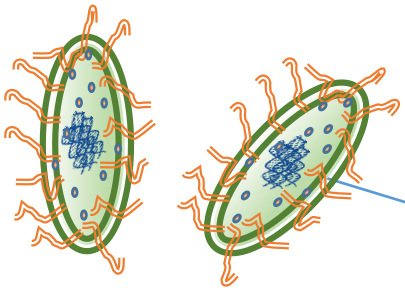
Cloning, expression, purification, and biophysical characterization of SSB from pathogenic bacteria

Molecular Cloning of the *SSB* gene into the pET21a plasmid for protein expression.

Introduction – *SSB* gene was isolated from a specific species of pathogenic bacterium and cloned into the pUC57 plasmid.



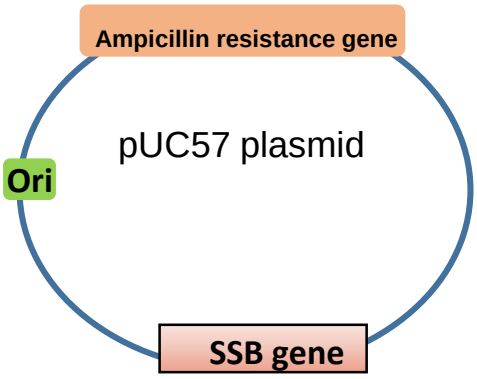
Pathogenic bacteria

Genomic DNA extracted from pathogenic bacteria

SSB gene

PCR using primers specific for the *SSB* gene was carried out to amplify SSB DNA

SSB gene was inserted into the pUC57 plasmid by molecular cloning



Molecular Cloning of the *SSB* gene into the pET21plasmid for protein expression.

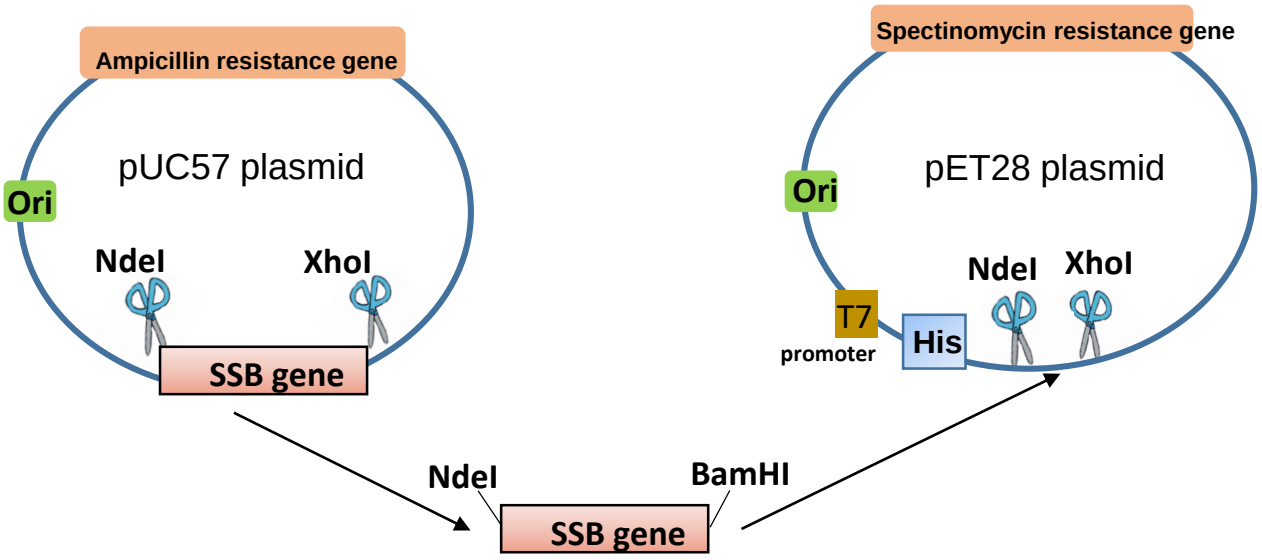
Introduction (cont'd.)

SSB gene is currently inserted in pUC57 plasmid. pUC57 plasmid lacks a T7 promoter, which is required for gene expression. Therefore, *SSB* gene has to be cut out from the pUC57 plasmid and inserted into the pET28a plasmid that carries the T7 promoter.

Step I - Restriction Digestion (Overview):

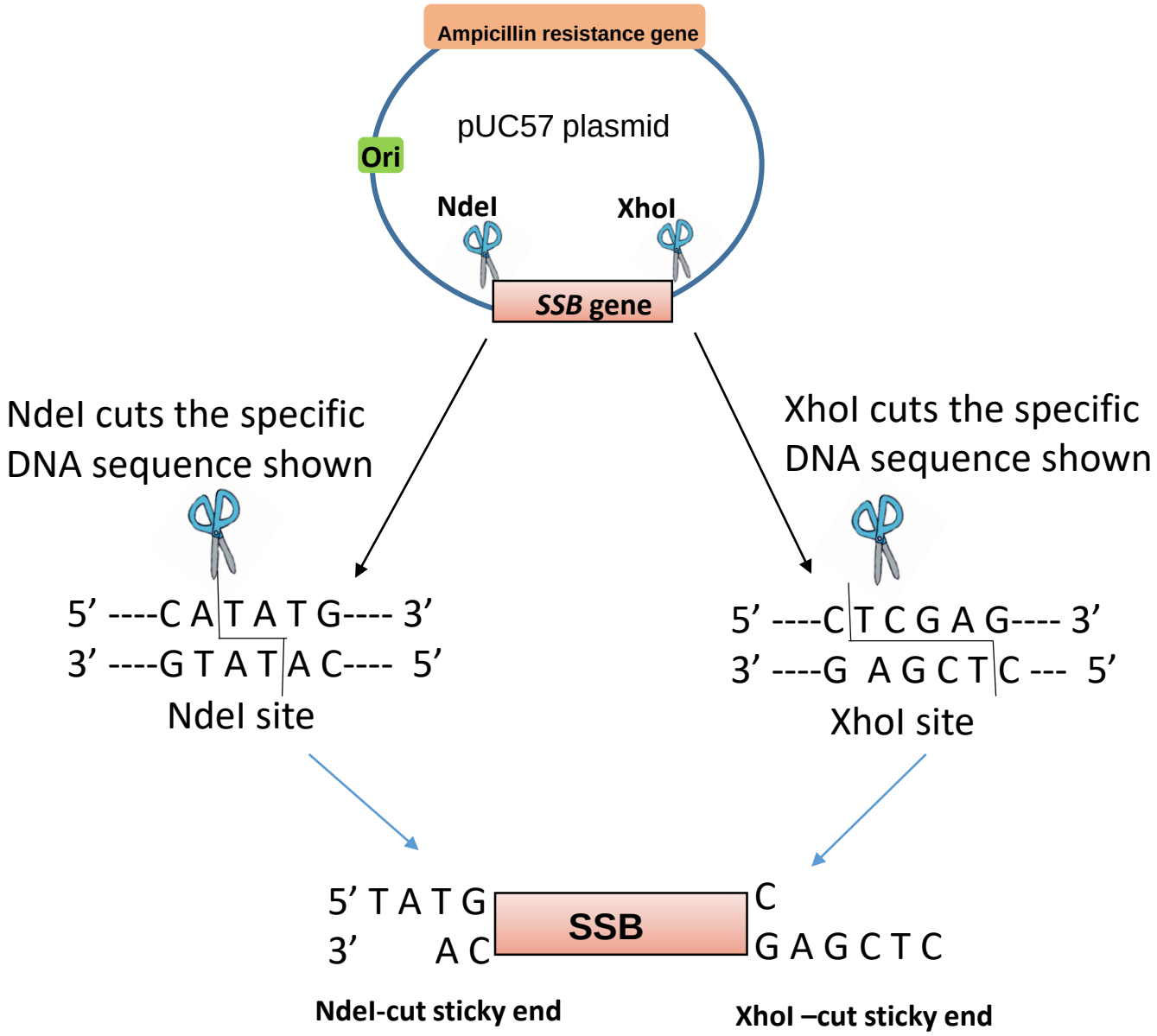
***SSB* gene- Insert** - The entire *SSB* gene has to be cut out from the pUC57 plasmid using restriction enzymes NdeI and XhoI that cut at specific restriction sites at the start and end of the SSB DNA respectively.

Vector – The vector-pET21a is also cut with the same enzymes - NdeI and XhoI to generate sticky ends that will be compatible with the restriction digested ends of SSB DNA. This will help in inserting SSB DNA into the pET28a vector.



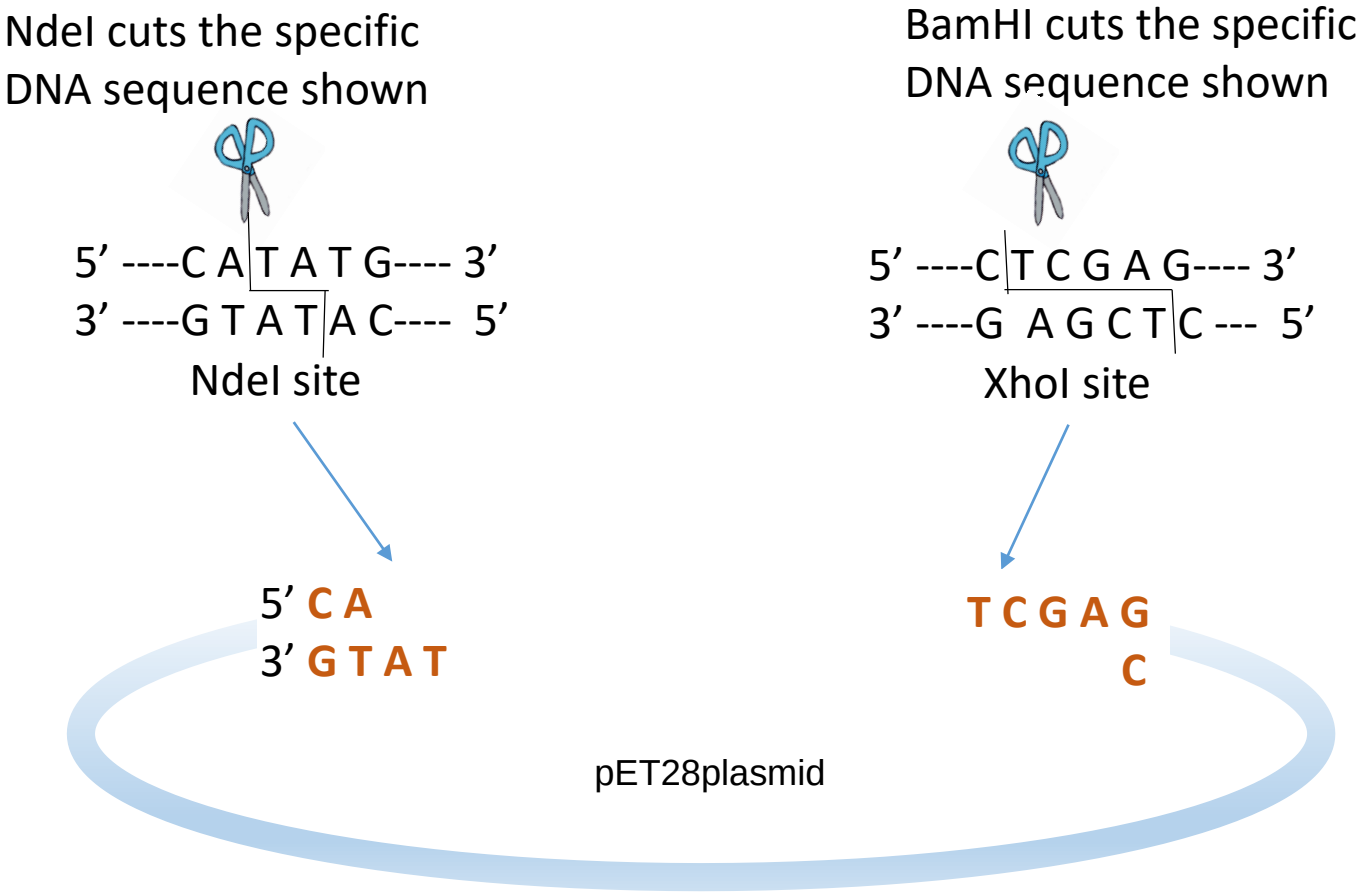
Step I - Restriction Digestion (overview-cont'd.):

SSB- Insert - The entire SSB gene has to be cut out from the pUC57 plasmid using restriction enzymes NdeI and XhoI that cut at specific restriction sites at the start and end of the SSB DNA respectively.



Step I - Restriction Digestion (Overview- cont'd.):

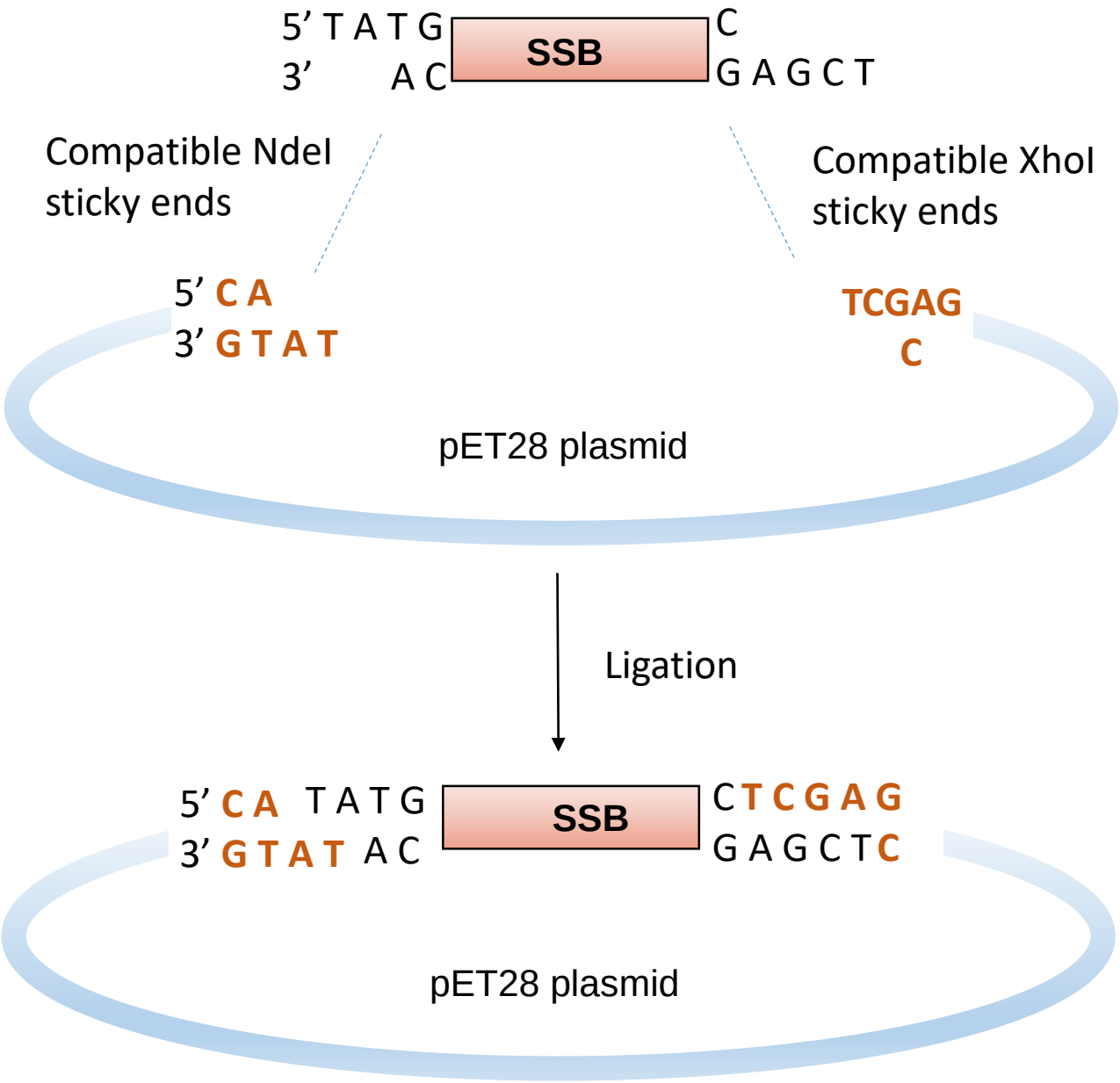
Vector – The vector-pET28 is also cut with the same enzymes NdeI and XhoI sites to generate sticky ends that will be compatible with the ends of the *SSB* gene insert and will help in inserting *SSB* into the vector.



Step I - Restriction Digestion (Overview – cont'd.):

SSB- Insert - The entire *SSB* gene has to be cut out from the pUC57 plasmid using restriction enzymes NdeI and BamHI that cut at respective restriction sites at the start and end of the SSB DNA.

Vector – The vector-pCDF is also cut with the same enzymes NdeI and BamHI sites to generate sticky ends that will be compatible with the ends of the SSB DNA insert and will help in inserting the gene into the pCDFvector.



Step 1- Restriction digestion protocol

Reaction I - restriction digestion of pET28-SSB vector

Label a 1.5mL microcentrifuge tube with your group name , and set up the following reaction:

pET28-SSB plasmid DNA – 10 uL
*NdeI---------- 1 uL
*XhoI---------- 1 uL
CutSmart buffer 10X --- 5 uL
DI water ---------- 33 uL

50uL (total)

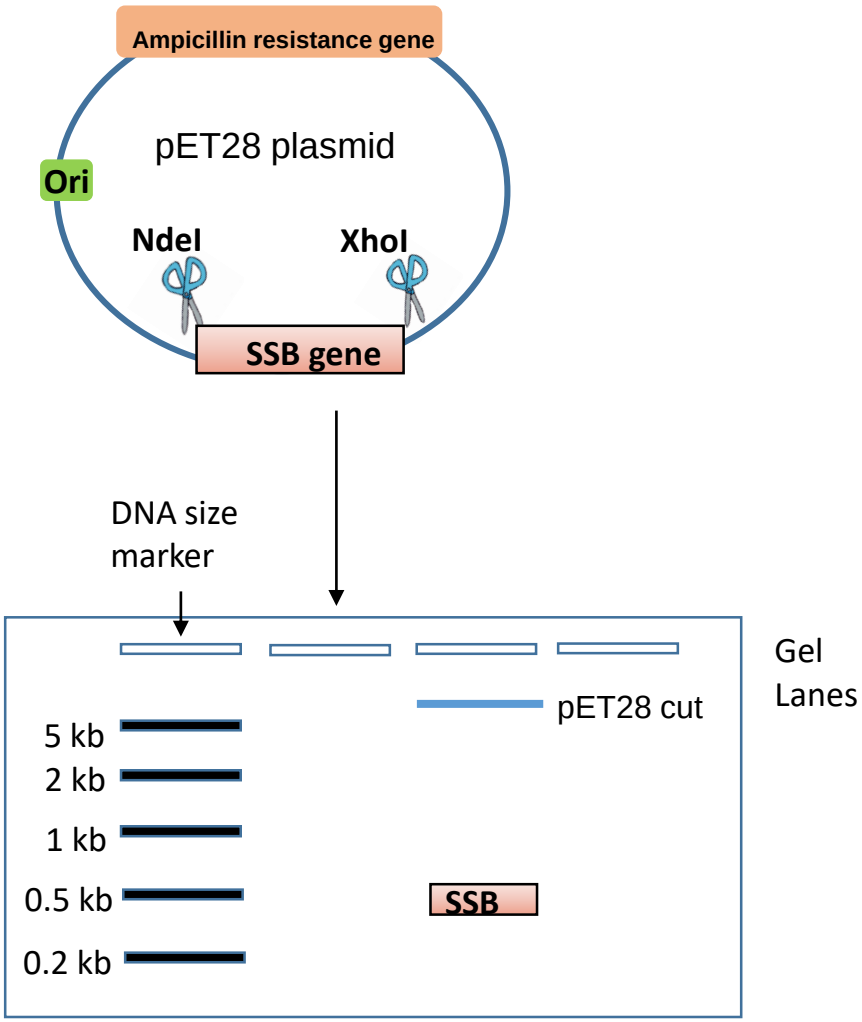
*Add enzymes- NdeI and XhoI last!

Gently mix the reaction and incubate at 37°C for 1 hr!

Step II : Agarose gel electrophoresis (Overview)

- After restriction digestion, SSB DNA is separated from the pET28 plasmid using agarose gel electrophoresis.
- pET28 cut plasmid is separated from any uncut plasmid using agarose gel electrophoresis.

pET plasmid DNA (cut) size : **5.4 kb**
SSB gene size is : approximately **0.5kb**
Any uncut plasmid DNA is supercoiled –will not run at expected size and will run faster than cut plasmid DNA.



Step II - Agarose Gel Electrophoresis protocol to analyze digested products

Prepare 1% agarose gel:

1. Add 0.5 g of agarose to 50 mL of 1X TAE buffer in a 200mL beaker.
2. Microwave solution till agarose is dissolved. (caution –hot!)
3. Once the beaker is cool to the touch – add 5 µL of EtBr Stain.
4. Mix gently and pour into the casting tray.
5. Place the comb and wait for gel to polymerize (solidify).

Prepare sample to be loaded onto the gel:

1. Add 8ul of 6X loading dye to reaction tube.

Loading reactions onto the gel:

1. Load 5uL of DNA marker in gel lane 1
2. Load 20 ul of sample onto gel 3
3. Run at 120V for 45min to 1hr.