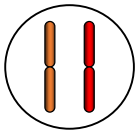
Phenotype: the visible characteristics EG seed wrinkle or coat colour

Genotype: the genetic make-up & alleles present

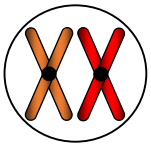
Homozygous: the same EG SS or ss alleles

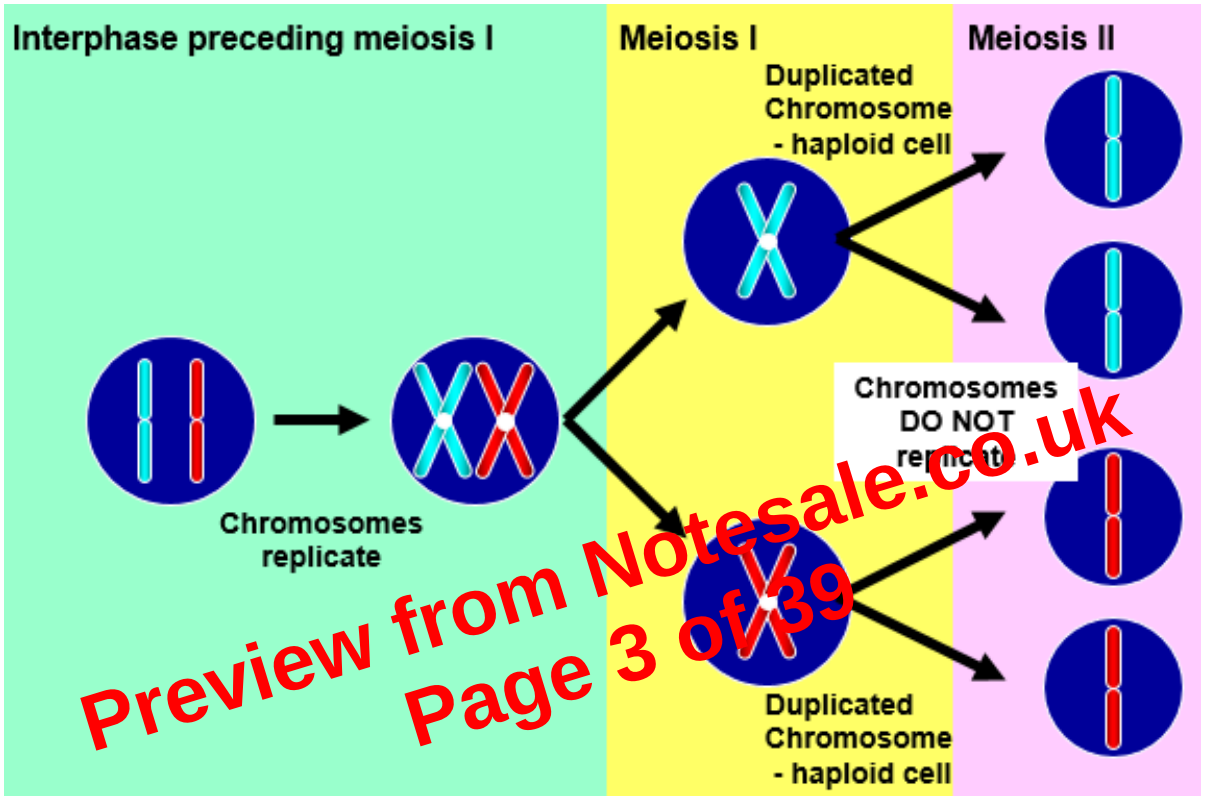
Heterozygous: different EG Ss alleles



Homologous Pair

Sister Chromatids

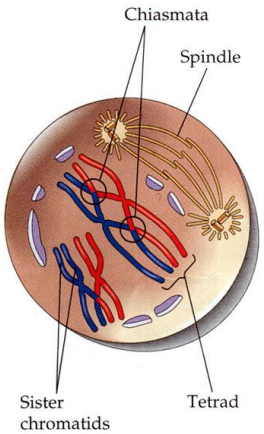




Meiosis I

- **The homologous chromosomes line up together, homologue to homologue, these homologues then separate**

Prophase I

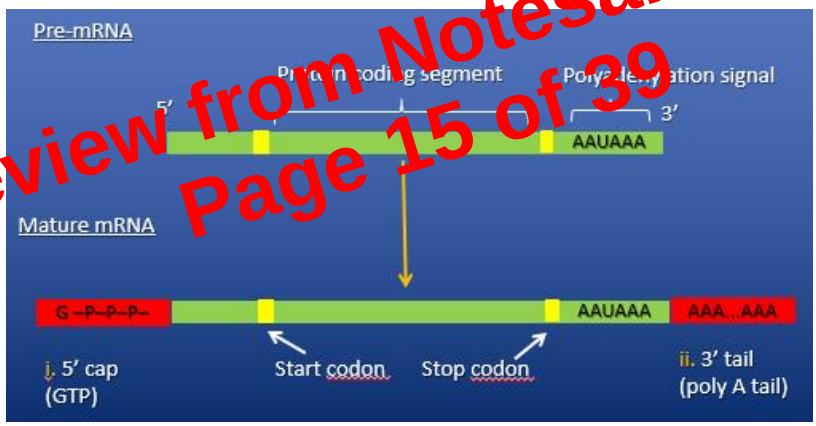


- 90% of meiotic division
- Homologues pair together
- Crossing over occurs at chiasmata
- The segments swap and exchange genetic information

RNA Processing

Characteristic	Prokaryotes	Eukaryotes
Site of transcription & Translation	Cytoplasm	Transcription – nucleus; Translation - cytoplasm
Gene structure	Complementary to protein structure	Noncoding sequences "introns"
Modification of mRNA after transcription before translation	None	A) Additions to mRNA ends B) Intron removal

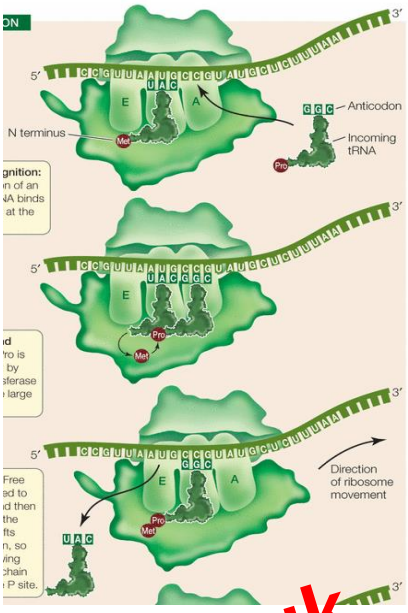
- **A)** additions to mRNA ends
  - **I.** 5' cap
    - This is added to the 5' end of the pre-mRNA (whilst it is still being transcribed)
    - It is a chemically modified molecule of GTP (guanosine triphosphate)
    - It facilitates the binding of mRNA to a ribosome enzyme for translation and also protects mRNA from being digested by ribonucleases
  - **II.** 3' tail
    - this is added immediately after the mRNA transcript has been released from RNA polymerase
    - Called a 'Poly A Tail' which is 100-300 adenine nucleotides long
    - Enables the mRNA to be exported from the nucleus to the cytoplasm
    - Helps to promote mRNA stability and prevent degradation



- **B)** intron removal (splicing)
  - Eukaryotic gene sequences contain non-coding sections that don't code for a protein
  - These are transcribed into mRNA sequences which produces introns
  - **Small nuclear Ribonucleoproteins (snRNPs):** these are proteins that perform the splicing
  - RNA polymerase transcribes both the introns and exons, this material is called the pre-mRNA
  - For the mRNA to leave the nucleus for translation to occur in the cytoplasm the introns need to be removed

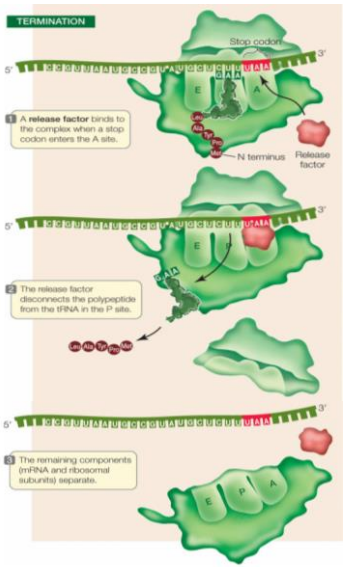
    c. E: exit
  - Methionine charged tRNA occupies the site
  - The A site is aligned with the next mRNA codon (CCG)

- Elongation
  1. Codon recognition:
     - The anticodon of an incoming tRNA (GGC) binds to the codon at the A site (CCG) of the ribosome
  2. Peptide bond formation:
     - Proline is linked to methionine by peptidyl transferase activity of the ribosome large subunit
     - Peptidyl transferase has catalytic activity for 2 reactions:
       a. Breaks the bond between the amino acid and its tRNA P site
       b. Forms a bond between that amino acid and the new amino acid attached to its tRNA in the A site



  3. Elongation:
     - Free tRNA (without amino acid) is moved to the E site and released as the ribosome moves along to the next codon
     - The growing polypeptide chain moves to the P site
  4. The process then repeats
     - The free site A attracts its complementary charged anticodon (AUA) for the codon in the A site
     - The bond between the amino acids and tRNA in the P site breaks
     - These amino acids bond to the new amino acid on the tRNA in the A site
     - The ribosome then moves along the mRNA to the next codon, the old tRNA is release and the new tRNA is in the P site

- Termination
  1. A release factor binds to the complex when a stop codon enters the A site
     - Stop codons do not correspond to any amino acids or bind to any tRNAs
     - Stop codons only bind the release factor
  2. The release factor disconnects the polypeptide from the tRNA in the P site
  3. The remaining components (mRNA, ribosome subunits) separate

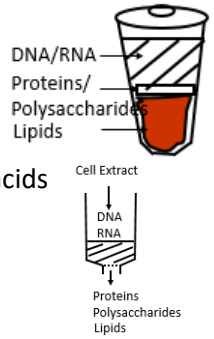


- Bacteriophages
- Viruses

24.11.15 Analytical Methods in Molecular Genetics II

Nucleic acids Isolation and Purification

- Tissue disruption
  - Mechanical methods: grinding tissues in a mortar
  - Enzymatic methods: using lysozyme to destroy bacterial cell walls
- Tissue homogenisation
  - Buffer systems: used to solubilise and protect nucleic acids
- Purification
  - Differential (phase) extraction: uses a combination of aqueous and organic solvents to separate nucleic acids from other substances
  - Ion exchange cartridges: use charged resins that retain nucleic acids which are eluted after all the contaminants are washed

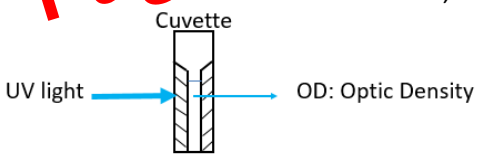


Nucleic Acid Quantification

- Spectrophotometry: light absorbance at different wavelengths
  - Nucleic acids absorb light at 260nm (the UV range)
  - Allows you to calculate the amount/concentration of nucleic acids (DNA/RNA)

OD x EC = χ µg/µl

  - You can study the secondary structure of DNA and the pairing of single stranded DNA
  - You can check the purity of DNA & RNA
    - This is determined by the A260/280 ratio
      - The OD of nucleic acids is 260 and the OD of proteins is 280
      - If this ratio is between 1.6 and 2.4, the preparation is good

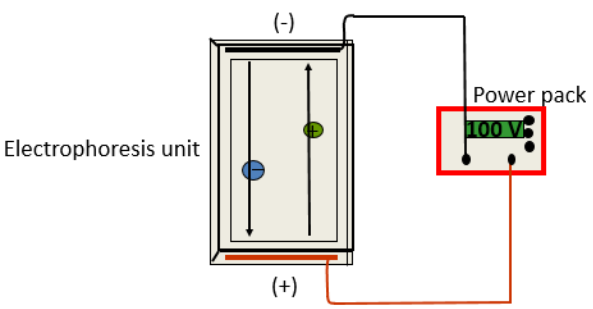


- The extinction coefficient (EC) at 260 nm varies depending on which DNA you have: (Light absorbance (the optic density) in a 1ml solution in a 1cm path):
  - Double stranded DNA: 50 µg/µl
  - Single stranded DNA: 33 µg/µl
  - RNA: 40 µg/µl

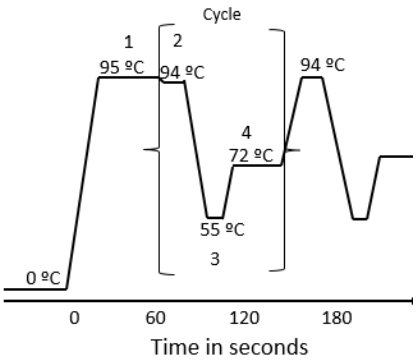
Produces 1 optic density

Nucleic Acid Separation

- **Electrophoresis:** the motion of charged particles into fluids under the influence of an electric field
- DNA & RNA are separated based on their size, they are uniformly negative charged and will move towards the positive anode

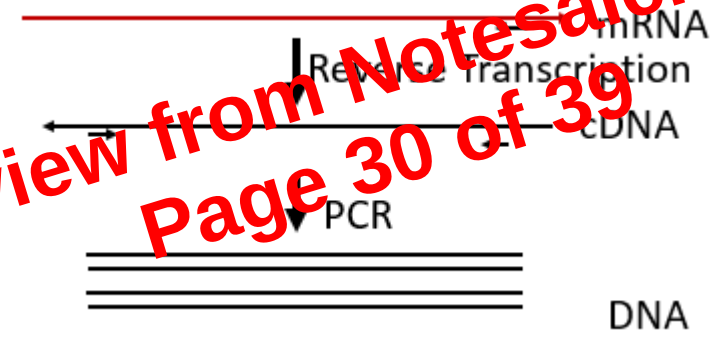


- **Temperature Cycle (PCR)**
  1. Initial denaturation
  2. Denaturation time is dependent on the type of template: it must be kept at a minimum
  3. Annealing temperature and time are depended of the type of primer
  4. The extension time is dependent on the fragment size & on the enzyme used



- **Thermal cyclers**
  - Control the temperature
  - Rapid ramping: the time it takes to change from one temperature to another
  - Simple to program
  - Low cost to run
  - Heat pumps: Pelletier effect
  - Heater air: very rapid cycles, the circulation of heated air into a chamber containing the PCR tubes
- **PCR specificity**
  - Primer sequence
  - Template quality and concentration
  - Stringency of the annealing of the primers
  - Ionic force [Mg^+]: high [Mg+] ----> low stringency
    Temperature: high temperature ----> high stringency

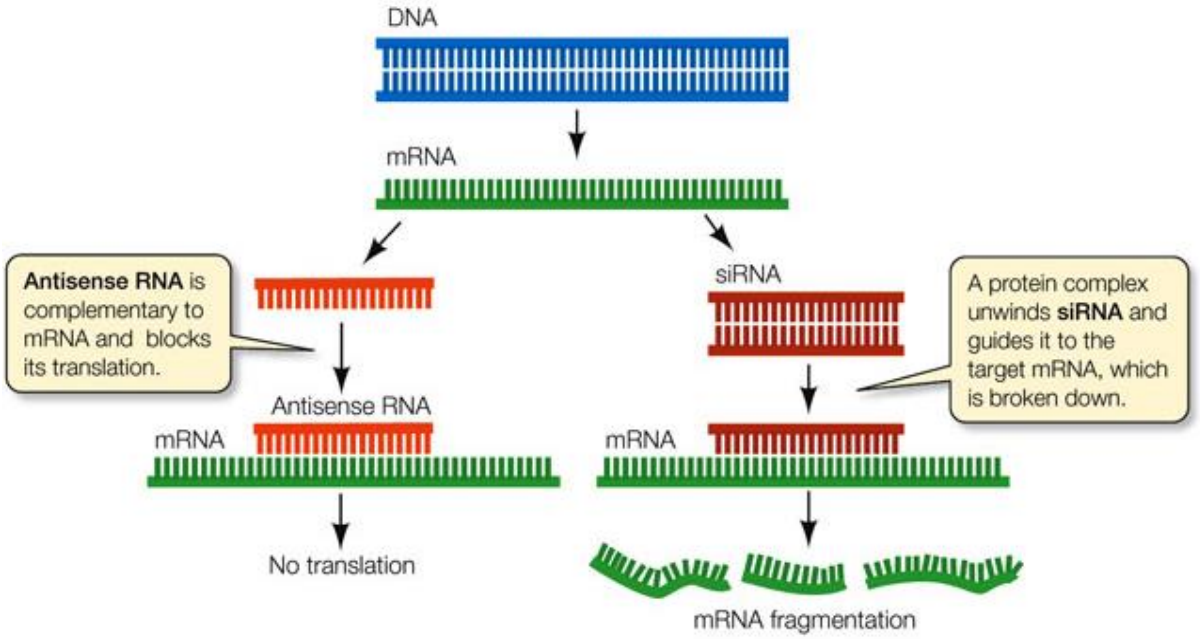
- **Reverse transcription PCR: RNA templates**



- **Applications of PCR**
  - Gene cloning
  - Monitoring gene expression
  - Mutagenesis
  - Detection of hereditary diseases
  - Detection of microorganisms and viruses
  - Detection and quantification of GMos
  - Fingerprinting polymorphism
  - Ancient DNA

Whole genome amplification (WGA)

- Amplification of the entire genome or entire DNA in a sample
- It produces a high yield, can produce a mg of DNA from less than a pg of template
- Needed:
  - Phi 29 DNA polymerase
  - Pyrophosphatase
  - Exonuclease resistant random primer (binds randomly to DNA)
  - All reactions done at 30°C
- Applications: used to amplify traces of DNA and ancient DNA



- Knock-out Technology
  - Knock-out are collections of insertional mutants produced by rDNA technology
  - A vector is used to transfer randomly or specifically a tDNA to a location in the genome to alter a gene at that locus
  - Each mutant has an alteration in a single gene
  - By tracing the tDNA it is possible to find which gene was affected
  - By identifying the change in the phenotype it is possible to link gene with function

4.12.15 Recombinant DNA Technology II

Transgenesis

- The transfer of a gene from one species to another species
  - Direct transfer
  - Semi-direct transfer
  - Mediated transfer