1 Normalization

RPKM is a very important normalization tool.
not too sure about tpm, need to double check on that

2 Single Cell

Goals:

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

2.1 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

2.2 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????)

2.3 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

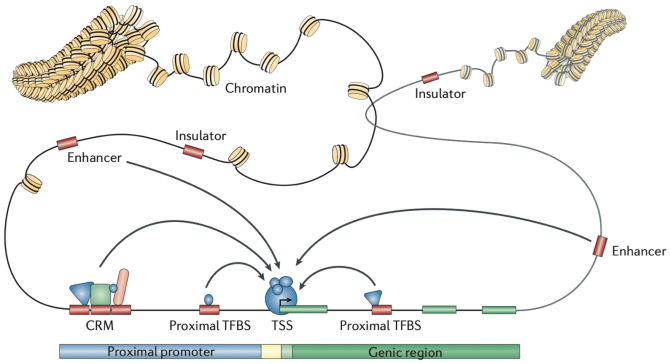
2.3.1 Additional NN architecture: Siamese

- 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

3 ChIP-Seq

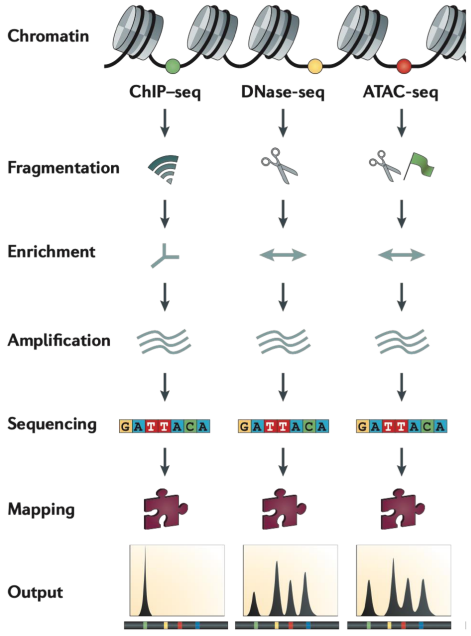
3.1 Background

- 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



3.2 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?

3.3 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

4 cis-regulatory motif

5 Haplotype inference

5.1 Phase

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

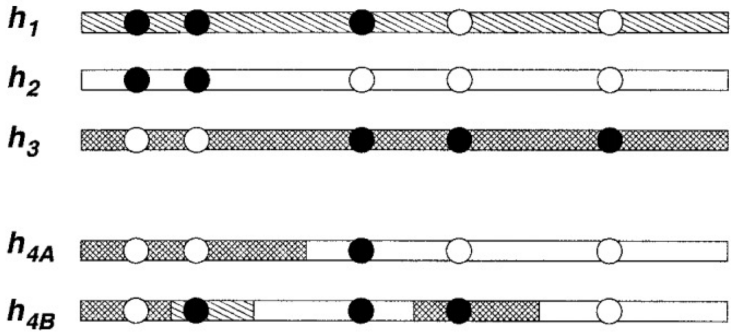
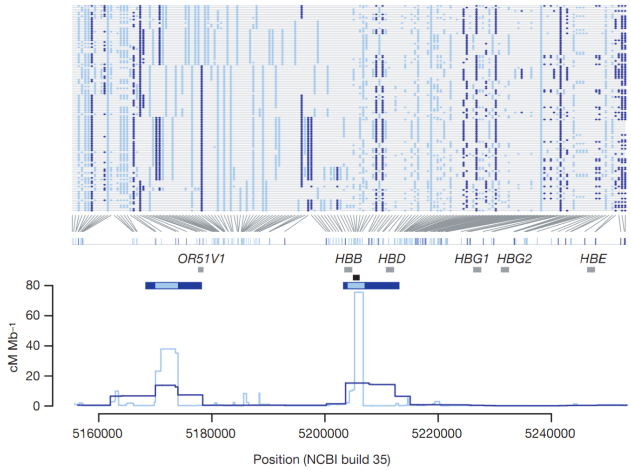


Figure 1

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

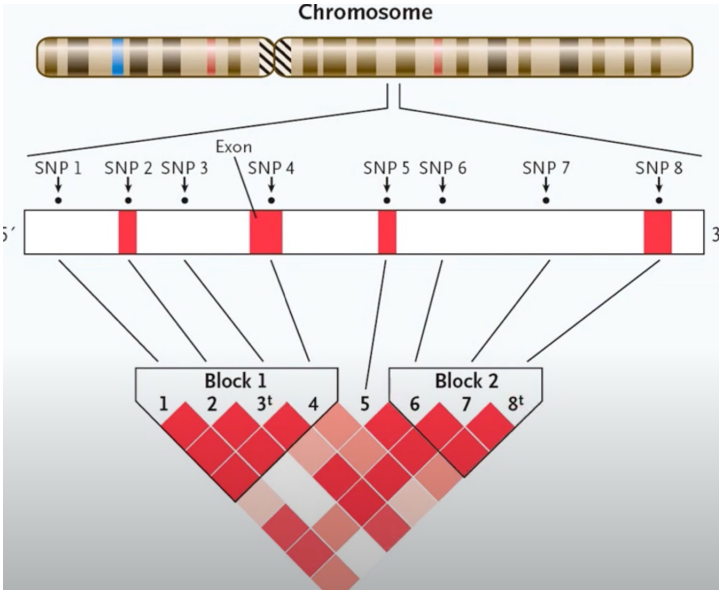


- Genotype inputation
  - Given: SNP array genotype data
  - some has missing data / untyped SNPs
  - much cheaper
  - use HMM
  - more data, more acurate for inputation
- Beagle / SHAPE-IT are most commonly used software

5.2 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

6 Population structure

6.1 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

6.2 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

-