

## **Setting up of ERIC PCR and gel documentation and analysis**

Murugadas Vaiyapuri, Sreejith V. N., Vineetha Das and Toms C Joseph

### **Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)**

Highly conserved repetitive DNA sequences used for the typing of bacteria are the ERIC sequences that occurs in the intergenic (between genes coding for a protein) regions of polycistronic operons or present upstream or downstream of the untranslated regions of the bacterial genome. The ERIC sequences are a 126-bp imperfect palindromic sequences that shows similarity to REP sequences in many features. They are detected mainly in *E. coli* and *Salmonella typhimurium*. However, the typing method based on ERIC pattern is now expanded to the other bacteria within *Enterobacteriaceae* (Sharples et al., 1990).

ERIC PCR and analysis has three steps

### **Setting up of PCR with *E. coli* culture**

Perform ERIC-PCR reactions in duplicate for each isolate in 25 µl volume containing 3 µl of *E. coli* genomic DNA, 2.5mM MgCl<sub>2</sub>, 1U Taq polymerase, 0.2mM dNTPs, 1X PCR buffer, 1µM of each primer (ERIC 1 and ERIC 2), and final volume adjusted with nuclease-free water.

Carry out reaction in 0.2 mL PCR tubes, always in same thermal cycler (Nemoy et al. 2005; Mohapatra et al. 2007).

### **Gel documentation**

Perform the gel electrophoresis of PCR product in 3% agarose gel with 120V for 3h, and gel images are captured in the gel documentation system. Document twice for each gel with low and high exposure of trans UV light.

## **Bioinformatic analysis**

Using GelJ software normalise the banding pattern visually. Construct the phylogenetic tree of the selected isolates with GelJ software. Keep the DNA ladder (100bp) for the normalization of banding position. Construct the phylogenetic tree based on the similarity calculated by Pearson correlation between the fingerprints with the tolerance of 1%, and grouping of the fingerprints with the help of the unweighted pair group method using arithmetic averages algorithm (UPGMA) (Rasschaert et al. 2005).

\*\*\*\*\*