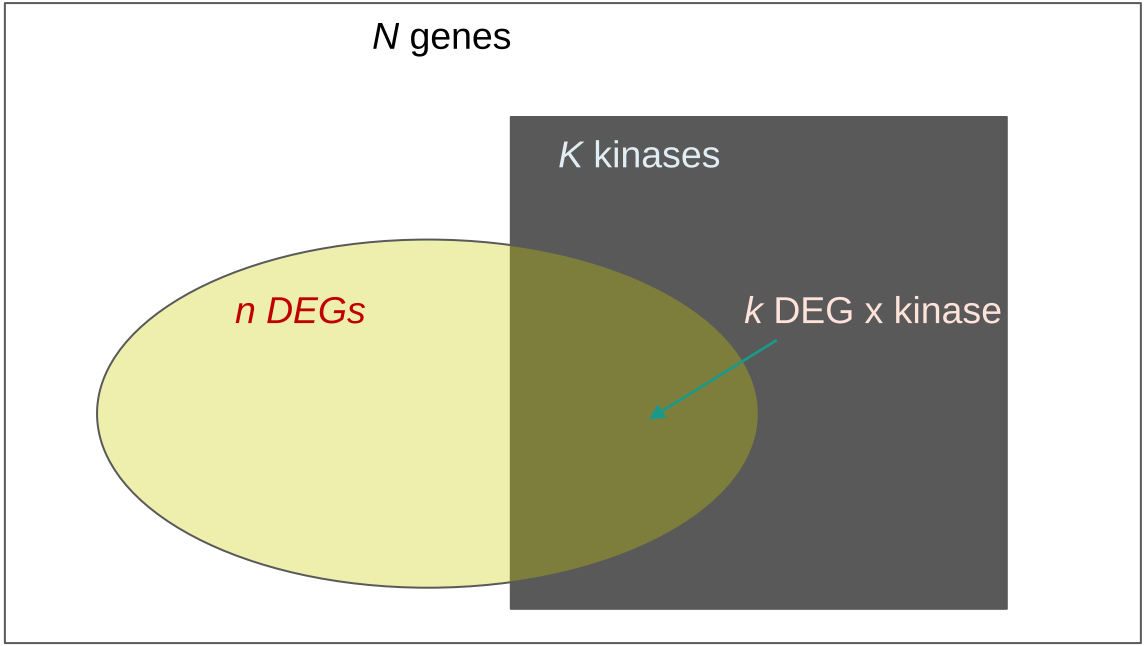
- Poisson distribution
  - Independent events occurring with expected count = λ
  - Probability of observing exactly k events = $\frac{\lambda^k e^{-\lambda}}{k!}$
  - Expected number of events = λ

Derivation of Poisson distribution

- Binomial model
  - *N* independent discrete trials with probability of success *p*

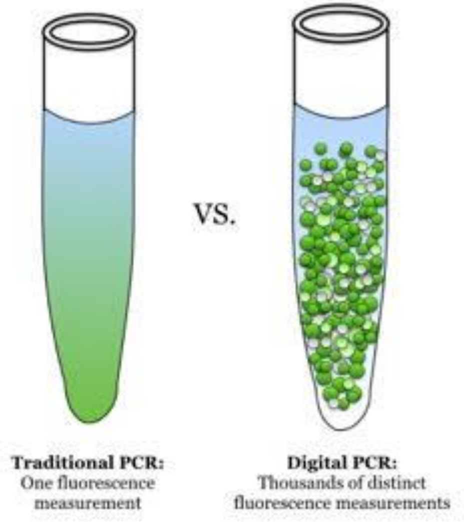
- Poisson = Binomial with *N* → infinity
  - Events have the probability to occur continuously
  - Probability of success *p* = $\frac{\lambda}{N}$
  - $\lim_{N\to\infty} \binom{N}{k} \left(\frac{\lambda}{N}\right)^{k} \left(1-\frac{\lambda}{N}\right)^{N-k} = \lim_{N\to\infty} \frac{N!}{N^{k}(N-k)!} \frac{\lambda^{k}}{k!} \left(1-\frac{\lambda}{N}\right)^{N} \left(1-\frac{\lambda}{N}\right)^{-k} = \frac{\lambda^{k} e^{-\lambda}}{k!}$

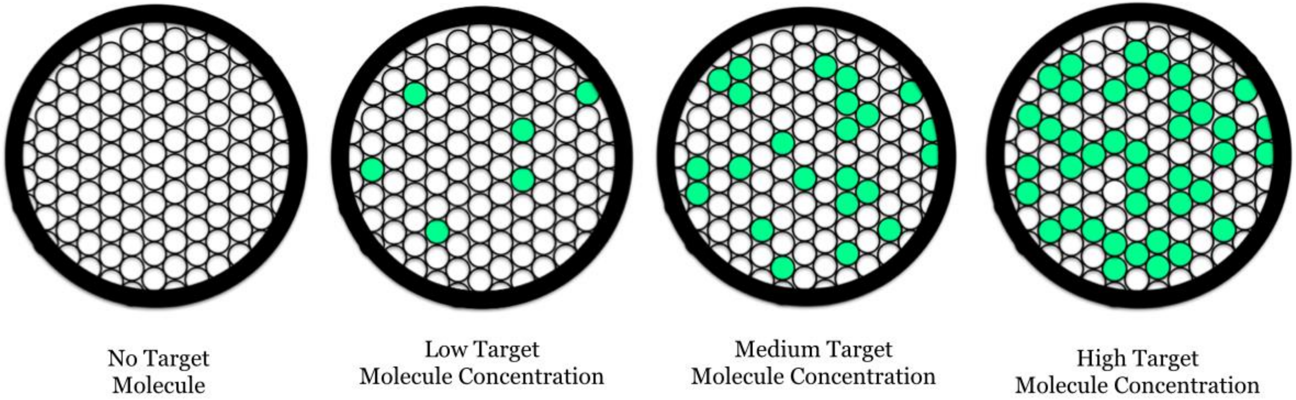
Hypergeometric distribution



- Why does the probability = $\frac{\binom{K}{k}\binom{N-K}{n-k}}{\binom{N}{n}}$?

Example 1: Digital Droplet PCR


VS.
Traditional PCR:
One fluorescence measurement
Digital PCR:
Thousands of distinct fluorescence measurements


No Target Molecule
Low Target Molecule Concentration
Medium Target Molecule Concentration
High Target Molecule Concentration

https://en.wikipedia.org/wiki/Digital_polymerase_chain_reaction

- M total DNA molecules: M_a of allele a and M_A of allele A
- N droplets, k are positive for allele a
- Likelihood ratio test of $\dfrac{\mathrm{P}(k \text{ positive droplets} \mid \text{patient genotype } AA)}{\mathrm{P}(k \text{ positive droplets} \mid \text{patient genotype } Aa)}$

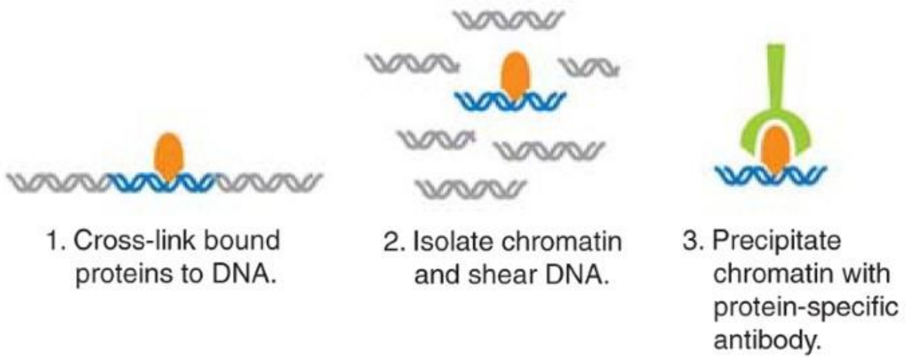
Digital Droplet PCR

- Fluorescent signal is a “success” of PCR probe binding to a molecule of allele *a*
- *M* total DNA molecules: M_a of allele *a* and M_A of allele *A*
- *N* droplets, *k* are positive for allele *a*

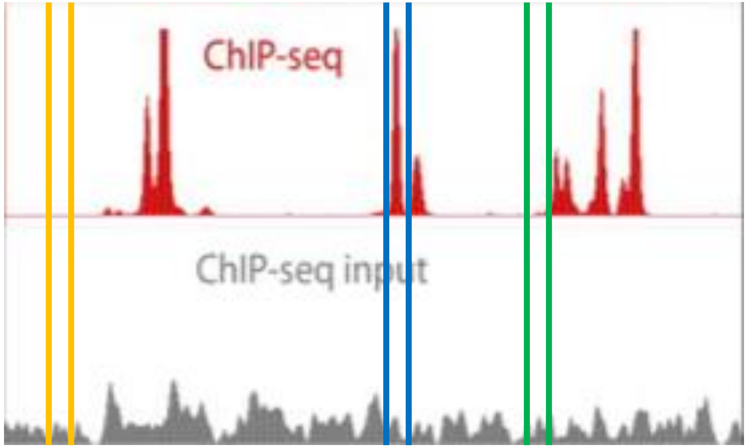
- Q1: Which distribution captures the number of “success” in each droplet?
- Q2: What is the expected number of “successes” in each droplet?
- Q3: What is the probability of observing a positive droplet (≥1 success)?

- Q4: Which distribution captures the number of positive droplets?
- Q5: What is the probability of observing *k* positive droplets in this experiment?

Example 2: ChIP-seq peak assessment


1. Cross-link bound proteins to DNA.
2. Isolate chromatin and shear DNA.
3. Precipitate chromatin with protein-specific antibody.

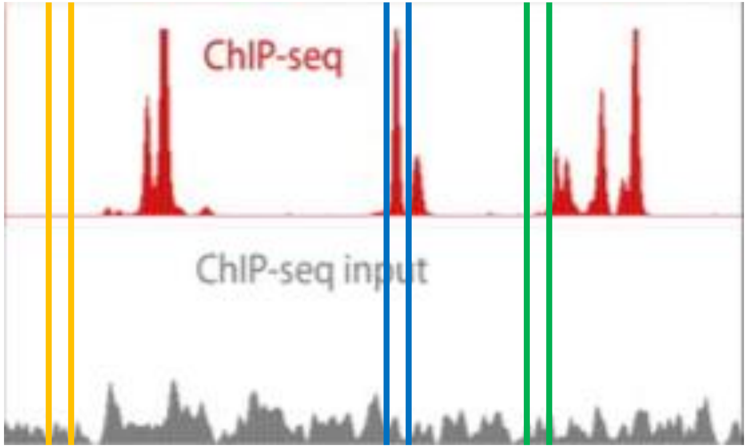
Shah, A. Nature Methods 6:i-ii (2009)



Park et al. Nat Rev Genet 10:669-680 (2009)

- ChIP-seq peak = enrichment of DNA read at certain genomic position
  - Imply protein binding or histone modification
- Does the peak arise from bias in DNA sequencing?

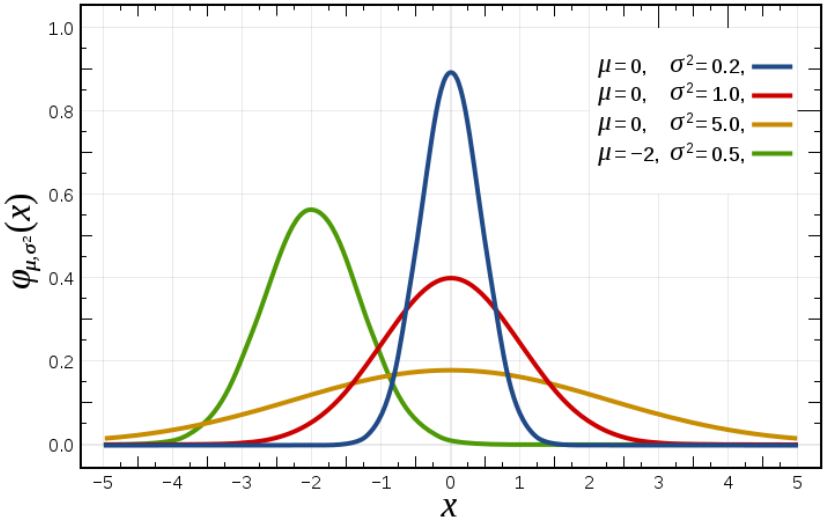
ChIP-seq peak assessment



Park et al. Nat Rev Genet 10:669-680 (2009)

- Estimate expected number λ_g of reads at a genomic position *g* from a control
  - Also called ChIP-seq input, without immunoprecipitation
- Probability of observing *k* reads at a position *g* = Poisson(λ_g)

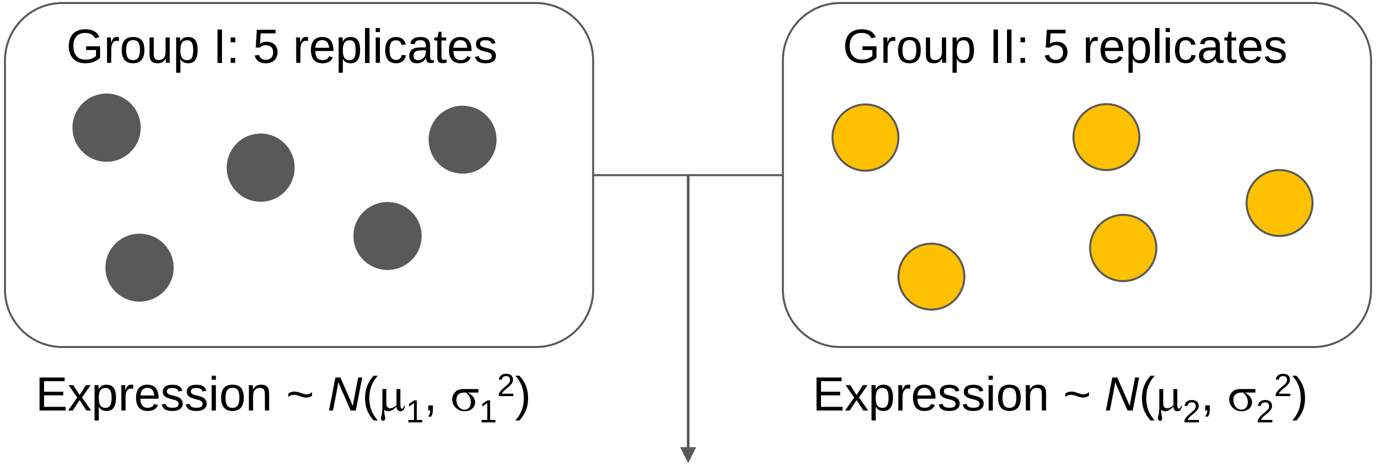
Continuous distributions



Images from https://en.wikipedia.org/wiki/Normal_distribution

- Normal or Gaussian distribution
- Defined by location (mean) and spread (variance)
  - $N(\mu, \sigma^2) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{\sigma^2}}$

Properties of normal distribution



Group I: 5 replicates

Group II: 5 replicates

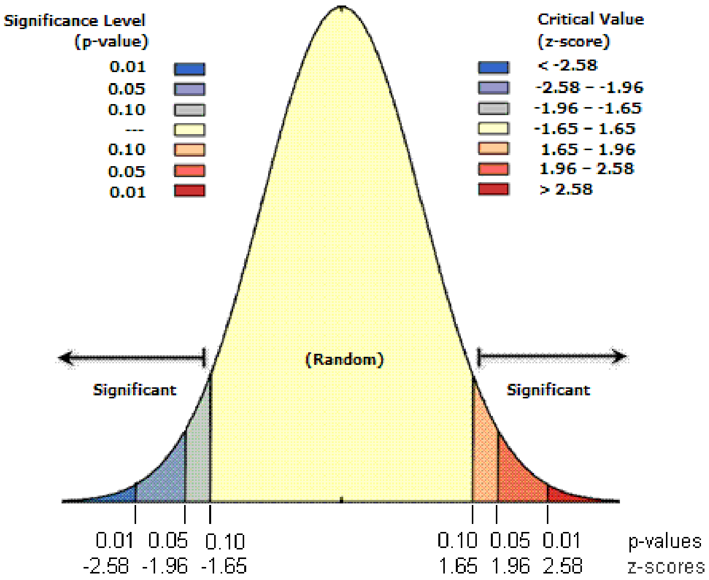
Expression ~ $N(\mu_1, \sigma_1^2)$

Expression ~ $N(\mu_2, \sigma_2^2)$

Difference ~ $N(\mu_1 - \mu_2, \sigma_1^2 + \sigma_2^2)$

- $N(\mu, \sigma^2) + \mathrm{c} = N(\mu + c, \sigma^2)$
- $N(\mu, \sigma^2) \times \mathrm{c} = N(c\mu, (c\sigma)^2)$

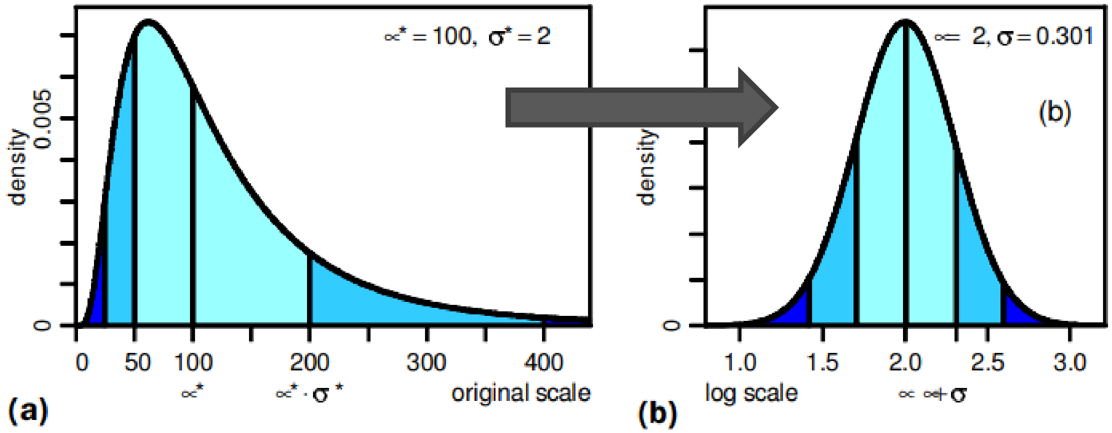
Standard normal distribution and Z-score



https://desktop.arcgis.com/en/arcmap/10.4/tools/spatial-statistics-toolbox/what-is-a-z-score-what-is-a-p-value.htm

- Z-score = $\frac{x - \text{mean}}{\text{standard deviation}}$
- If the data came from a normal distribution, $N(\mu, \sigma^2)$, Z-score is the transformation to standard normal distribution, $N(0, 1)$
- Z-score can be converted to p-value

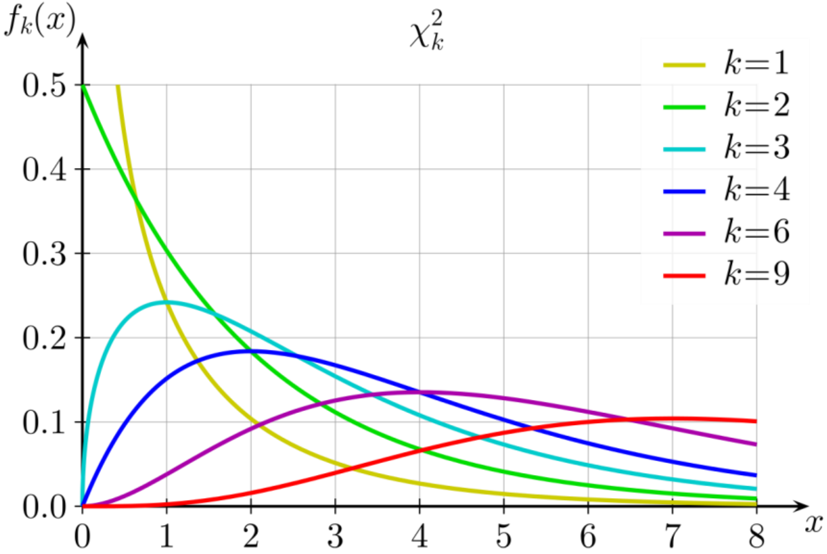
Log-normal distribution



Limpert, Stahel, and Abbt. BioScience 2001.

- Some data, especially intensity, are not normally distributed but their log are
  - Fluorescence intensity
  - Gene expression from microarray
  - Peptide abundance from mass spectrometry

Chi-squared distribution



Images from https://en.wikipedia.org/wiki/Chi-squared_distribution

- Equal to $\sum_{i=1}^{k} Z_i{}^2$ where Z_i are standard normal (this may seem unnatural but is useful in many statistical tests)

Statistical tests related to Chi-squared

Genotype	*A/A*	*A/a*	*a/a*
Expected frequency	200	120	70
Observed frequency	90	210	80

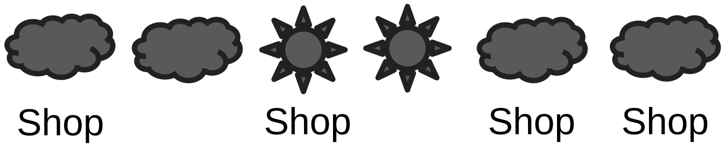
- $\sum_i \frac{(O_i - E_i)^2}{{E_i}^2}$ follows Chi-squared distribution
- Test of nested models, such as multiple mutation rate models
  - Likelihood ratios = $\frac{\mathrm{P(data \mid complex\ model)}}{\mathrm{P(data \mid simple\ model)}}$
  - −2 x Log Likelihood ratio follows Chi-squared distribution

Statistics explains things that already happened

- Gene A has two alleles, *A* and *a*. A study of 1,000 Thai individuals found 700 with genotype *AA*, 200 with genotype *Aa*, and 100 with genotype *aa*. What is the estimated allele frequency of *a*?

- In a study of 5 pancreatic cancer patients, they survived for 1, 5, 3, 4, and 5 years. What is the estimated yearly survival rate?

Let’s review some terminology



- P(A) = probability that A occurs

Joint probability

- P(A, B) = probability that A and B occurs

Conditional probability

- P(A | B) = probability that A occurs given that B already occurred
- P(A | B) = P(A, B) / P(B)
- P(A, B) = P(A | B) P(B) = P(B | A) P(A)

- P(Shopping | Sunny) = ? How about P(Sunny | Shopping)?

Maximum likelihood principle

- Likelihood = P(data | model), or P(data | hypothesis)
- Find model that maximize likelihood

- Gene A has two alleles, *A* and *a*. A study of 1,000 Thai individuals found 700 with genotype *AA*, 200 with genotype *Aa*, and 100 with genotype *aa*. What is the estimated allele frequency of *a*?
  - Let's set the allele frequencies $f_A = p$ and $f_a = 1 - p$
  - $P(AA) = p^2$, $P(Aa) = 2p(1 - p)$, and $P(aa) = (1 - p)^2$
  - Likelihood = $P(AA)^{700} P(Aa)^{200} P(aa)^{100} = p^{1400} 2^{200} p^{200} (1 - p)^{200} (1 - p)^{200}$

    $= 2^{200} p^{1600} (1 - p)^{400}$
  - Which p maximize the likelihood?
    - Solve the equation $\frac{dLikelihood}{dp} = 0$ → $p_{MLE} = 0.8$

Maximum likelihood principle

- In a study of 5 pancreatic cancer patients, they passed away after 1, 5, 3, 4, and 5 years, respectively. What is the estimated yearly survival rate?
  - Let's set the yearly survival rate = r
  - P(survive exactly k years) = $r^k(1 - r)$
  - P(data | r) = $r^1(1 - r)\, r^5(1 - r)\, r^3(1 - r)\, r^4(1 - r)\, r^5(1 - r) = r^{18}(1 - r)^5$
  - Which r maximize the likelihood?
    - Solve the equation $\frac{dLikelihood}{dr} = 0$ → r_{MLE} = 18/23

Bayes' rule

- P(A | B) / P(A) = P(B | A) / P(B)
- Just rearranging what we knew!

- Why is Bayesian powerful?
  - Data is already collected. But the model is yet to be determined.
  - Shouldn't we want P(model | data) instead of likelihood?
  - But, how to compute

- P(model | data) = P(data | model) * P(model) / ~~P(data)~~
  - Prior belief, P(model), can be integrated with likelihood to get a better estimate
  - If we have no prior information, reduce back to likelihood

Likelihood ratio test

- Likelihood ratio = $\frac{\mathrm{P(data \mid model\ 1)}}{\mathrm{P(data \mid model\ 2)}}$
  - If LR is high, reject model 2. If LR is low, reject model 1

- Example:
  - Test for viral spreading rate: $\frac{\mathrm{P(COVID{-}19\ infection \mid spreading\ rate > 1.5)}}{\mathrm{P(COVID{-}19\ infection \mid spreading\ rate < 1.5)}}$
  - Test for impact of treatment: $\frac{\mathrm{P(gene\ expression \mid control\ and\ treatment\ differ)}}{\mathrm{P(gene\ expression \mid all\ samples\ are\ the\ same)}}$

- This is theoretically the most powerful test (Neyman-Pearson Lemma)

Nested model testing

- **Simple model**: Omicron and Delta have the same spreading rate
  - One parameter
- **Complex model**: Omicron and Delta have different spreading rates
  - Two parameters

- Complex model always achieve higher likelihood
  - Find two spreading rates that fit the data better than a single rate

- But is the additional complexity worth it?
  - $\frac{\text{P(data | complex model)}}{\text{P(data | simple model)}}$ must be much greater than 1 to reject the simple model
  - Akaike information criterion (AIC): 2 x # parameters – log likelihood

Null hypothesis-based testing

- **Alternative hypothesis**: Omicron BA.5 spreads more easily than other strains
- **Null hypothesis**: Omicron BA.5 spreads at the same rate as other strains

- Data = Rise of COVID-19 cases with frequency of BA.5 in population

- Likelihood under alternative hypothesis = P(Data | spread rates for all strains)
  - We want to show that this is more likely than null hypothesis
  - But difficult to calculate!

- Likelihood under null hypothesis = P(Data | same spread rate)
  - Easier to calculate
  - We will try to show that this is unlikely instead

P-value

- Probability of observing the same or more extreme result, given that the null hypothesis is true

- How to quantify the same or more extreme?
  - If BA.5 has the same spread rate as other strains, rise in BA.5 frequency shouldn’t increase the number of new daily infections
  - What if we measure the rate of increase in daily infections?

P-value example

- Before BA.5, a study estimated the rate of daily increase in COVID-19 infections with a normal distribution $N(1.3, 0.01)$

- After BA.5, data show that the rate of daily increase in COVID-19 infections is 1.5

- P-value = P(daily rate ≥1.5 | BA.5 has the same spread rate as prior strains)
  = P(getting value ≥1.5 from $N(1.3, 0.01)$)
  = P-value of Z-score of 2 = 0.02275

- Reject null hypothesis

P-value caution

- Before BA.5, a study estimated the rate of daily increase in COVID-19 infections with a normal distribution $N(1.3, 0.01)$

- After BA.5, data show that the rate of daily increase in COVID-19 infections is 1.4

- P-value = P(daily rate ≥1.4 | BA.5 has the same spread rate as prior strains)
  = P(getting value ≥1.4 from $N(1.3, 0.01)$)
  = P-value of Z-score of 1 = 0.158655

- Do we accept null hypothesis?

Test statistics

- A measure, or score, of the same or more extreme result

- In one-sample *t*-test of whether the mean $\bar{x}$ of data {x_1, x_2, ..., x_n} is equal to β, the test statistics is $t = \frac{\bar{x}-\beta}{\frac{SD}{\sqrt{n}}}$
  - Measure how close is $\bar{x}$ to β, subject to the variability of the data
  - Correspond to the null hypothesis that the mean of the data is β
  - The more *t* deviates from zero, the more extreme the result

- P-value = P($t \geq t_{\text{observed}}$ | the data is normally distributed with mean β)
  - *t* follows N(0, 1) by Central Limit Theorem

Test statistics behind popular tests

- Mann-Whitney U test:

$$U=\sum_{i=1}^{n}\sum_{j=1}^{m}S(X_i,Y_j),\quad S(X,Y)=\begin{cases}1, & \text{if } X>Y,\\ \frac{1}{2}, & \text{if } Y=X,\\ 0, & \text{if } X<Y.\end{cases}$$

- Wilcoxon rank-sum test:

1. Compute $|X_1|,\ldots,|X_n|$.
2. Sort $|X_1|,\ldots,|X_n|$, and use this sorted list to assign ranks $R_1,\ldots,R_n$

$$T=\sum_{i=1}^{N}\operatorname{sgn}(X_i)R_i.$$

- Sign test: Assume that each observation is equally likely to be positive or negative
  - Probability of *k* positive values out of *N* observations = Binomial(*N*, *k*, *p* = 0.5)

Impact of null hypothesis choices

Cell	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7
C1	0.701	0.503	0.991	0.827	0.623	0.728	0.596
C2	0.691	0.478	0.905	0.739	0.589	0.719	0.508

- Are gene expressions up-regulated in cell C1?
  - Unpaired Student’s t-test p-value = 0.5687
  - Mann-Whitney U test p-value = 0.6101
  - Paired Student’s t-test p-value = 0.0137
  - Wilcoxon signed rank test p-value = 0.0156
  - Sign test p-value = 0.00815

Null hypothesis-based testing framework

- Propose null hypothesis
  - The data are normally distributed with mean = β
- Design the test statistic $t = \frac{\bar{x} - \beta}{\frac{SD}{\sqrt{n}}}$
- Derive the distribution of test statistic under the null hypothesis
  - This is where probability knowledge comes in
- Specify the significance level α to reject null hypothesis (e.g., 0.05)
- Calculate p-value: $P(t \geq t_{\text{observed}} \mid \text{null hypothesis})$
- By following this framework, new tests can be created!

Correlation

Cell	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7
C1	0.701	0.503	0.991	0.827	0.623	0.728	0.596
C2	0.691	0.478	0.905	0.739	0.589	0.719	0.508

- Correlation of gene expression between C1 and C2 = 0.9746
- How significant is this?
  - We have only one dataset and no information about the underlying distribution
- Null hypothesis
  - Gene expression between C1 and C2 are uncorrelated
  - C2 data can be shuffled and still give the same correlation score

Permutation test

- **Alternative hypothesis**: The observed property of the data, such as high correlation, is due to some structure, such as the pairing of genes, in the data

- **Null hypothesis**: That structure in the data does not contribute to the property of interest

- P-value = Probability that the shuffled data has the same or more extreme property than the original data

- Shuffle data in such a way that the structure of interest is disrupted
- Calculate the property of interest and compared to the original score

Permutation test

Cell	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7
C1	0.701	0.503	0.991	0.827	0.623	0.728	0.596
C2	0.691	0.478	0.905	0.739	0.589	0.719	0.508

Correlation = 0.97

1,000 times

Correlation = 0.24

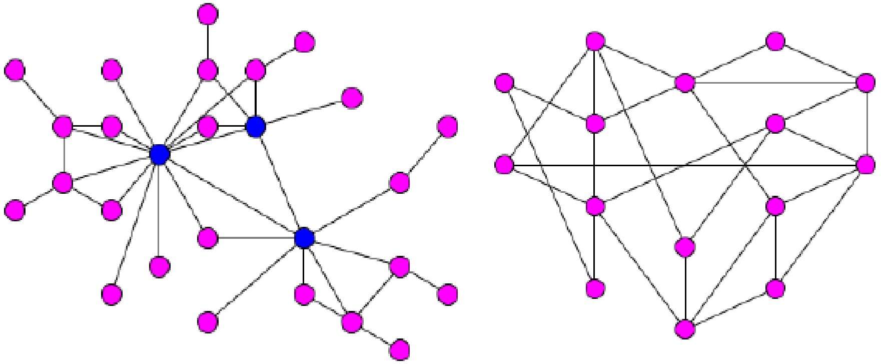
Correlation = -0.30

C2	0.719	0.691	0.739	0.589	0.508	0.905	0.478

Correlation = 0.32

Permutation test for network data

Biological network has hubs that serve as shortcut between other nodes



Random network is not well-connected

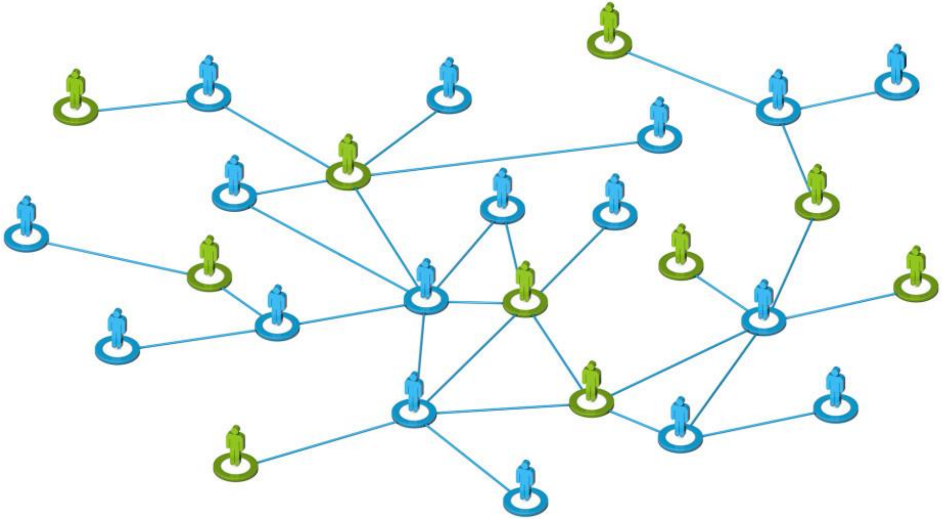
Source: Segura-Cebrera *et al*. Analysis of Protein Interaction Networks to Prioritize Drug Targets of Neglected-Diseases Pathogens

- **Null hypothesis**: The small diameter of biological network can be achieved by chance in networks with the same number of nodes and edges

- **Permutation test**: Generate 1,000 random networks with the same number of nodes and edges and compute the diameter of these networks

Permutation test for network data

Same-gender FB friendship occur more easily than different-gender ones



- **Null hypothesis**: The high number of same-gender edge of Facebook friendship network can be achieved by chance in random networks with the same number of nodes and edges

Correction for multiple testings

- P-value cutoff of 0.05 means that under the null hypothesis, there is only 5% chance of observing the same or more extreme result

- Applying the same test 1,000 times will result in 50 tests on average with smaller p-value than 0.05 just by chance
  - Differential expression analysis tests thousands of gene at once

- This in unacceptable if a conclusion relies on multiple tests
  - Functional enrichment analysis assumes that all input DEGs are true

Bonferroni method

- Divide the p-value cutoff by the number of test
- Adjusted p-value cutoff = 0.05 / 1000 = 0.00005

- Applying the same test 1,000 times will result in 0.05 tests on average with smaller p-value than 0.00005 just by chance

- Easy but lose power

False discovery rate (FDR)

- P-value operates under the null hypothesis

- But in practice, we want to control the number of errors in the output
    - The number of DEGs that were incorrectly proposed

- FDR = Probability of getting a false positive
  = # false positive / # all predicted positives

- But FDR involves alternative hypothesis, which is difficult to calculate

- We can control FDR somewhat through p-value!

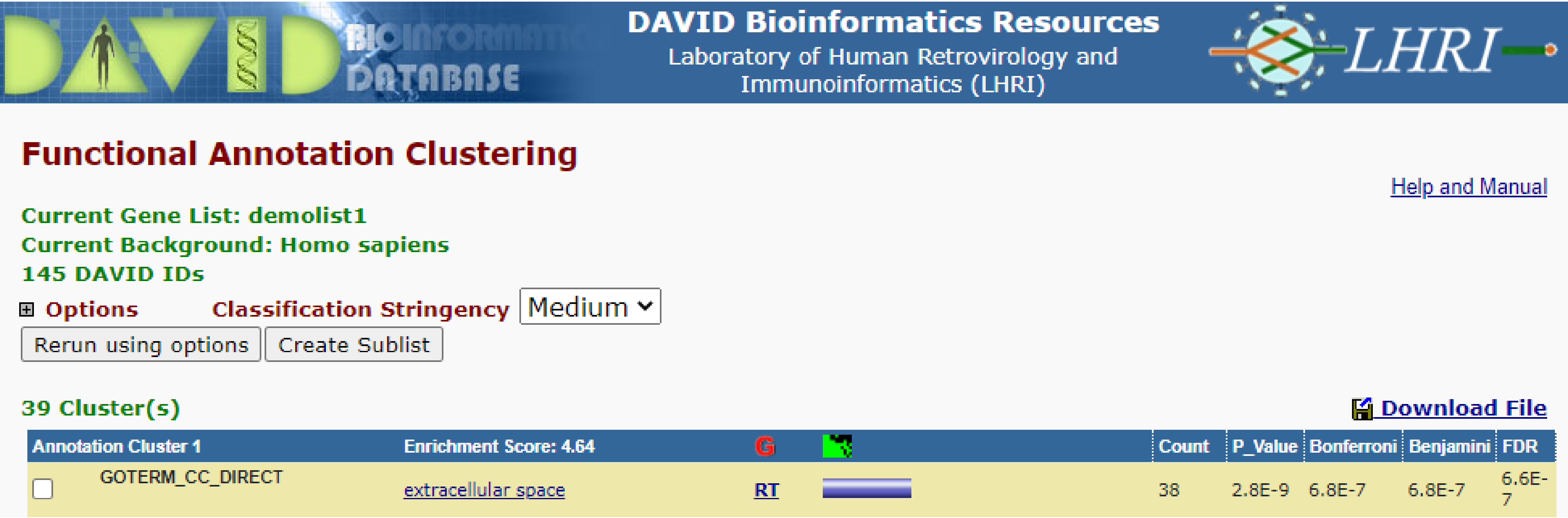
Benjamini-Hochberg procedure

- Valid under broad assumption (independent tests, etc.)

- Given a series of tests with p-values, $p_1, p_2, \ldots, p_n$

- To control FDR to be within 0.05
  - Sort p-values from low to high, $p'_1, p'_2, \ldots, p'_n$
  - Find largest *k* such that $p'_k \leq 0.05 \times k / n$
    - For the smallest p-value, this is equivalent to Bonferroni
    - For other p-values, this technique gradually loosens the cutoff
  - Reject null hypothesis for tests corresponding to $p'_1, p'_2, \ldots, p'_k$

Example of functional enrichment report



DAVID
BIOINFORMATICS DATABASE
DAVID Bioinformatics Resources
Laboratory of Human Retrovirology and Immunoinformatics (LHRI)
LHRI

Functional Annotation Clustering

Help and Manual

Current Gene List: demolist1

Current Background: Homo sapiens

145 DAVID IDs

⊞ Options Classification Stringency Medium

Rerun using options | Create Sublist

39 Cluster(s)

Download File

Annotation Cluster 1	Enrichment Score: 4.64	G	🟩	Count	P_Value	Bonferroni	Benjamini	FDR
☐ GOTERM_CC_DIRECT	extracellular space	RT	▬	38	2.8E-9	6.8E-7	6.8E-7	6.6E-7

Any question?

- See you on August 24th