Advancing Transfusion Medicine

What is Damned Nucleic Acid (DNA)… and why should I care?

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Future of Blood Transfusion Medicine Symposium
London, November 2008

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The title…what was I thinking?

1. August 11, 2008. Not thinking. HSF Grant application panic.
2. Didn't like "DNA for Dummies". Wasn't feeling all that smart myself.
3. How much you understand about DNA depends on when you were trained and if you use the information.

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Why should you care?

- You value learning.
- DNA technology currently in use in some aspects of transfusion medicine
  - Detection and classification of disease-associated mutations (e.g. hemophilia)
  - Screening of donated blood for infectious diseases
  - HLA typing for transplantation

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DNA-based testing is very rapid…?

Source: Wikipedia Commons Images

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Objectives

- Explain structure and biology of DNA
- Explore an example of familial genetic variation using DNA-based technology
- Extend the example to ID screening
- Complete DNA survey by mentioning recombinant proteins
- Provide background to understand some of the developments we expect in the future.

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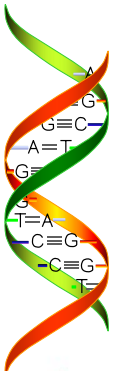
Deoxyribonucleic Acid
Damned Nucleic Acid

- "Deoxy" because is has one less O than RNA (ribonucleic acid)
- 2-stranded polymer made up of nucleotides
- Nucleotides contain deoxyribose sugars, phosphates and bases
- Repository of (almost) all genetic information
- Watson and Crick



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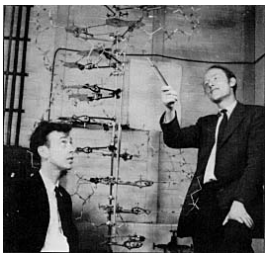

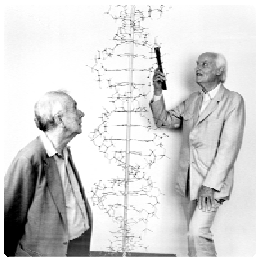

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.)..... This structure has novel features which are of considerable biological interest....
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

J. D. WATSON F. H. C. CRICK

Nature VOL 171, page737, 1953

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Possible copying mechanism?

- Only the bases in DNA differ
- Only 4 "flavours"
- A, C, G, or T
- Adenine, cytosine, guanine, thymine
- Across the strands, A binds to T and C binds to G (always)

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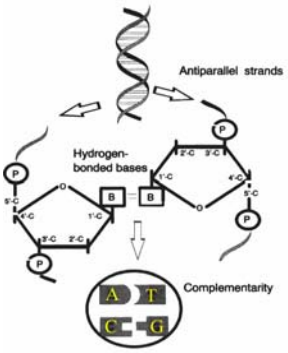
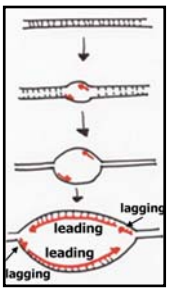


- DNA has direction defined by the sugar phosphate bonds
- RULES
  - Only copied 5' to 3'
  - Must be "primed"

Source: **Sheffield WP**. Concepts and techniques in molecular biology: An overview. Trans Med Rev 11:209-223, 1997.

- DNA serves as the TEMPLATE for new DNA synthesis
- Each strand is copied by enzymes called polymerases
- Progeny cells get same DNA (or combination of parental DNA at fertilisation)



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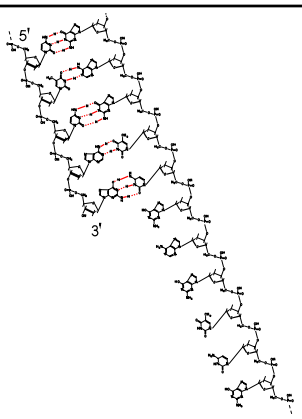


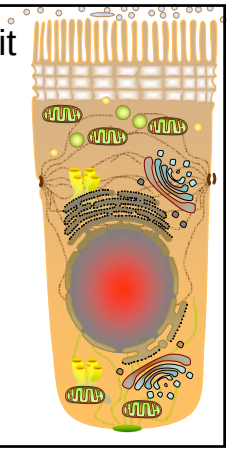
DNA replication – "linguistic" representation

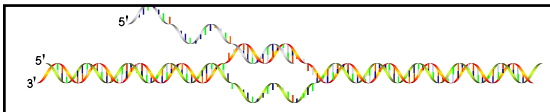
5'-ACGTAATTCCGATCTT-3'
3'-TGCATTAAGGCTAGAA-5'
3'-TGCATTAAGGCTAGAA-5'

5'-ACGTAATTCCGATCTT-3'
3'-TGCATTAAGGCTAGAA-5'

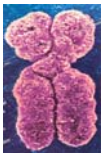
DNA: Where we keep it

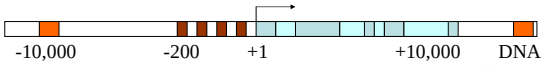
- In nucleus (6 billion bp)
- In mitochondria (16,569 bp)
- Nuclear DNA is organized into chromosomes
- Each chromosome is a very long double-stranded DNA molecule (Chr1 245 MBp).
- Body cell has 46 chromosomes (22 pairs of autosomes and two sex chromosomes XX or XY)
- Egg or sperm cell has 23





- **GENE**: A stretch of DNA transcribed into RNA.
- Genes that are transcribed into messenger RNA encode proteins.
- Messenger RNA is translated into protein by ribosomes.
- A/C/G/T (DNA) to A/C/G/U (RNA) "read" by ribosome in 3 letter "words"
- 4 X 4 X 4 = 64 possible ways to encode 20 amino acids
- (40-48%) of the genome does not encode anything!





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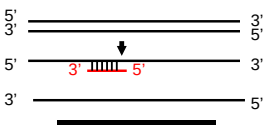
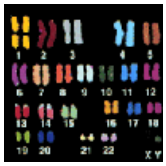
Exploiting DNA properties

- Natural DNA can be unwound and bound to synthetic DNA for copying (PCR) or detection (FISH; fluorescent in situ hybridization).
- PCR = polymerase chain reaction





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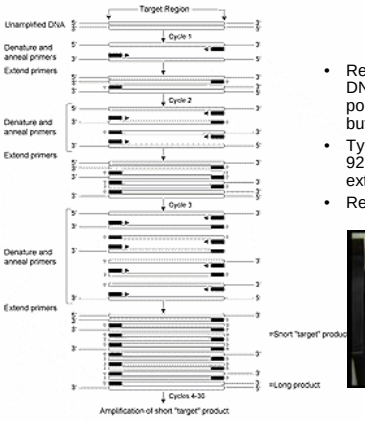
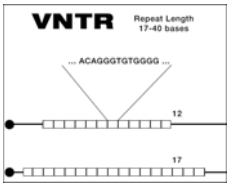


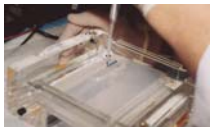
PCR

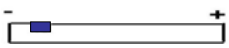
- Requires two synthetic DNA primers, heat-stable polymerase, dNTPs, buffers, thermal cyclers
- Typical cycle: denature at 92 degrees, anneal at 55, extend at 72
- Repeat cycle 30-40 times

"DNA fingerprinting"

- Most human DNA is identical
- Need difference to ID individuals
- VNTR: Variable nucleotide tandem repeat (non-coding)
- DS 180: A VNTR position on Chromosome 1
- Every human has (14 - 40 X) 16 bp repeats
- Flanking DNA not variable; design primers to bind here
- PCR products 200 - 700 bp long
- We have 2 Chromosome 1s, one from Mom and one from Dad
- Use agarose gel electrophoresis to size the products







DNA Isolation Procedure

- Buccal swab/mouthwash
- Cells lysed
- DNA isolated using commercial lysis/DNA binding filter kits
- DNA bound to a positively charged filter
- Washed repeatedly with ethanol-containing solutions
- Eluted using low salt

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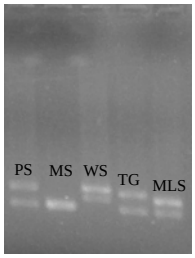
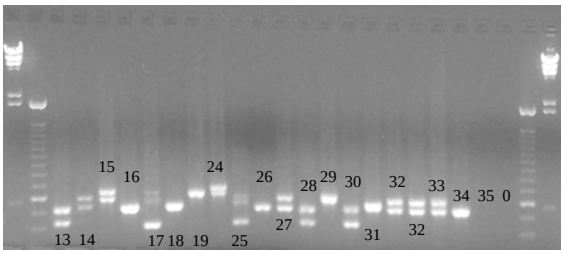
Inheritance at DS180 locus in my family

PLEASE NOTE:
FAMILY IMAGES
REMOVED.



AD=TG
WS = MS
PS MLS

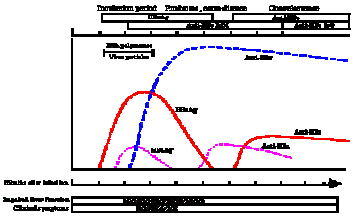
DS180 Variability in Grade 12 students



13=SS, 14 = RIP, 15 = WPS*, 16=LB, 17=VW, 18=SJ, 19=LC, 24=AL, 25=KM, 26=MT, 27=LL, 28=JU, 29=RA, 30=RR, 31=RD, 32=DM, 33=AD, 40 = SC

NAT

- NAT = nucleic acid testing
- Refers to practice of looking for viral RNA or DNA in blood donations ± anti-viral Ab
- Enhances transfusion safety by limiting "window period"



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Blood Donor Screening

- Donor testing
  - HIV1/2 — Antibody (Ab) and nucleic acid testing (NAT)
  - HBV — HBsAg, anti-HBc
  - HCV — Ab and NAT
  - HTLV1/2 — Ab
  - WNV — NAT
  - Syphilis — Ab
  - CMV — Ab (selected units)

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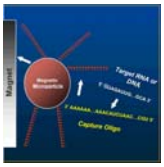
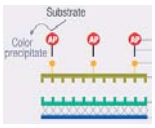
Features of NAT

- Viral RNA extracted, converted to DNA by Reverse Transcriptase
- PCR using biotinylated dNTPs
- Capture of products on oligonucleotide-coated magnetic beads
- Quantification of captured DNA using avidin-horse radish peroxidase and chromogenic substrate
- Read-out is in OD!!





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Recombinant proteins

- Protein production from a DNA template = protein expression
- A protein made using recombinant DNA
- Recombinant DNA: DNA molecules containing sequences assembled in an order not found in nature… usually to direct cells to make new proteins

Source: www.artes-biotechnology.com

Source: Wikipedia Commons Images

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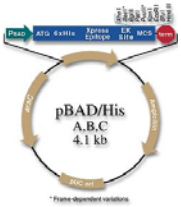
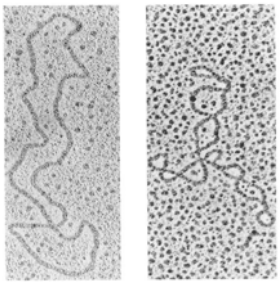
How to tell a cell to make a new protein



Source: www.invitrogen.com



Source: Becker WM, Kleinsmith LJ, Hardin, J. The World of the Cell, 4th edition, 2000. Benjamin/Cummings Pub. Co., Don Mills ON.

- Use a plasmid
- Small, double-stranded, circular DNA
- Propagated in bacteria (E. coli)
- Purified plasmid DNA transferred into genome of target cells

Why make recombinant proteins?

- From a clinical point of view…
- Safety (e.g. vs. purifying FVIII from pooled plasma of 1000s of donors)
- Natural human material not available due to abundance or source (e.g. G-CSF [filgastrim/Neupogen], erythropoietin [Epotin], fVIIa [Niastase], tissue plasminogen activator [Activase])
- Cost?

Recombinant CBS products

- Factor VIII (Kogenate [FS], Advate, Recombinate)
- Factor IX (Benefix)
- Factor VIIa (Niastase)
- Palivizumab (Synagis)
  - Humanized MAb for prevention of respiratory syncytial virus (RSV) LRT infections in at-risk infants

Why make recombinant proteins?

- From a research point of view…
- Test ideas about protein structure and function by making single amino acid substitutions (or larger changes)
- Make new protein drugs
- …Has become increasingly “easy” and “inexpensive”

Stuff we’re working on

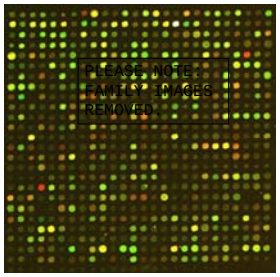
- Recombinant human albumin altered to carry a plasmin-activatable coagulation inhibitor
- Recombinant human albumin altered to become incorporated into a thrombus and weaken it

Source: Wikipedia Commons Images

Source: Wikipedia Commons Images

DNA Conclusions

- Not damned but very cool
- Conceptually simple
- Double-stranded, complementary structure is the key to finding, binding, and copying
- DS180 inheritance
- NAT
- Using DNA to make recombinant proteins for research and therapy
- Human Genome Project, $1000 genomes, microarrays, genotyping vs. serotyping, “DNA computers”…



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