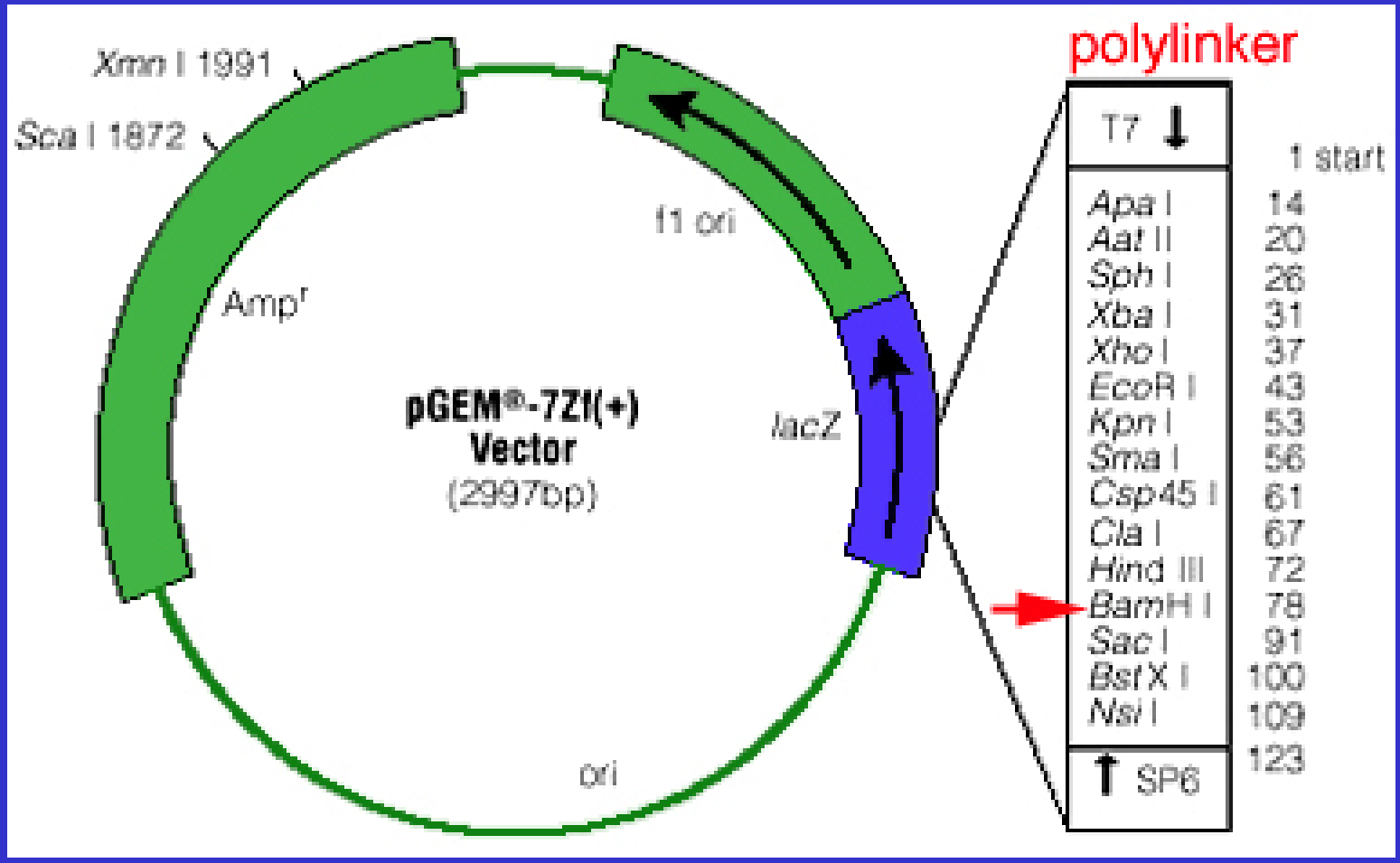
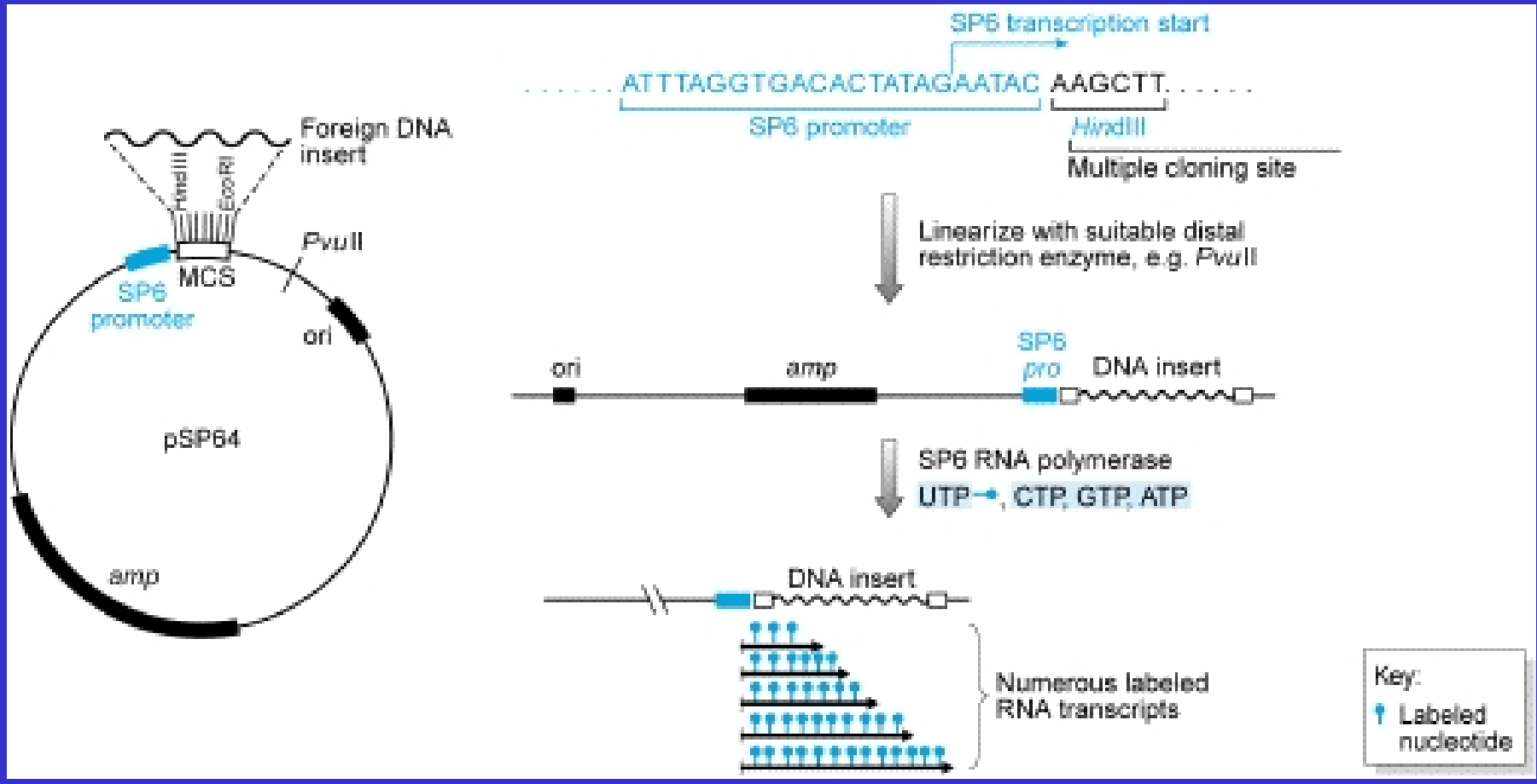
# *Recombinant DNA Technology*

## *In Vitro TRANCRIPTION AND TRANSLATION*

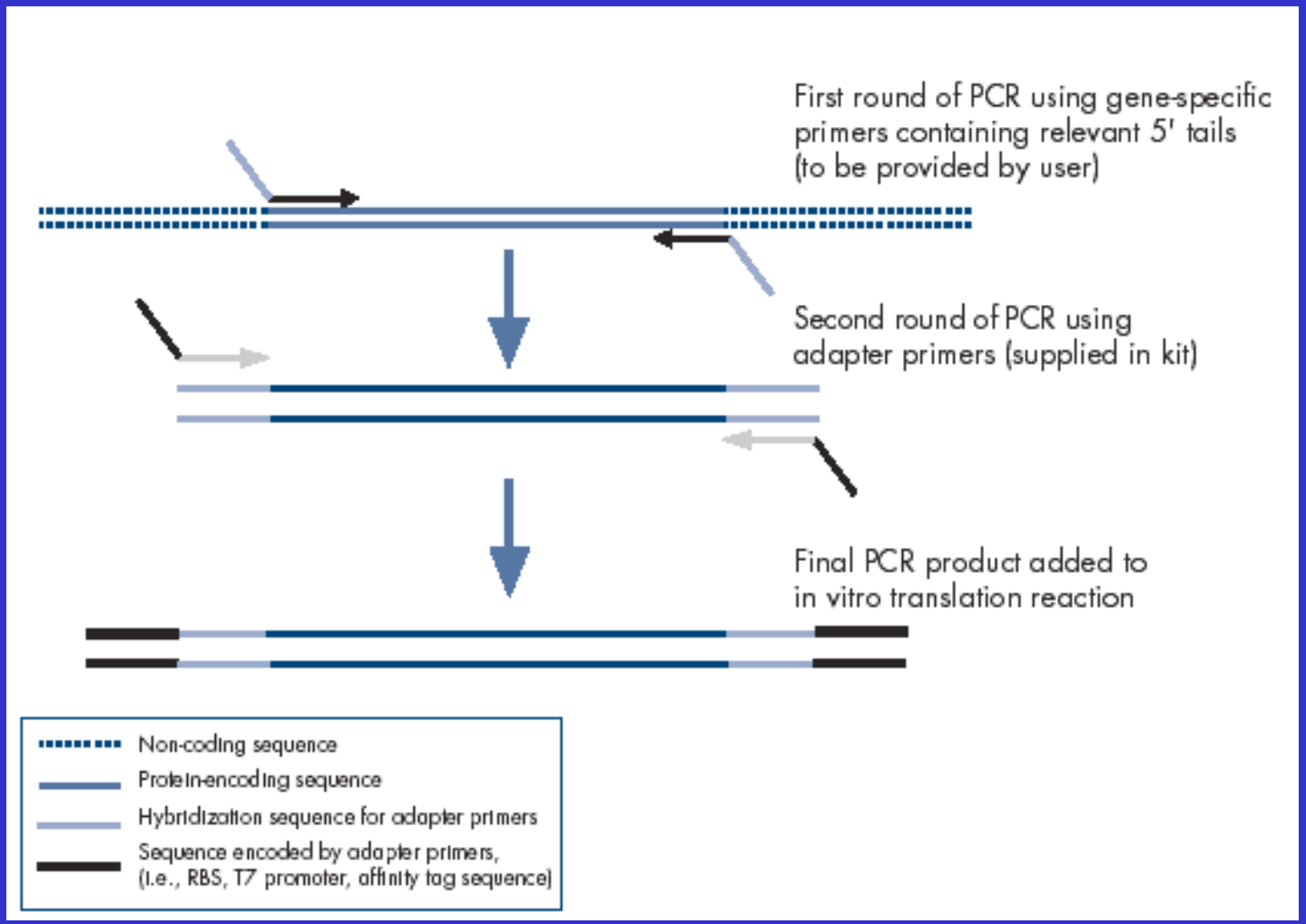
# *Plasmid vectors may contain promoters recognized by the T7 or SP6 RNA polymerases*

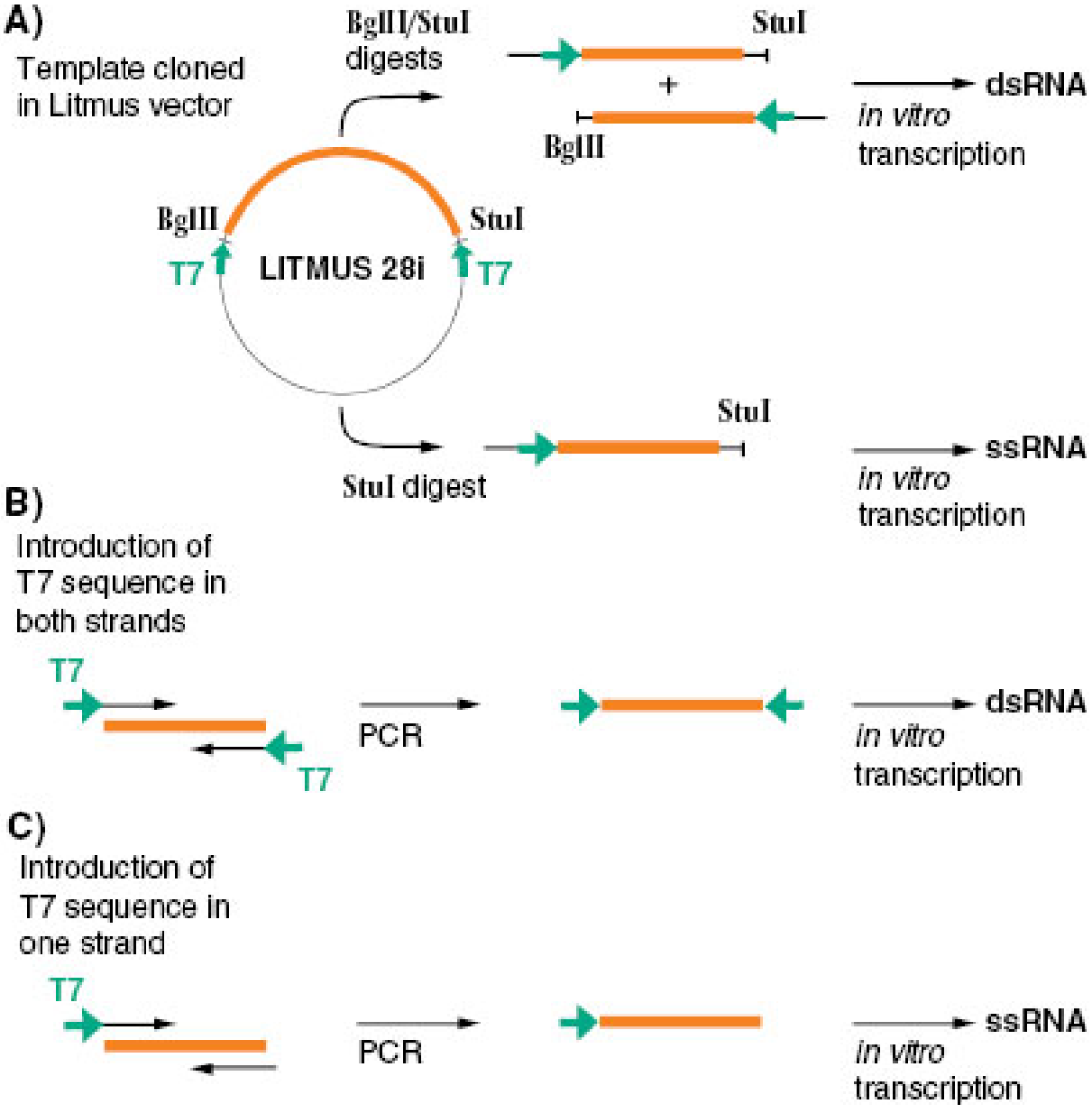


# In vitro Transcription, production of RNA probes

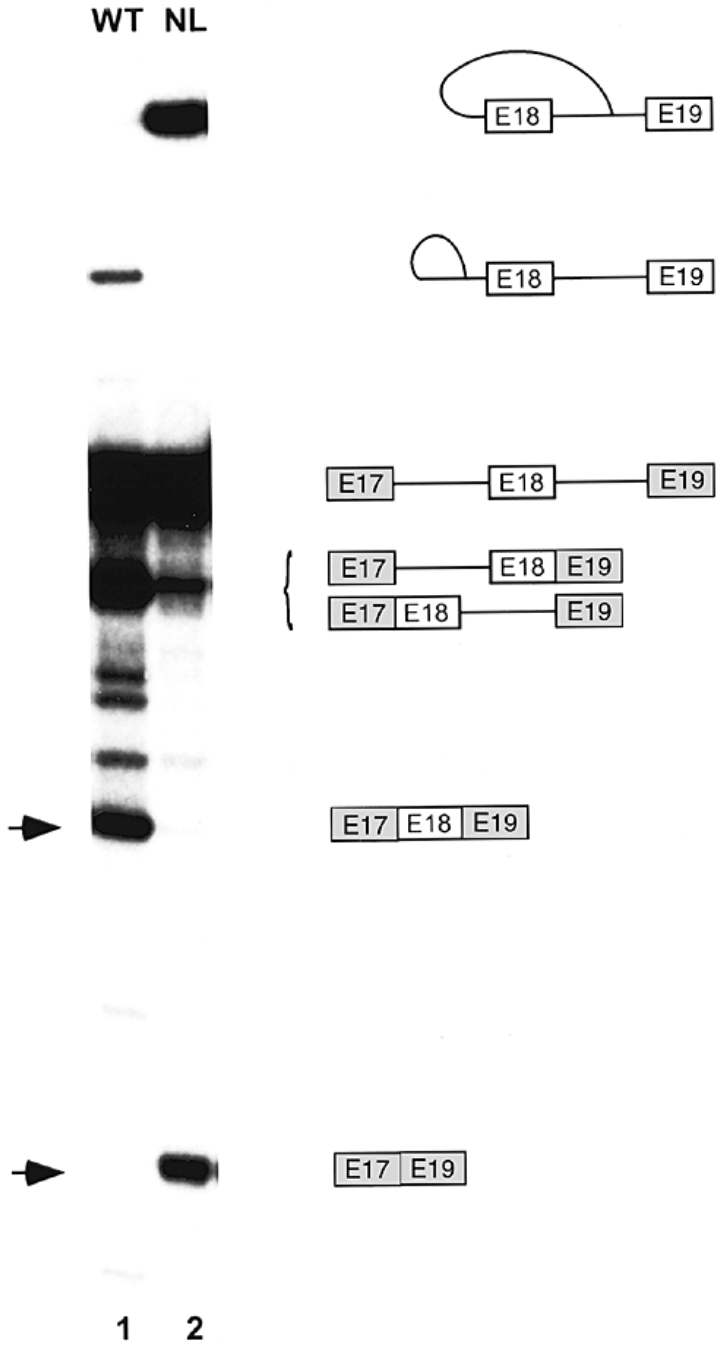


# *We can also directly transcribe and translate coding sequences via PCR amplification*

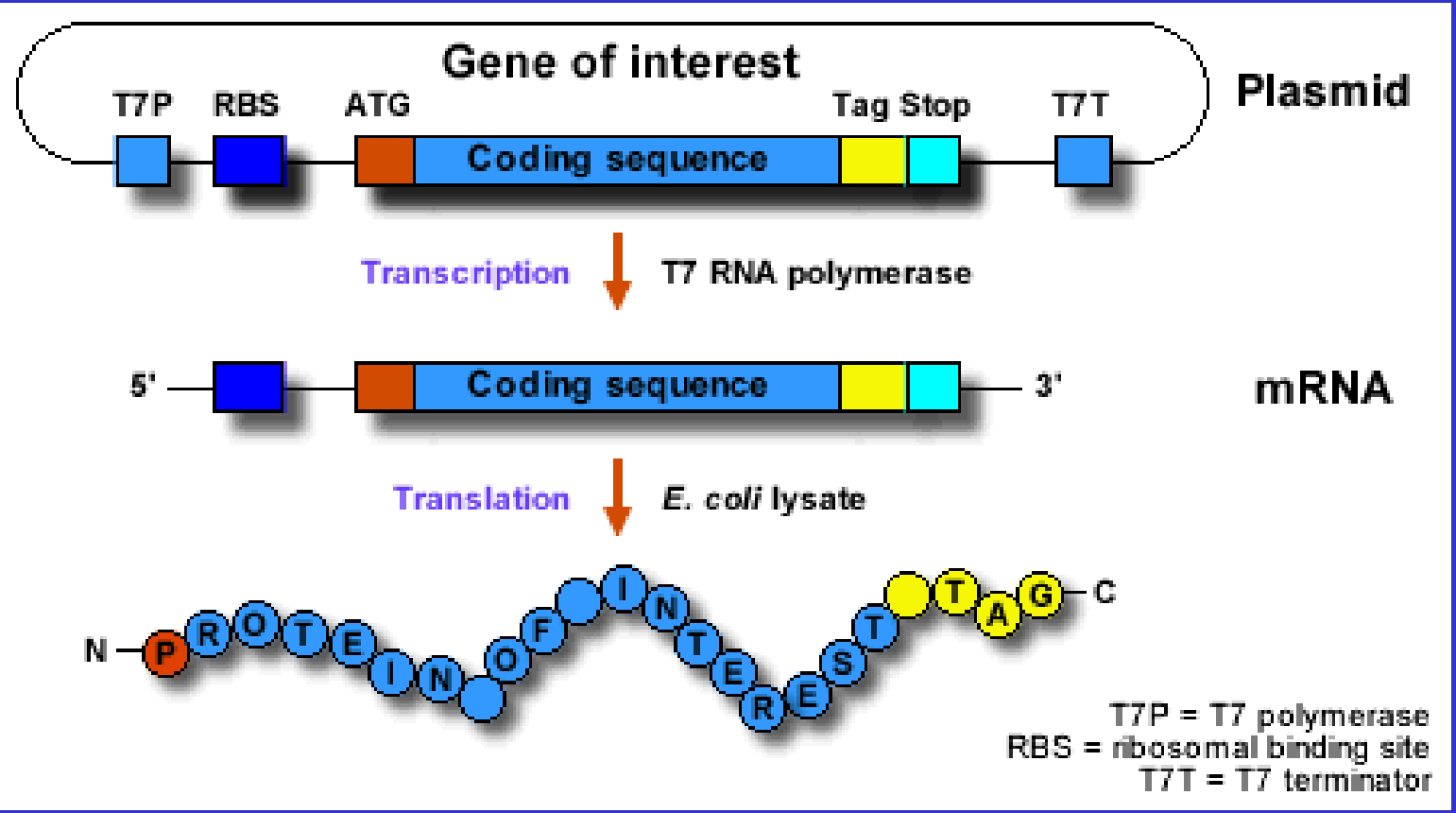



A)
Template cloned in Litmus vector
BglII/StuI digests
StuI
+
BglII
dsRNA
in vitro transcription
BglII
StuI
T7
LITMUS 28i
T7
StuI
StuI digest
ssRNA
in vitro transcription
B)
Introduction of T7 sequence in both strands
T7
T7
PCR
dsRNA
in vitro transcription
C)
Introduction of T7 sequence in one strand
T7
PCR
ssRNA
in vitro transcription

In vitro transcribed eukaryotic mRNA might be used to study the splicing mechanisms

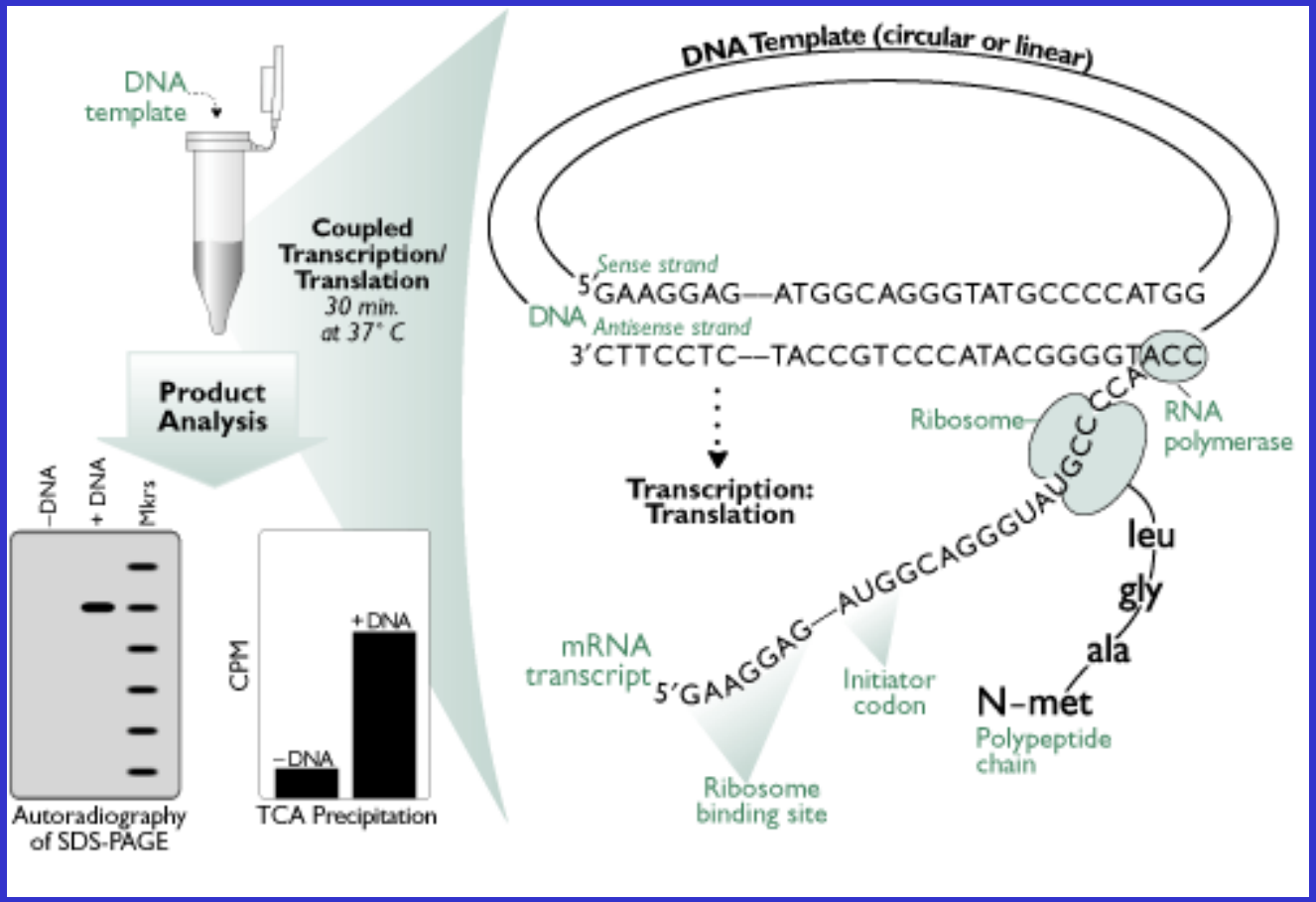


# *Coupled Transcription:Translation in E.Coli*



# Coupled Transcription:Translation

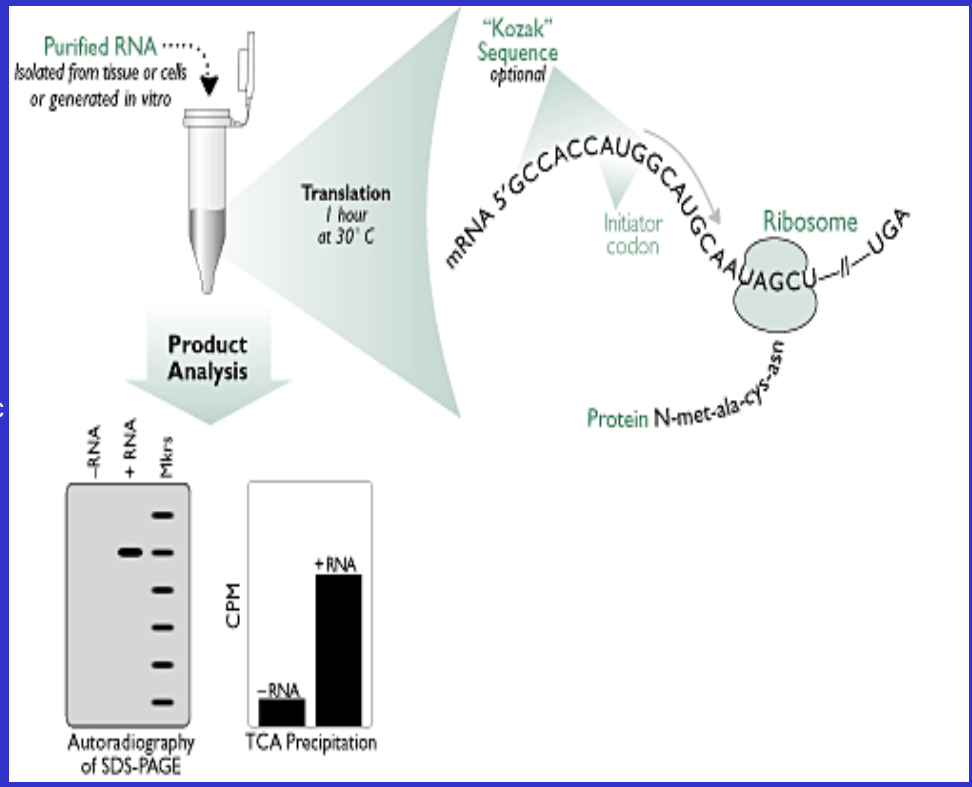
Unlike eukaryotic systems where transcription and translation occur sequentially, in *E. coli*, transcription and translation occur simultaneously within the cell. In vitro *E. coli* translation systems are thus performed the same way, coupled, in the same tube under the same reaction conditions. During transcription, the 5' end of the RNA becomes available for ribosomal binding and undergoes translation while its 3' end is still being transcribed. This early binding of ribosomes to the RNA maintains transcript stability and promotes efficient translation. **This bacterial translation system gives efficient expression of either prokaryotic or eukaryotic gene products in a short amount of time.** For the highest protein yield and the best initiation fidelity, make sure the DNA template has a Shine-Dalgarno ribosome binding site upstream of the initiator codon. Capping of eukaryotic RNA is not required. Use of *E.coli* extract also eliminates cross-reactivity or other problems associated with endogenous proteins in eukaryotic lysates. Also, the *E. coli* S30 extract system allows expression from DNA vectors containing natural *E. coli* promoter sequences (such as *lac* or *tac*).



# In Vitro Translation Systems

## *E. coli* Cell-Free System

*E. coli* cell-free systems consist of a crude extract that is rich in endogenous mRNA. The extract is incubated during preparation so that this endogenous mRNA is translated and subsequently degraded. Because the levels of endogenous mRNA in the prepared lysate is low, the exogenous product is easily identified. In comparison to eukaryotic systems, the *E.coli* extract has a relatively simple translational apparatus with less complicated control at the initiation level, allowing this system to be very efficient in protein synthesis. *E.coli* extracts are ideal for coupled transcription:translation from DNA templates.



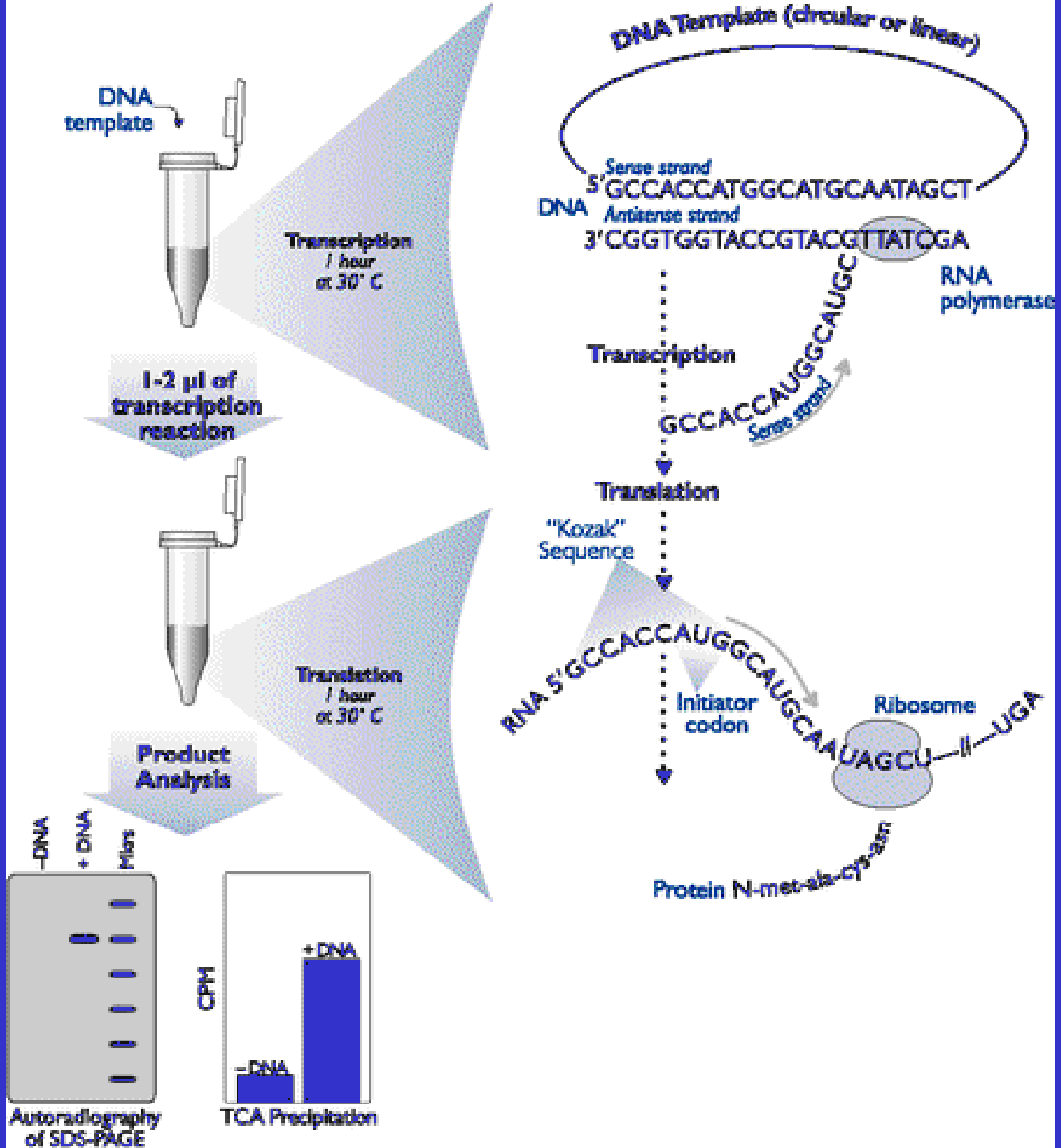

## Eukaryotic Systems:

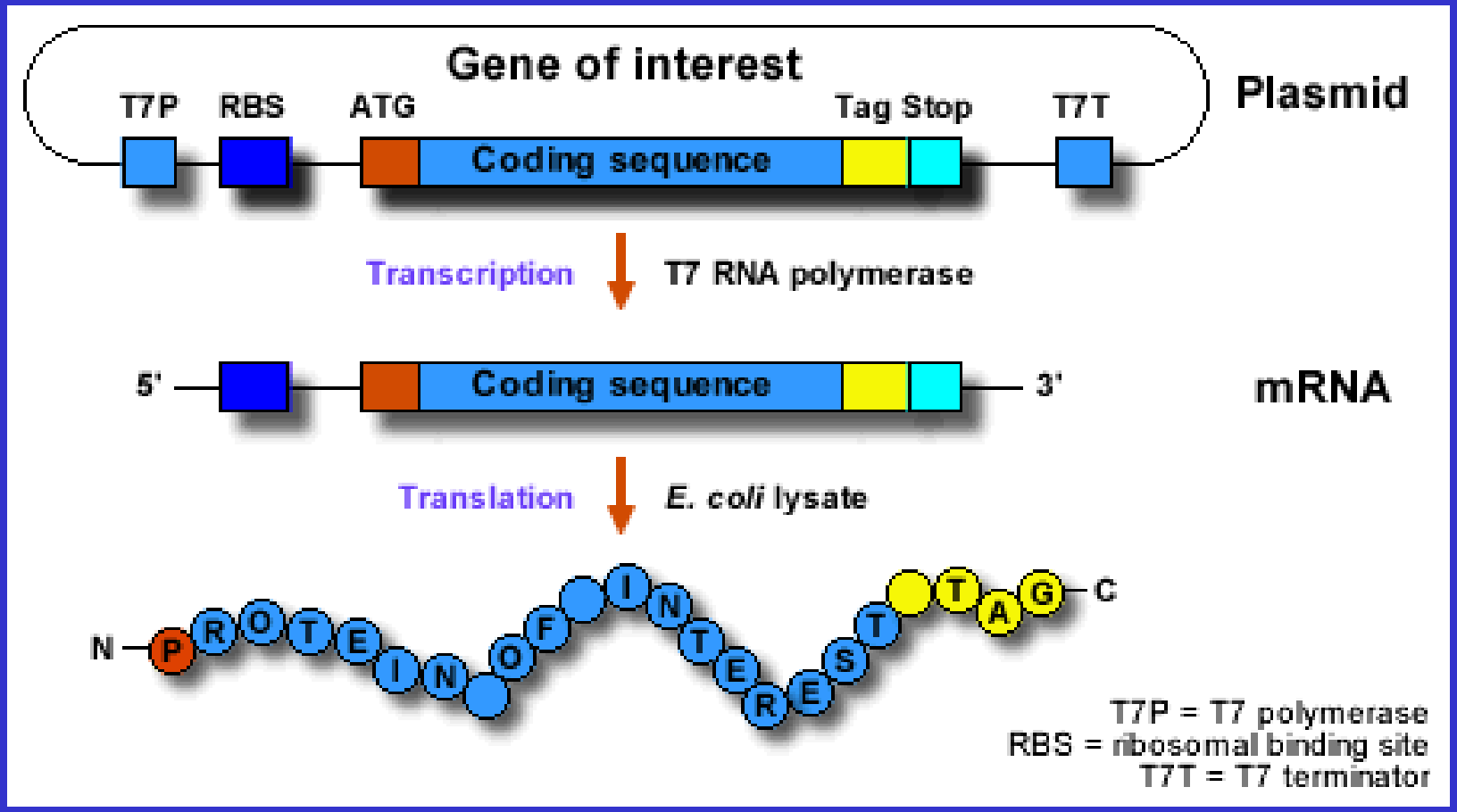
### 1) Rabbit Reticulocyte Lysate

Rabbit reticulocyte lysate is a highly efficient in vitro eukaryotic protein synthesis system used for translation of exogenous RNAs (either natural or generated in vitro).

### 2) Wheat Germ Extract

Wheat germ extract is a convenient alternative to the rabbit reticulocyte system. This extract has low background incorporation due to its low level of endogenous mRNA. Wheat germ extracts translate RNA isolated from cells and tissue or those generated by in vitro transcription. When using RNA synthesized in vitro, the presence of a 5' cap structure may enhance translational activity. Typically, translation by wheat germ extracts is more cap-dependent than translation by retic extracts. If capping of the RNA is impossible and the protein yield from an uncapped mRNA is low, the coding sequence can be subcloned into a prokaryotic vector and expressed directly from a DNA template in an E.coli cell-free system.


DNA template
Transcription
1 hour
at 30° C
1-2 µl of transcription reaction
Translation
1 hour
at 30° C
Product Analysis
-DNA
+ DNA
Mars
Autoradiography of SDS-PAGE
CPM
+DNA
-DNA
TCA Precipitation
DNA Template (circular or linear)
Sense strand
5' GCCACCATGGCATGCAATAGCT
DNA
Antisense strand
3' CGGTGGTACCGTACGTTATCGA
RNA polymerase
Transcription
GCCACCAUGGCAUGC
Sense strand
Translation
"Kozak" Sequence
RNA 5' GCCACCAUGGCAUGCAAUAGCU
Initiator codon
Ribosome
UGA
Protein N-met-ala-cys-asn


Gene of interest
T7P
RBS
ATG
Coding sequence
Tag Stop
T7T
Plasmid
Transcription
T7 RNA polymerase
5'
Coding sequence
3'
mRNA
Translation
E. coli lysate
N
PROTEIN OF INTEREST
TAG
C
T7P = T7 polymerase
RBS = ribosomal binding site
T7T = T7 terminator