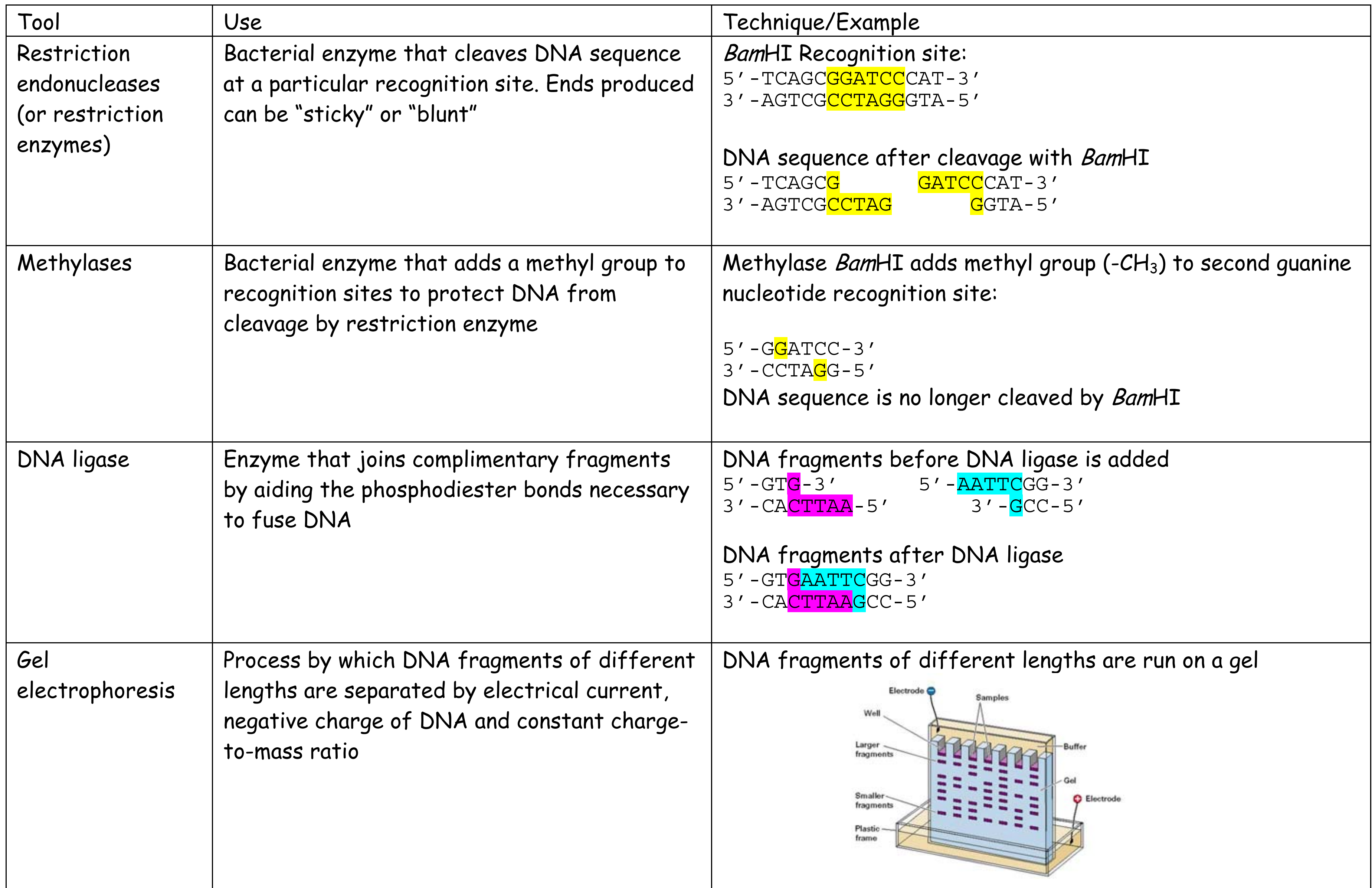
<u>Tools and Techniques in Molecular Genetics</u>

Tool	Use	Technique/Example
Restriction endonucleases (or restriction enzymes)	Bacterial enzyme that cleaves DNA sequence at a particular recognition site. Ends produced can be "sticky" or "blunt"	*Bam*HI Recognition site: 5'-TCAGCGGATCCCAT-3' 3'-AGTCGCCTAGGGTA-5' DNA sequence after cleavage with *Bam*HI 5'-TCAGCG GATCCCAT-3' 3'-AGTCGCCTAG GGTA-5'
Methylases	Bacterial enzyme that adds a methyl group to recognition sites to protect DNA from cleavage by restriction enzyme	Methylase *Bam*HI adds methyl group (-CH_3) to second guanine nucleotide recognition site: 5'-GGATCC-3' 3'-CCTAGG-5' DNA sequence is no longer cleaved by *Bam*HI
DNA ligase	Enzyme that joins complimentary fragments by aiding the phosphodiester bonds necessary to fuse DNA	DNA fragments before DNA ligase is added 5'-GTG-3' 5'-AATTCGG-3' 3'-CACTTAA-5' 3'-GCC-5' DNA fragments after DNA ligase 5'-GTGAATTCGG-3' 3'-CACTTAAGCC-5'
Gel electrophoresis	Process by which DNA fragments of different lengths are separated by electrical current, negative charge of DNA and constant charge-to-mass ratio	DNA fragments of different lengths are run on a gel 

Plasmid	Small circular DNA that has the ability to enter and replicate in bacterial cells, and therefore can be used as a vector to introduce new genes into a bacterial cell	Using the bacteria's own restriction enzymes, a section of target DNA can be attached into a plasmid, which is then inserted into a bacterial cell. Once foreign DNA is incorporated into the bacterial DNA the bacteria will replicate it along with its own DNA. The bacteria can also create the target protein for medical use.
Polymerase Chain Reaction (PCR)	Purpose is to create many copies of a particular target gene in order to use it for research, treatment, or forensic investigations. Amplifies a small amount of DNA more easily and rapidly than cloning can.	1. DNA sequence is identified 2. Sequence is heated to 90°C to separate strands 3. Temperature is lowered to 60°C so primers can anneal 4. Temperature is raised to 70°C so *Taq* polymerase can elongate complementary strand. 5. Cycle is repeated until enough strands are produced
Restriction Fragment Length Polymorphism	Purpose is to differentiate between individuals. Useful in forensics and to determine relationships between individuals (ex/ paternity tests)	1. Restriction endonucleases are used to cleave genome at places unique to the individual. 2. Sample is sent through gel electrophoresis 3. Banding pattern is produced (through Southern Blot) 4. Patterns can be compared.
DNA Sequencing	Purpose of DNA sequencing is to determine actual sequence of gene or entire genome. Dideoxy nucleoside triphosphates (ddNTPs) can be tagged with fluorescent ink so that a computer can read the sequence rather than relying on a human to do so (since sequences can be very long)