

Inactivation of three RG(S/T)GR pentapeptide-containing negative regulators of HetR results in lethal differentiation of *Anabaena* PCC 7120

Supplemental Figures

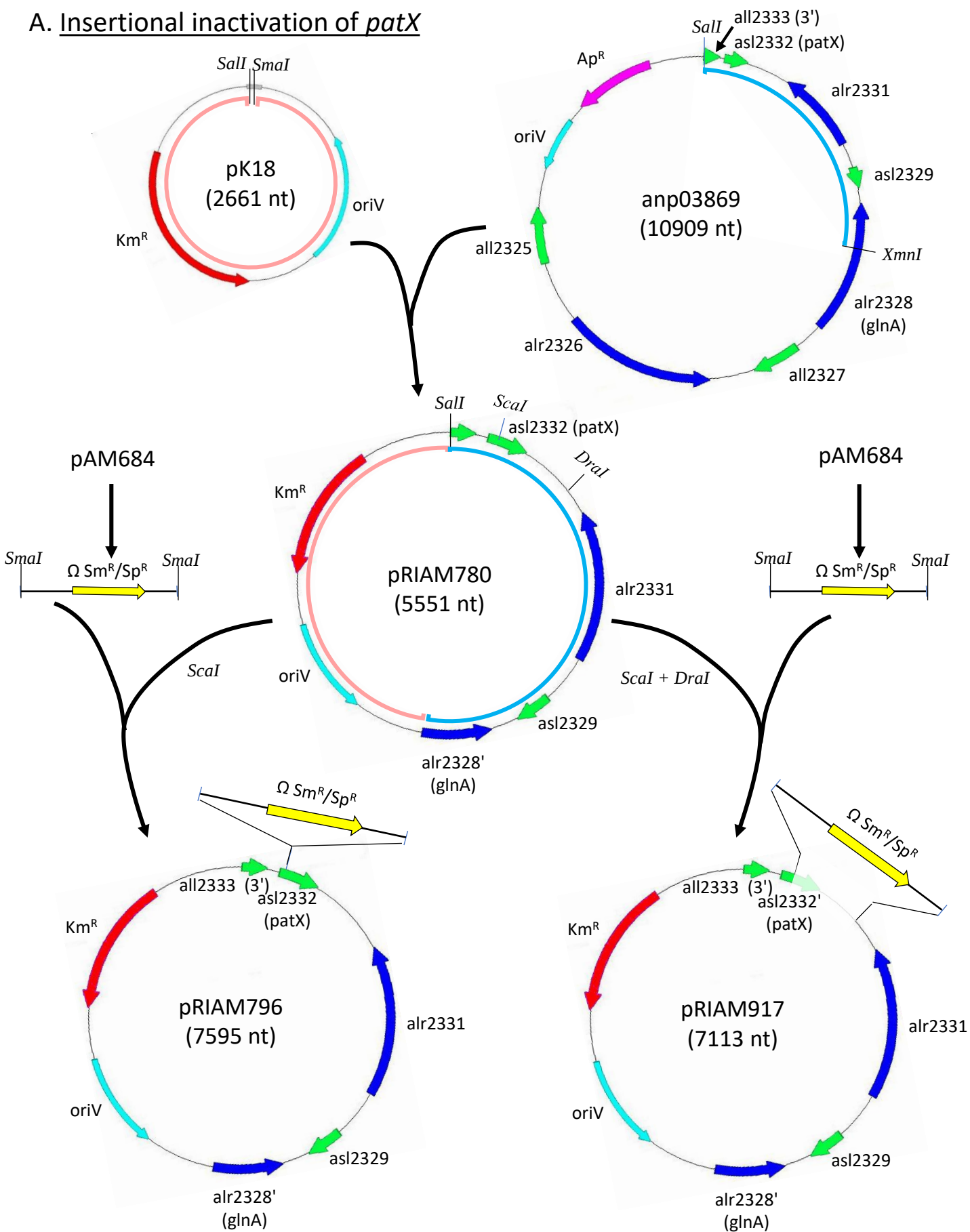
Supplemental Figure 1: Construction of pRIAM929 and pRIAM931 ($\Delta patX$)

Supplemental Figure 2: Construction of pRIAM1177 ($\Delta patS$)

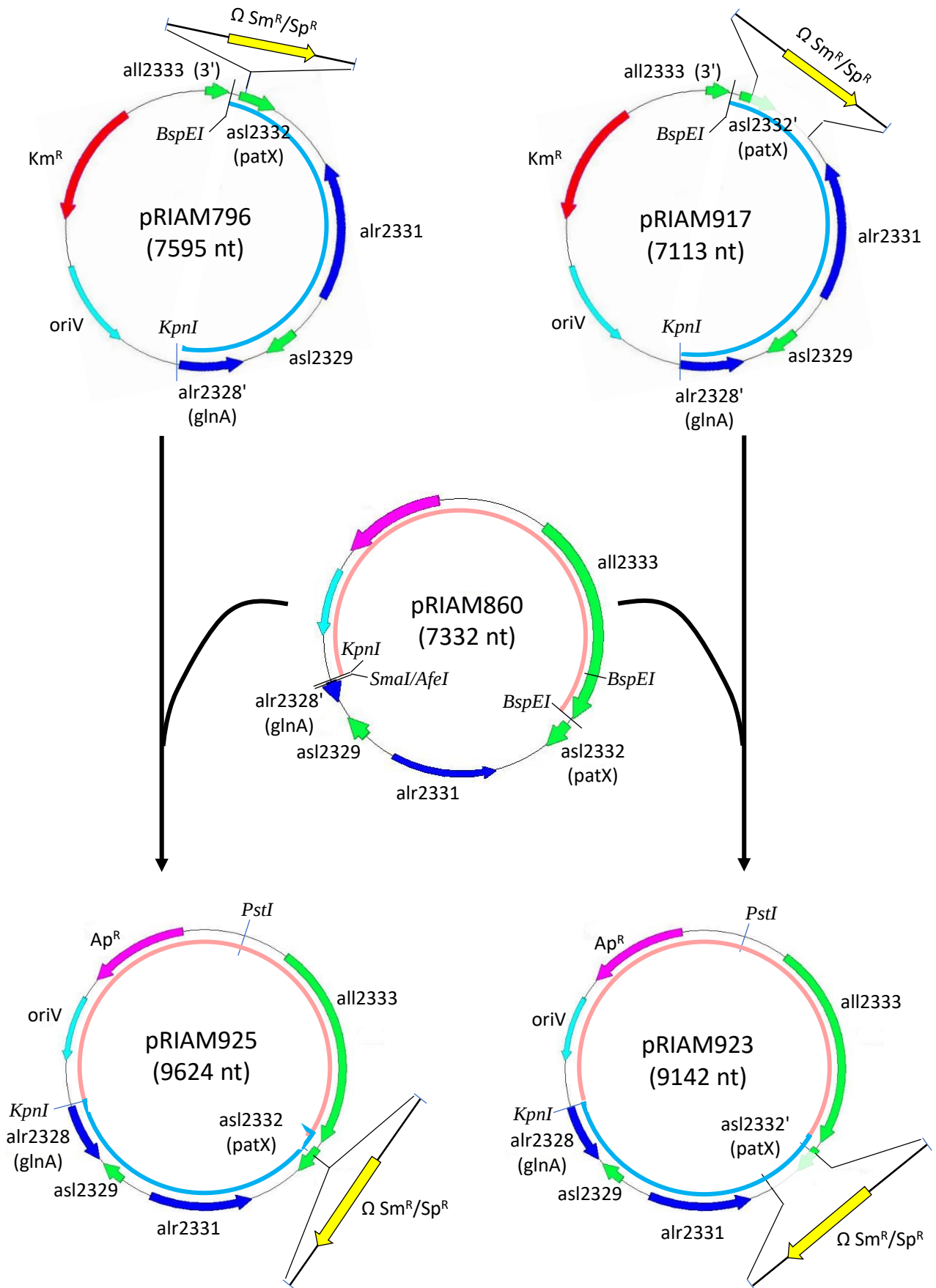
Supplemental Figure 3: Segregation of mutations

Supplemental Figure S1. Construction of pRIAM929 and pRIAM931 ($\Delta patX$)

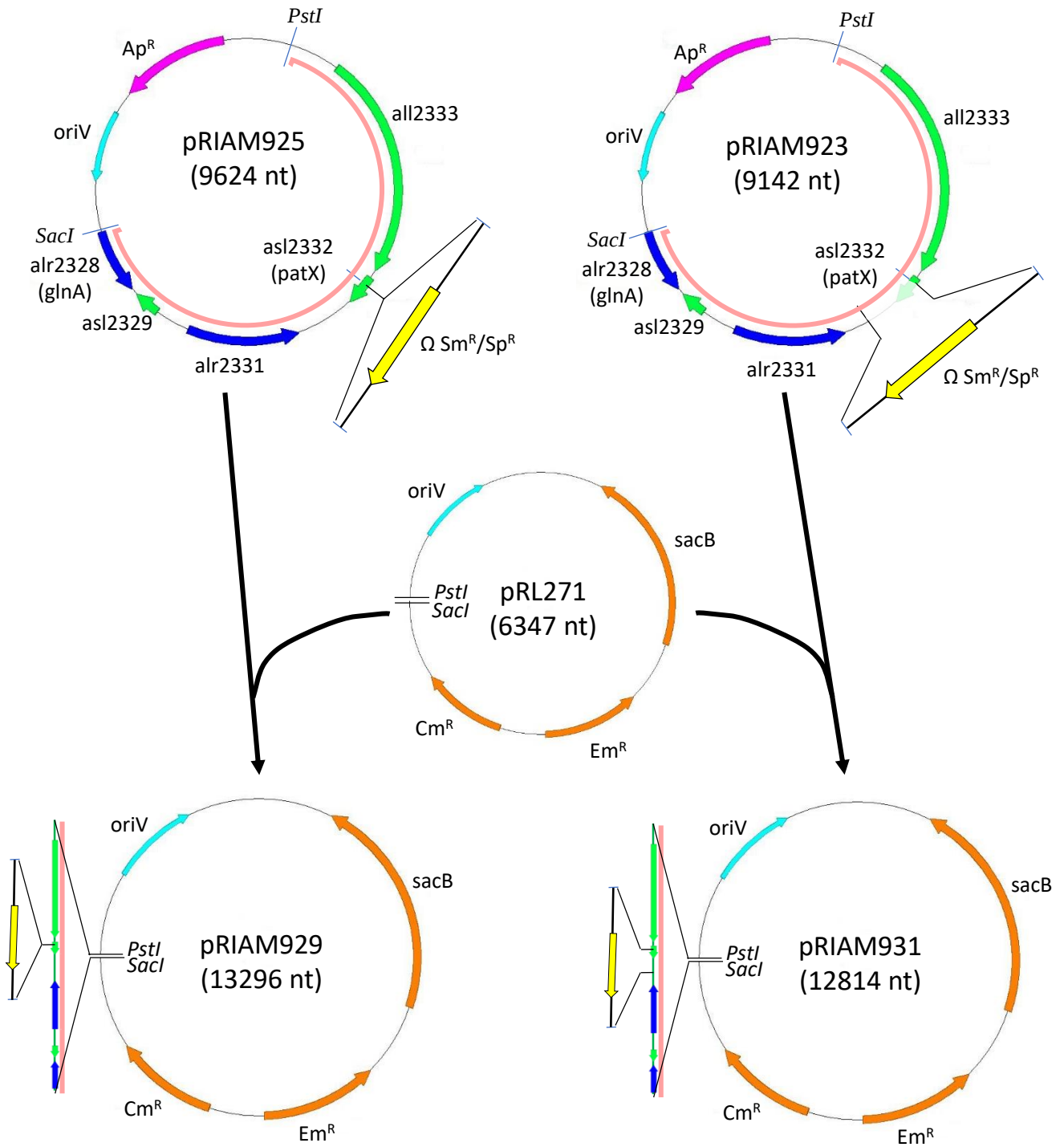
A. Insertional inactivation of *patX*



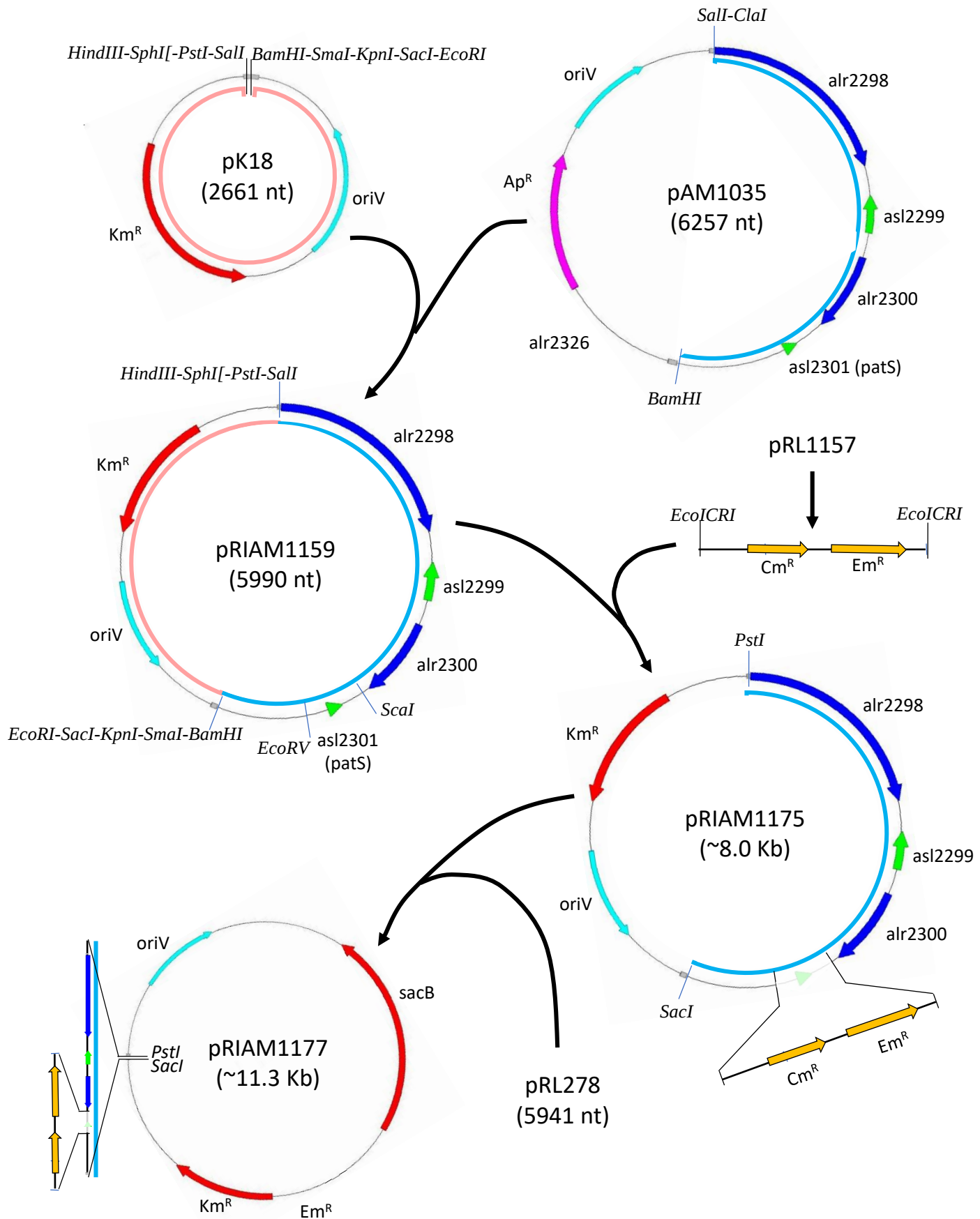
B. Reconstruction of 5' flanking region



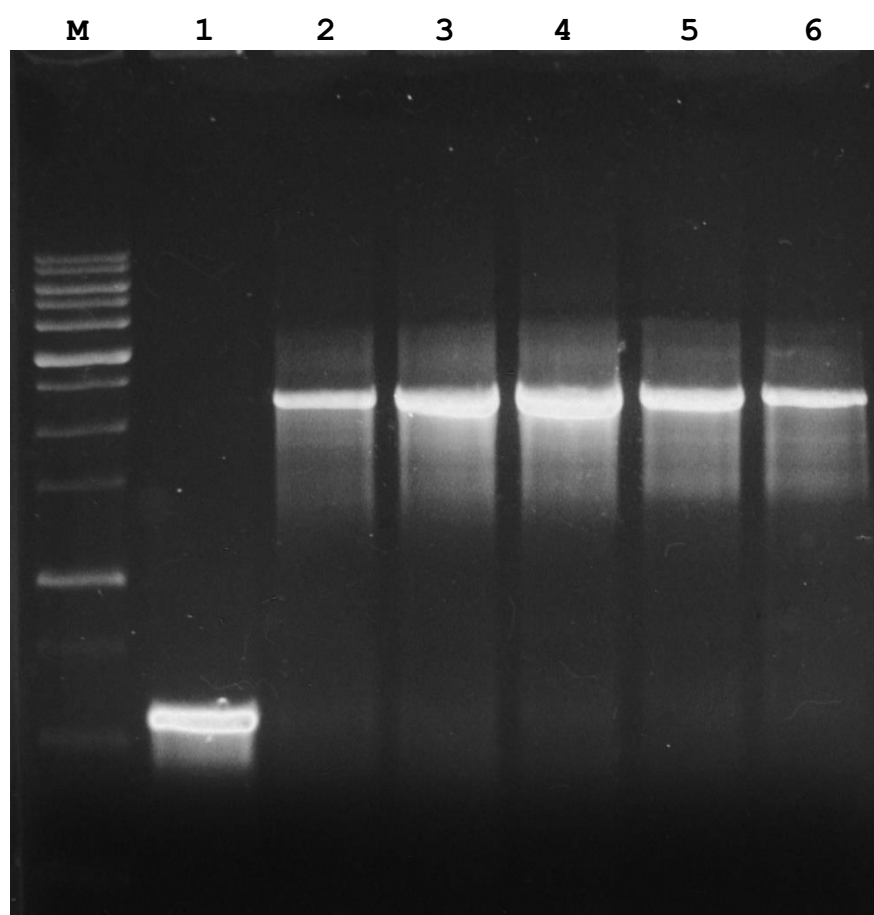
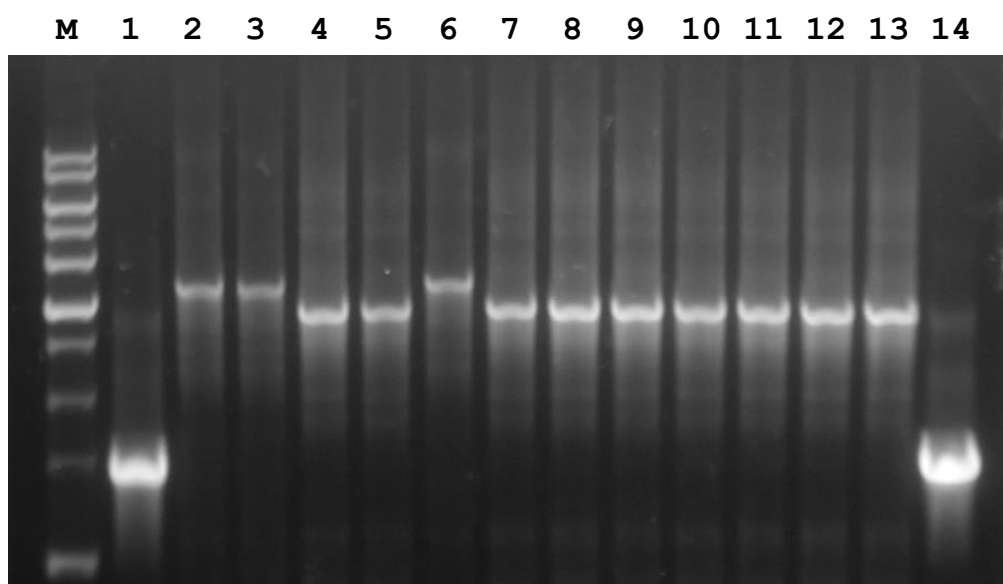
C. Transfer of construct to suicide vector



Supplemental Figure S2. Construction of pRIAM1177 ($\Delta patS$)



Supplemental Figure S3. Segregation of mutations



(top) **Segregation of *patX* mutations in constructed *patX* mutant strains.**

Primers 2332-F2 TATTGACACCATGCACACTT and 2331-F1

TCTCAGATGGAGCTCGTCATGCC were used for PCR using chromosomal DNAs from the following strains:

- M** molecular markers (1kb: 10, 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1)
- 1** wild type *Anabaena* PCC 7120
- 2,3** different clones of RIAM1238, *patX*:: Ω
- 4,5** different clones of RIAM1239, Δ *patX*:: Ω
- 6** RIAM1241, *P_{petE}-hetN patX*:: Ω
- 7** RIAM1242, *P_{petE}-hetN Δ patX*:: Ω
- 8,9** different clones of RIAM1243, Δ *patS Δ patX*:: Ω (pAM1714)
- 10,11** different clones of RIAM1245, *P_{petE}-hetN patX*:: Ω Δ *patS*::C.CE3
- 13,14** different clones of RIAM1248, *P_{petE}-hetN Δ patX*:: Ω Δ *patS*::C.CE3
- 15** wild type *Anabaena* PCC 7120

(bottom) **Segregation of *patS* mutations in constructed *patS* mutant strains.**

Primers 2299-F1 GTCTGCTGTAAGCCTTATCAGC and 2299-R1

CACCATTCAATTGCACCATC were used for PCR using chromosomal DNAs from the following strains:

- M** molecular markers (1kb: 10, 8, 6, 5, 4, 3, 2.5, 2, 1.5, 1, 0.75, 0.5, 0.25)
- 1** wild type *Anabaena* PCC 7120
- 3** RIAM1245, *P_{petE}-hetN patX*:: Ω Δ *patS*::C.CE3
- 3,4** different clones of RIAM1248, *P_{petE}-hetN Δ patX*:: Ω Δ *patS*::C.CE3
- 5,6** RIAM1249 and RIAM1250, different clones of *P_{petE}-hetN Δ patS*::C.CE3