GLOSSARY OF TERMS and REAGENTS

Agar: a gelatinous substance used in plate media for growth of bacteria. It is extracted from the cell walls of certain species of algae or seaweed and is a mixture of two un-branched polysaccharides, both of which are polymers of galactose. **Other uses:** Laxative, thickener in jellies or ice cream and MacDonald's milkshakes.

Agarose: one of the polysaccharides in agar. Agarose is an unmodified polysaccharide of galactose resulting in its neutral charge which is essential to prevent interactions with charged DNA and protein molecules. It forms large pores which is useful for size separation of DNA and proteins.

Cell Spreader: to be made from supplied thin glass pipettes

Competent cells: prepared *E.coli* cells that have been treated to make the cell permeable to small DNA molecules. Stored at -80C in the dry ice box.

DNA ligase: enzyme that joins the sugar-phosphate backbone of two DNA ends. Involved in DNA replication and repair *in vivo*. Coupled with the breakdown of ATP.

DNA marker: A standard DNA size marker for determining the size of PCR and restriction DNA fragments.

DNA Polymerase (*Taq*): Thermostable DNA polymerase for use in temperature cycling. Derived from the thermophilic bacterium *Thermus aquaticus.*

EcoRI: restriction enzyme with recognition sequence GAATTC. Should cut on either side of the KanR insert following the miniprep to yield two DNA bands on the agarose gel.

Ethidium Bromide: Shows orange fluorescence in UV light after binding double-stranded DNA. Toxic and carcinogenic, handle with gloves and place all gloves, tips and gel in the biohazard waste bag provided.

Gel electrophoresis: For separation of DNA (and protein) on the basis of their net charge and mass by migration through an agarose (or polyacrylamide) gel in an electric field. DNA will migrate away from the negative to the positive pole due to the negative charge on the phosphate backbone. Smaller fragments will travel faster through the gel.

Glop: cellular waste such as the cell membrane, other cellular components and proteins

GMO: Genetically modified organisms, usually modified by insertion of a gene(s) from another species.

Isopropanol: DNA is insoluble in alcohol so isopropanol is used to precipitate DNA.
Kanamycin agarose plates: white agarose plates containing the kanamycin

antibiotic. Only bacterial cells containing the vector with the KanR gene successfully inserted with grow.

Ligation: Joining of two DNA molecules using DNA ligase

Loading Dye: contains a dense chemical called Ficol that increases the density of the DNA sample so it will sink into the wells in the agarose gel. Also contains charged dyes (blue colour) that will migrate through the gel with the DNA for monitoring the progress of the gel.

Lysozyme: Acts as an antibacterial reagent by disrupting the bacterial cell walls. Used to break open bacterial for DNA extraction. Found in animal secretions such as tears and egg whites.

MacConkey Plate: Red plates. Colonies which ferment lactose are red. This reaction is due to the action of acids produced by fermentation of lactose on the bile salts and the subsequent absorption of neutral red, a pH indicator, from the medium. The vector contains a gene required for this fermentation and thus colonies with the vector alone will appear red. Colonies of non-fermenters of lactose appear colorless. The KanR insert will interrupt the gene for fermentation in the plasmid so colonies with an insert-containing plasmid (successful ligation) will be white.

Plasmid: small circular DNA molecule that replicates independently of the chromosome in bacterial and yeasts. Used as vectors to carry and clone other DNA fragments (your favourite gene).

Polymerase Chain Reaction (PCR): technique for rapid amplification of a selected DNA fragment. Used for cloning, DNA fingerprinting and many other applications.

Restriction digest: used to determine the structure/identity of a DNA fragment by cutting with restriction enzymes.

Restriction enzyme: enzymes that recognize palindromic DNA sequences to cut DNA at a particular location within the sequence, e.g. EcoRI

RNase: An enzyme that degrades RNA

SOC media: non-selective bacterial growth medium

STET Buffer: Buffer for digestion of bacteria with lysozyme.

Supernatant: Top liquid fraction after precipitation of a solution.

Transformation: genetically modifying bacteria achieved by adding DNA to the cell. Transformation techniques are also used to genetically modify other organisms.

Vector: A plasmid (circular) DNA into which other DNA fragments can be inserted for transformation into bacteria.