- Detecting the presence of a gene/protein:
  - Immunofluorescence
  - RNA in-situ hybridisation
- Study of gene function: in-vivo vs in-vitro:
  - Knockout
  - Conditional knockout
  - Transgenic organism
  - RNAi kockdown
  - Overexpression

- RNA in-situ hybridisation (wholemount in-situ hybridisation = examination of the whole animal)
  - Genetic sequence of the gene in interest is acquired through sequencing or genetic database
  - Artificial manufacture of antisense mRNA sequence, which contains a probe
  - Addition of antibody, recognises and binds to the probe.
  - Antibody attached to an enzyme or fluorophore, able to catalyse reactions to release signal.

- Protein detection:
  - Primary antibody specific to protein of interest added, bind to protein
  - Secondary antibody added, with a fluorophore attached bind to primaey antibody, release signal.

- Knockout experiments (KO) see if the gene function is necessary in an organism. Creation of genetic KO organism:
  - A vector gene is created by inserting a selection gene into the target gene exon to disrupt its function
  - The vector gene is then added to be cultured with the cell line.
  - Selector gene, flanked by the exon fragments of the interested gene, can be incorporated into the cell genome via homologous recombination. Successfully introduced cells would express selector gene.
  - If non-homologous insertion (random insertion) occur, other genes on the vector would act as another selector, can be distinguished.
  - Cells with target mutation is selected and transplanted into organism embryo to create chimeric animals.
  - Chimeric animals (M/m) is then crossed with an wild type animal (M/M), half of the offspring will have heterozygous KO gene, can be identified through PCR
  - The heterozygous individuals can then be crossed together to produce 1/4 homozygous KO organism.

- Conditional KO: allow time-specific, tissue specific KO, in case the homozygous mutation is lethal.
  - Cre-Lox system:
    - Similar to creating KO organisms, the target gene is introduced into organism via homologous recombination
    - However, instead of disrupting the gene, it is flanked with two LoxP sites.
    - Cre recombinase gene is then introduced to the organism downstream of a promotor. The promotor only promote expression at a specific developmental stage or in specific tissues.
    - Under right condition, Cre recombinase is expressed, which will recombinate the two LoxP sites together, excising the target gene.
  - Flp-FRT system
    - Analogous to the Cre-Lox system, target gene is introduced to the organism flanked by two FRT sites
    - Flipase is expressed conditionally, which will invert the sequence between the two FRT sites
    - This can cause conditional KO or conditional expression if a FRT-flanked STOP codon is introduced

upstream to the target gene.

- Gain of function analysis:
  - Construction of a vector sequence containing a tissue specific promotor and target gene.
  - Inject the vector sequence into the early embryo, allowing random insertion of the sequence
  - Genetic screening for the genotype.

- Transient expression / inhibition:
  - Electroporation: injection of DNA followed by electric shock to induce pore opening
  - Entry of the DNA segment, transient expression of the gene.
- RNAi: First discovered in C.elegans.
  - Introduction of RNAi precursur into the cell (siRNA is double stranded)
  - siRNA bind with protein to form RNA-induced silencing complex (RISC), recognise complementary target mRNA.
  - RISC bind to target mRNA, argonaute protein in the complex induce cleavage of the mRNA
  - Transient response, longer lasting if using viral vectors to introduce shRNA into the nucleus