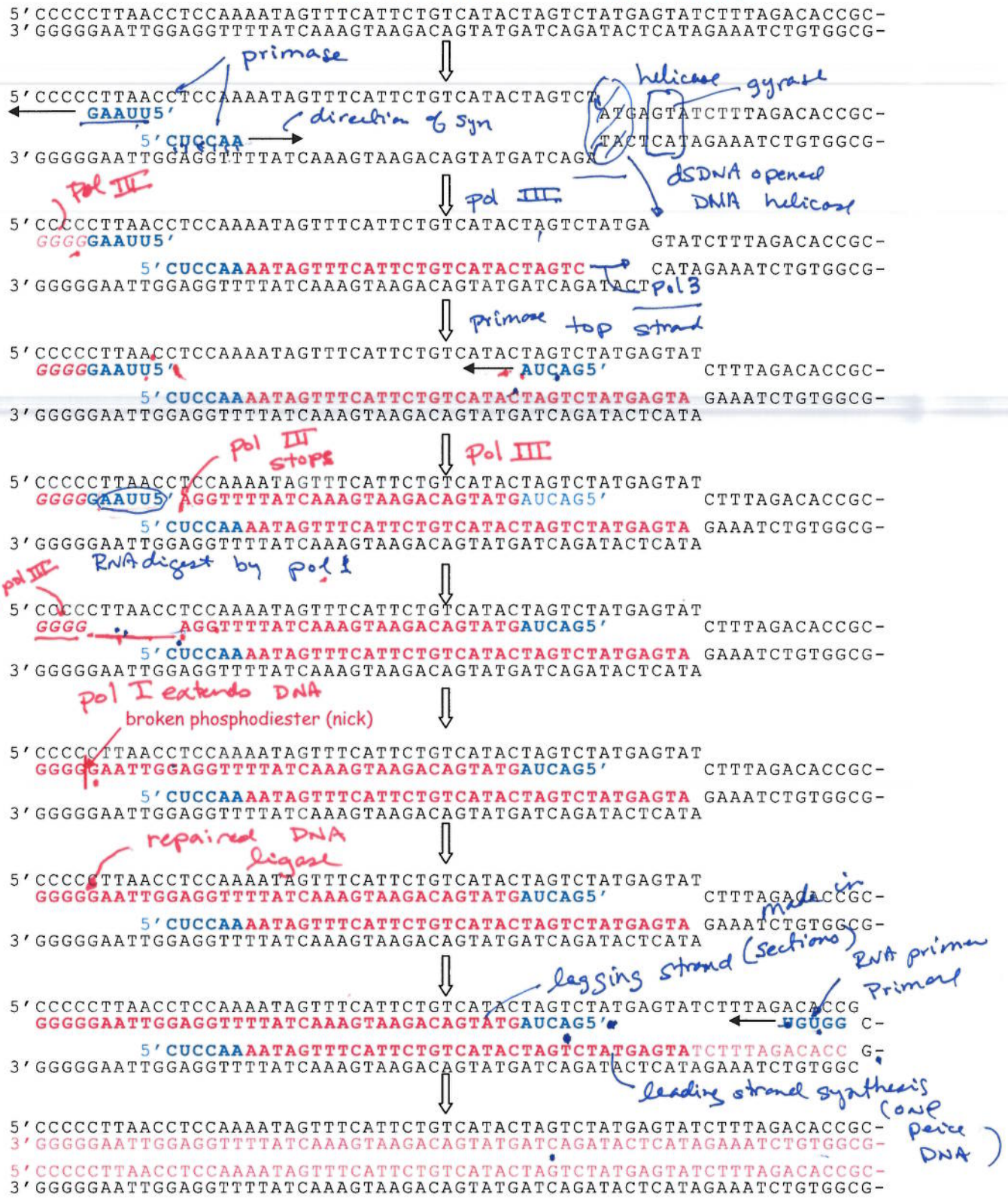
**Lecture 23: Replication, Transformation, mRNA Processing, Carbohydrates.**

**Lagging strand synthesis** – Replicated in sections, with replacement of multiple RNA primers by DNA pol I and joining of segments by DNA ligase.

Identify the differences between each image – what happened and who did it?

Black = template Blue = RNA primers Red = New DNA



**Online material to help with DNA Replication:** https://oli.cmu.edu/

- Register using course key: DNAREP-D
- Complete pre-quiz, view material, complete post-quiz (2 pts bonus for course).
- Send comments/suggestions to me (rule@andrew.cmu.edu) for improvements.

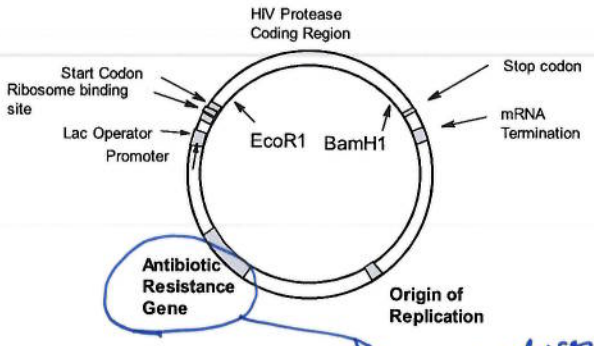
## Bacterial Transformation & Antibiotic Resistance

**Transformation** – process of putting plasmid DNA into bacteria.

**Selectable marker** – gene contained on the plasmid that can produce a protein that makes the bacteria resistant to antibiotics.

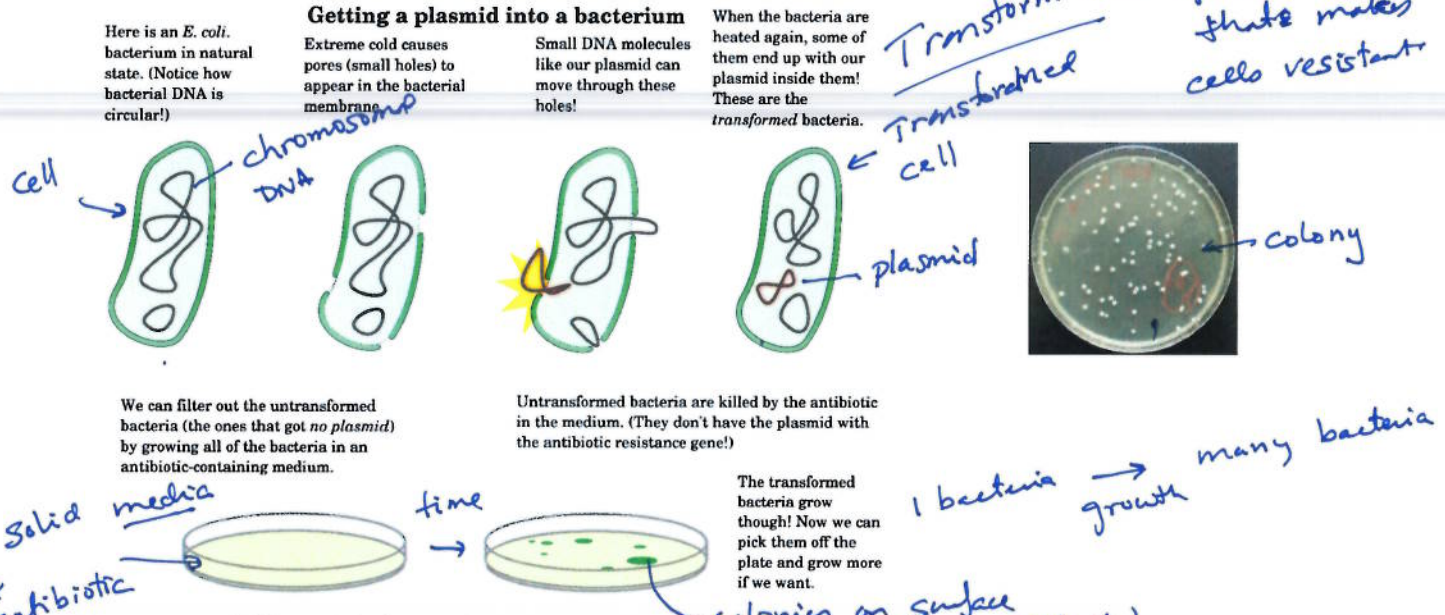
- Growth of transformed cells in the presence of the antibiotic is referred to as selection, because it selects for those bacteria that contain the plasmid.
- It is necessary to have the antibiotic present at all times, otherwise the plasmid will be lost from the cells.

**(image from http://2012.igem.org/Team:St_Andrews/Public-outreach)**




Transformed / Transformation

produces protein that makes cells resistant




### Summary of Plasmid elements:

**Restriction sites** (EcoR1 and BamH1): Used to insert coding region into plasmid via sticky ends and DNA ligase.

mRNA production:
- **Promoter** – Binding site for RNA polymerase, generates mRNA from DNA sequence
- **Lac Operator** – Lac repressor binds here, on/off switch for mRNA production.
- **mRNA termination** – end of mRNA

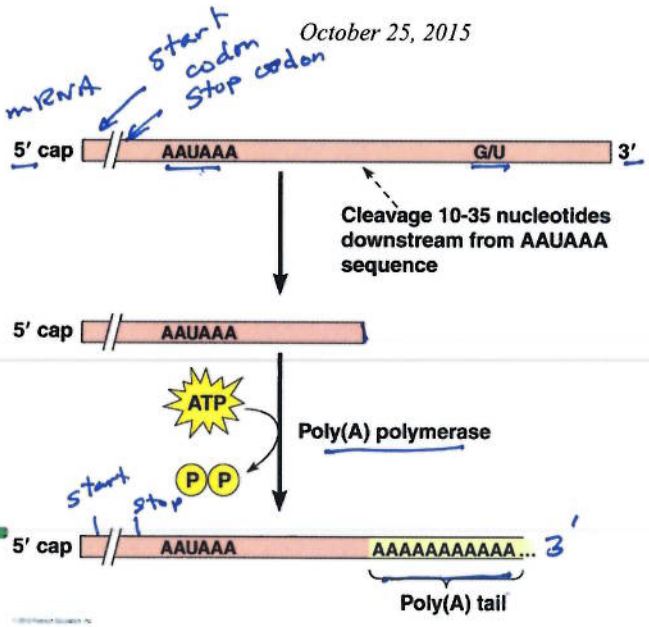
Protein Synthesis:
- **Ribosome binding site** – binds mRNA to ribosome, positions start codon in P-site.
- **Start codon** – first codon, recognized by $tRNA^{fMET}$, all prokaryotic proteins begin with a modified methionine residue.
- **Codons** – coding for the amino acid sequence of our desired protein, anything can be made.
- **Stop codon** – Signals protein release factor to release completed protein from ribosome, breaking bond between the last tRNA and the new protein.

Essential Components of any Plasmid:
- **Origin of replication** – so that the plasmid will be replicated and passed on to daughter cells.
- **Antibiotic resistance gene** – so that we can select for cells that have our plasmid.

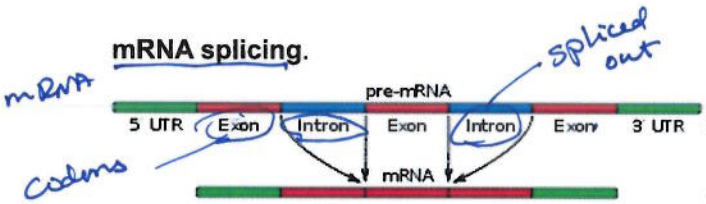
## mRNA processing in Eukaryotic Cells

**Poly A addition.** A series of A residues are added to the end of the mRNA by specialized enzymes. This is important for:

- Nuclear export
- Translation (protein synthesis)
- enhancing the stability of mRNA.

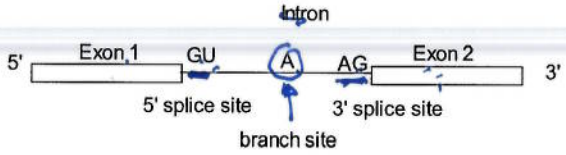



**mRNA splicing.**

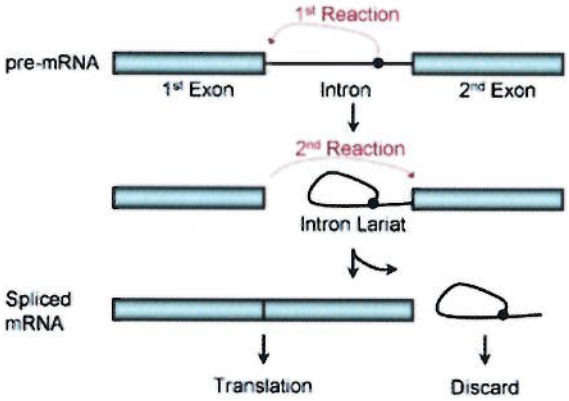



The initial transcript is composed of:

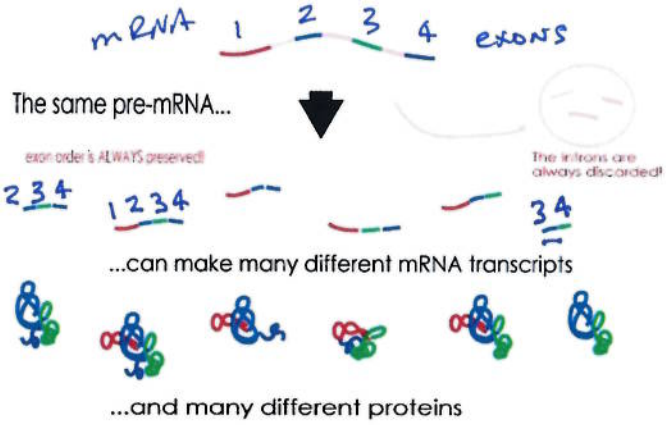
- Exons that code for amino acids
- Introns are intragenic regions that are removed during splicing.
- Splicing requires the following sequences in the intron to guide the splicing machinery:
  - i. a 5' donor site
  - ii. a 3' acceptor site
  - iii. a branch sequence within the intron




- Steps:
  - i. A in branch breaks phosphodiester at 5' splice site
  - ii. 3' OH at 5' splice site forms new phosphodiester with 3'splice site




**Alternative splicing** is common, with different exons retained in different tissues. This allows the same gene to produce many different proteins.




**Genetic Diseases:**

- Mutations in the splicing machinery can cause wide-spread problems in mRNA splicing.
- Mutations in the donor or acceptor site can cause incorrect splicing of individual mRNAs.