BOX-PCR

Preparation of samples to be amplified:

1. Grow overnight bacterial cultures and transfer 0.75 ml of *E. coli* or 1.5 ml of enterococci culture to labeled 1.5 ml microcentrifuge tubes.
2. Pellet cells by centrifugation for 1 minute.
3. Discard the supernatant and wash cells in 0.5 ml of deionized sterile water.
4. Repeat steps 2 and 3 so that the cells are washed a total of 2 times.
5. For *E. coli* , resuspend cells in 0.5 ml deionized sterile water. For enterococci, resuspend cells in 0.25 ml deionized sterile water.
6. Use 1 ul of the cell suspension for PCR.

PCR:

1. Count the number of samples to amplify and prepare master PCR mix. Multiply the following by the number of samples and combine in order, on ice:

	Stock Conc	Volume	Final Conc
Water		18.1 ul	
10X Buffer	10X	2.5 ul	1 X
$MgCl_2$	25 mM	3 ul	4.5 mM*
dNTPs	25 mM ea	0.25 ul	0.25 mM ea
Primer BOX A 1R	100 uM	0.5 ul	2 uM
JumpStart DNA Polymerase	2.5U/ul	0.4 ul	1U/reaction
Total		24 ul	

* The 10X buffer contains 15 mM $MgCl_2$; other components include 100 mM Tris (pH 8.3 at 25˚C), 500 mM KCl_2 and 0.01 (w/v) gelatin.

2. Add 24 ul of the master mix to 1 ul of cells in 0.2 ml PCR tubes. Cap and then mix well by tapping gently.

3. Spin tubes briefly to pellet liquid to the bottom and use the following thermal cycle program to amplify DNA:

   Single step at 95˚C for 2 min

   35 cycles of: 94˚C for 3 sec
   92˚C for 30 sec
   50˚C for 60 sec
   65˚C for 8 min

   Single step at 65˚C for 8 min.

The sequence of the PCR primer (BOX A 1R) is CTA CGG CAA GGC GAC GCT GAC G.