Gene construct

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- In molecular terms, a gene commonly is defined as *the entire nucleic acid sequence that is necessary for the synthesis of a functional polypeptide.*

- According to this definition, a gene includes more than the nucleotides encoding the amino acid sequence of a protein, referred to as the *coding region.*

- A gene also includes all the DNA sequences required for synthesis of a particular **RNA** transcript.

- In some prokaryotic genes, DNA sequences controlling the initiation of transcription by **RNA polymerase** can lie thousands of base pairs from the coding region.

- In **eukaryotic** genes, transcription-control regions known as enhancers can lie 50 kb or more from the coding region.
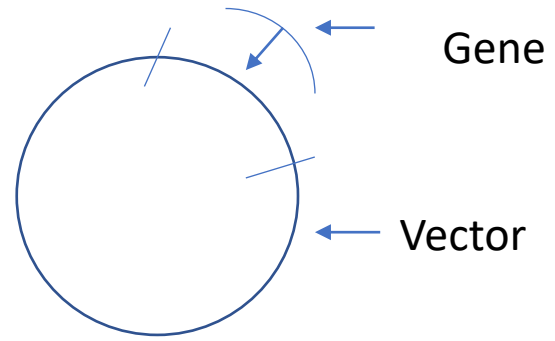
Gene construct

- A gene construct is a gene of interest (GOI) associated with the regulatory sequences which is inserted in the right orientation in an expression vector to make the protein *in vivo* or *in vitro*.

- So what would we require?

- Regulatory sequences with the gene that will help the host cell to make the mRNA and then protein of it . The construct should include

- A promoter

- A ribosome binding site

- A terminator

- The gene in between

- And some markers to indicate the insertion of gene as well as its proper functioning. So we will have all the cloning vectors markers and a reporter gene to indicate the transcription and translation of the gene you have inserted
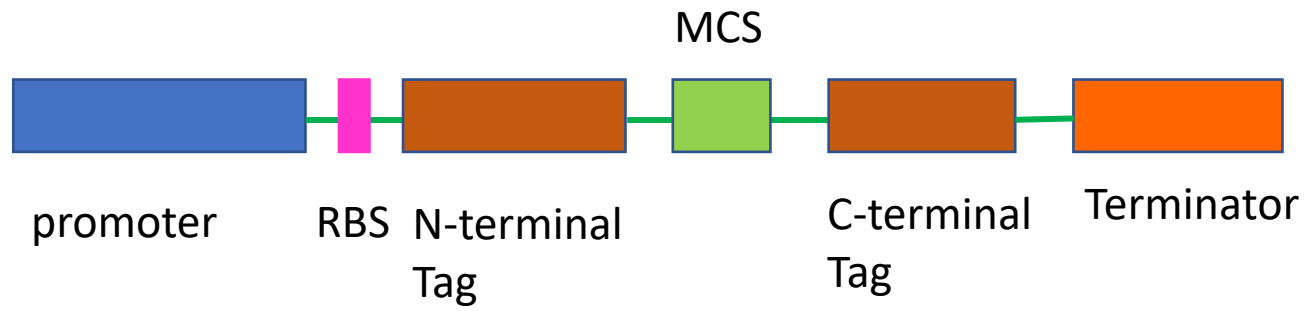
Typical structure of gene Construct

- Lets now look at the elements and recall from our mol bio class their function
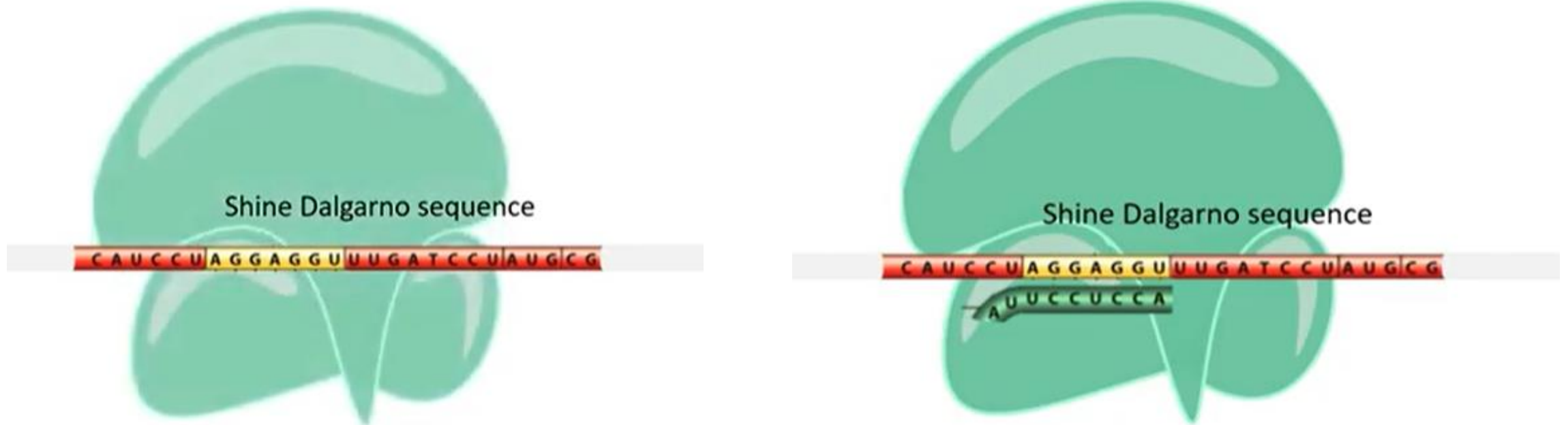
Promoter. We require a promoter so that the polymerase can identify the position of the gene . Here we use an inducible promoter. You remember how lac operon is induced? Right by allolactose.
So we use an inducible promoter to help RNA polymerase to recognise the position of the gene.
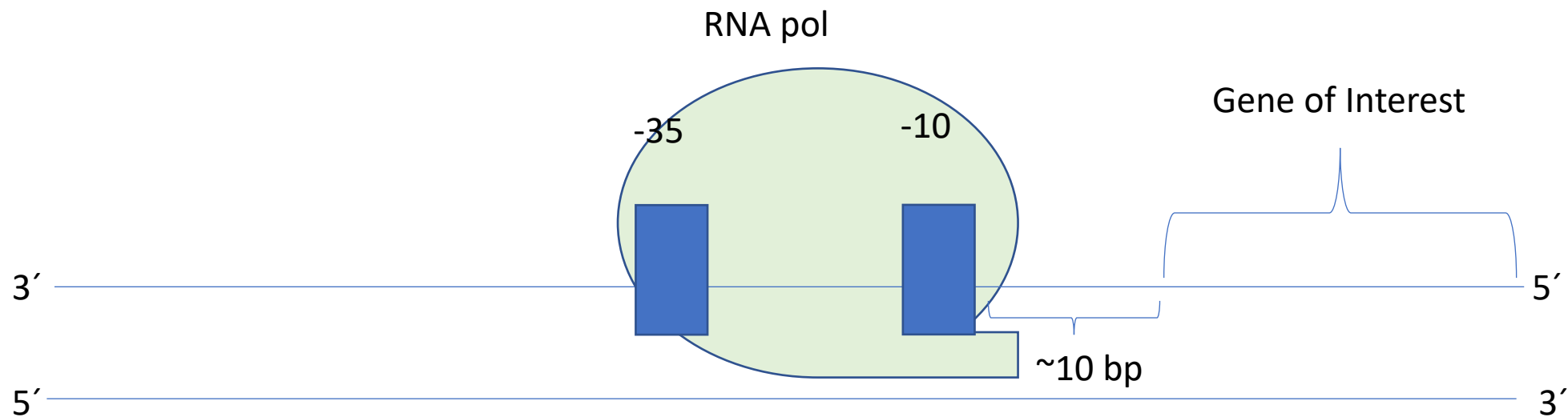
- Inducible promoters are used as we want the gene to be transcribed and translated (Expressed) when we want it to. We may use the promoter of lac operon that is induced by …….?? Here we use IPTG (find out what is IPTG and memorize that) to induce our gene expression.

- We then require a terminator at the 5´end of the template sequence. Recall which two terminators we have studied in Mol bio. Nearly all common bacterial expression plasmids use Rho-independent terminators, which include naturally occurring terminators, such as T7, as well as engineered high-efficiency terminators such as T0. Rho-independent termination is also known as intrinsic termination (see the mol bio Transcription PPT), and it relies on the formation of a GC-rich hairpin in the RNA transcript.

Just after the promoter (down stream) we require a signal for ribosome binding.
The **Shine**-**Dalgarno** (SD) **Sequence** is a ribosomal binding site in bacterial and archaeal
messenger RNA, generally located around 8 bases upstream of the start codon AUG. The
RNA **sequence** helps recruit the ribosome to the messenger RNA (mRNA) to initiate protein
synthesis by aligning the ribosome with the start codon.

Shine Dalgarno sequence

CAUCCUAGGAGGUUUGATCCUAUGCG

Shine Dalgarno sequence

CAUCCUAGGAGGUUUGATCCUAUGCG
AUUCCUCCA

- Then we require a cloning site where we can add our gene. So this site must have an array of restriction sites. This site is often known as multiple cloning site or MCS.
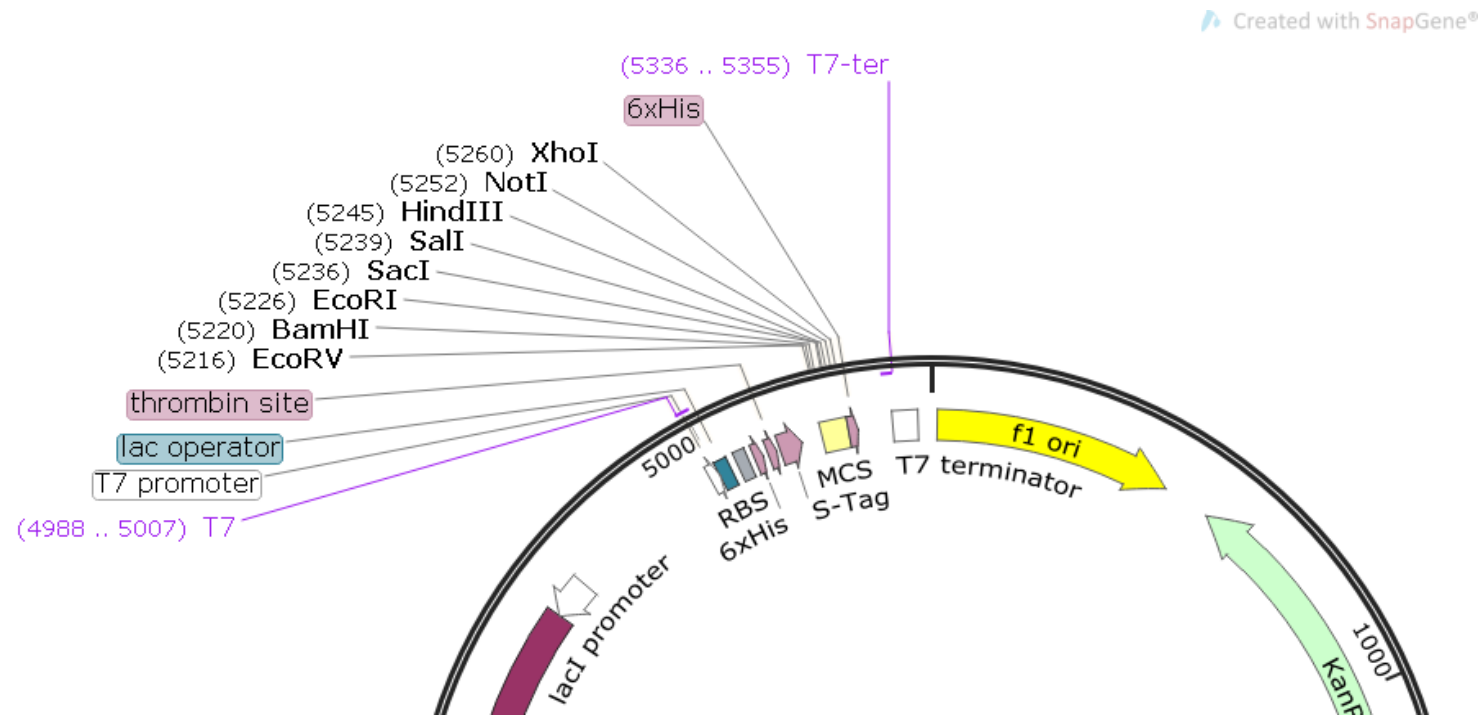
Markers

- We also require some markers for indicating

- Transformation : Antibiotic resistance gene

- Recombination or insertion: lacz ´ selection

- Gene expression: Reporter gene e.g green fluorescent protein (GFP) or sone affinity tags
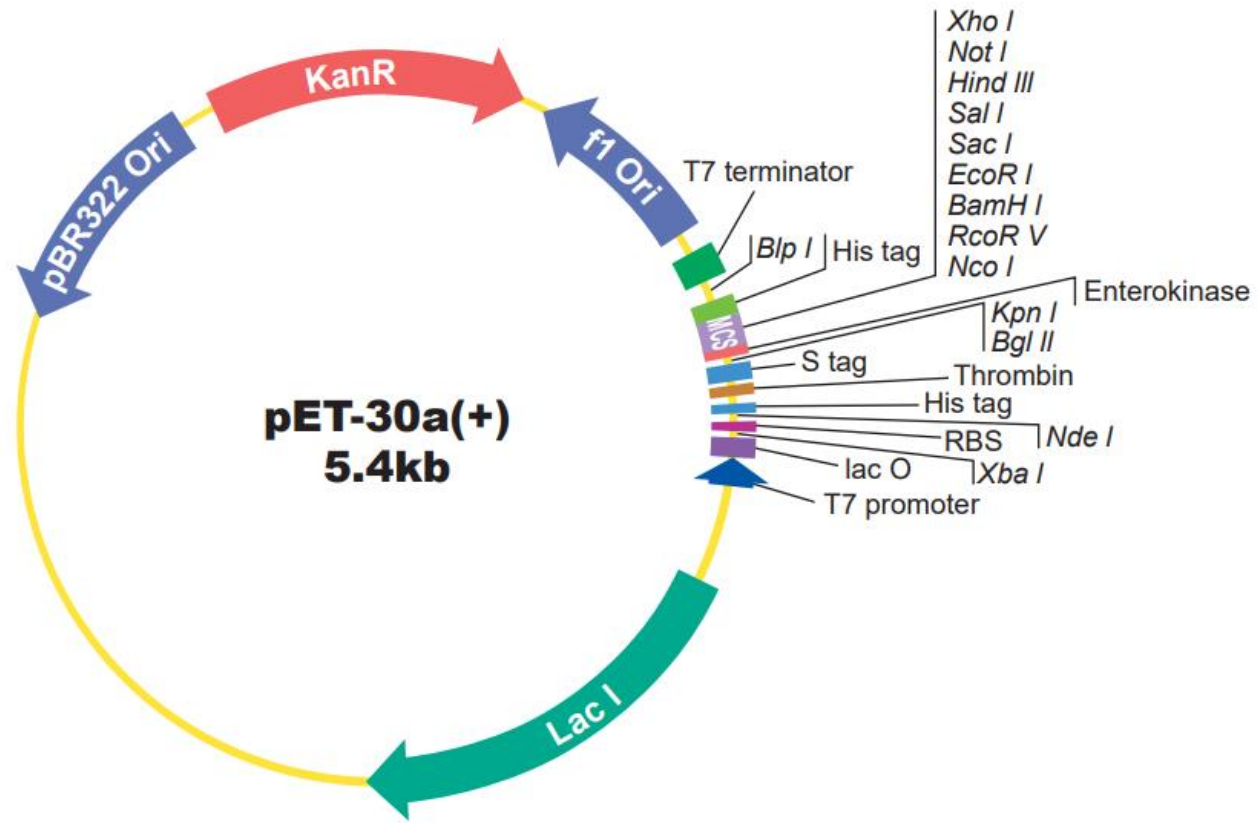
Earlier – blue white selection

Now additional markers
Reporter gene

- Consult the picture I have mailed to you

pET-30a(+)
5.4kb

KanR

pBR322 Ori

f1 Ori

Lac I

T7 terminator

Blp I | His tag

Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
RcoR V
Nco I

Enterokinase

Kpn I
Bgl II

S tag

Thrombin

His tag

RBS

Nde I

lac O

Xba I

T7 promoter

Resources used

- Free web resources