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 $X \rightarrow X' = X - \overline{X}$
        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
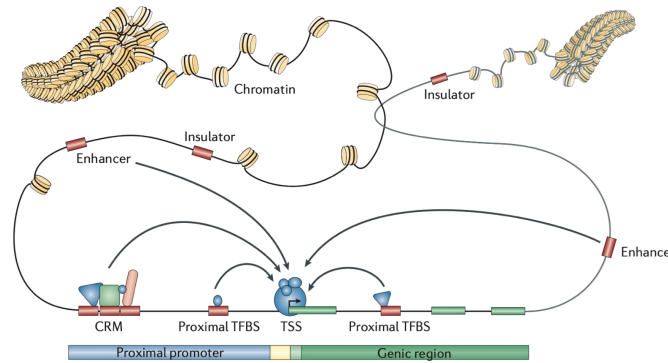
☐ Transcription start site (TSS)
☐ Transcription factor binding sites (TFBS)
☐ Cis-regulatory module (CRM)
☐ Proximal promoter and distal enhancer

- 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
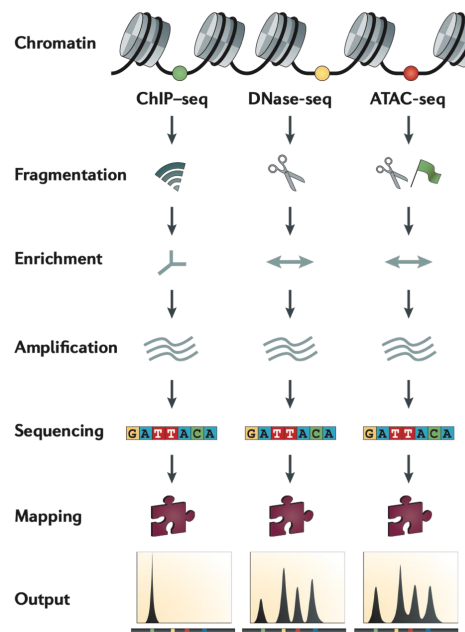
3.2 Questions related to TF binding

- 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)

- How to identify where they bind

- How is it involved in gene regulation

- Is it useful for gene regulatory network?

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4 cis-regulatory motif