

Recombinant DNA Technology Activity

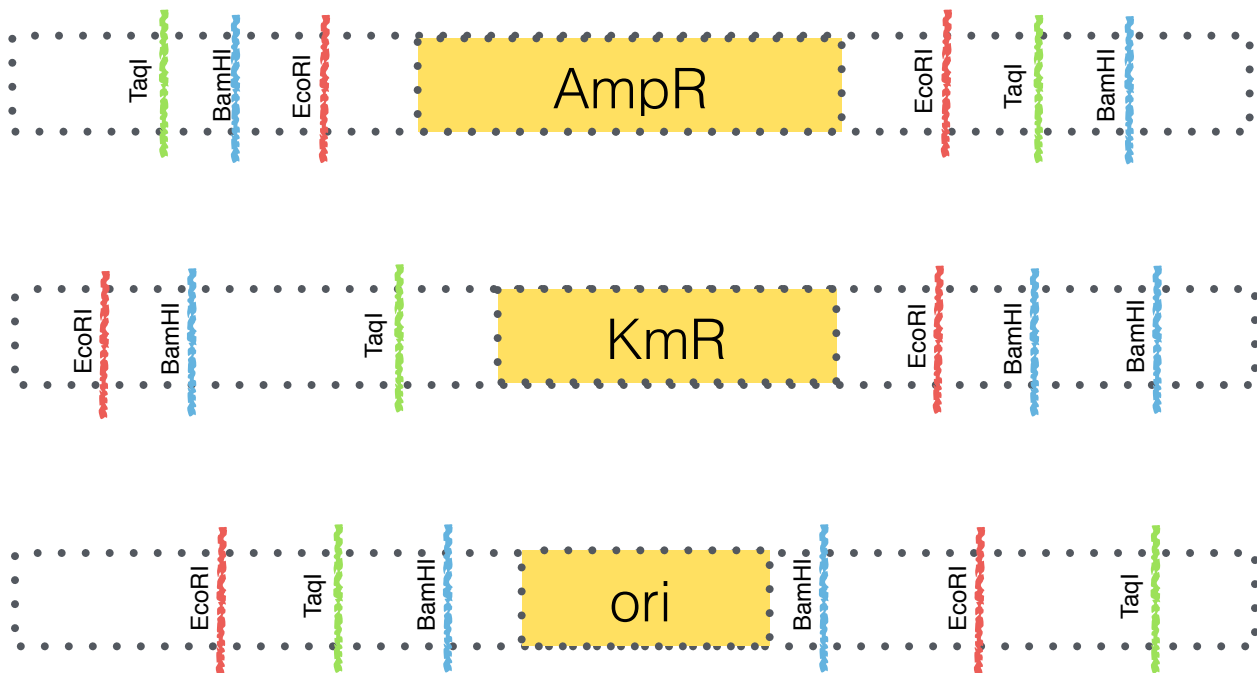
Recombinant DNA technology involves the cutting and recombining of DNA fragments from different sources and organisms. Fragments of DNA are typically cloned into small circular DNA fragments called plasmids which are originally isolated from bacteria. The genetically modified plasmids are then transformed into a bacterial host such as *E. coli* which can then transcribe and translate the genes into proteins. Several components of restriction enzymes can be found below.

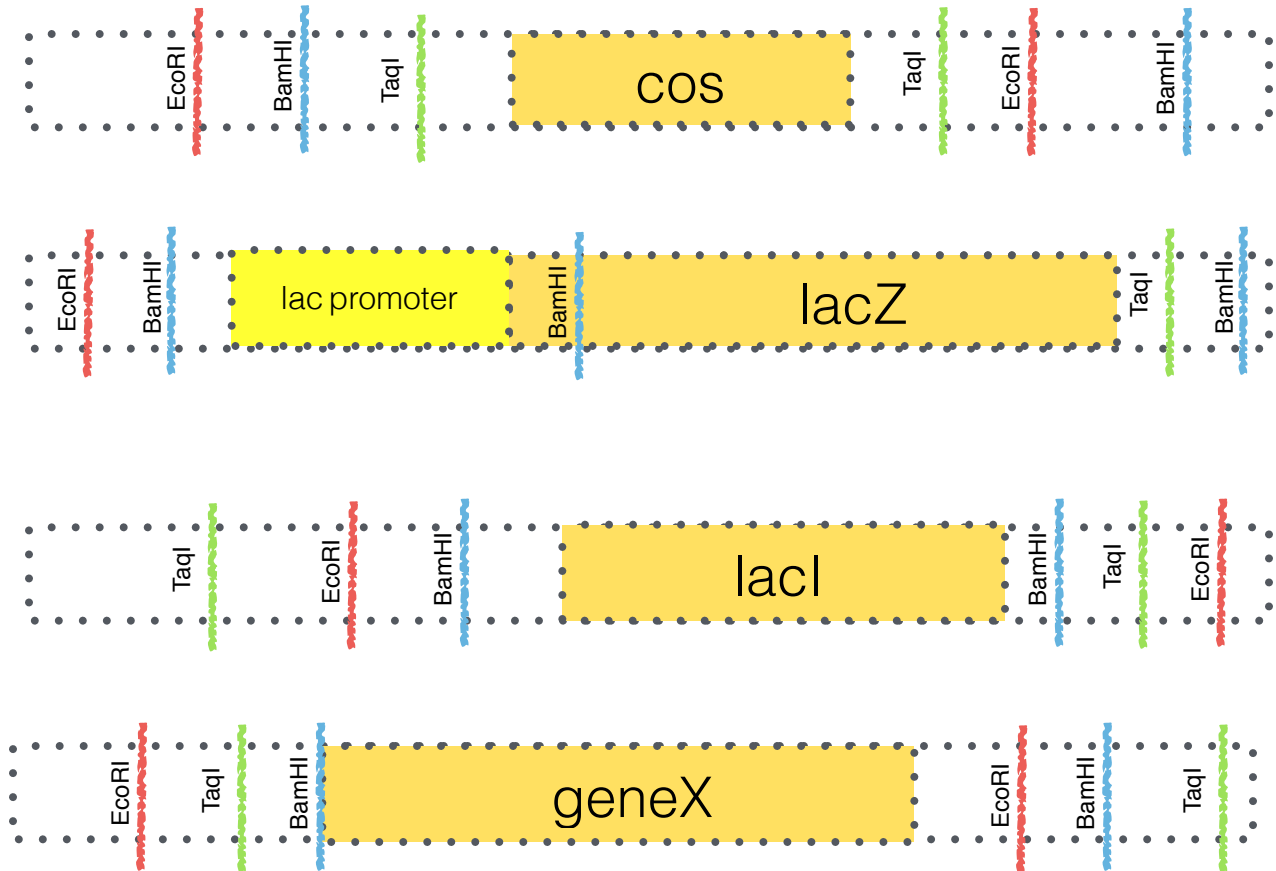
Using scissors and clear tape, cut out and paste together the *minimal set of components needed to create a plasmid which can express *geneX* in *E. coli*.**

Components will be needed that make it possible to:

- select for colonies that have the plasmid
- screen for colonies that have *geneX* cloned into the plasmid
- allow the plasmid to replicate in *E. coli*
- allow *geneX* to be induced by addition of IPTG

*Not all components below are necessary. Cut and "paste" (or ligate) together the components that you think are needed.





Amp^R is a gene which can be transcribed and translated into a protein that makes bacteria resistant to the antibiotic ampicillin. It is one example of a "marker" which allows selection of bacteria that have this gene on a plasmid.

Km^R is a gene which can be transcribed and translated into a protein that makes bacteria resistant to the antibiotic kanamycin.

cos represents the sequences necessary for packaging of DNA within a bacteriophage (a virus that infects bacteria).

ori represents the origin of replication sequence needed for the plasmid to be replicated in bacteria. Many different origin of replication sequences have been discovered on different plasmids.

lacZ is transcribed and translated into a protein called beta galactosidase which metabolizes lactose. It also converts X-gal (a structural analog of lactose) into a blue compound. The lac promoter (with operator) is also present to allow transcription and regulation of transcription.

lacI represses transcription of the lac operon in the absence of lactose (allolactose) or a structural analog of allolactose called IPTG.

geneX is our hypothetical gene that we want to transcribe and translate in *E. coli*.

Additional Information and Hints:

- Restriction Enzymes (or Restriction Endonuclease) are named after the bacteria from which they are isolated. EcoRI for example is named after the bacteria *Escherichia coli* (strain **R**) and the **I** (roman numeral I) since it was the first restriction enzyme discovered from that strain. See examples in the table below:
- Hundreds of different restriction enzymes have been discovered, isolated and are available to scientists. Each restriction enzyme cuts at a specific recognition sequence.

| Restriction Enzyme | Bacterial Source | Recognition Sequence |
|--------------------|-----------------------------------|----------------------|
| EcoRI | <i>Escherichia coli</i> | GAATTC |
| BamHI | <i>Bacillus amyloliquifaciens</i> | GGATTC |
| TaqI | <i>Thermus aquaticus</i> | TCGA |
| HindIII | <i>Haemophilus influenzae</i> | GANTC |

(N in a recognition sequence refers to either A,T,C or G.)

- LacI is a protein which binds to the lac operator and represses transcription. The inducer, lactose, or an analog called IPTG can be added which binds to the repressor and removes it from the operator. This induces, or "turns on" transcription of the operon.
- LacZ is a protein that metabolizes lactose. A compound called X-gal can also be added which turns blue when metabolized by LacZ. Colonies turn blue if LacZ is functional, but remain white if the *lacZ* gene has been disrupted by the insertion of another DNA fragment. This allows screening for colonies that have a plasmid with a successful insert into the plasmid.