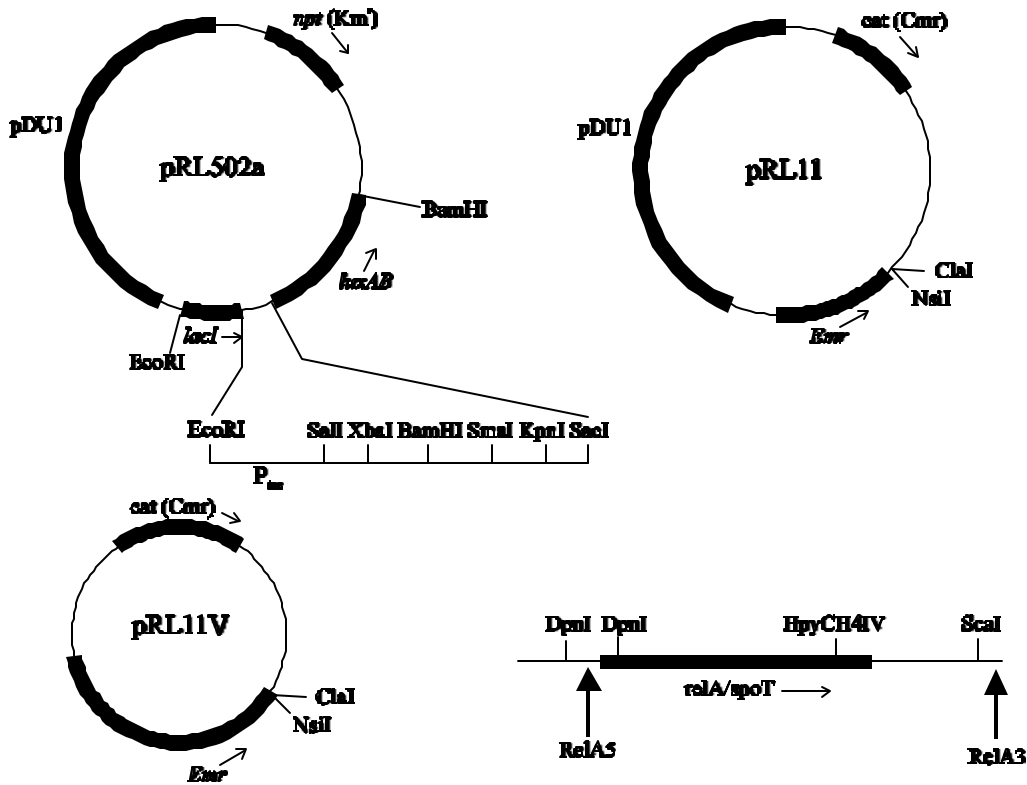# Bacterial Development and Pathogenesis (Spring 2001)
Maps for cloning of *relA* from *Anabaena* PCC 7120
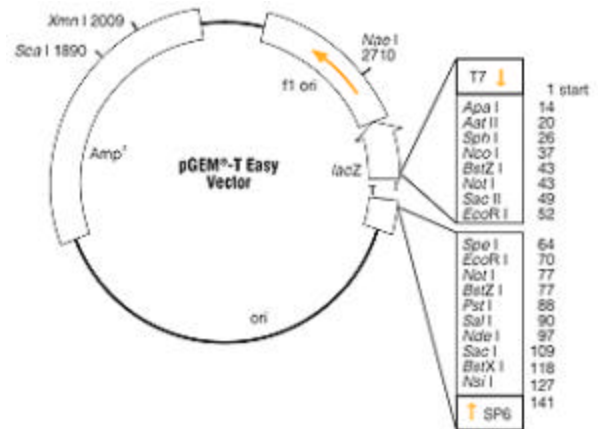

Primers

RelA5:   5'-**ACCTCTTCTATTGTATGAGCAG**-3'

RelA3:   5'-GTTAATCGCCTCATCTGGGTT-3'
         3'-**CAATTAGCGGAGTAGACCCAA**–5'

Coordinates
relA: 12171-14426
RelA5: 12157-12178
RelA3: 14843-14863
DpnI: 12201
PvuII: 12433
HpyCH4IV: 12294,14290
ScaI: 14764

Objectives

1. **Intact *relA* in pRL502a, sense orientation (KTY)**

   Clone in pGEM-T Easy, choose orientation parallel to *lacZ*, cut out with SalI+ScaI (blunt), clone in SalI-SmaI(blunt) hole of pRL502a.

2. **Intact *relA* in pRL502a, antisense orientation (JE)**

   Clone in pGEM-T Easy, choose orientation antiparallel to *lacZ,* cut out with SacI+ScaI (blunt), clone in SmaI(blunt)-SacI hole of pRL502a.

3. **Intact *relA* in pRL11, sense orientation (KL)**

   Clone in pGEM-T Easy, choose orientation parallel to *lacZ*, cut out with SacI+ScaI, clone in SacI-EcoRV hole of pBluescriptKS, cut out with PstI-ClaI, clone in NsiI-ClaI hole of pRL11 (could be improved)

4. **5' end of *relA* in pRL502a, antisense orientation (JR)**

   Clone in pGEM-T Easy, choose orientation antiparallel to *lacZ,* cut out with DpnI(blunt)+SacI, clone in SmaI-SacI hole of pRL502a

5. **Internal *relA* fragment in pRL11V (CP)**

   Clone in pGEM-T Easy, cut out with PvuII+SacI, subclone in SmaI-SacI hole of pBluescriptKS, cut out with PstI+HpyCH4IV, clone in NsiI-ClaI hole of pRL11V