15-499:Algorithms and Applications

Computational Biology IV
- Sequencing the "Genome"

Thanks to: Dannie Durand for some of the slides.
Various figures borrowed from the web.

Tools of the Trade

Cutting:

Arber, Nathans, and Smith, **Nobel Prize in Medicine** (1978) for "the discovery of restriction enzymes and their application to problems of molecular genetics".

Copying:

Mullis, **Nobel Prize in Chemistry** (1993) for "his invention of the polymerase chain reaction (PCR) method"

Reading: (sequencing)

Gilbert and Sanger, **Nobel Prize in Chemistry** (1980) for "contributions concerning the determination of base sequences in nucleic acids"

Cutting

Cutting:

- Restriction Enzines:
  Cut at particular sites, e.g. ACTTCTAGAT
- Chemical, physical or radiation cuts
  Cut at random locations

Copying

Copying:

Cloning a strand of DNA
- Cosmids: clones sequences up to 40K bps
- BAC, PAC: up to about 200K bps
- YAC (yeast artificial chromosones): up to 1 M

Copying between two specific sites
- PCR (polymerase chain reaction): 500 bps

Cloning (copying fragments)

Isolate DNA

Isolate DNA

fragmentation

Isolate DNA

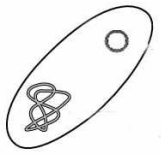
fragmentation

plasmid

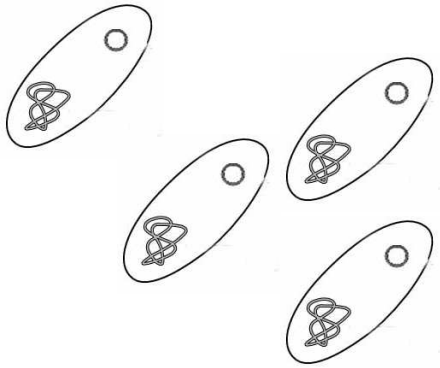
+

insert fragments

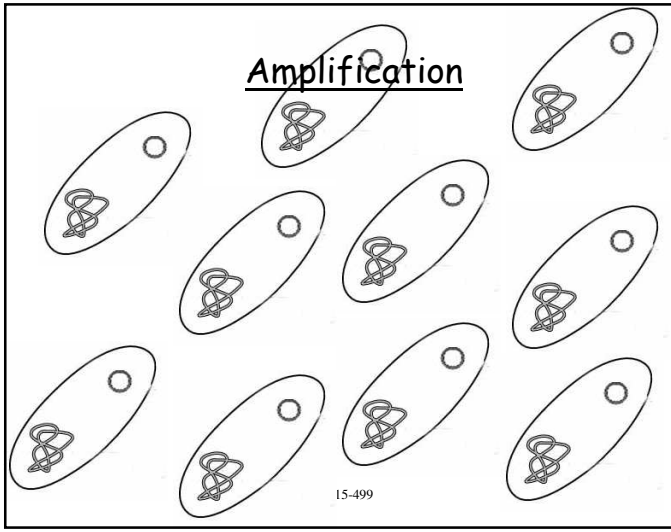
Amplification



Amplification



Amplification



PCR (Polymerase chain reaction)

Select two sequences that appear in the DNA sequence (e.g ATACTTAATG and TCTAAGATAG)

Design two synthetic "**primers**" identical to sequences

REPEAT:

1. **Denature:** Heat DNA to split into two strands
2. **Anneal:** cool and let primers attach
3. **Replicate:** let DNA attach in both directions

Note: cells copy DNA strands character by character

PCR (Polymerase chain reaction)

Reading: sequencing a fragment

Currently too expensive to actually read each bp.

Finding the length is cheap.

- The speed of a fragment in a gel when an electric charge is applied is proportional to its length (DNA has slight negative charge at one end).

Lengths are what are **used in Forensic DNA** analysis and for DNA "fingerprints"

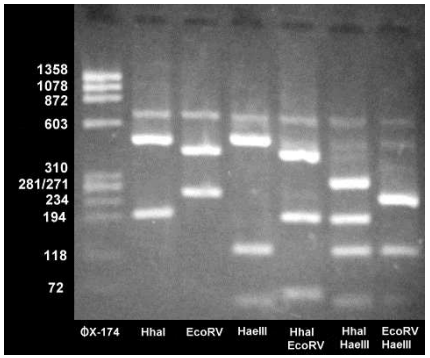
Gilbert and Sanger got the Nobel Prize for figuring out how to **use lengths to "read" a DNA strand** from one end.

Currently only good for about **500** bp.

Forensic DNA Analysis

For the two samples, and some "control" DNA

1. Copy using PCR if sample is small
2. Use restriction enzines to cut up DNA at particular sites (e.g. AATGATGGA)
3. Tag DNA with radioactive (or florescent) tracer This is a strand that will attach to particular sites of the cut DNA.
4. Put each sample on its own track on a gel
5. Apply charge for fixed time
6. Expose film to see pattern of lengths

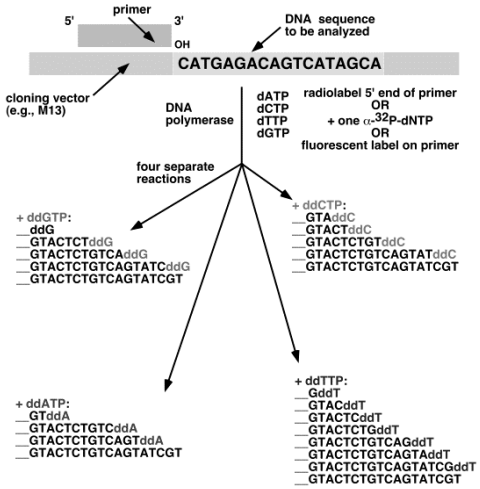


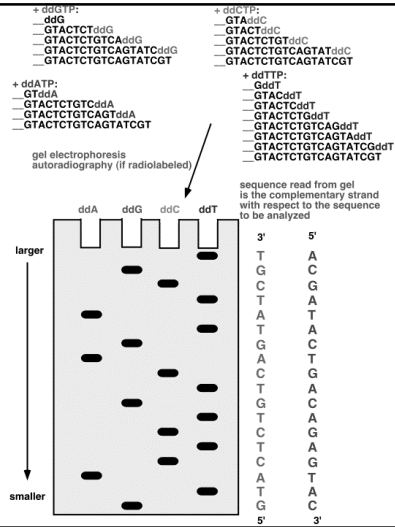
7 different DNA sequences and their "fingerprints"

Reading using lengths

Can use special base-pairs that stop growth: DDC, DDA, DDT, DDG.

Will generate all prefixes that end in A, T, C or G.





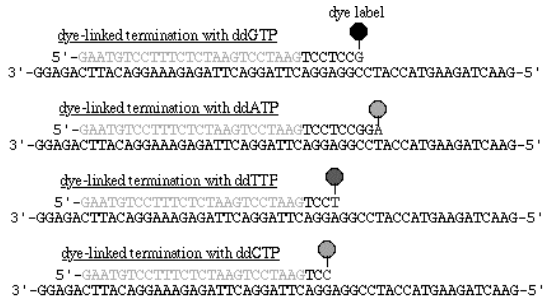
Improvements

Use fluorescent dies on the base pairs and laser to excite the die as it passes a certain point on the gel.

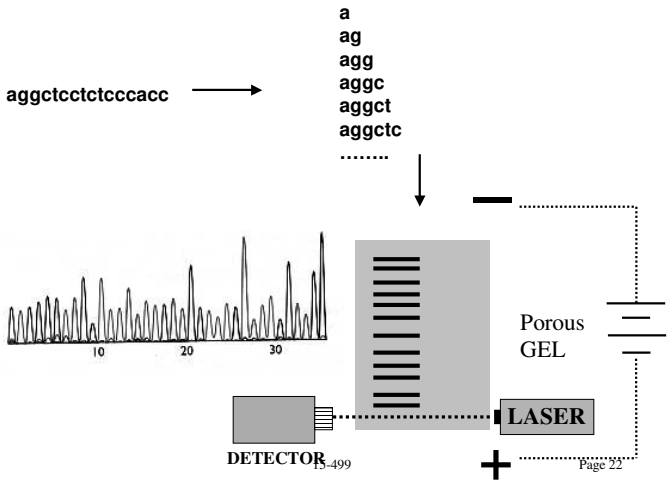
Improvements (1)

4 "test tubes", single track.

Improvements (2)



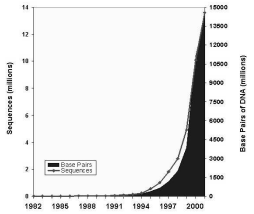
Single "test tube", single track



ABI 3700 sequencer

History of Sequencing

1971 Nobel prize for restriction enzymes
1973 First recombinant DNA
1980 Nobel prize for DNA sequencing
1988 Congress establishes Genbank
1995 First genomic sequence
1998 First multicellular organism
2000 Fly genome
2000 First plant genome
2001 Human genome
2003 Mouse genome



22 million sequences

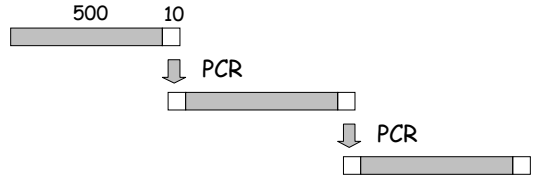
28 billion base pairs

Suquencing the Whole Genome

Problem: we only know how to sequence about 500 bps at a time in the lab.

1. Linear sequencing
2. The shotgun method
3. The double-barreled shotgun method.
4. Mapping

Linear Sequencing



Each step takes too long. Requires "wet" runs.

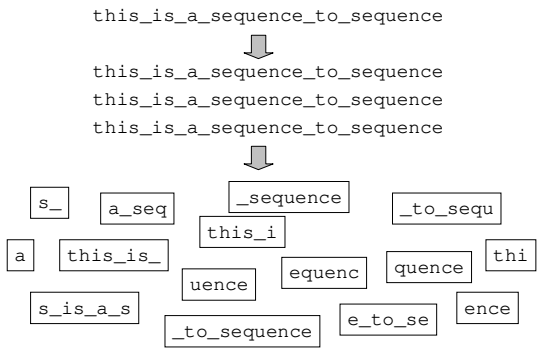
e.g. if each step took 4 hours, sequencing the human genome would take
$4 \times 3 \times 10^9/500$ hours = 3000 years

Also no interesting Computer Science ☺

The Shotgun Method

1. Make multiple copies of the sequence.
2. Randomly break sequences into parts (e.g. using radiation or chemicals).
3. Throw away parts that are too small or too large.
4. Read about 500bp from the end of each part
5. Try to put the information together to reconstruct the original sequence

Example



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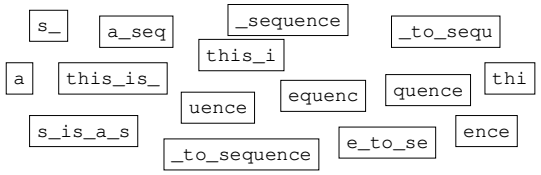
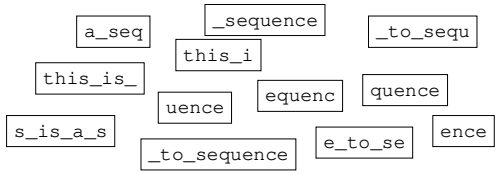


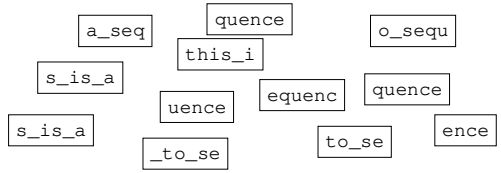
Remove strands that are too short (or too long)

Example



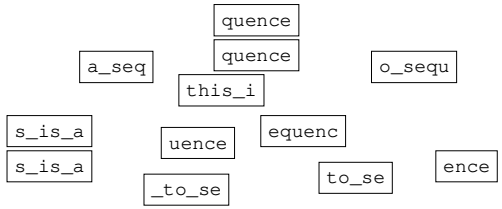
Sequence k characters from each (e.g. 6), from either end.

Example



Find overlaps

Example



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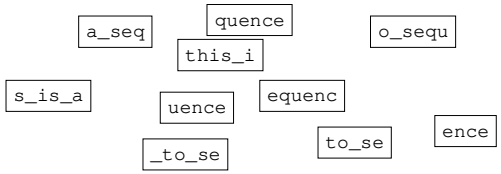

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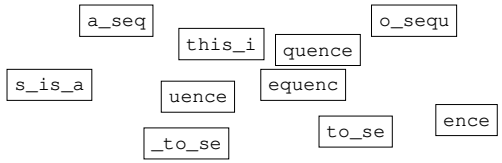

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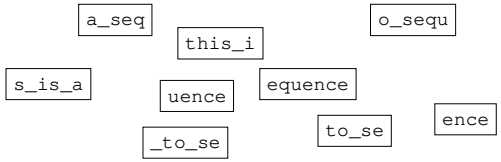

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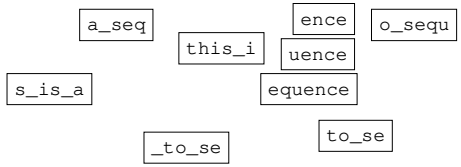

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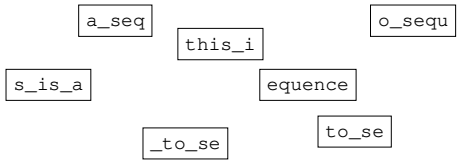

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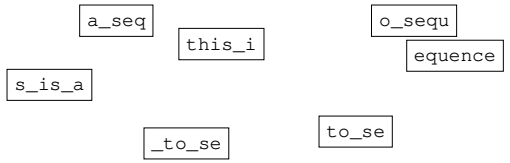

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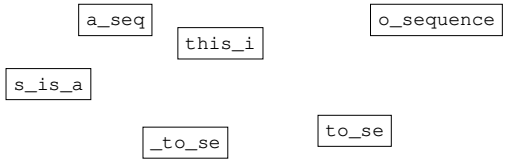

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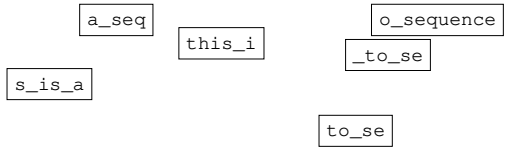

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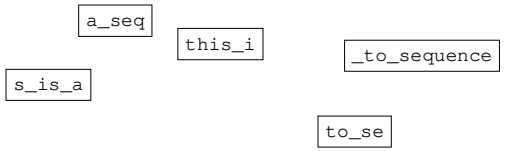

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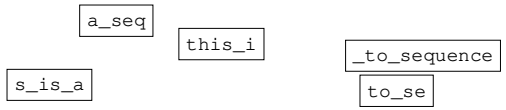

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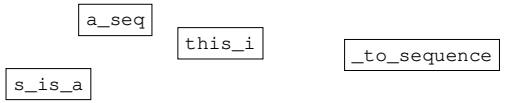
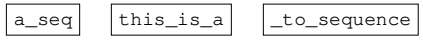


Example

Example

Having a single character overlap might not be enough to assume they overlap.

Example



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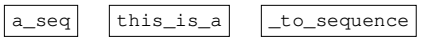

We are left with **gaps**, and unsure matches.

Each covered region (e.g. `this_is_a`) is called a **contig**

Is there a systematic way to find or even define a "best solution"?

The SSP: an attempt

The shortest superstring problem: given a set of strings $s_1, s_2, \ldots, s_n$ find the shortest string S that contains all s_i.

NP-Hard, but can be reduced to TSP and solved approximately (nearly optimally in practice).

Even if easy to solve, are we done?

Our example gives:

```
this_is_a_seq_to_sequence
```

but this is the best we can do given the data.

This problem is caused by repeats.

Other problems?

Problems

In practice the data is noisy.

- Reads have up to a 1% error rate
- Samples could have contaminants
- Fragments can sometimes join up

The reads could be in either direction (front-to-back or back-to-front). Cannot distinguish.

Assembly in Practice

gatcgat_ga

attgactactatg

Score all suffix-prefix pairs

- This can use a variant of the global alignment prob. It is the most expensive step (n^2 scores).

Repeat:

- Select best score and check for consistency
- If score is too low, quit
- If there is a good overlap, merge the two.

Determine consensus:

- We know the ordering among strands, but since matches are approximate, we need to select bps. Can use, e.g., multiple alignment over windows.

Some Programs for Assembly

Phrap
SEQAID
CAP
TIGR
Celera assembler
ARACHNE

After using one of these programs to generate a set of "contigs" with some gaps, one can use the linear method to fill in the gaps (assuming they are small).

atgattagccagtacgttt tcagcatcccagtacgttatgcac ttagccaga