<table>
<thead>
<tr>
<th>Vectors</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>Long lasting gene expression</td>
<td>Only infects dividing cells</td>
<td>~37% of Gene Therapy Trials</td>
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<td></td>
<td>Efficiently enters cell</td>
<td>Low yield (hard to produce)</td>
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<td>Potential insertional mutagenesis</td>
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<tr>
<td>Lentivirus</td>
<td>Long lasting gene expression</td>
<td>Potential insertional mutagenesis</td>
<td>~10% of Gene Therapy Trials</td>
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<td>Will infect dividing and non-dividing cells</td>
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<tr>
<td>Adenovirus</td>
<td>Efficiently enters cell</td>
<td>Immunogenic – rapidly cleared from the body</td>
<td>~20% of Gene Therapy Trials</td>
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<td></td>
<td>High delivery rate</td>
<td>Can cause inflammation and tissue damage</td>
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<td></td>
<td>No chromosomal integration</td>
<td></td>
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<tr>
<td>Adeno-associated Virus</td>
<td>Long term expression</td>
<td>Difficult to produce in high quantities</td>
<td>1% of Gene Therapy Trials</td>
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<td></td>
<td>Wide host cell range</td>
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<tr>
<td>Herpes Simplex Virus</td>
<td>Produced at high levels</td>
<td>Immunogenic – rapidly cleared from the body</td>
<td>~6% of Gene Therapy Trials</td>
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<td></td>
<td>Can carry lots of DNA</td>
<td>Potentially toxic</td>
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<tr>
<td>Liposome</td>
<td>Not immunogenic</td>
<td>Low rate of delivery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can carry lots of DNA</td>
<td>Transient expression</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>No viral component</td>
<td>Transient expression</td>
<td>3% of Gene Therapy Trials</td>
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<td>Difficult to target specific tissues</td>
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- Emerging Approaches
  - **Targeted Cell Delivery** - integrative technique of cell mediated transfection
    - Specific antibodies are used to bind DNA to target cells
  - **Transposons**
    - DNA injected into the bloodstream in lipid capsules
    - Lipid capsules enter cells
    - Cells make transposase enzyme from the gene
    - Transposase cuts out a gene and inserts into a random spot on the genome
  - **Antisence technology** – antisense drug disrupts translation to result in the prevention of protein synthesis
DNA Fingerprinting
- Testing for – parentage verification, ID, forensics
  - Can replace conventional methods
    - Blood grouping
    - Ear tagging
- Advantages
  - Uses very little amounts of DNA
- Limitations
  - Initial development requires detailed DNA sequence information

Method
- **Sample is collected** and transported
- DNA/RNA is **extracted** from the sample
- **PCR** – determination of normality/affected from visualisation of:
  - Presence or absence of PCR Product
  - Variation in PCR product length or composition
  - Number of PCR products
- **PCR process**
  - Temperature raised to **denature** DNA into two single strands
  - **Primers** are designed to test for specific pathogens and bind to matches on the target DNA sequence
    - **Primers** mark sequences to be copied
    - **PCR product is cut** by restriction enzymes depending on the presence of certain sequences
  - **PCR amplifies** an affected segment of DNA
    - **Polymerase** add nucleotides to form two strands
    - Strands are denatured in another cycle
    - **Process is repeated several times**
    - Thus producing different lengths of PCR products
      - Ie – normal PCR is cut, affected isn’t cut

PERSONALISED MEDICINE
- Assumptions in drug development and treatment
  - **Dose, efficacy and treatment is determined by a mean response**
- Individuals will respond differently to drugs
- Disease progression may have subtle differences between patients
- Drug development is expensive thus are made for mass production and depend on demand

- **Personalised Medicine** – utilising genomic and proteomic technologies to **determine the most appropriate treatment** rather than generate a new drug for an individual
  - Screen for predisposition
  - Specifically diagnose and characterise the correct disease
  - Monitor effectiveness of treatment and disease progression

Example – Cancer Treatment
- Diagnosis – cancer is determined by tumor morphological characteristics
  - Acute Lymphoblastic Leukemia – lymphoblast origin cells
  - Acute Myeloid Leukemia – myeloid origin cells
  - Both forms of Leukemia decrease blood cell production
- Treatment – chemotherapies used for maximal efficacy and minimal toxicity
  - Treatments for ALL and AML are different
  - Incorrect treatment decreases efficacy and increases side effects
- Therefore Personalised Medicine is seen to be concerned with the correct diagnosis

- **Molecular Characterisation** – differences in gene expression on protein and mRNA levels to allow differentiation between diseases
  - Gene expression is characterised through the use of a Microarray
  - Aggregate pattern of expression between diseases are different
    - Different diseases have up and down regulation of different genes
    - Red is up-regulation, blue is down-regulation
  - Allows classification between disease types and thus the correct treatment can be applied

- Platforms
• Transcription is reduced and cells appear superficially to be dead
• Cells exit the cell cycle and stall at the G0 stage to become suitable donors

**Recycling**
• Nucleus from a cultured cell is transferred to an enucleated oocyte
• Embryo is cultured in vitro to the morula or blastocyst stage
• Embryo is disaggregated and the nuclei are transferred to new enucleated oocytes
• Recycling is repeated for additional cycles

**Nuclear Transfer Method**
- **Enucleated Oocyte** – no nucleus in the oocyte
- **Cell grows in tissue culture**
- **Cell is transferred** from culture with an injection pipette so that the cell and oocyte membranes touch
- **Electric pulse** is applied to fuse the membranes
- **Cell nucleus enters the oocyte**
- **Electric pulse** activates cell division

**Applications** of Cloning
- **Elite Animals** – propagated
- **Regeneration** of animals from cells in tissue culture – potential to modify, select and specifically isolate events
  - **Gene insertion and expression**
  - **Gene knockouts** via homologous recombination – gene function can be analysed and used for xenotransplantation
- **Xenotransplantation** – transplantation of genes between species
  - Galactose alpha 1,3 galactose – disaccharide impeding xenotransplantation of pig organs into humans causing hyperacute rejection
  - Human immune system recognise galactose as foreign to induce an immune response
  - Therefore by knocking out the enzyme causing rejection, transplantation will work

**Problems in Clones**
- **Reduced life span**
  - Inbred mice used in a study – essentially genetically identical
  - 85% of naturally reproduced mice survived 800 days
  - 20% nuclear transfer mice survived 800 days
- **Inefficient** - Only a small percentage (0-5%) survive to birth
- **High perinatal mortality** (30-100%)
- **Patterns of gene expression can be abnormal**
  - Eg – X-inactivation abnormality causes death
    - Production of XIST
    - XIST – “X-inactive specific transcript” non-coding RNA inactivating one X chromosome to prevent further transcription
    - Healthy cloned animals have regular X-inactivation, identical to non-cloned animals
    - Unhealthy cloned animals have an abnormal X-inactivation pattern

**RECOMBINANT DNA**
- **Recombination** – natural process of exchange of DNA
  - **Eukaryotic** – occurs during meiosis between chromosomes
  - **Prokaryotic** – occurs during conjugation
- **Recombinant DNA Technology** – new combinations of DNA fragments are created (unnatural process)
- **Key Aspects**
  - **Cutting** and **Joining** DNA Fragments
  - **Vectors** containing DNA
  - **Hosts** for propagating vectors
- **Restriction Enzymes** – cut DNA sequences to form fragments
  - Restriction endonuclease recognise specific sequences in DNA and cut in or near the recognition sequence
    - Ie - EcoRI cuts between G-A
  - Restriction enzymes have recognition sites 4, 6, or 8 nucleotides long
    - 4 Base – very common, expected to cut DNA into small pieces every 256 base pairs
REASONS FOR RECOMBINANT DNA

- **Analysis** – cloning and characterisation of genes and genomes to determine their DNA sequence
  - Genomic sequences, libraries, large insert clones, expressed sequences, cDNA libraries, expression arrays
- **Protein Expression** – expression of protein in other organisms or systems of gene regulation

ANALYSIS OF EXPRESSED GENES

- **Production of cDNA**
  - Primer **binds** to the Poly-A tail of mRNA
  - **Reverse transcriptase** synthesises a single strand of DNA that is complementary to RNA
  - **DNA Polymerase** synthesises a 2nd strand of DNA to form cDNA
  - cDNA **cloned into a plasmid or phage vector**
  - All expressed genes will be present in a library of cDNA clones
  - Fragments of **randomly chosen clones are sequenced** to allow identification as Expressed Sequence Tags (ESTs)
- **Expression Arrays**
  - **Composition** – a set of identified DNA fragments on a glass microarray slide
  - **Function** – used for analysing changes in expression of genes due to environmental or genetic modification
- **Measuring levels of expression**
  - mRNA **collected** and treated
  - cDNA **synthesised**
    - Control cDNA labelled with **red** dye
    - Treated experimental cDNA labelled with **green** dye
  - **Hybridisation (expression) of cDNA into an expression array**
    - Spots with increased expression appear green
    - Decreased expression appear red
    - Unchanged expression appear yellow-brown
    - Intensity of colour on each array is proportional to the number of cDNA molecules
- **Proteomics**
  - Alternative to expression arrays
  - **Function** – qualitative and quantitative analysis of protein expression
  - Proteins resolved as unique spots by electrophoresis
  - Analysis of charge and size by mass spectrometry allows identification of spots
- **Protein Expression Systems**
  - DNA Recombinant technology allows the expression of eukaryotic genes in prokaryotic species (and vice versa)
  - **Obstacles**
    - Prokaryotes do not recognise introns, signal peptides (sequences) and other signals
    - Intronless Prokaryotic genes do not function in higher eukaryotes
  - **Bacterial Expression Vector**
    - **PI** – Promoter controlled by a repressor protein
    - **EK** – Enterokinase cleavage site allowing protein of interest to be cleaved from the fusion protein
      - **Ie** – unwanted sequence of thioredoxin is removed by cleavage at this site
      - Proteins of interest are between EK and **Term**
    - **Positive Selectable Marker** – **Amp**
    - **Bacterial cells produce protein**
    - **Cells burst and release proteins**
• Cancer Cells
  o Often have cytogenic abnormalities
  o Characteristic - identified by a capacity to multiply
    ▪ Cell proliferation – normal physiological process resulting from cellular mechanisms regulating cell cycle and cell survival
      • Cell proliferation requires multiple mutations before cancerous self-proliferation is noticeable or dangerous
    ▪ Progenitor cells are responsible for renewal of tissues thus most cancers arise from progenitor stem cells
  ▪ Cells undergo cell cycles – natural life span concludes with apoptosis
    • Failure to undergo apoptosis or senescence is a feature of neoplasia
    • Cell survival is controlled by anti-apoptotic or pro-apoptotic factors
      • Cancer cells often over-express telomerase
• Changes in cells favouring malignancy
  o Uncontrolled proliferation due to:
    ▪ Self-sufficient growth signals
    ▪ Insensitivity to growth-inhibition signals
    ▪ Evasion of apoptosis
  o Sustained angiogenesis
  o Invasion and metastasis
  o Escape from tumour immunity
  o Defects in DNA repair
• Uncontrolled proliferation is caused by:
  o Hyperactive Oncogenes (growth stimulatory genes)
    ▪ Oncogenes – promote autonomous cell growth by removing the need for growth factors or mitogenic signals
      • Eg – RAS – G-Proteins involved in growth factor signal transduction
      • Eg – myc – transcription factor localised to the nucleus before associating with target genes to become a transcription activator
  ▪ Protooncogenes – counterpart from which oncogenes are derived from producing regulators of cell proliferation and differentiation
    ▪ Oncoproteins – proteins coded by oncogenes
      • Growth Factors
      • Signal Transducers – G-Proteins
      • Transcription Factors
      • Cell-Cycle – CDKs and Cyclins
  o Inactive growth inhibitory genes (tumour suppressing)
    ▪ Function – genes inhibiting cell proliferation
      • Absence causes cells to become insensitive to growth-inhibitory signals
    ▪ Mutations are recessive – thus both alleles must be damaged for mutation to occur
    ▪ Mutations can be inherited through the germ line
    ▪ Eg – rb – normally a checkpoint from G to S-Phase
      • RB dissociates from E2F transcription factor
      • Genes needed for S-Phase are then transcribed
      • Cells continue to divide without growth factors after S-Phase
      • RB prevents G1-S transition
    ▪ Eg - p53 - DNA binding protein arresting cells in G1 after genetic damage to prevent cells from dividing incorrectly
      • Acts as a transcription factor to stimulate genes in cell-cycle arrest and apoptosis

CELLULAR BIOLOGY OF DISEASE, THERAPY AND PERFORMANCE – GENERAL ANAESTHESIA

• Neurophysical changes
  o Unconsciousness – hypnosis
  o Graded reduction of motor cortex activity
  o Graded reduction of sensory cortex activity
    ▪ Graded reduction of cortex activity - dependant upon the amount of drug administered
Methodology

1. **Physical Examination** – determination of heart rate, blood pressure, respiratory function, weight, last meal, liver and kidney function
   i. **Weight** – used to determine amount of anaesthetic required
   ii. **Last meal** – important in case of possible regurgitation
   iii. **Liver function** – used to determine ability to eliminate and filter anaesthetic agent
2. **Premedication**
3. **Induction** – patient goes from a state of consciousness to unconsciousness
   i. Injectable or inhaled
4. **Maintenance** – duration of anaesthetised state
5. **Recovery** – Regaining control, consciousness, etc.

Safe Induction Agents must be:
- Predictable and reliable
- Have minimal pain on induction
- Fast acting
- Minimal side effects on other body systems
- Have a wide safety margin
- Fast and smooth recovery

**EXAMPLE: THIOPENTONE**

- **Structure and Characteristics**
  - **Sidechains** on barbiturates - responsible for hypnosis
    - *Increased sidechain length increases potency*
    - Replacement of oxygen atom with a sulphur atom increases the rate of action
  - **Lipophilic barbiturate** – allows agent to pass through the blood brain barrier
  - **Non-ionised at body pH** – facilitates diffusion through membranes
  - **Weak base** – binds to alphal-acid glycoprotein, haemoglobin, lipoproteins and other globulins
    - Majority (>90%) bind to plasma proteins
  - Application - Injected intravenously as a bolus induction agent
- **Unbound form of the drug is active**
  - **Amount is dependant upon:**
    - Total drug concentration
    - Plasma-protein concentration
    - Affinity of proteins for the drug
    - Plasma pH

**Bound form of the drug acts as a reservoir**

**Course of Action**
- Unbound thiopentone is distributed throughout the body to the highest perfused organs first – heart, kidney, liver, brain
- **Brain** – thiopentone is able to diffuse through the blood-brain barrier and binds to specific GABA receptors at neurons
  - Inhibits propagation of action potentials in neurons
  - Hypnotic effect occurs within 10-20 seconds
- **Concentration of thiopentone in the CNS constantly decreases**
  - **CNS** - Unbound drug is being slowly metabolised with every pass through the liver
  - **Muscle** - Drug diffuses down the concentration gradient from the highly perfused CNS to lesser perfused muscle tissue
  - **Fat** - Diffuses further to fat where it accumulates due to the lipophilic nature of thiopentone
  - Fat slowly releases thiopentone back into circulation where it is metabolised by the liver
- **Metabolism** – metabolised in the liver to become hydrophilic then excreted in aqueous urine
  - **Catalysed** by cytochrome P450 system
  - **Side chain is oxidised and oxygen replaced by sulphur**
  - **Non-lipophilic form cannot cross the blood-brain barrier**
  - Rates of elimination
    - T1/2 at 2-6 minutes – diffusion into tissues of high blood flow
    - T1/2 at 30-60 minutes – diffusion into adipose tissues
    - T ½ at 5-10 hours – elimination phase
- **Thiopentone is a good induction agent but poor maintenance agent**
  - due to accumulative effects
  - Requires constant readministration to maintain hypnosis
  - Causes vasodilation of blood vessels
  - Respiratory depression
  - Prolonged recovery