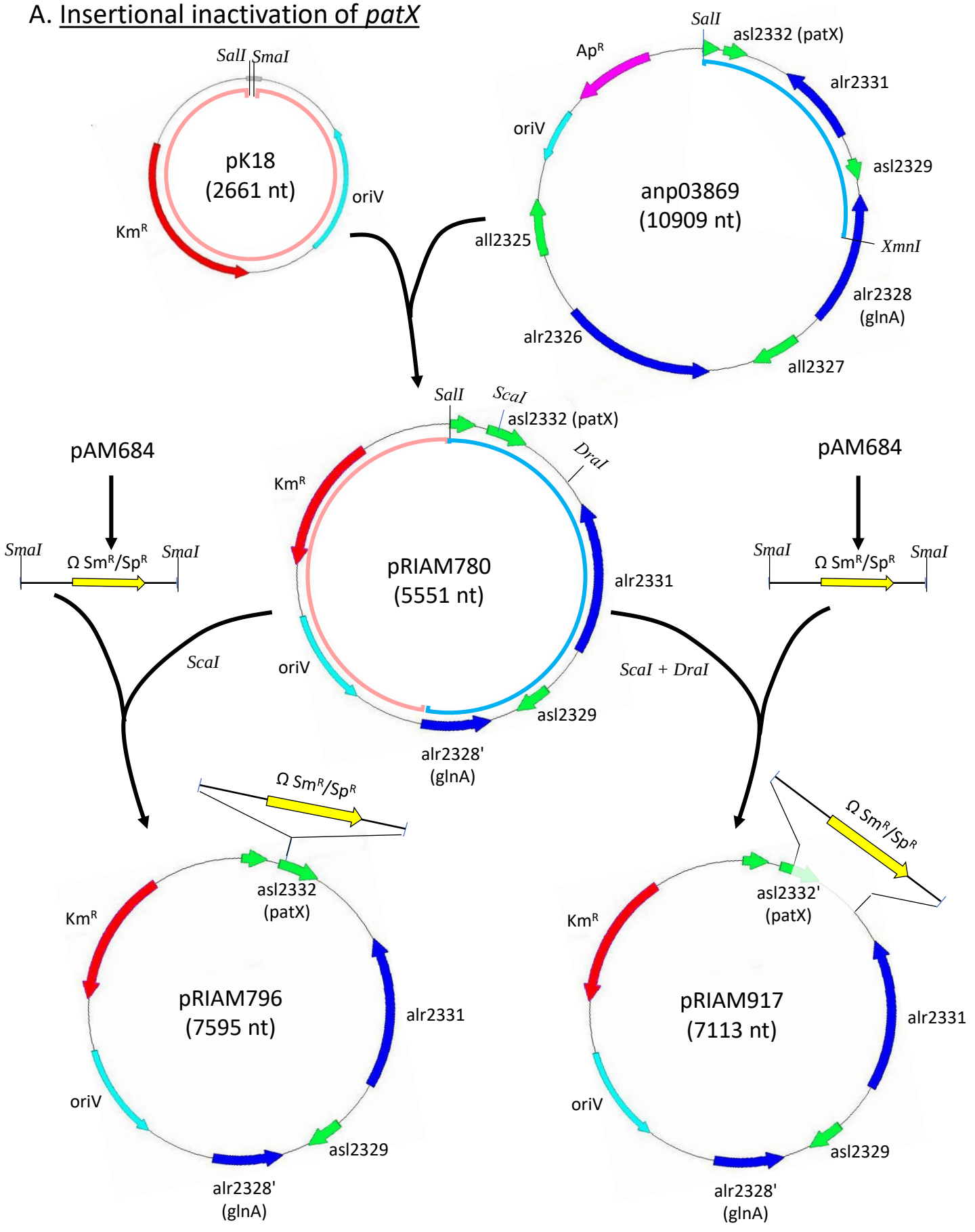
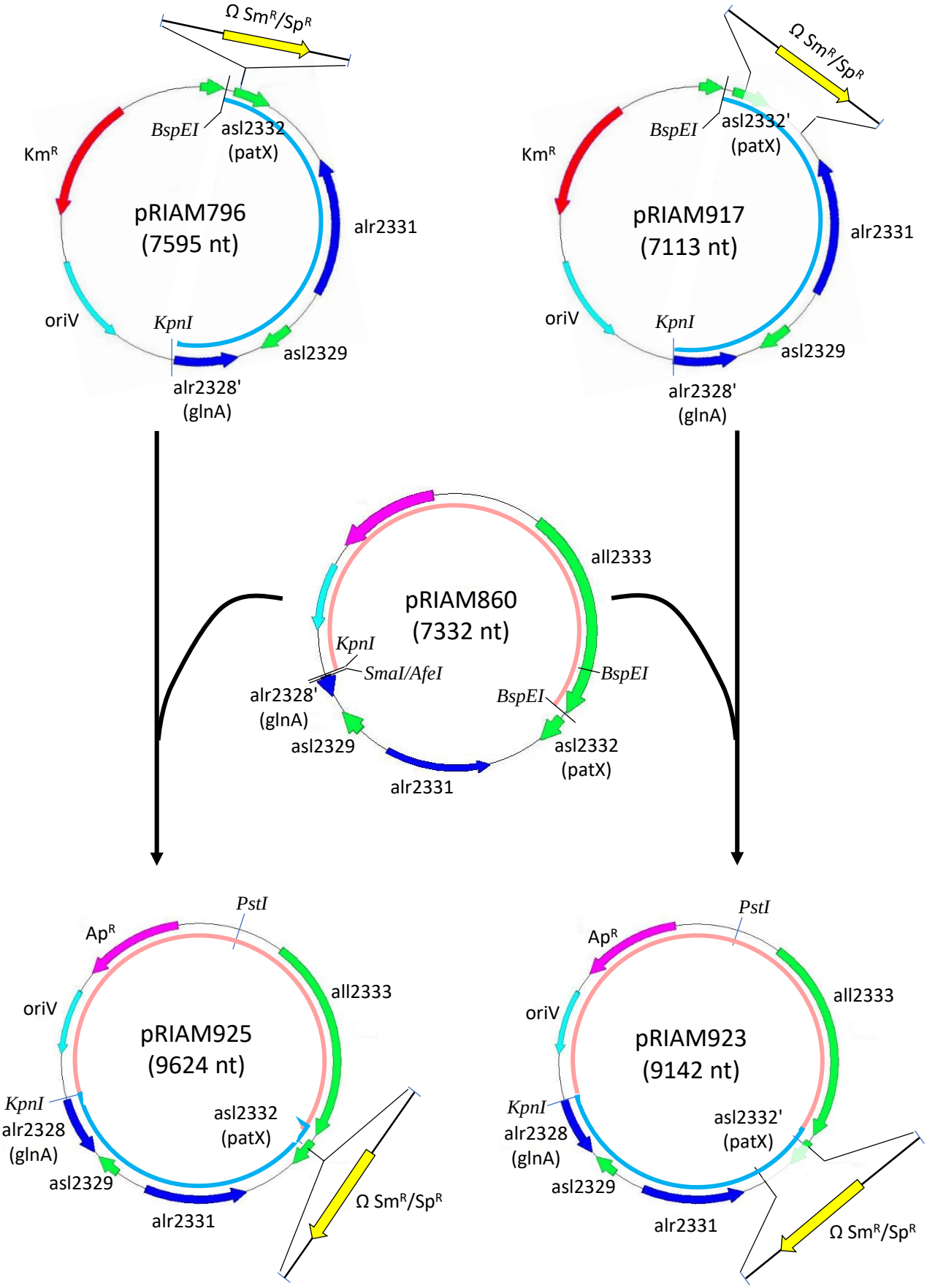
Inactivation of three RG(S/T)GR pentapeptide-containing negative regulators of HetR results in lethal differentiation of *Anabaena* PCC 7120

Supplemental Figures

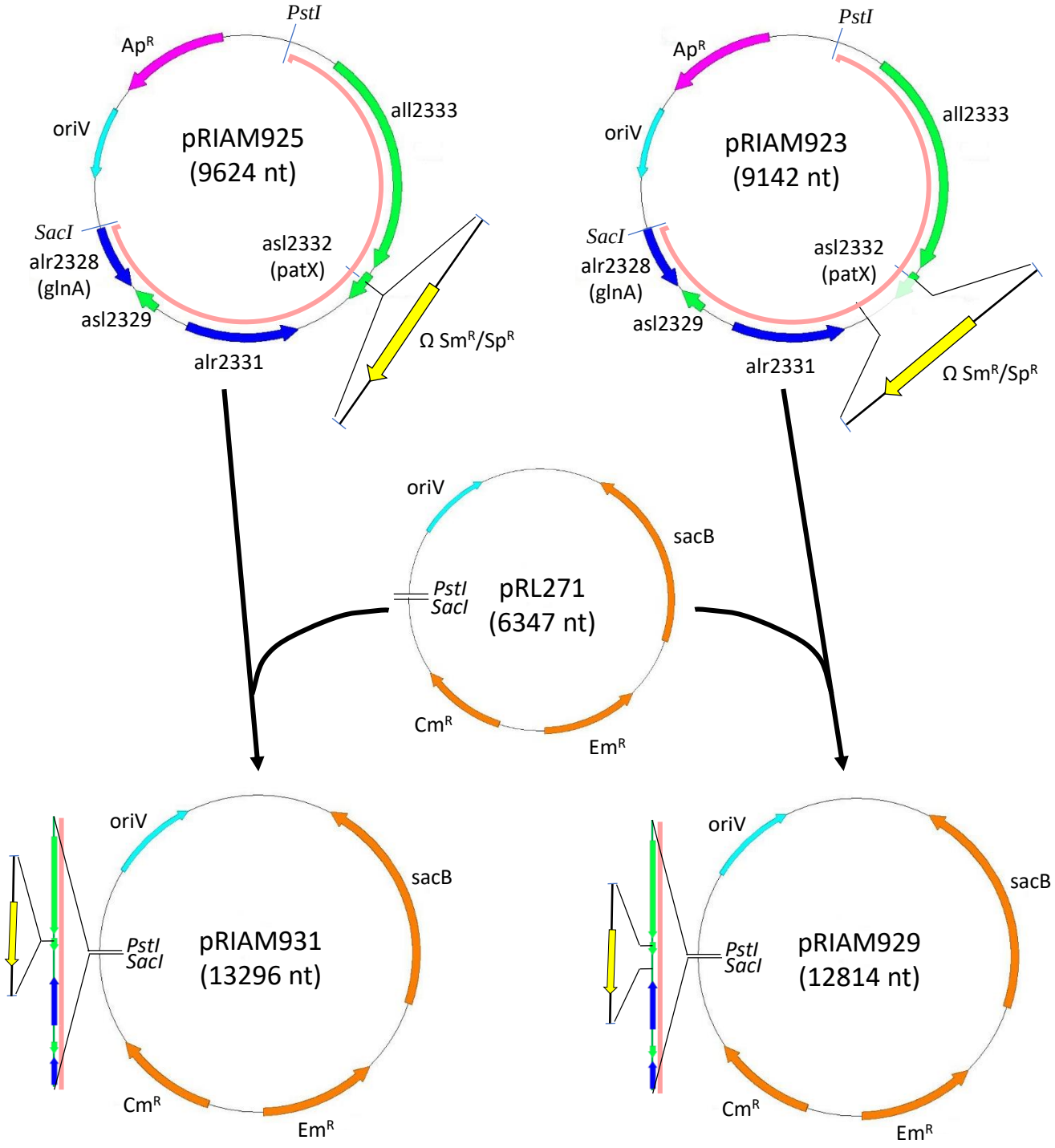
Supplemental Figure S1. Construction of pRIAM929 and pRIAM931 (Δ*patX*)



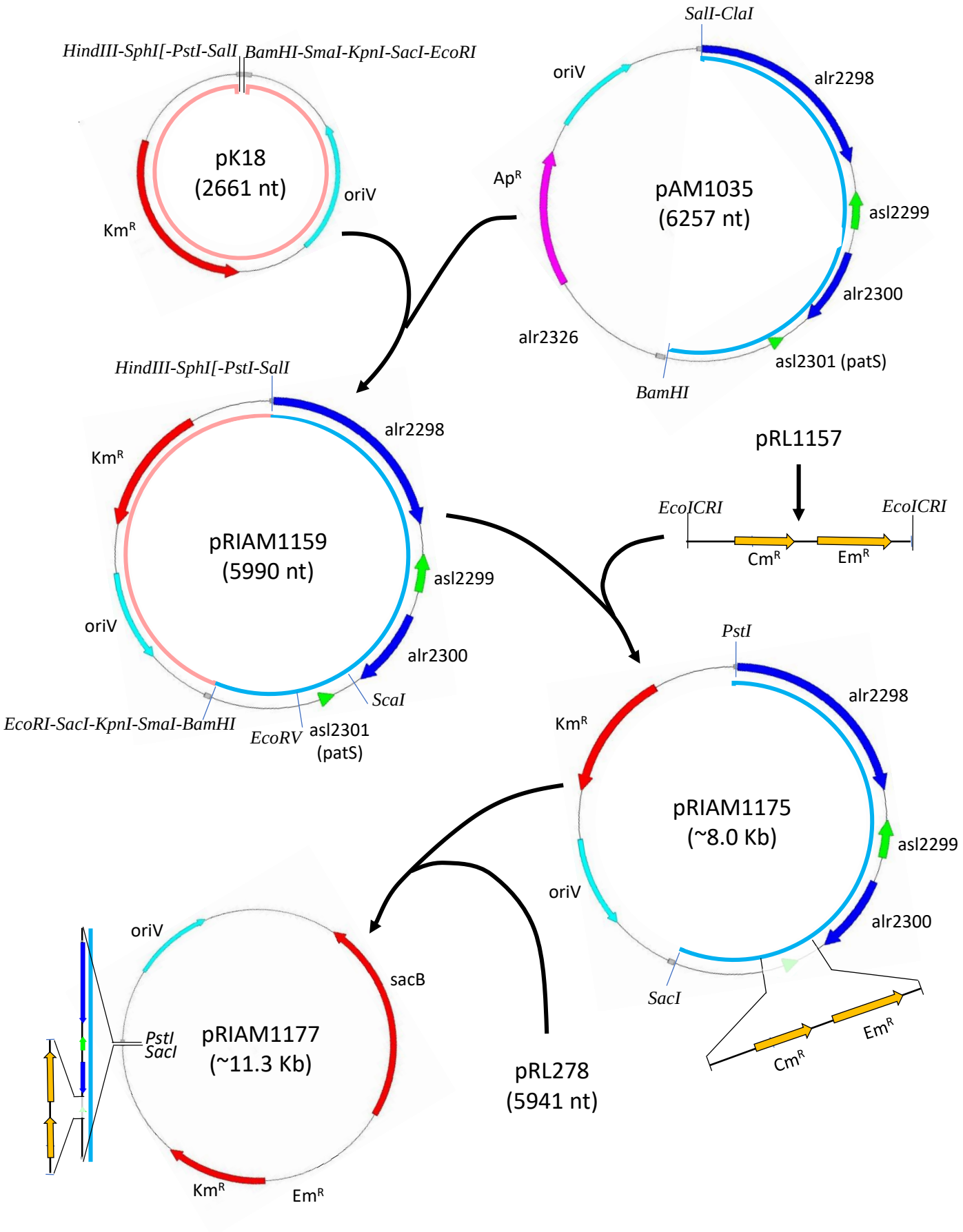
B. Reconstruction of 5' flanking region



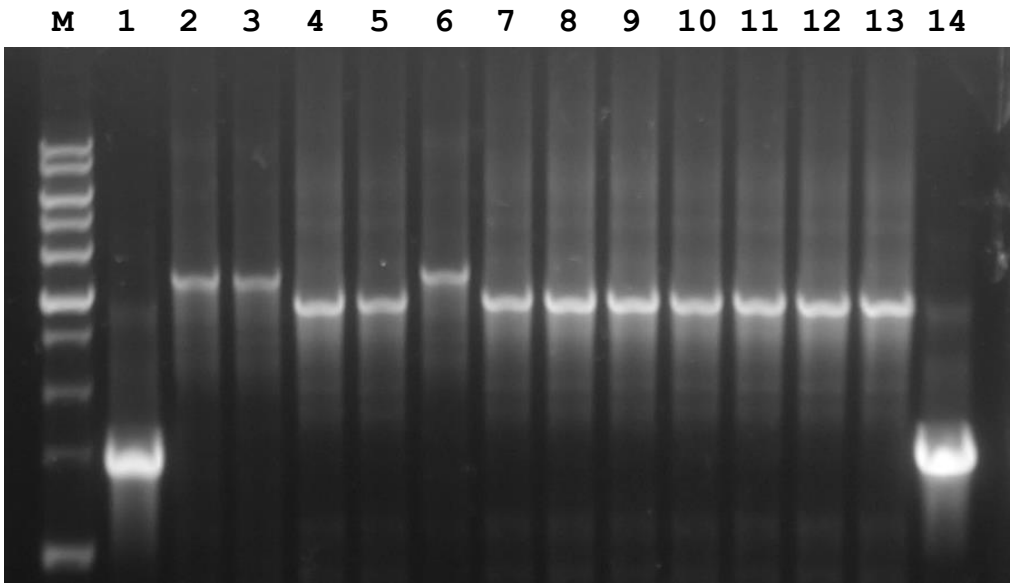
C. Transfer of construct to suicide vector

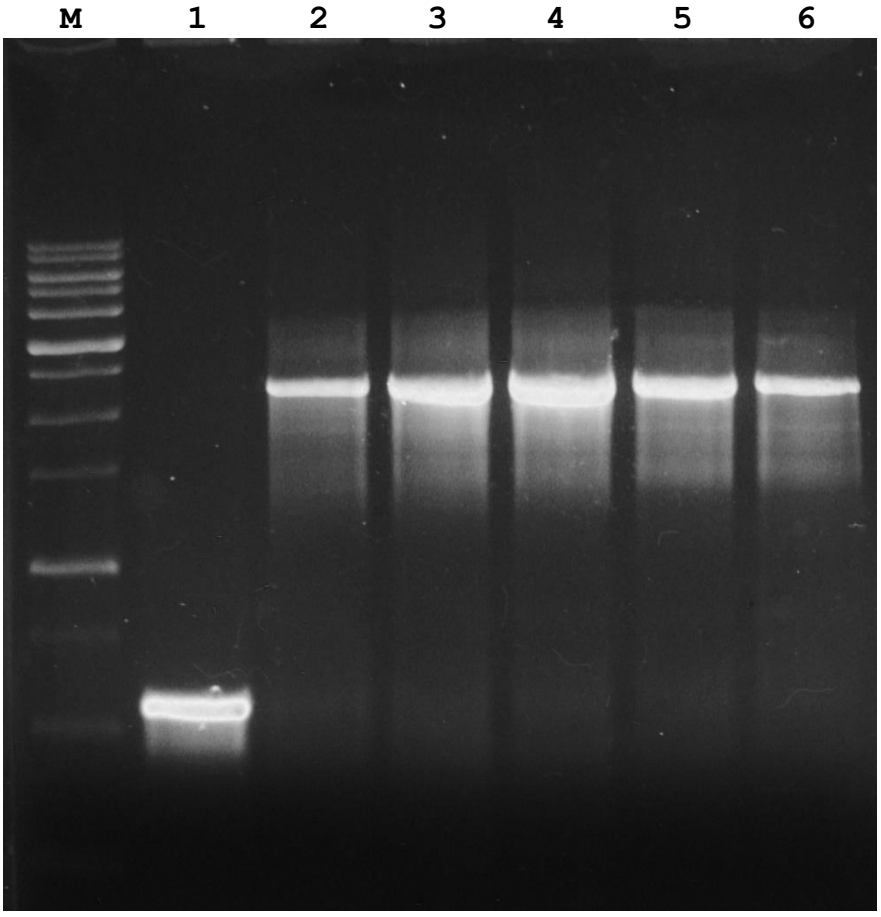


Supplemental Figure S2. Construction of pRIAM1177 (Δ*patS*)



Supplemental Figure S3. Segregation of mutations





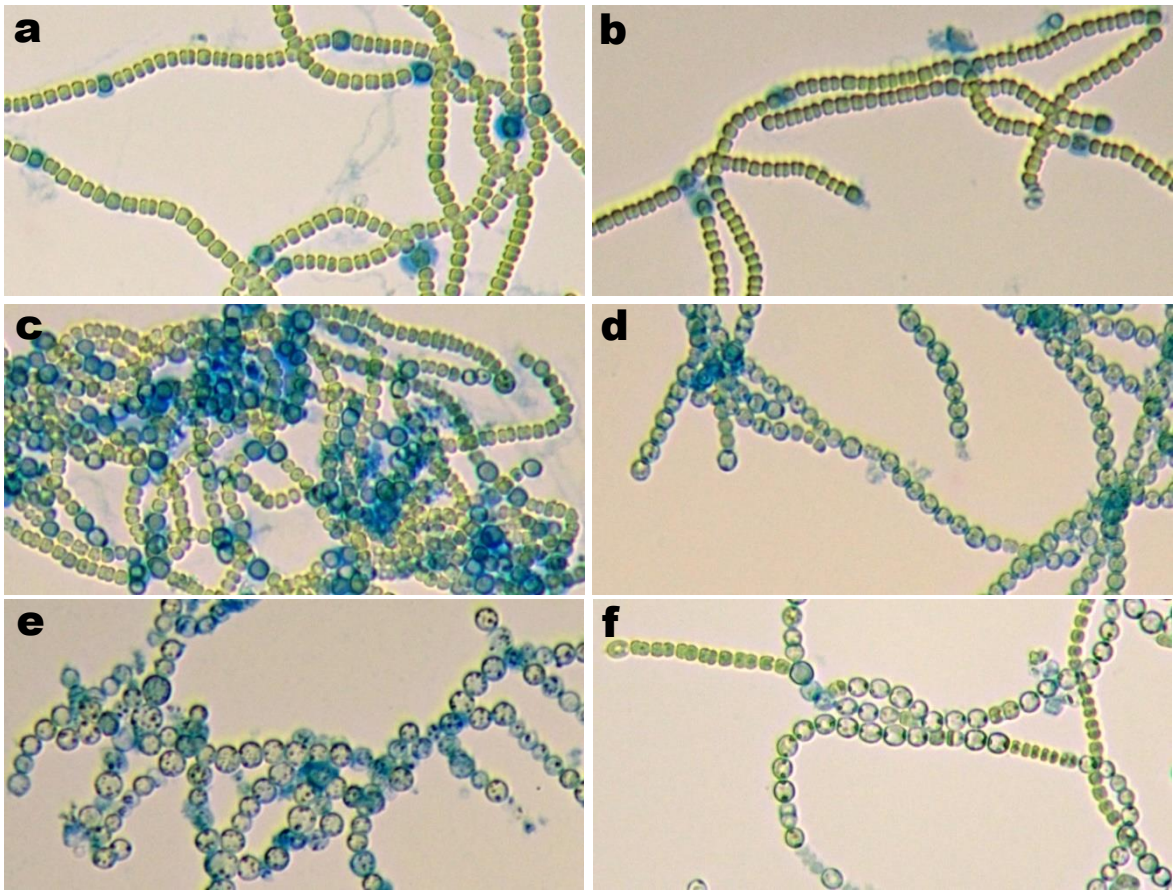
(top) **Segregation of *patX* mutations in constructed *patX* mutant strains.**
Primers 2332-F2 TATTGACACCATGCACACTT and 2331-F1 TCTCAGATGGAGCTCGTCATGCC were used for PCR using chromosomal DNAs from the following strains:

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

Supplemental Figure S4. Differentiation of mutant strains



Alcian blue staining of mutant strains PN (a), RIAM1242 (b), RIAM1250 (c), RIAM1248 (d), RIAM1243 (e), and RIAM1250 (f). Figures (a-d), strains 1 day after transfer to copper-free nitrogen-free BG-11_0 medium; (e) RIAM1243 3 days after transfer in copper-free nitrogen-replete BG-11 medium; (f) RIAM1250 3 days after transfer to copper-free, nitrogen-free BG-11_0 medium from a selective BG-11 Sp Sm Em plate (after several transfers on that medium). Segments of vegetative cells in filaments of differentiated heterocysts start to appear.