2 Sequence Alignment

3 Short read assembly

4 Sequencing

5 Normalization

Assumption

Assume the subset of the genes in the sample are expressed at the same total level across all cells / samples

5.1 Methods

- **RPKM - read per kilobase of transcript per million reads of library**
  - Corrects for coverage, gene length
  - 1 RPKM 0.3 -1 transcript / cell
  - Comparable between different genes within the same dataset
  - packages: TopHat / Cufflinks

$$RPKM = \frac{\text{no. of reads mapped to gene G} \cdot 10^9}{\text{total no. of mapped reads} \cdot \text{length of gene G}}$$

- **FPKM - fragments Per Kilobase of exon model per Million mapped fragments**
  - similar to FPKM
  - for pair-end seq, $FPKM = 2RPKM$

$$FPKM = \frac{\text{no. of fragments mapped to the exons} \cdot 10^9}{\text{total no. of mapped reads} \cdot \text{length of the exon}}$$

- **TPM - transcript per million**
  - Normalizes to transcript copies instead of reads
  - Longer transcripts have more reads
  - RSEM, HTSeq
  - steps:

1. $RPK = \frac{\text{read count of gene}}{\text{length of gene}}$
2. scaling factor $= \sum RPK/10^6$
3. $TPM = \frac{RPK}{\text{scalling factor}}$

- additional assumptions

1. values are exactly the same between runs (genes could have variable values)
   → quantile normalization
2. values are normally distributed w same mean and var across samples
   → scale factor normalization
3. assume some genes have stable values over runs (rank invariant)
   → invariant set normalization

- **TMM - Trim Mean of M**
  - high level idea: remove extreme values before normalizing
  - intuition:
    sample A and B both have 100 genes sequenced to the same depth, 90 genes in A and B are expressed at about the same level, last 10 genes expressed at extremely high level in B
    → could appear as the first 90 genes expressed twice as high in A than in B, which does not make sense
    Reason: fixed amount of sequencing real estate

- observed coutns of gene g in experiment k: $E[Y_{g,k}] = \frac{\mu_{g,k} L_g}{S_k} N_k$, where
  - $S_k = \sum_g \mu_{g,k} L_g$
  - $\mu_{g,k}$ true expression level of gene g in experiment k
  - L_g length of gene g
  - N_k total no. of reads for experiment k
- $M_g = \log \frac{Y_{g,k}/N_k}{Y_{g,k'}/N_{k'}}$
- $A_g = \log Y_{g,k}/N_k + \log Y_{g,k'}/N_{k'}$
- trim off genes with extreme M and A values (in the paper, took 5% and 30% as cutoff)
- compute ratio based on all other genes

5.2 Transformation

- While ratios are useful, not symmetric
  $\rightarrow$ hard to visualize different changes
- use a log transform, and focus on the log ratio

$$y_i = \log \frac{R_i}{G_i}$$

- Empirical studies have also shown that in microarray experiments the log ratio of (most) genes tends to be normally distributed

6 DE gene

6.1 Problem

- log-fold change isn't ideal (there are problems)
- noise from technology too
- expression being double the amount → how reliable? → variation estimation
- easy to estimation variation if large biological models, but often only 2-3 replicate available
- Solution: often assume some mathematical model

6.2 Statistical models

- Gaussian
  - mean μ, var σ^2

A couple methods based on counts...

- Binomial
  - $$P(x=k) = \binom{n}{k} p^k (1-p)^{n-k}$$
  - mean: np, var: $np(1-p)$
- Poisson
  - $$P(x=k) = \frac{\lambda^k e^{-\lambda}}{k!}$$
  - mean = var = λ
- Negative Binomial
  - common when data has variance >> mean (overdispersed)
  - defined as the number of successed in a seq of Bernoulli trials before some number of event r occurs, eg. no. of trials until 3 heads
  - Thus appropriate for modelling biological replicates
  - $$P(x=n) = \binom{n-1}{k} p^k (1-p)^r$$
    where r: no. of failures, k: no. of successes until r failures, and $n = k + r$
  - mean: $rp/(1-p)$, var = $rp/(1-p)^2$
  - can be re-written in a format similar to Poisson dist.
  - Poisson assumes same mean and variance, whereas NB assumes larger variance
  - → dispersion parameter α s.t.
    $$\text{mean} = \lambda, \ \text{var} = \lambda + \alpha\lambda^2$$
  - NB = Poisson when $\alpha = 0$

6.3 Hypothesis testing

- H_0: mean expression of the gene under two conditions are the same
- p-value: how likely it is to see the data we observe under H_0
- eg. one sample t test, two sample t test, non-parametric rank test, χ^2 **test**

6.4 log-likelihood ratio test

- Compute the likelihood under H_0 and H_1. ie.

$$\Pi_{i \in A} P(x = i | \text{model param}) \Pi_{i \in B} P(x = i | \text{model param})$$

- log-likelihood ratio $= \log \frac{L1}{L0}$ (note we are assuming equal variance under both hypothesis)
- degree of freedom: no. of free parameters - 1

6.5 limitations

- assume specific probabilistic model
- need many replicates
- multiple hypothesis testing issue

6.6 Multiple hypothesis testing

- eg. 1 trillion monkeys, one of the randomly typed out shakespear
- Bonferroni Correction
  - very conservative
  - may cause us to miss out genes
  - adjusted p-val = original pval / no. of genes testing
- FDR
  - 100 genes identified w p-val 0.05, then 5 genes are probably falsely discovered
  - more stuff from hw2???
- Permutation based methods
  - idea is to determine the prob. of seeing the real data in a random sample
  - divide by no. of permutations done
  - can be a problem when the no. of samples small
- time series data are often too hard to be considered, not always possible

7 Clustering

8 Classification

9 Single Cell

Goals:

1. cells differentiates into sub-celltypes
2. unknown celltype discovery

9.1 Dimensionality reduction

Motivation:

1. high dim data often has lower dim representation w/o much reconstruction error
2. lower dim representation can often represent info about high dim pairwise dist.

Types of dim-red:

- Global methods
  1. all pairwise dist equally impt
  2. lower dim pairwise dist fit high-dim ones
  3. often use magnitude or rank order
- Local methods
  1. only local dist reliable in high dim
  2. more weight on modelling local dist correctly

Methods:

- PCA
  - finds directions with largest variance
  - minimize squared reconstruction error
  - equiv to liner autoencoders
  - Steps of PCA
    1. $\overline{X}$: mean of all samples(usually rows), adjust $X \to X' = X - \overline{X}$
    2. covar matrix $C = X'^T X'$
    3. find eigenvectors and eigenvalues of C, ie. all pair of $\vec{v}, \lambda$ st. $C\vec{v} = \lambda\vec{v}$
    4. eigenvalues can be used to calculate percentage of total variance for each component

$$v_j = 100 \frac{\lambda_j}{total \quad eigenvalue}$$

  This is non-parametric method, do not insist on a parametric encoding function
- Multi-Dimensional Scaling
  - arrange low dim points to minimize diff between pairwise distances in the high and low D space
  - a possible approach: start w a random vector, perform gradient decent
  - is there something to do with PCA? then we don't need iterative method
- Sammon (non-linear autoencoder)
  - with extra layers, much more powerful than PCA, but can be slow to converge, and can get stuck on local optima
  - Multi-Dimensional Scaling(MDS) can be made non-linear by giving higher weights to smaller distances, a popular formula is

$$cost = \sum_{ij} \left(\frac{|x_i - x_j| - |y_i - y_j|}{|x_i - x_j|} \right)^2$$

    where x is high-dim dist, and y is low-dim dist
  - still slow and get stuck on local optima

9.2 Graph-basd method

- address uniform circularity
- Isomap is a dim-red technique based on graphs
  - each datapoint is connected to k nearest neighbor in high-dim
  - edge weights = euclidean dist
  - approx of distance = shortest path in contracted graph
- Probabilistic local MDS
  - local distances are more impt than non-local ones
  - in this way all local distances are given equal importance
- stochastic neighbor embedding(SNE) has a probabilistic way to decide if a distance is local
  - convert global distances into probability of one datapoint picking another datapoint as its neighbor (what defines a neighbor tho) - still about isomaps?
  - each point in high-dim has a conditional probability of picking any other point as its neighbor
  - distribution (some sort of Gaussian) is over high-dim distances (if high-dim coords unavailable, a similarity / dissimilarity matrix may be used)
    $p_{j|i}$ is the prob. of picking j given starting at i in high-dim.

$$P_{j|i} = \frac{e^{-2d_{ij}^2/2\sigma_i^2}}{\sum_k e^{-d_{ik}^2/2\sigma_i^2}}$$

  - having the probabilities potentially allow us to throw away the raw high-dimensional data
  - evaluation done using pairwise distance in low dimensional map (shows how well the lower dim representation models high-dim data ig)
    $q_{j|i}$ is the prob of picking j given starting at i in low-dim
  - compute the Kullback-Leibler divergence between prob. in the high-dim and low-dim spaces (why not just use dist in high dim?) - more space / time efficient
  - nearby pts in high-dim should be close in low-dim
- picking σ used to compute p - the radius of the gaussian
  - different radius is needed in different parts of the space to keep the no. of neighbors constant
  - big radius $\rightarrow$ high entropy for distribution over i's neighbors
  - small radius $\rightarrow$ low entropy
- Symmetric SNE
  - simpler than stochastic
  - works best if different procedures are used for computing p's and q's.
  - compromise: no longer guarantees that if using same dimension will produce optimal solution
  - turn conditional prob into symmetric pairwise probabilities
- Optimization methods for SNE
  - simulated annealing could lead to better global optimization
  - add Gaussian noise to y location in each update
  - spend longer time at noise level where global structure start to form
  - t-SNE - use Gaussian at many (infinite) spatial scales, cheaper as we don't have to exponentiate anymore (why????)

9.3 Supervised dim-red: Neural networks

- last few layers have much fewer values than inputs
- use intermediate layers as lower-dim representations
- can easily add prior biological knowledge, such as protein interactions or transcription factors
- essentially, some nodes in the hidden layer are same as before, others are based on biological info

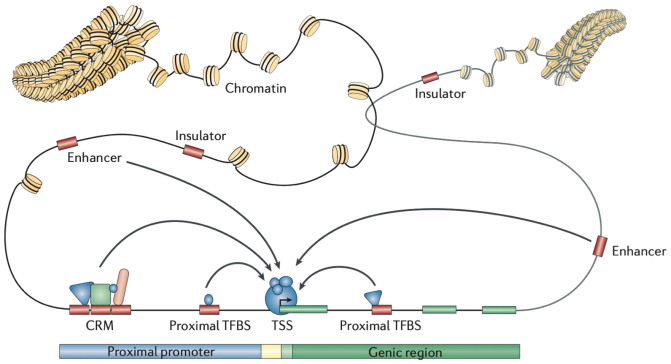
9.3.1 Additional NN architecture: Siamese

- supervised, but not trying to maximize training accuracy
- input: whether each pair is similar
- output: binary label of similar / not similar
- thus directly optimize dim-red layer for KNN

10 ChIP-Seq

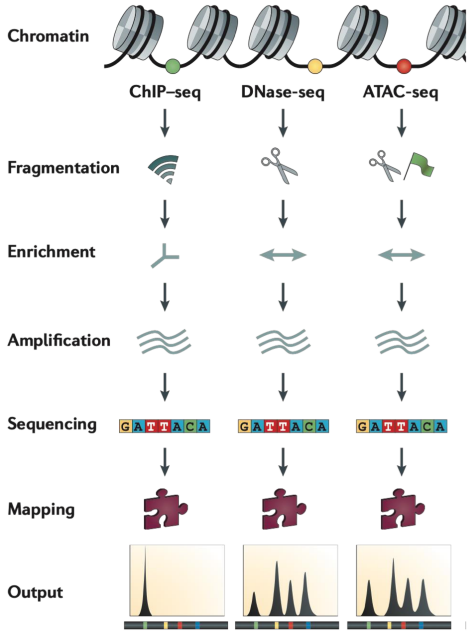
10.1 Background

- transcription factors bind to specific locations on the chromosome
- the binding is highly specific
- this has impact on gene regulation



- Transcription start site (TSS)
- Transcription factor binding sites (TFBS)
- Cis-regulatory module (CRM)
- Proximal promoter and distal enhancer

- The core promotor regions has about 300 TF,
  (general transcription machinery, required for the transcription of most things),
  another 1500 TFs for others (proximal enhancer/promotor/silencer, only affect some genes)
- comparisons of different whole genome enrichment technologies



10.2 Questions related to TF binding

- Where do they bind
  - some almost always bind to proximal promotors
  - others could bind to many regions
- How does specific binding work
  - exists a consensus motif (most common sequence)
  - looks like this

  - shows which nucleotide is most abundant at each position, represented as Position Weight Matrix (PWM)
  - Motif: a recurring sequence with biological importance
    - can be constant or have a feww variable elements
    - often serves as binding site for TFs /proteins
    - often very short (5-25)
    - often distant from gene
    - often has inexact repeating pattern (challenge for identification)
  - sometimes also an effect of protein-protein interactions,
    ie. protein conformation change upon interactions etc?
  - to determine binding site, often uses Protein Weight Matrix, but it is over-simplifying
- How to identify where they bind
  - genes regulated by the same TF are likely to have the same motif in regulatory region
  - to find genes regulated by the same TF → comparative genomics could be very useful
  - eg. knock-off of specific TFs → lower expression of a set of genes
  - group of genes that are co-expressed across many set of experiments are also likely to be regulated by same TF
  - challenges
    - we don't know the exact motif seq
    - we don't know how far it will be
    - motifs can differ slightly across
    - how to distinguish it from just "random" sequences that happen to repeat?
  - Multiple sequence alignment (MSA)
  - motif-finding based on EM algorithm (MEME-suite - Multiple EM for Motif Elucidation)
- How is it involved in gene regulation
- Is it useful for gene regulatory network?

10.3 ChIP-seq

- technology:
  ChIP: chromatin immunoprecipitation
  studies protein interaction with DNA
  able to map global binding site precisely for any protein of interests

  1. chromatin immunoprecipitation + high throughput sequencing
  2. detect genome-wide location of TF and other binding proteins in labs
  3. find all DNA seq bound by TF-X
  4. try to learn the regulatory mechanisms of a TF or DNA-binding protein

- General strategies to call ChIP-seq peaks
  - ChIP-seq yields distributions for tags from forward and reverse strands
  - overlap of the two can be observed
  - actual binding site of TF should be between the 2 distributions
  - from the difference between the 2 peaks → formulate a single peak summit

- MACS: model-based analysis for ChIP-seq

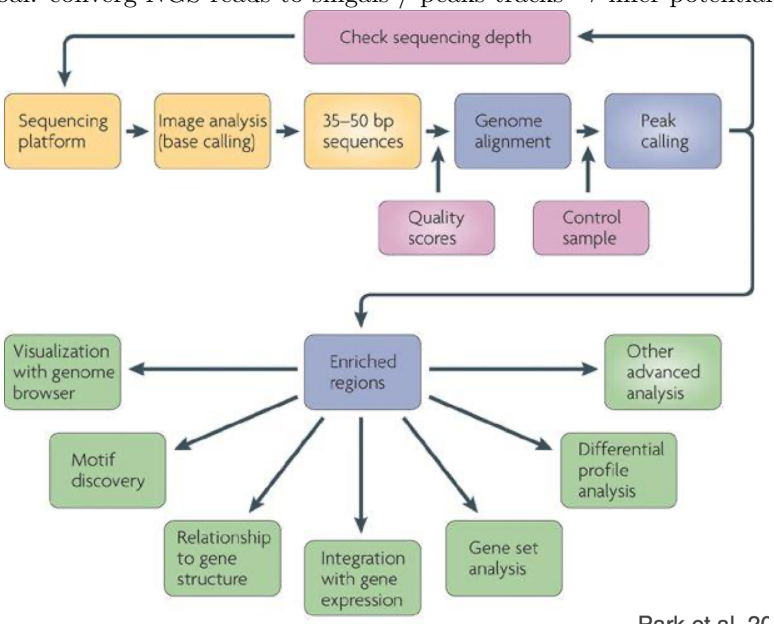
  1. map reads using Bowtie2
  2. get ChIP-seq reads around but may not contain binding site
  3. sequence are from ends of randomly chopped segments $->$ hopefully overlap at binding site
  4. produce 2 adj. set of read peaks located about 2× fragment length away
  5. shift distance: dist between read peaks at which will find true peak
  6. automatically subtract control to define a final set of peaks

  - input:
    bandwidth : sonication size
    mfold: high-confidence fold enrichment
  - slides 2× bandwidth window across genome to find regions with tags $> mfold$ enriched compared to a random tag
  - random sample 1000 high quality peaks, separate their Watson Crick tags, and align by midpoint $->$ 2 peaks, shifts $= d/2$
  - tag distribution $Poisson$
  - MACS uses a dynamic parameter λ_{local}
  - P-val of peaks?
  - FDR?

- Downstream analysis
  - to identify motif for TF using ChIP-seq peaks – MEME
  - to find out what the sequence motifs resembles – TomTom
  - to find peak regions of known motifs – FIMO
  - look up biological pathways or functions of target genes of TF –GREAT

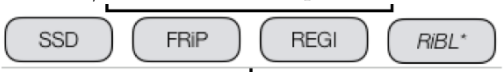
- Practical pipeline
  - overview: TF-binding → co-factors → DNA histone modifications → DNA DS break

- goal: converg NGS reads to singals / peaks tracks → infer potential TF binding regions



- fastq → FASTQC → bam/alignment
  → wig:
  - narrow → narrow peak caller
  —-calculate QC matrics

  - broad / mixed → broad peak caller —-calculate QC matrics



- QC good → differential enrichment
- QC bad → combine replicates (IDR)

- Steps: (eg. ENCODE)

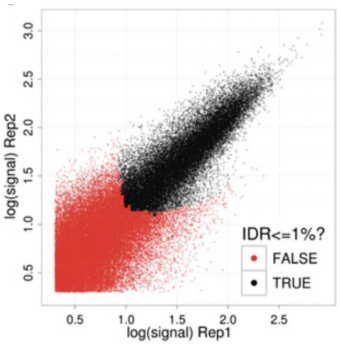
0. experiment design
   - replicates (more replicates >> greater coverage)
   if more TF targets → multiple hypothesis testing??? - choice of control
   - open-chromatin regions fragmented more easily than closed ones
   - copy number variation in cancer samples
   - potential non-specific antibody binding
   - repetitive regions could cause alignment errors
   - MOST DO CONTROL AS PART OF THE INPUT
   - some also do mock IP as control (DNA obtained from IP by a mock antibody such as IgG)

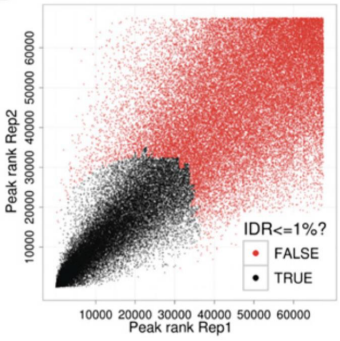
   * **matched control** is required for downstream analysis

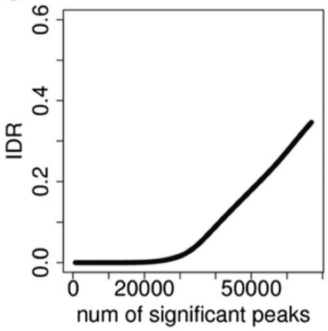
1. input data & QC
   - fasta format → FastQC
2. Aignment and filtering
   - alignment tools: bowtie, bwa→output bam
   - QC = % mapped
   - filtering tools: samtools, picard

  - QC = non-redundant fraction

3. Peak calling
   - narrow peak
   - broad peak
   - QC: fraction of reads in peak (FRiP)
     * FRiP values correlate positively and linearly with the number of called regions
   - QC: strand cross-correlation
     * shift vectors with each other and calculate correlations
     * plot shift size w correlation
   - Irreproduncibility discovery rate (IDR)
     * avoid of choices of initial peak caller cutoffs
     * modelling peaks pairs from replicates as belonging to two groups: reproducible group and irreproducible group







   - visualization
     * UCSC genome browser etc
     * IGV genome browser

11 cis-regulatory motif

11.1 Background of transcription factor binding motif analysis

- basic for TF binding, where they bind
- Position weight matrix (PWM)
- *De novo* motif binding
  - comparative genomic approach → find motifs within same species

11.2 Finding regulatory motifs

11.3 Motif finding based on EM

11.4 Transform a PWM into log likelihoods

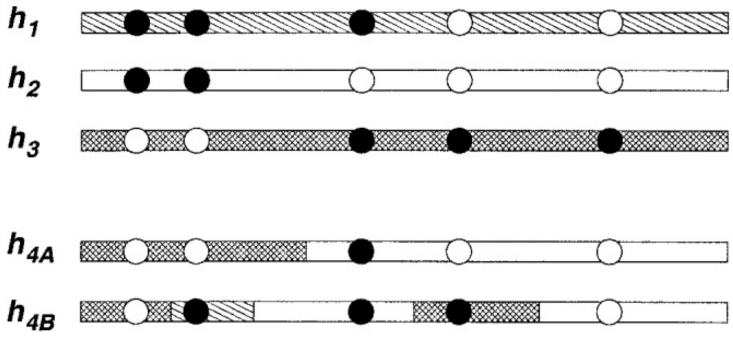
11.5 Comparative genomics - PhyloCon

- Align two profiles
- each col → base count $(n_A, n_C, n_G, n_T) \rightarrow (f_A, f_C, f_G, f_T)$
- log-likelihood ratio = $\sum_{\text{base } b} n_{bj} \ln(f_{bi}/p_b)$ * not all regulatory elements are conserved

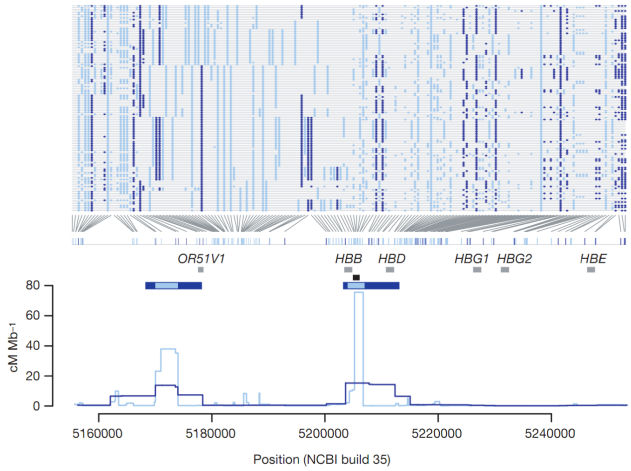
12 Haplotype inference

12.1 Phase

- h_1, h_2, h_3: unobserved ancestral haplotypes
- h_{4A}, h_{4B}: unobserved haplotypes for individuals
- Circles: alleles, mutations



Haplotype Structure and Recombination Rate Estimates: HapMap I vs. HapMap II

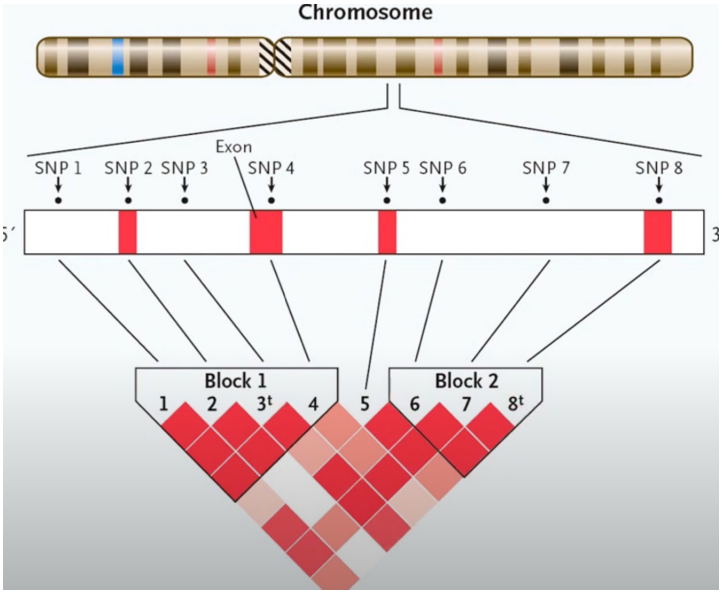


- Genotype inputation
  - Given: SNP array genotype data
  - some has missing data / untyped SNPs
  - much cheaper
  - use HMM
  - more data, more acurate for inputation
- Beagle / SHAPE-IT are most commonly used software

12.2 Linkage disequilibrium (LD)

- reflect rs between alleles at diff loci
  - linkage equilibrium: no linkage, not coupled

  - disequilibrium → linkage
- LD: allelic association meansure
- calculating LD:
  - assume independence, calculate expected frequency
  - $D_{AB} = P_{AB} - P_A P_B$
- calculate for all loci (SNPs)
- spot-light figures



- neighboring loci shows more linkage
  there could be LP blocks on the chromosome, where there's stronger linkage within block and weaker across block
  each block is like a voting district / Marker regions
- They are Tag SNPs
- pretty standard practice
- dense genotyping more expensive
  - get the LP
  - use inference on sparser genotyping
  - multi-phase procedure

13 Population structure

13.1 Background

- def: set of individuals with distinct genetic variations
- eg. ancestral history, lactose intolerance
- Hardy-Weinburg equilibrium
  - Under random mating, both allele and genotype frequency remain const
  - Current gen:
    - $D + H + R = 1$
    - $p = \frac{2D+H}{2}$
    - $q = \frac{2R+H}{2}$
  - Next gen:
    - $D' = p^2$
    - $H' = 2pq$
    - $R' = q^2$
    - $p' = \frac{2p^2+2pq}{2} = p^2 + pq = p$
    - $q' = \frac{2p^2+2pq}{2} = q^2 + pq = q$
  - given population data, can test if it holds (often chi-square test)
    - testing is recommended
    - if fail, means something is going on
    - or genotyping error
    - want HWE to hold for control group

    1. compute allele freq from observed data
    2. compute expected genotype freq
    3. compute test statistic (deg of freedom 1)
  - Due to Genetic drift, even when assumptions of HWE hold, HWE may not hold
- genetic drift
  - change in allele freq due to random sampling
  - all mutations eventually drift to 0 or 1 eventually
  - is neutral
- Wright's F_{ST} (Wright-Fisher model)

$$\binom{2N}{k} p^k q^{2N-k}$$

- Ways how populations evolve
  - population divergence
    separated into subpopulations with independent selection and drift
  - admixture
    mixing of population

13.2 Infering pop structure from genotype data (nowadays mostly just PCA)

- Mixture model
  - cluster individual into K populations
  - does not model admixture
  - cluster individual into populations
  - probability model for mixture of C Gaussians

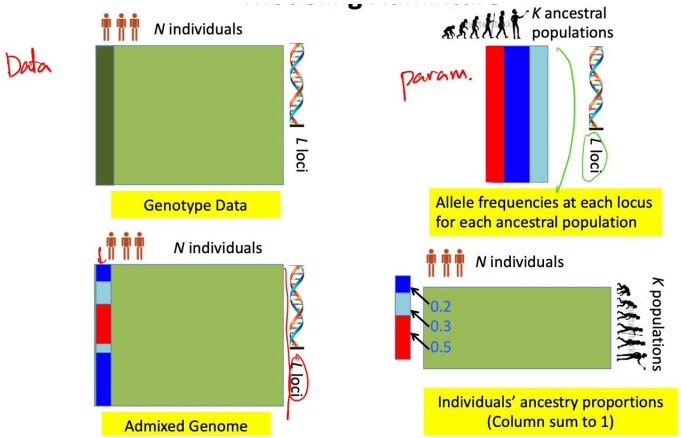
$$p(x) = \sum_{i=1}^{k} p(x|c=i) \cdot p(c=i)$$

  - c: labels, $p(c)$ label freq, multinuilli
  - x: genotype / alleles,

$$p(x|c) = \Pi_{i=1}^{j} p(x_i|c)$$

    , assume independence
  - can then learn the model with EM
  - Inference: $p(c|x)$ to infer cluster label

- Admixture model
  - modern species are mixture of ancestral populations
  - genome consists of contributions from multiple ancestral populations, eg. Asian, African, Caucassians
  - want to model it as a mosaic of variant
  - Assumptiosn
    - no linkage disequilibrium
    - SNPs are iid



  - for each individual $i : 1 \to n$:
    - sample θ_i from Dirichlet(α)
    - for each loci $j : 1 \to L$
      - sample $Z_{j,i}$ from multinomial θ_n
      - sample $X_{j,i}$ from β_{k_j} for k chosen by $k = Z_{j,i}$
  - still have to go back to β
  - instead of sampling p_c of population it gets p_c for each individual

- Evaluations
  - probabilistic model
  - is generative process
  - explanatory, descriptive, and interpretable
  - computation can be very expensive
- PCA
  - fast, although not too much information
  - input: $N \times L$ for N individuals and L loci
  - good: easy visualization
  - bad: doesn't give intuition about what is going on, no allele frequency info

14 Linkage Analysis, GWAS(Genome Wide Association Study)

14.1 Background

- Genome polymorphisms
  - human genealogy →SNPs →haplotypes
  - useful markers for studying disease association
  - finding genetic markers that are likely to be linked with the disease locus, instead of finding the disease locus itself (cuz it's hard)
  - making use of linkage (dis)equilibrium, find those loci with $r < 0.05$
- linkage analysis
  - family data
  - more likely to have linkage data
  - Effective for rare diseases
  - Low resolution on the genomes (only a few recombinations)
  - **Parametric linkage analysis:**
    - need to specify disease model
    - Highly effective for Mendelian disease caused by a single locus
    - usually large pedigree
    - Founder probabilities (founders: parents not in population)
      - thus need to assign distributions to their genotypes
      - often done with Hardy-Weinburg
      - genotype of the founder couple assumed independence
    - children get their genes according to **Mendel's law**
  - from genotype →phenotype
    - complete vs incomplete penetrance

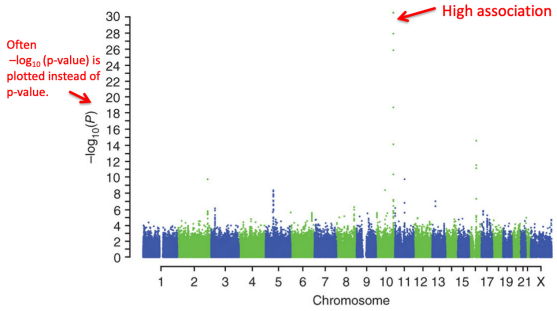
$$\Pr[complete|DD] = 1$$
$$\Pr[incomplete|DD] < 1$$

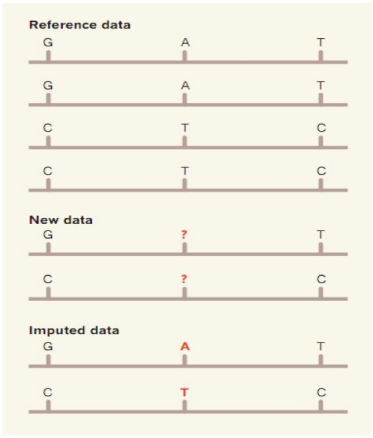
    -

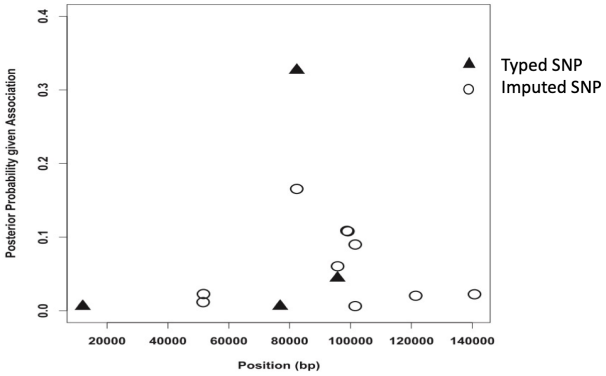
14.2 GWAS

14.3 single SNP association analysis

- unrelated individuals
- Easier to find a large number of affected individuals
- Effective for common diseases
- Relatively high resolution for pinpointing the locus linked to the phenotype







14.4 multimarker association test

14.5 using reference datasets for genotype imputation

- reference data: dense SNP data - from dataset eg 1000 genome project
- new data = sparser SNP
- leverage LD - data after imputation w ref data

- imputation based method - everyone is doing it rn
- association to rare variants (GWAS only mostly apply to common variants)
  at least a lot more confident when allele is common
  ppl often combine multiple rare alleles along a gene and compare gene
- underlying: common allele - common disease / common hypothesis – assumption
  usually 5% considered "common", 0.5% - 5% considered "low frequency variant", else "rare variant"

- still quite a lot of variant to analyze
- feasibility of detecting disease loci

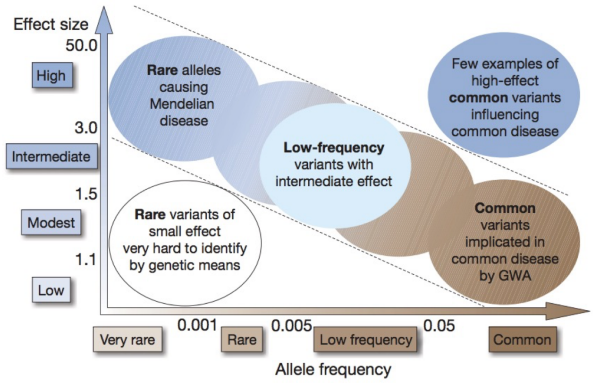
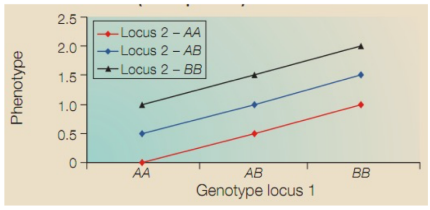


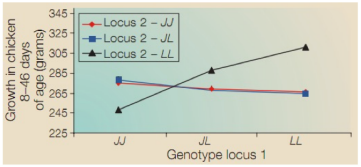
Figure 1

14.6 Epistasis

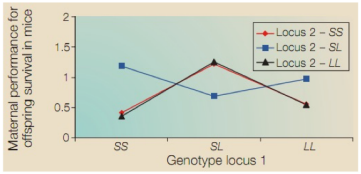
- effect of one locus masks another loci



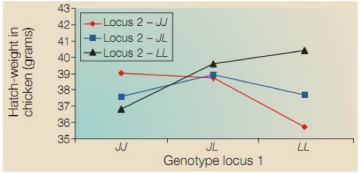
(a) no epistasis



- Dominant epistasis
- One locus in a dominant way suppresses the allelic effects of a second locus



- Dominance-by-dominance epistasis
- Double heterozygote (LS, LS) deviates from the phenotype that is expected from the phenotypes of the other heterozygotes.
- Double heterozygotes have a lower phenotype than expected.



- Co-adaptive epistasis
- Genotypes that are homozygous for alleles of the two loci that originate from the same line (JJ with JJ, or LL with LL) show enhanced performance.
- Almost no marginal effects: average effect of JJ, JL, LL do not differ

(b) epistasis

- hard to detect - only if the interacting SNPs are considered jointly
- also suffer from multiple testing problem

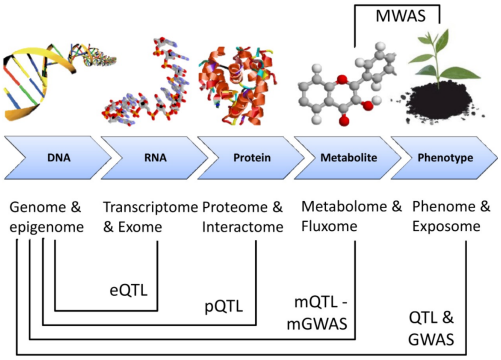
14.7 Population struture and association analysis

- pop structure can cause false positives
  - samples in case are often more related
  - Any SNPs more prevalent in the case population will be found significantly with the case
  - may capture both disease and population related SNPs
  - ideally have equal proportion of population in case and control
- computationally solve the problem
  - poputation based method
  - Eigenstrat: PCA based method
    1. inferring ancestry - PCA applied
       infer continuous axis
    2. removing ancestry effects
       genotype at candidate SNP adjust by amounts attributable to ancestry along each axis
       scaling factor: regression on genotype - ancestral info
       missing eqns from notes
    3. association tests
  - linear mixed model

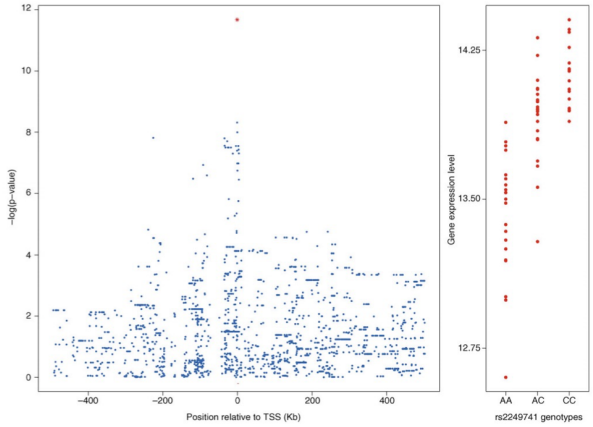
15 eQTL (expression quantitative trait locus)

15.1 limitation of likage analysis and GWAS

- we often know the genetic loci
- but don't know the molecular mechanism



- find connection between DNA and mRNA
- eQTL mapping



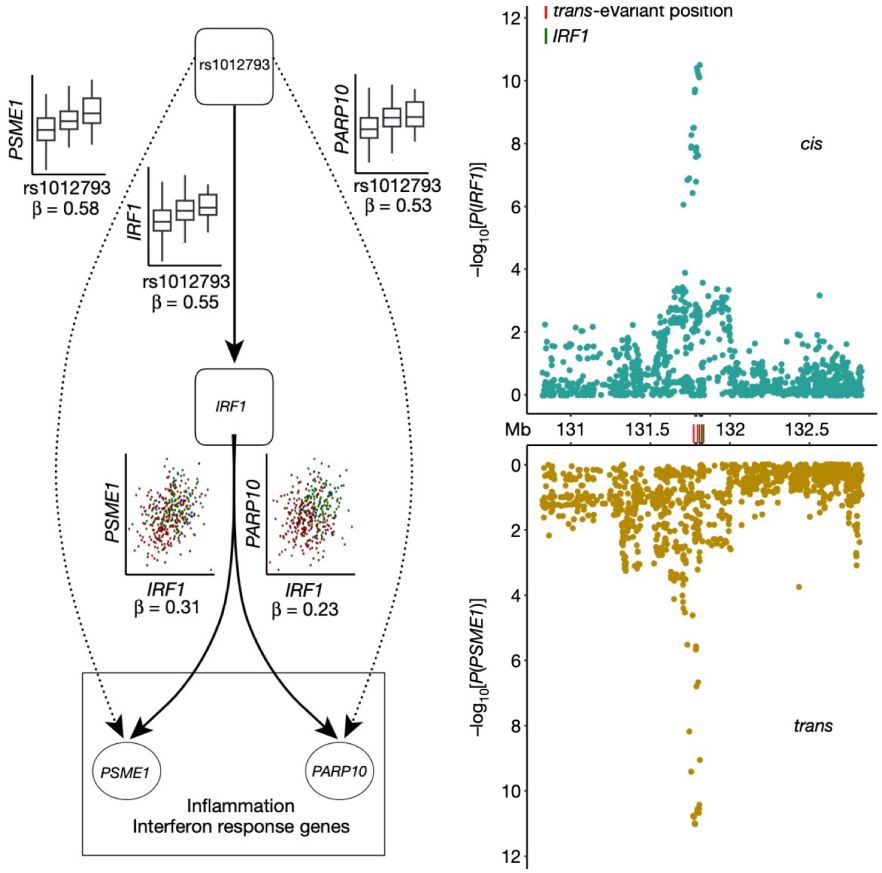
eQTLs for HLA-C gene expression in HapMap European samples

- genometype tissue expression (GTEx) project
  - goal: characterize molecular function of human genome
  - postmortem samples from normal, non-disease tissues -¿ reference data
- popular tool to study genetic basis of expression for
  - diff tissue types
  - diff diseases

15.2 terminologies

- eGene genes whose expression is affected by eQTLs

- cis eQTL in the genome, the eQTL is located near the eGene
  – E.g.,mutations in the promoter region of a gene influence the expression level of the gene
  – but enhancers can be far away, may look like trans
- trans eQTL eQTL is located far away (or on a different chromosome)
  – E.g.,mutationsinthetranscriptionfactorgene
  * cis regulatory can have downstream effect -¿ trans



- eg. From GTEx thyroid expression levels
  • SNP rs1012793 affects expression of IRF1 in cis and PSME1 and PARP10 in trans

15.3 human vs model organism

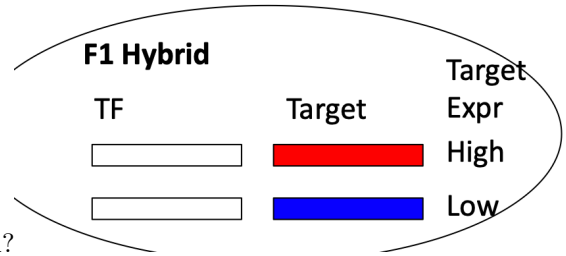
- human harder to collect data
- model organisms can be cultured in a lab
- Yeast recombinant inbred lines, instead of population of unrelated individuals
  – two founder strains, BY and RM
    – Mate the founders in a lab for generations
    – Genotype/expression profile for 110 progenies
    – In a follow-up study
    —- The same founders, 1000 progenies, whole-genome sequencing, RNA-seq
- effect of recombination
  – schuffle genome through mating
  – More generations of mating means more shuffling through recombinations and higher resolution for eQTL mapping

15.4 allele-specific eQTL mapping

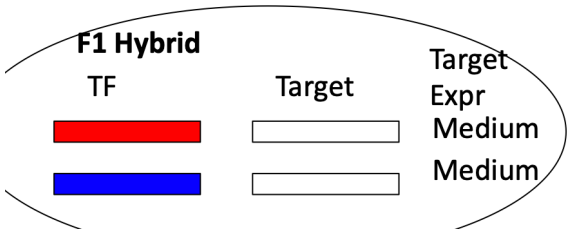
- cis-acting elements - DNA sequences in the regulatory region of the gene(e.g.,TF binding sites)
- trans-acting elements - RNA and proteins that interact w cis
- problem:
  - assumed cis = local and trans = distant, but not the case
  - exists nearby trans and distant cis
- fix: allele-specifc expression quantification w transcriptome RNA-seq
  - diploid: maternal / paternal allele diff, RNA-seq can capture this
- idea: between two species
  - Studied cis/trans regulatory divergence between two species – First study that makes use of allele-specific gene expressions to learn about gene regulatory network – Examines genome / transcriptome data for two parent species and their F1 progeny
- idea: extension to population genome / transcriptome data

15.5 allele specific eQTL mapping

- cis-trans regulatory divergence in two species
- cis / trans, but location not very helpful
- Only cis-regulatory divergence between two species



  - only in each chromosomes? local?
  - no cross regulation → high and low
- trans-regulatory divergence:



  -
  - both are medium → got averaged out
  - since there are trans-regulation
  - one transcription factor is less efficient than the other
- ratio between the high and low in parents vs. ratio in F1 progeny
- ratio = 1 → trans-regulatory divergence
- $y = x$ → cis-regulatory divergence

- most genes are influced by a mix of the two
- regulatory genes tend to be trans-acting
- structural genes: Usually terminal nodes with no trans- acting effects

15.6 limitations

- only examine 2 species and offspring (sample size too small)
- can do on population
- Population-based study: allele-specific eQTL mapping
- Cis-acting eQTLs = cis-regulatory variation
  - linear model wrt alleles (eg, A, C, T, G)
- Trans-acting eQTLs = trans-regulatory variation
  - linear model wrt genotypes (eg, AA, Aa, CG)

16 scRNA-seq & CRISPR

16.1 Background

- experimental validation of eQTL w CRISPR perturbation
  - genome editing w CRISPR, followed by scRNA
- scRNA made possible
  - Cell–type specific eQTLs
  - Co-expression eQTLs

16.2 co-expression eQTLs

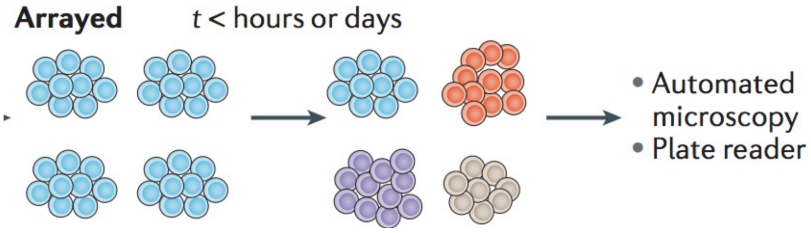
- one vector for expression and one vector for genotype
- coexpression eQTL: study eQTLs that influence "co-expression" between two genes
- → personal gene regulatory network

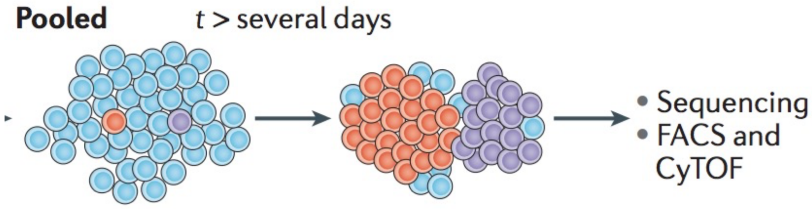
16.3 CRISPR

- genetic screnning
- three components
  - Perturb:Knockout,RNAi,CRISPR
  - Model:primary cells,organoids,mice
  - Assay:measuring phenotypes of interest,RNA-seq,scRNA-seq
- Cas9 molecule
  - acomponentofthetypeIICRISPR bacterial adaptive immune system
  - DNAdouble-strandbreak(DSB)by Cas9 is repaired by the endogenous DNA DSB repair pathways
- sgRNA (single guide RNA)
  - guide Cas9 to the correct place
  - 20bp complimentary to the target DNA
- CRISPR knockout
- CRISPR interference
  - repress gene of interest
- CRISPR activation
  - overexpress the gene of interest

16.4 array vs pooled screening

- array screening





- pooled screening
- viability screens
  - negative screen
    Perturbations that reduce cell fitness will be depleted by the end of the screen
  - positive screen
    Perturbations that give survival advantage to the cells, e.g., alternative culture conditions, small molecules