DNA Technology

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The following are some of the most important molecular methods we will be using in this course. They will be used, among other things, for

- Sequencing genes and genomes
- PCR
- Making recombinant DNA
- Detecting mutations at the molecular level
- Studying the control of gene action
DNA Sequencing

**Reminder:** DNA polymerase adds nucleotides, one at a time, to a single nucleotide strand, in the 5' to 3' direction, using a DNA template and RNA or DNA primers.

- Polymerase + DNA template + pair of DNA primers --> copy DNA sequence between the primers.
- Interrupt reaction by dideoxynucleoside triphosphates which can have labels attached to them. You don’t need to know the method; sequencing is done for you at facilities at UA and elsewhere.
- Sequence both strands.
- Proofread sequences.

Cambridge biochemist Fred Sanger won Nobel Prizes for sequencing proteins *and* DNA.
DNA Denaturation and Renaturation
(Melting and Annealing)
DNA Denaturation and Renaturation (Melting and Annealing)

Molecule A has 30% GC.
Molecule B has 45% GC.
Which one will melt at a lower temperature?
A pre-defined DNA sequence in the genome can be greatly amplified by repeated Polymerization cycles using 2 primers which hybridize to the ends of the target DNA. In each cycle, the amount of target DNA is doubled. After 10, 20 and 30 cycles, there is a 1000-, million- and billion-fold amplification respectively.
Polymerase Chain Reaction (PCR)-2

Each PCR cycle has 3 steps-

a. Melting of DNA (e.g. 94°)

b. Hybridization of primer (e.g. 40° (ca. 20 bp long))

c. DNA synthesis (e.g. 68°)
PCR Requires Thermostable Polymerase

*Thermus aquaticus*: bacterium found in hot springs of Yellowstone National Park in 1969. Survives at up to 80° C.

DNA polymerase from *Thermus aquaticus* = Taq DNA Polymerase has optimum temperature 80°.

Monetary value of natural habitats and organisms. If we don’t conserve them, we won’t be able to exploit them.
DNA is negatively charged due to phosphates on its surface. As a result, it moves towards the positive pole.
Distance migrated by a DNA fragment in a gel is related to $\log_{10}$ of its size.
Gel Electrophoresis to Verify Amplification

PCR very powerful, amplifies tiny amounts of contaminating DNA.

Should have negative control = all reagents but no added DNA.

If we amplified the correct DNA segment, we should get a product of the size of that segment.
Question: A pair of PCR primers are designed to be complementary to sites 120-135 and 440-465 of the human β-globin gene. What is the expected size of the amplification product if these primers are used with a human DNA template?
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Answer: 120 - 465 +1 = 346 bp

Question: If the template is DNA from a baboon, do we expect to get the same size?
Q & A

Question: A pair of PCR primers are designed to be complementary to sites 120-135 and 440-465 of the human β-globin gene. What is the expected size of the amplification product if these primers are used with a human DNA template?

Answer: 120 - 465 = 345 bp

Question: If the template is DNA from a baboon, what size product do we expect?

Answer: Similar but not necessarily identical, because the genes of these closely-related animals are similar but not identical and can differ in length as well as in sequence.
Memorize and understand:

- \(2^1 = 2\)
- \(2^2 = 4\)
- \(2^3 = 8\)
- \(2^4 = 16\)
- \(2^5 = 32\)
- \(2^6 = 64\)
- \(2^7 = 128\)
- \(2^8 = 256\)
- \(2^9 = 512\)
- \(2^{10} = 1024 \approx 10^3\)
- \(2^{11} \approx 2 \times 10^3\)
- \(2^{12} \approx 4 \times 10^3\)
- \(2^{20} \approx 10^6\)

### Powers of 2

Useful in thinking about:

- Anything that doubles in number: PCR, increase in cell number during binary fission
- Dilution series in the lab
- Genetic ratios 1:1, 3:1, 9:3:3:1 etc., etc.
Engineering DNA

The same enzymes that are used in DNA replication can be used to rearrange DNA molecules at will.

**Endonuclease**: breaks 5'-3' phosphodiester bonds in backbone of DNA; "nicks" DNA.

**Restriction endonucleases** are enzymes made by bacteria. They recognize and cleave specific sequences. These sequences are usually palindromes. E.g.:

- **EcoRI**
  
  5' G\(\text{I}\)A A T T C 3'
  
  3' C T T A A \(\text{I}G\) 5'

  6-cutter, staggered

- **AvaII**
  
  5' G\(\text{I}\)G W C C 3'
  
  W = A or T
  
  3' C C W G\(\text{I}\)G 5'

  5-cutter, staggered, degenerate

- **Sau3A**
  
  5' G\(\text{I}\)A T C 3'
  
  3' C T A G\(\text{I}\) 5'

  4-cutter, staggered

- **AluI**
  
  5' A G\(\text{I}\)C T 3'
  
  3' T C\(\text{I}\)G A 5'

  4-cutter, blunt end
Engineering DNA

How *EcoRI* cuts

```
3'AGTGAAACCGTTTCTTTAAAGTGATGTGTGTCACGTCCAGCCGAAATAGTCTTTC
5'TCACTTTGGCAAAAGAATTCACCCCCACCAGTGCAAGGTGCTCTATCAGAAAG
```

<table>
<thead>
<tr>
<th>8 H-bonds break</th>
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```
3'AGTGAAACCGTTTCTTTAAAGTGATGTGTGTCACGTCCAGCCGAAATAGTCTTTC
5'TCACTTTGGCAAAAGAATTCACCCCCACCAGTGCAAGGTGCTCTATCAGAAAG
```

```
3'AGTGAAACCGTTTCTTTAAAGTGATGTGTGTCACGTCCAGCCGAAATAGTCTTTC
5'TCACTTTGGCAAAAGAATTCACCCCCACCAGTGCAAGGTGCTCTATCAGAAAG
```
Reminder

**Ligase**: makes 5'-3' phosphodiester bonds in backbone of DNA; "ligates" DNA.

Blunt-end ligation:
Recombinant DNA

DNA A

5' ATGGTGCACCTGAATTCCTGAGGAGACTG6G6GCAAGGTGAAC 3'
3' TACCACGTGGACTTAAGGACTCCTCTG6CAACCCGCTTCCACTTG 5'

DNA B

5' GGCTAGATCTTTGAATTCAGAGTACCAGTTTCATTAGAGGGACCCCA 3'
3' CCGATCTAGAACCTTAAGTCTCATGGTCAAAAGTAATCTCCTGGGT 5'

DNA A restricted with EcoRI

5' ATGGTGCACCTG AATTCCCTGAGGAGACTG6G6GCAAGGTGAAC 3'
3' TACCACGTGGACT TAA GGACTCCTCTG6CAACCCGCTTCCACTTG 5'

DNA B restricted with EcoRI

5' GGCTAGATCTTTG AATTCAAGAGTACCAGTTTCATTAGAGGGACCCCA 3'
3' CCGATCTAGAACCT TAA GTCTCATGGTCAAAAGTAATCTCCTGGGT 5'

5' fragment of DNA A + 3' fragment of DNA B

5' ATGGTGCACCTG AATTCAAGAGTACCAGTTTCATTAGAGGGACCCCA 3'
3' TACCACGTGGACT TAA GTCTCATGGTCAAAAGTAATCTCCTGGGT 5'

ligase

5' ATGGTGCACCTGAATTCAGAGTACCAGTTTCATTAGAGGGACCCCA 3'
3' TACCACGTGGACT TAAAGTCTCATGGTCAAAAGTAATCTCCTGGGT 5'

DNA A + DNA B = recombinant DNA
Cloning DNA: Making Cells Amplify DNA for US

Plasmid = small DNA molecule that can replicate inside bacterial cell independently of cell chromosome to achieve high copy number.
Cloning DNA

Cloning vector needs:
• Replication origin
• Selectable genes, e.g. antibiotic resistance
• Restriction sites in genes

Selecting cells with vector + insert:
• Join DNA cut with restriction enzyme to plasmid cut with same enzyme, e.g. \textit{BamH}1
• Transform bacteria with DNA ->

cells
cells + plasmid
cells + plasmid + insert

Antibiotic sensitive/resistant phenotypes?
Cloning DNA

Cloning vector needs:
• Replication origin
• Selectable genes, e.g. antibiotic resistance
• Restriction sites in genes

Selecting cells with vector + insert:
• Join DNA cut with restriction enzyme to plasmid cut with same enzyme, e.g.\textit{BamH}1
• Transform bacteria with DNA ->
cells \hspace{1cm} \text{amp} \text{S} \hspace{1cm} \text{tet} \text{S}
cells + plasmid \hspace{1cm} \text{amp} \text{R} \hspace{1cm} \text{tet} \text{R}
cells + plasmid + insert \hspace{1cm} \text{amp} \text{R} \hspace{1cm} \text{tet} \text{S}

• How would I select cells + plasmid + insert?