Computational Biology

Lecture 2

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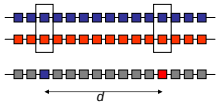
Genetic mapping

- Single chromosome with n genes
- Single recombination point that occurs uniformly at random
- Probability of recombination between two genes at distance d is $p=d/(n+1)$
- Estimate p (and therefore d) by observing the frequency of different phenotypes



- Problems
  - Too many chromosomes
  - Not all genes have phenotypes that can be observed
  - We usually don't know what we are looking for

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RFLP: Restriction Fragment Length Polymorphism

- A *restriction enzyme* cuts the DNA molecules at every occurrence of a particular sequence, called *restriction site*.
- For example, *Hin*dII enzyme cuts at GTGCAC or GTTAAC
- If we apply a restriction enzyme on DNA, it is cut at every occurrence of the restriction site into a million *restriction fragments*, each a few thousands nucleotides long.
- Any mutation of a single nucleotide may destroy or create the site (CTGCAC or CTTAAC for *Hin*dII) and alter the length of the corresponding fragment.
- RFLP analysis is the detection of the change in the length of the restriction fragments.

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Gel-Electrophoresis

- DNA is cut into fragments using an enzyme
- The cut DNA is put on a Gel material
- An electric current is applied on the Gel
- DNA is negatively charge
- DNA fragments will start moving towards the positively charged side
- Smaller fragments move faster
- After some time, we have a separation of the different fragment lengths

DNA Sample

- Some cells are obtained
- The cells are immersed in a nutritious solution on a plate and left to grow and multiply
- The cells are gathered and frozen for future use
- Liquidized DNA is obtained from these cells

Restriction Enzyme

- A restriction enzyme is used to cut the DNA into fragments
- Hind III restriction site is A | AGCTT

Apply Enzyme

- DNA sample and Hind III are put together in a tube
- The tube is shaken by rotation for DNA and Hind III to mix

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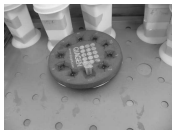
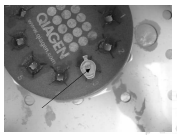
Water Bath

- The tube is put on a plate floating on water at 37^{o}C
- It is left for 30 minutes
- This is needed for the Hind III reaction to take place





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Preparing the Gel

- In the meantime, we prepare the Gel
- Agarose powder is the basic substance for making the Gel

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Preparing the Gel

- The powder is mixed with water in a container

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Preparing the Gel

- The container is heated (in a microwave if you want) until the powder completely dissolves in the water
- The solution becomes clear

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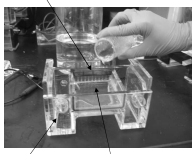
Preparing the Gel

- The liquid Gel is poured into the inner box
- A comb like piece is put at the edge of the inner box
- The liquid Gel is left to cool and solidify (you can use a fridge)
- When the Gel solidifies, the comb will create *wells* for the DNA samples to be put



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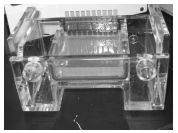
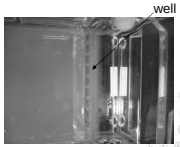
Gel Ready

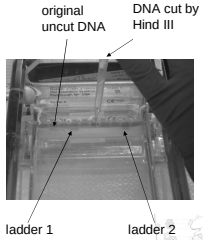
- Gel ready
- Fill the H shaped container with water
- Remove comb





Putting DNA on the Gel

- DNA samples mixed with colored solution and UV reactive solution
- DNA samples inserted into wells
- A sample DNA containing only specific fragments (called ladder) can be used for comparison

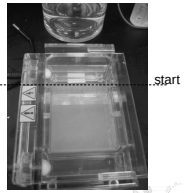


Run the Gel

- Apply electric current
- DNA is negatively charged
- Fragments will migrate toward the positive charge
- Small fragments move faster

DNA Fragments Move

- The colored solution provides an indication to how much the DNA has traveled on the Gel

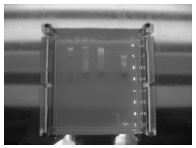


Viewing

- Gel can be viewed under UV light

Viewing

- Original uncut DNA sample makes a sharp band at the beginning (one big fragment)
- DNA sample cut with Hind III makes s smear (lots of fragments of all sizes)
- Ladders are used for comparison (they contain specific fragments)
- We could run it for a longer time to achieve better separation

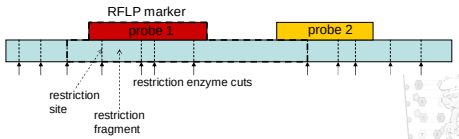


Hybridization

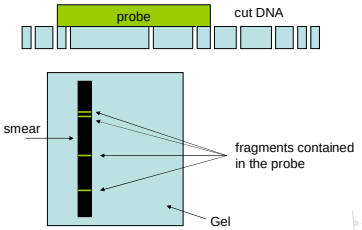
- In a hybridization experiment, we try to verify whether a specific sequence known as **probe** binds (or hybridizes) with a DNA fragment.
- If the binding occurs, this means that the DNA fragment contains the sequence complementary to the probe sequence (or parts of it).

RFLP Markers

- We apply a number of probes in turn on the gel
- Each probe is mixed with a radioactive material
- Each probe hybridizes with a portion of the original DNA
- After cutting, the probe will hybridize with the fragments belonging to that portion
- These fragment can now be observed due to the radioactive material
- **RFLP marker** is defined by a probe and the set of lengths (unordered) of fragments that hybridize with the probe.
- Use analysis of recombination to order RFLP markers on the chromosome



Illustration



First RFLP map in 1987

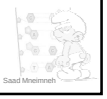
- Donis-Keller et al. constructed the first RFLP map of the human genome, positioning one RFLP marker per approximately 10 million nucleotides.
- RFLP markers (probes) need to be long enough to span the whole DNA.
- 393 random probes where used to study RFLP in 21 families over 3 generations.
- Computational analysis of recombination lead to the ordering of RFLP markers on the chromosome.

RFLP and Gene Finding

- Using the ordering of RFLP markers on a chromosome, we can approximately determine the location of a gene.
  - How?
  - Find the difference between the RFLP markers of family members with the disease and family members not having the disease.
  - It is likely that the RFLP marker that consistently differ is on the gene responsible for the disease, since family members have more or less the same genetic characteristics.
  - But we still don't know where and what the exact gene is.

Physical Mapping

- Genetic mapping and RFLP
  - (1) do not tell the actual distance in base pairs
  - (2) if genes (or markers) are very close, one cannot resolve their order, because the observed recombination frequencies will be zero.
- Physical mapping reflect actual distances
  - Hybridization Mapping
  - Restriction Mapping



Hybridization Mapping

- Break several copies of DNA into fragments (using different restriction enzymes).
- Obtain many copies of each fragment (cloning, incorporating a fragment into a replicating host), forming a clone library.
- Clones may overlap (cutting DNA with distinct enzymes), and we want them to (we will see why).
- *Fingerprinting* the clones: Now use DNA probes, and for every clone determine the list of probes that hybridize with the clone
- When two clones have substantial overlap, their fingerprints will be similar.
- Reconstruct the relative order of the clones using the overlap information (this order is unknown in RFLP)

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Hybridization Mapping

- For n clones, and m probes, the hybridization data consists of an n x m matrix D, such that d_{ij}=1 if clone C_i contains probe p_j.
- Let S be a string over the alphabet of probes $p_1 \ldots p_m$. S covers a clone C if there exists a substring of S containing exactly the same set of probes as C (order and multiplicity are ignored)
- A *simple approximation* of physical mapping is the **Shortest Covering String**.

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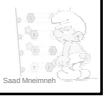
Illustration

No overlap
No information

Covering String:



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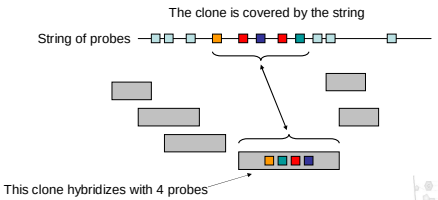
Covering string



Shortest Covering String

clones \ probes	A	B	C	D	E	F	G
1	1		1				
2	1	1			1		1
3		1	1	1	1	1	1
4		1	1	1		1	1
5			1	1		1	1
6	1			1		1	1
7	1	1		1	1		1
8	1	1		1			1
9				1			

A covering string: S= AC ABEG BCDEFG BCDFG CDFG ADFG ABDEG ABDG D

A shortest covering string (max overlap): S= C A E B G C F D A G E B A G D

Shortest Covering String: NP-hard Problem in general. If probes are unique, a polynomial algorithm exists.

Unique/Non-Unique Probes

- Non-unique probes: probes are short random sequences that can occur many times in the DNA. Therefore, a probe can hybridize with distant clones.
- Unique probes: probes are sufficiently long and are unlikely to occur twice in the DNA. Therefore, a probe will hybridize with close clones.
- Advantages of non-unique probes: probe generation is cheap and straight-forward.

Restriction Mapping

- Before using the list of probes in a clone as a fingerprint, biologists used the order of restriction fragments in a clone.
- Restriction map as Fingerprinting: If two clones share several consecutive fragments, they are likely to overlap.
- **Restriction map** of a clone: an ordered list of its restriction fragments (Hard Problem).

Double Digest

- Cut the DNA fragment with enzyme A, then enzyme B, then both
- Obtain a multiset of lengths in each case (using Gel electrophoresis)
- Using this information, construct an order of the lengths

- A: {2,2,3}
- B: {3,4}
- A+B: {1,2,2,2}

Partial Digestion

- Instead of obtaining lengths of restriction fragments, the DNA is digested in such a way that fragments are formed by every two cuts and the lengths of all fragments are obtained.
- The problem often might be formulated as recovering positions of points on a line when only some pairwise distances between points are known. (why?)
- Many mapping techniques lead to the following problem: X is a set of points, ΔX is the multiset of all pairwise distances between points in X: $\Delta X=\{|x_1-x_2| : x_1, x_2 \in X\}$, $E \subseteq X$ is given. Reconstruct X from knowing E alone.
- Partial Digest Problem. Given ΔX, reconstruct X ($E=\Delta X$). Also known as the *turnpike* problem in computer science, construct the geography of the highway from knowing the distance between every two exits.
- No polynomial time algorithm for this problem is yet known, but in practice, efficient algorithms exist.