Detecting and Interpreting Genetic Homology: Lecture Notes on Alignment

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After lots of time spent on improving the quality of the genomic sequence, you have a large piece of DNA. How are you going to make sense of it?

Table of Contents

1. Annotation

Defn: genome annotation is the process of determining the biological structure and function of genomic sequence.

- Where are the genes?
- What do they do?
- Are there regions of functional non-coding DNA?
- What is the evolutionary history of the sequence?

We'll introduce one method of identifying features in the genome: *comparative annotation*.

2. Rationale of Comparative Annotation

How can we assign meaning to parts of a DNA sequence? In particular, how do we discover regions of potential function?

- How can we exploit our ability to quickly generate lots of DNA sequence data?

- **Fact 1**: all DNA is subject to mutations.
- **Fact 2**: most functional regions are under negative selection (i.e., mutations are often deleterious).
- Conclude that pieces of DNA with a specific function (especially genes!) tend to be *conserved* against mutation more strongly than pieces with no specific function.
- Conservation implies that, if you compare two homologous functional regions, you will see *extensional similarity*. That is, the DNA sequences, considered as raw character strings, look similar to each other.

This makes sense so far, but what is the implication for annotation?

- Computer scientists are good at detecting extensional similarity between sequences.
- We'd like to reason: if two sequences are unusually similar, they exhibit evidence of conservation (derivation from common ancestor) …
- … hence exhibit evidence of negative selection …
- … hence exhibit evidence of conserved function!
- This chain of inference provides evidence that two sequences are functionally homologous based on fact that they're similar.
- **Comparative annotation** is the process of discovering similarity, and then following the above chain of inference to assign sequences a putative function.

Unfortunately, evidence is not proof. What are some caveats?

- Extensional similarity can arise purely by chance. If I flip a fair coin 10 times, then 10 more times, the chance that the first and second sequences of 10 flips are identical is about 1 in 1000. Two unrelated DNA bases have at least a one in four chance of being the same.
- Conservation (derivation from a common ancestor) need not imply strong negative selection. Other possibilities include:
  - recent genomic duplications
  - pseudogenes
  - intergenic DNA of closely related organisms (e.g., human/chimp)
- Negative selection may indicate that compared sequences and/or their structures are functionally important, but may not indicate that the compared sequences and/or structures have a common function. For instance:
  - The *adh1* and *adh2* genes in yeast are close paralogs (alcohol dehydrogenase), but catalyze opposite metabolic reactions.
  - Structural crystallins in the vertebrate eye lens function as enzymes (with minor variation) in other tissues.

3. What Does Similarity Mean?

What is a biologically sensible notion of “extensional similarity?”

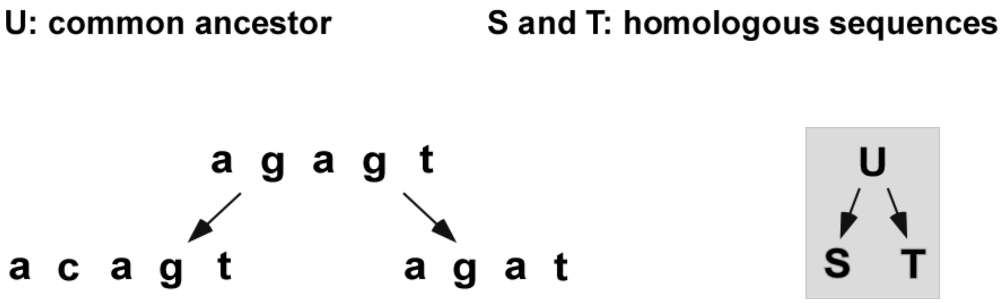
- Exact match? That’s just a crude approximation: biosequences mutate.
- Inexact match? But there are many ways to define what an “inexact match” means.

The following is an intuitive description of and justification for a particular definition of similarity.

- Our notion of similarity should be rooted in an underlying process of evolution.
- Define a set of *permissible mutation events*.
    - *Substitution*: replace one character by another
    - *Insertion*: add one character somewhere in a sequence
    - *Deletion*: remove one character somewhere from a sequence
- (Note: an insertion or deletion is sometimes called an *indel*)
- **Assumption**: biosequences change over time only by a series of permissible mutation events.

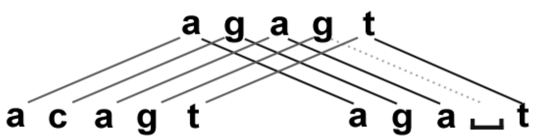
How does a set of mutation events lead to a measure of similarity?

- Let *S, T* be two homologous sequences.



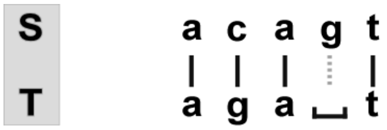
- At some time in the past, *S* and *T* had a common ancestor *U*.
- Each base of *S* or *T* represents one of
    1. a conserved base from *U*,
    2. a base from *U* that underwent substitution(s),
    3. or a base inserted (maybe changed) after the split from *U*.

If we could draw the correspondence between the bases of *S*, *T*, and *U*, we'd have a *tree alignment*.



Tree alignment

- If we omit the ancestral sequence *U*, we get a sequence alignment of *S* with *T*.



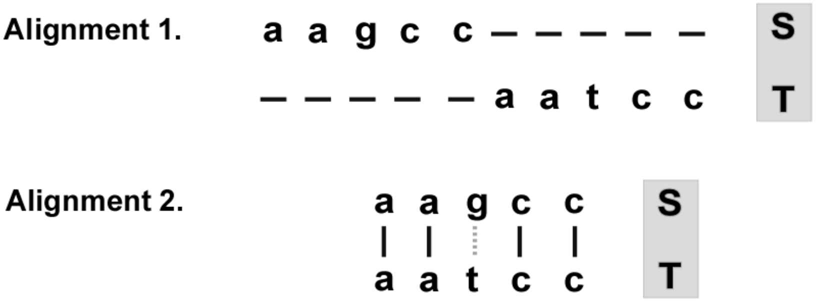
Sequence alignment of S with T

- An alignment shows which bases of *S* and *T* came from the same base in the (unspecified) ancestor *U*.
- To measure similarity of *S* and *T*, measure the fraction of positions (aka *columns*) in the alignment that remain identical.
- This measure is *fractional identity*, a.k.a. *percent identity*

The above is a rational basis for measuring similarity, but we have a problem …

- The ancestor *U* and the mutation history are unknown!
- All we have are the modern sequences *S*, *T*.
- **Idea**: guess a "plausible" alignment and measure its identity.
- Below are two possible alignments of "aagcc" with "aatcc":

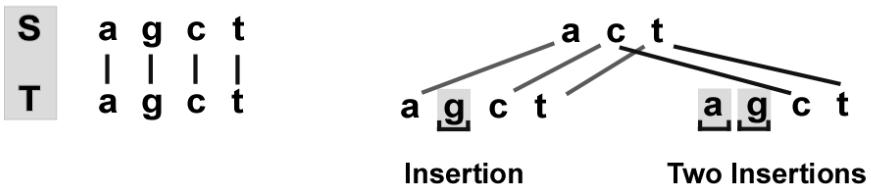
Plausible Alignment of S with T



- Alignment 1 is intuitively silly. Why?

- *Pluralitas non est ponenda sine neccesitate*. (Ockham)
- This is also known as the "principle of parsimony."
- Don't postulate more mutations than necessary to align *S* with *T*.
- **Defn**: *an optimal (global) alignment of S* with *T* is one that requires the minimum number of mutations.
- Note that the optimal alignment may not be historically true.
- Consider two identical sequences of 'agct'. It is possible that one or more mutations did occur historically that do not correspond to the optimal alignment.

Sequence Similarity vs. Mutation History

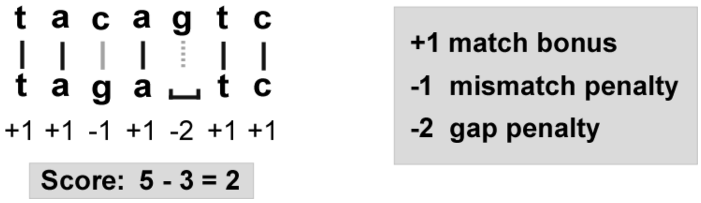


- In the absence of historical truth, *similarity of two sequences is defined as similarity of an optimal alignment between them.*

Real measures of similarity are a bit more sophisticated than just counting mutations.

- Some substitutions are more likely than others (e.g., transitions vs transversions).
- Substitutions and indels are not equally common.
- *General solution*: we assign a score to each position of an alignment.
- Matches receive a (base-dependent) bonus. Mismatches or gaps receive a (base-dependent) penalty.
- The *score* of an alignment is the total score of all its positions.

Score of an Alignment



- Alignments with higher scores provide stronger evidence of conservation.

In practice, tools like BLAST seek *portions* of two sequences that form high-scoring alignments. For example, we might want to isolate alignments between the exons of two genes, while ignoring the much less conserved introns between them. A correspondence between portions of two sequences is called a *local alignment*.

- I'm resolutely ignoring the question of how optimal alignments are computed efficiently.

4. Alignments in Context: Database Search

Given two sequences, we now know how to measure their similarity. What the heck does this have to do with database search?

- Annotation tools must compare a *query* sequence to a large database of potentially matching sequences.
- For each sequence in the database, it is possible to compute an optimal local alignment with the query sequence.
- So, which alignments do we report?
- Higher alignment scores are better, so we could just report the *k* highest-scoring alignments between query and database.
- But are these alignments interesting ? Maybe they indicate chance matches between unrelated sequences, rather than real conservation.
- We need a way to assess whether a given alignment score is meaningful in the context of a search.

BLAST and related tools use the idea of *E-values* to rate how interesting a pair of aligned sequences is. Here's where E-values come from …

- Suppose we have sequences *S* and *T* whose best local alignment has some score σ.
- *Null Hypothesis*: *S* and *T* are unrelated sequences.
- Under this hypothesis, would we expect *S* and *T* to align with a score as high as σ purely by chance?
- To answer this question, we need a formal mathematical model *M* of what a pair of unrelated sequences looks like.
- **Model**: a DNA sequence is an *i.i.d. random sequence of bases*. i.i.d. means "independent and identically distributed." In other words, we build a sequence by randomly choosing its first base, then independently choosing its second base, and so forth.

- How do we choose each base? For each base *b* in the sequence alphabet, choose *b* with probability proportional to its observed frequency. For example, if ‘a’ is more common in database than ‘c’, our model *M* picks ‘a’s more often than ‘c’s.
- Now we can formalize our question a bit more. *Given sequences S, T generated at random from model M, what is the probability p(σ) that S and T will align with score at least σ?*
- If *p(σ)* is very small, we reject the null hypothesis as unlikely and call the alignment interesting.
- An easier quantity to compute is *E(σ)*, the expected number of times an unrelated sequence from the database would align to the query with score at least *σ*, assuming that query and database were generated according to the null hypothesis.
- The “BLAST E-value” for an alignment with score σ is this *E(σ)*.
- E(σ) depends on database size. It should be very small (much less than 1) before we believe that an alignment with score σ is interesting.

A few caveats about E-values...

- The null model makes the very strong assumption of i.i.d. random sequences.
- The bases of real DNA sequences do not occur independently, even when the sequence has no biological function.
- *Example*: loss of CpG dinucleotides
- Also, the database may have heterogeneous base composition, which violates the assumption of identical distribution.
- *Example:* AT-rich and GC-rich regions within a single genome.
- For these reasons, small E-values should be taken with a grain of salt.
- Rejecting the null model with E = 0.01 is *not* the same as saying “*S* and *T* are 99% likely to be homologous”!
- When using E-values to judge the likelihood that a match is interesting, use a large margin of safety — be very suspicious of *E(σ)* > 10^{-5}, or possibly even 10^{-10}.