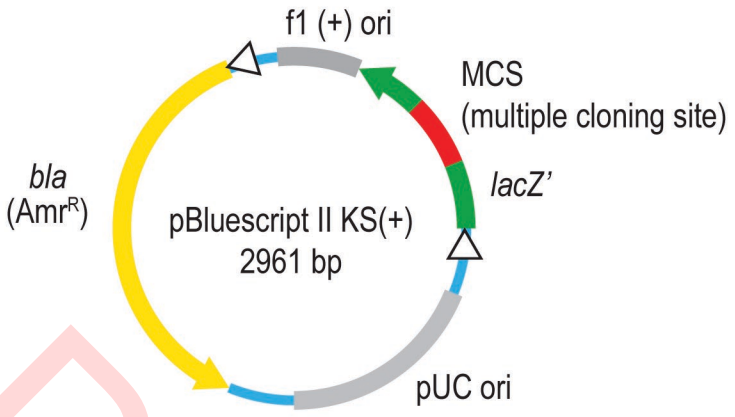
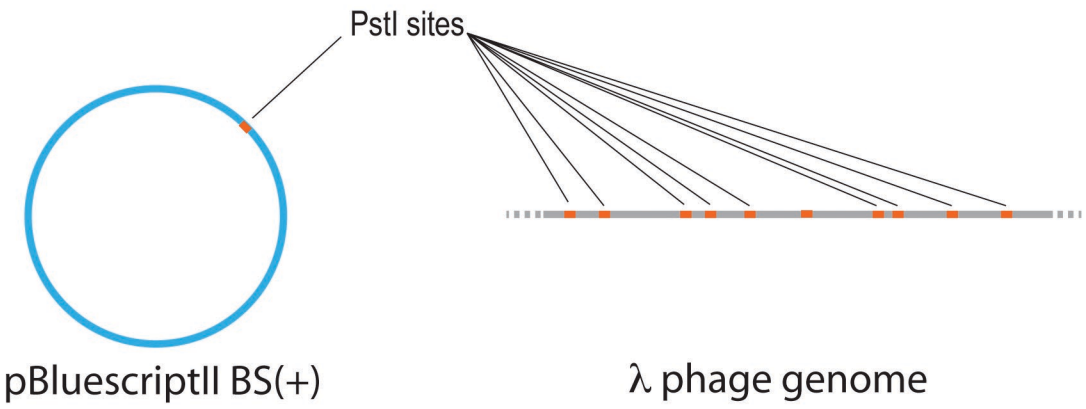
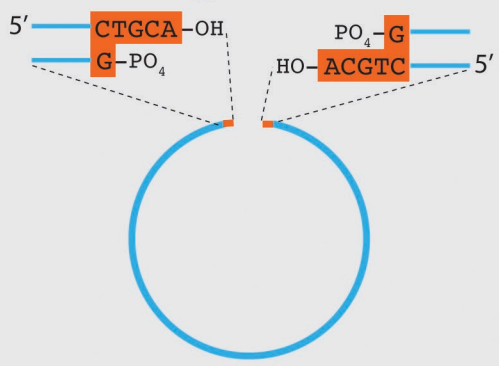
Shotgun cloning



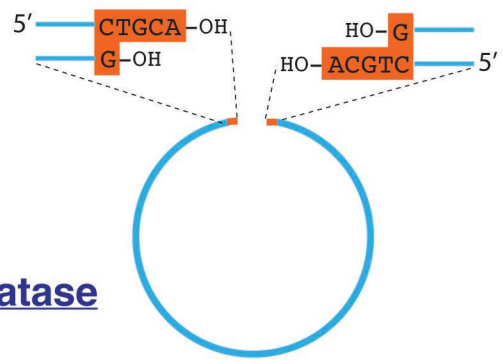


1 λ genome fragmentation and vector digest with PstI



- digest pBluescriptII vector and λ DNA with **PstI** overnight

2 Vector dephosphorylation

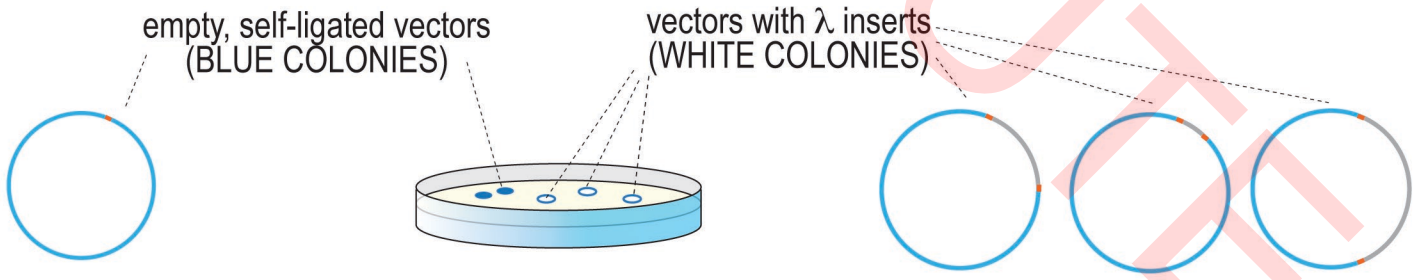


- the vector is additionally dephosphorylated by **alkaline phosphatase**

3 Ligation of λ fragments into the vector

- test the fragmentation by agarose gel electrophoresis
- ligate λ PstI-generated DNA fragments into the cut vector using **T4 DNA ligase**
- heat-inactivate the ligase for 10 min at 65°C

4 Transformation and blue-white selection



- transform the ligation mix into chemically competent *E.coli* XL-1 Blue
- plate onto LB/Amp plates containing X-Gal and IPTG and grow at 37°C overnight

5 Determination of the insert size

- inoculate liquid LB/Amp with single colonies
- grow liquid cultures at 37°C overnight
- isolate plasmid DNA
- perform restriction analysis to determine the size of λ DNA fragments in the isolated clone