**DNA Notes for Forensics:**

**Central Dogma of Molecular Biology**, which states that:



**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

 **DNA Polymerase:**

**DNA**

**DNA**

 **RNA Polymerase: uses only the one coding DNA strand**

 **Transcription into mRNA**

 **RNA**

**tRNA**

**mRNA**

**(ribosome translation)**

**rRNA (L&S)**

**Ribosomes**

 **Translation at the ribosome into protein**

**24 AA**

**Protein**

**ez**

**Functional (e.g. muscles)**

**Structural (e.g. cell wall)**

**DNA Theory**

**Double Stranded DNA**

 anti-parallel strands



 5’ and 3’ are ends of the sugars connected by phosphates





**Nitrogenous Bases: N is in the ring**

**Purine Bases: Pyrimidine Bases:
 guanine & adenine cytosine & thymine**

No sugar and no phosphate (PO4-3)

Now add sugar = NUCLEOSIDE

Now add phosphate = NUCLEOTIDE

Now you can make Nucleic Acid, DNA or RNA

**Make Large DNA molecule for whole class**

 40 copies of A,T,C,G needs 120 white sugar and phosphate

Scissors, and tape

Assemble and attach to walls

This took a good 30-40 min with everyone on task

**Restriction Enzymes cut as molecular scissors at restriction sites which are palindromes**

**This is a useful tool for molecular biologists that enable them to insert pieces of DNA into plasmids and replicate the DNA many times by growing bacteria that contain these DNA inserted plasmids.**

**These palindromes are the same Forward and Backward**

🡪🡨

**BOB**

**MADAM**

**RACECAR**

**Nucleotide Palindrome example: TAAT**

**If the restriction ez makes a Blunt cut in the following: GGC CGG**

 **CCG GCC**

**Resulting in DNA with blunt ends \_\_\_\_\_GGC CGG \_\_\_\_\_**

 **\_\_\_\_\_CCG GCC\_\_\_\_\_**

**or a Staggered Cut that LEAVES STICKY ENDS after cutting the double stranded DNA**

**CCCAATTCGA**

**GGGTTAAGCT**

**RESULTing in 2 sticky ends**

**CCCAATT AND CGA**

**GGG TTAAGCT**

**Blunt Cut** Example: EZ # 1 HindIII

A G C C T T A G C C T T A G C C T T

T C G G A A T C G G A A T C G G A A

**Sticky Ends**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_ A G C

 T C G \_\_\_\_\_\_\_\_\_\_\_\_\_

Overhang, Restriction Enzymes, Molecular Scissors

Examples of Restriction Enzymes: **Hind III
 EcoRI
 BamHI**

**Plasmid DNA** is circular.



More Examples:



**Gel**

Lane 1: linear 🡪 cut DNA Lane 2: circular 🡪 cut DNA

Three pieces: Two Pieces:
50 Kb 130 Kb
110 Kb 50 Kb
80 Kb



**Pipette Challenges**

**1st do the P200 Micropipette challenge**

**Use a 1.5 ml microcentrifuge tube to add the following:**

1. **25 ul green**
2. **50.0 ul blue**
3. **35 ul yellow**
4. **45 ul red**
5. **Total volume \_\_\_\_\_\_\_\_\_\_\_\_\_\_**
6. **Now set pipette to total volume and see how close you get, repeat 2X**

**2nd do the P20 Micropipette challenge**

**Practice Try 2x Add the following to waxed paper**

**3 uL red**

**4 uL blue**

**2 uL green**

**1 uL yellow**

**\_\_\_\_\_ total volume**

**Grey pipetteman DO NOT use 2-20**

 **0.5 uL – 10 uL**

**1) Gilson Blue Pipet 20-200 ul use Yellow tip’s**

**2) Grey Eppendorf 20-200 uL use Yellow tip’s**

**3) Gilson Blue Pipet 2-20 ul use small white tip**

. . **Gel Electrophoresis of DYES, discover the unknown contents**

1. **Do not plug anything into an electrical outlet until your teacher tells you too.**
2. **When pouring 1X TBE over solidified gel, pour into the gel box not on top of the gel itself.**
3. **Do not pour solutions back into the original stock bottle. Pour excess into a labeled beaker (1X TBE)**
4. Tape trays with masking tape. Set comb into middle row.
5. Make 0.8 % agarose in 1 X TBE buffer

~ 0.40g agarose in 50.0mL 1 X TBE buffer

1. Microwave 1 min swirl; repeat for 30 s, make sure all is dissolved until uniform throughout

~ wait 10-15min until cool to touch (like a baby’s bottle)

1. Pour it into taped Gel tray- be sure comb is set at end of tray
2. After gel is set, add ~500.0 ml of 1x TBE to the gel box pouring into the box not on top of the gel
3. Do not touch the Pt wires ( I cannot fix if they are disconnected) i.e. do not dry the inside of the gel boxes ever!

(10)You will load gels with dyes next class.

TBE stands for Tris Borate EDTA.

People also use TAE (Tris Acetate EDTA). Make up a 10x stock using cheap reagents. Do not use expensive 'analytical grade' reageants. Cheap Tris base and boric acid can be bought in bulk.

Recipe for 2L of 10xTBE

* 218g Tris base
* 110g Boric acid
* 9.3g EDTA

Dissolve the ingredients in 1.9L of distilled water. pH to about 8.3 using NaOH and make up to 2L.

**PCR** with Polly Dornette from DESTINY

DNA

**RFLP** restriction fragment Length Polymorphism

STR SBI Short tandem repeats

VNTR: variable number tandem repeats, current analysis

Use **introns** (Old Junk DNA) in last 4-5 years recognize some of the DNA not junk can be: promoter regions etc, there are more

**Exons** are the genes

**PCR Mix: DNA of interest + Master Mix**

Containing:

* DNA polymerase i.e. TAQ Polymerase
* dNTP’s
* Mg+2 acts as coenzyme
* Buffer
* 2 primers forward and reverse

**PCR simulation**: new tube for each, change tips each all tubes are different colors

* Step 1 yellow put 20 ul Master Mix + primers into PCR tubes put into all 6 PCR tubes
* Step 2 is add Template to each tube
* Tube 1 DNA template labeled is the DNA from crime scene
* Tube 2 suspect 1
* Tube 3 suspect 2
* Tube 4 suspect 3
* Tube 5 suspect 4
* Tube 6 clear negative no DNA

Run to Red (Red is +)

20 uL dye

|  |  |  |
| --- | --- | --- |
|  | Dye Name |  |
| Explorer II |  |  |
| Explorer I |  |  |
| Green  |  |  |
| Orange  |  |  |
| Blue  |  |  |
| Yellow  |  |  |
|  |  |  |

 1st space well, skip the first well, if possible place 20 ul of

dyes with spaces apart from each other.

**REPEAT for DNA gels** except

1. Put combs at the black end of the gel not the middle
2. Add Carolina Blue stain – 2 drops per 50.0 ml gel after cooling before pouring the gel into the taped gel tray.
3. Add 16 drops or 640 ul of Carolina Blue stain (C30528) to 400 ml running buffer. Or 960 ul (24 drops) per 500 ml TBE buffer.
4. Run gels at 100 volts for ~ 60 min. Turn off power and remove gel to gel staining tray.
5. Stain gels by covering with Carolina Blue Final DNA Stain for 15- 20 min. rinse with distilled water (chlorinated water bleaches the gels).

**DNA Sequencing**

A T G C

**SEQUENCING**

**GEL**

**Radioactive Nucleotides dTTP, dGTP, dCTP, dATP:**

**Coding Strand:** A-T G-C

T-G-T-C-A-T-G-G-C-C-T-T-A-T-C

A C A G T

**Protein Synthesis**

**rRNA is ribosomal RNA, large and small subunits make up one ribosome where the protein is elongated (synthesized)**

**mRNA is messenger RNA which carries the message for proteins**

**tRNA’s have CODONS: 3 N.T. BASES CODE FOR AN AMINO ACID**

**All types of RNA use ribose and uracil instead of deoxyribose and thymine**

**DNA START CODON: STOP CODONS can be**

**AUG ----------------------------- ---------------------- UGA**

 **UAG**

 **UAA**

**AUG codes for Methionine which will be the first AA for any protein**

**20 AMINO Acids**

**Protein Synthesis**

 

**Legend:** Process whereby DNA encodes for the production of amino acids and proteins.

This process can be divided into two parts:  **1. Transcription**  Before the synthesis of a protein begins, the corresponding RNA molecule is produced by RNA transcription. One strand of the DNA double helix is used as a template by the RNA polymerase to synthesize a messenger RNA (mRNA). This mRNA migrates from the nucleus to the cytoplasm. During this step, mRNA goes through different types of maturation including one called [**splicing**](http://www.accessexcellence.org/RC/VL/GG/trans_of.php) when the non-coding sequences are eliminated. The coding mRNA sequence can be described as a unit of three nucleotides called a **codon**.

**2. Translation**  The ribosome binds to the mRNA at the **start codon** (AUG) that is recognized only by the initiator tRNA. The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes, composed of an amino acid linked to tRNA, sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA **anticodon**. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into polypeptidic sequences dictated by DNA and represented by mRNA. At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

One specific amino acid can correspond to more than one codon. The genetic code is said to be [degenerate](http://www.accessexcellence.org/RC/VL/GG/genetic.php).

