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# Cell and tissue engineering

## Quantum Information Processing and Genetic Engineering

DNA is condensed into chromatin and chromatin loops and then finally chromosomes.

Human to human we differ of one to two nucleotide pairs per 1000.

We have roughly 3,000 nucleotide pairs.

Mice quite genetically similar to a human. Mouse contains the same genetic material, just in a rearranged state.

Naturally genetic mutations occurring in human also occur in mice, and can result in same effects.

* **KIT gene**: gene required for maintenance of pigment. KIT genes in human and mice are Orthologs: same gene in different species.
* Paralog: duplicate gene within the same species.
* Paralog and orthologs are formed form homologs.

Genome size does not correlate with organism complexity.

Diploid: two copies of the genome one from the mother and one from of the father.

Although genome size varies, they contain the same number of functionally distinguished genes.

Gene is comprised of **exon**: code for segments of proteins, separated by introns. When gene is active, it is transcribed into RNA. Then the intronic RNA is spliced out, and **the exonic RNA** is assembled into mRNA. Non coding intronic RNA may serve regulatory role, interacting with DNA and RNA to help perform specific functions.

Most genes have under 20 exons.

Number of exons varies with the size of the protein and the size of the gene.

* **Genotype**: genetic make-up of the cell or the organism.
* **Phenotype**: function characteristics traits or behavior of the cell or organism. Determined by the genotype but also the environment.

Genes are only transcribed from one strand of DNA.

They are read from 3’ to 5’.

**Transcription**: DNA is transcribed to RNA via RNA polymerase (5’ to 3’). It happens on the anti-sense or non-coding strand. Process of transcription is facilitated by RNA polymerase which marches down the DNA strand, unwinding it to expose an active site, adding in a nucleotide.

RNA has directionality similar to DNA, denoted by 5’ and 3’ thus it is only read and translated into a protein in one direction. Unlike DNA which needs to be open for transcription, RNA is already opened for translation.

A direct RNA transcript is spliced to become mRNA used in **translation**.

Process of transcription from DNA to RNA occurs in the nucleus. Then the transcript moves to the cytoplasm where the nucleotide code is converted into amino acid.

In eukaryotes, translation occurs in across the membrane of the ER (endoplasmic reticulum). The process of decoding RNA is completed by a ribosome and tRNA. Step 2: the amino acid forms a peptide bond with the prior amino acid. Step 3: the mRNA moves a distance of 3 nucleotides through the small subunit, ejecting the spent tRNA molecule, the tRNA no longer carrying an amino acid.

**Codon**: 3 nucleotides in the mRNA transcript; anti-codon, its complement.

4 nucleotides: 4^3 = 64 possible combinations (amino acid) but only 20:the code is redundant.

**Restriction endonuclease:** to cut DNA; they are found in bacteria.

**DNA ligase**: to undo these cuts.

* **Hybridization**: used for related but not identical DNA sequences. Use DNA probe to look for related RNA sequence is one way to find out if a gene is being expressed. Hybridization technics can also be used to eliminate patterns of gene expression during the development of a disease.
* **DNA sequencing**: to determine the exact nucleotide sequence of the DNA to work with. **Sanger method** the most popular. DNA is made up of dNTPs. Each nucleotide is added together using an OH group. Sanger method uses synthetic dNTPs which only have an H group. This blocks the addition of nucleotides and therefore terminates the chain. After sufficient incubation, the samples run through gel electrophoresis separating the strands from largest to smallest. The colors are then read by computers for analysis.
* **DNA cloning**: to produce large quantities of a DNA sequence. PCR or vector chemistry.
* Also used to amplify trace amount of RNA. Scientists can investigate gene expression patterns and levels.
* **Vector chemistry or technique**: insertion of DNA of interest to the genome of a self-replicating genetic element, a virus or plasmid. Plasmid vector usually is a circular double-stranded DNA molecules derived from larger plasmids, which occur in bacteria and separate from the bacterial chromosome. Next step is to introduce the recombinant DNA to bacterial cells.
* As these cells grow and divide, they replicate the plasma in the sequence.

**DNA engineering**: mutation can mean overexpression of a gene, change of location of a gene, change of activity from one tissue to another, or changing the timing of that activity.

## Making a transgenic animal

* **Embryonic stem cell method**: cells can grow in culture, and desire genetic modification can be verified before cells are transformed and put into the blastocyst. Another advantage is targeting by homologous recombination.
* **Pronucleus method**: DNA construct directly injected into the pronucleus of a fertilized egg, prior to the fusion of the pronuclei which forms a diploid zygote nucleus. When the zygote is a two cells embryo it is implanted in the pseudo-pregnant mother just as method 1.

## Type of mutations

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### Random vs. Targeted Mutations

In order to knock-out or knock-in a gene you must be able to target the location of that gene in the genome.

Targeting strategy is easier in haploid systems and lower eukaryotes.

### Antisense RNA to knock out gene

* Only genetic material in (no need to get material out): integration site is much less important.
* Create a gene when is transcribed is complement of the RNA for the targeted gene.
* When the target RNA to the compliment antisense RNA, this hybridization blocks translation of the protein.
* Short anti sense RNA peptides can also be directly injected (a synthetic version is morpholino).
* Introducing double-stranded RNA containing both the sense and anti-sense strands, is more effective at knocking out gene expression: **RNA interference**:

Long double-stranded RNA is administered which is cleaved by a protein called **dicer**, into small interfering or SI RNA. These are then assembled into an RNA-induced silencing complex, RISC complex. In this RISC complex, it is unwound and directed to its mRNA complement. Binding to the complement results in splicing of the mRNA, degradation and ultimately gene silencing.