1. SCOPE

This SOP describes how to carry out ERIC PCR to identify strains of lactobacilli based on PCR of enterobacterial repetitive intergenic consensus (ERIC) sequences.

There was a lot of confusion, particularly around the PCR protocol. I present this protocol based on my person experiences. This is the protocol that for some reason has worked very well for me and is based on the protocol of Ventura and Zink (FEMS Microbiology Letters 2002).

2. PRINCIPLE

Enterobacterial repetitive intergenic consensus (ERIC) sequences are 127-bp imperfect palindromes that occur in multiple copies in the genomes of enteric bacteria and vibrios. Here we investigate the distribution of these elements in the complete genome sequences of nine *Escherichia coli* (including *Shigella* species) strains. There is a significant tendency for copies to be adjacent to more highly expressed genes. There is considerable variation among strains with respect to the presence of an element in any particular intergenic region, but some copies appear to have been conserved since before the divergence of E. coli and *Salmonella enterica*. In comparisons of orthologous copies between these species, ERIC sequences are surprisingly conserved, implying that they have acquired some function, perhaps related to mRNA stability. The relationships among copies within *E. coli* are consistent with a master copy mode of generation. Insertion of new copies seems to occur at, and involve duplication of, the dinucleotide TA. Two classes of inserts of about 70 bp each occur at different specific sites within ERIC sequences; these inserts evolve independently of the ERIC sequences. The small number of ERIC sequences in E. coli genomes indicates that a widely used bacterial fingerprinting method using primers based on ERIC sequences (ERIC-PCR) does not rely on the presence of ERIC sequences. (Portions copied verbatim from Wilson and Sharp, 2006, Mol Biol Evol 23(6)).

3. MATERIAL REQUIREMENTS

- Pure culture on solid media or in broth
- Sterile PBS or acceptable alternative
- Instagene matrix (BIORAD)
- Wide bore 200 µL tips
- Taq (enzyme 5U/µl, $MgCl_2$ (50mM), 10x buffer, dNTPs (10mM)
- ERIC1R primer (5-ATGTAAGCTCCTGGGGATTCAC-3) at 200 µM
- ERIC2 primer (5-AAGTAAGTGACTGGGGTGAGCG-3) at 200 µM
- Nuclease/DNA free H_2O
- Waterbath and/or heatblocks set to 56˚C and 100 ˚C

4. EXTRACTING DNA

1. Resuspend 1 colony in 1 mL of PBS **OR** transfer 200 µL of broth culture into 1.5 mL centrifuge tube.
2. Centrifuge 1 min @ ~5000 g
3. Discard supernatant and resuspend pellet in 200 µL of Instagene using wide bore tips and vortex well. **vortex Instagene thoroughly before use*
4. Transfer tubes to 56˚C for 20 min

5. Vortex well before transferring to 100˚C for 8 min. **Be careful, tubes may pop open due to heat, can put plastic closures on top to prevent this*
6. Centrifuge for 5 min @ ~16000g.
7. Use the supernatant as template. You can freeze without separating the pellet, but recentrifuge before use.

5. SETTING UP PCR

1. That all components on ice
2. Combine appropriate quantities below (add Taq last) into a mastermix and distribute 40 µL per tube.
3. Add 10 µL of the supernatant from the Instagene extraction.
4. Breifly Centrifuge to get rid of air bubbles

50 ul reaction	1 RXN (ul)
H2O	30.5
10x Buffer	5
dNTPs (10mM)	1
MgCl2 (50mM)	2.5
ERIC1R (200uM)	0.25
ERIC2 (200uM)	0.25
Taq (5U/ml)	0.5
Template (10ul)	10
Total	50

Table 5.1 PCR Master Mix for ERIC

6. RUNNING PCR

Run under the following parameters (ERICPCR on Eppendorf thermocycler in F3-127)
LID: 105 ˚C

3 min @ 94˚C

35 cycles of:
30 sec @ 94 ˚C
60 sec @ 48 ˚C
4 min @ 72 ˚C

Final elongation: 7 min @ 72˚ C

7. GEL ELECTROPHORESIS

I found, at least in lactobacilli, that small fragments (<3000 bp) tend to be most informative so I run 2% gels in TBE (better resolution of smaller fragments, though TAE would also be acceptable). I run 2 hours at 80V and load 20 µL with 2 µL of 10x loading dye (bromophenol blue) (Figure 7.1). Alternatively, a 3% gel, run over a very…very…very long gel can also be quite informative (Figure 7.2).

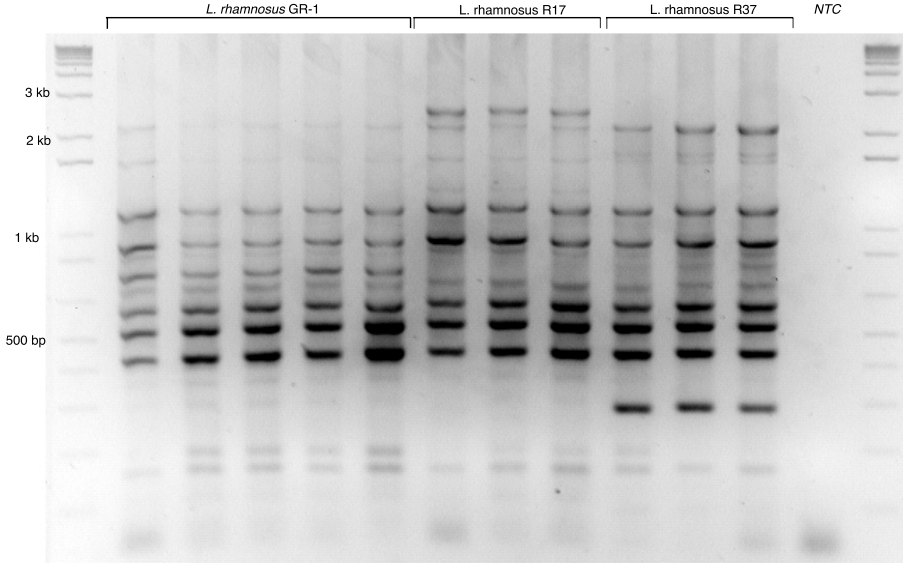

Figure 7.1 ERIC PCR of *L. rhamnosus* mutants. This work was done to ensure that transformed strains were the correct parent strain that this gel confirmed.

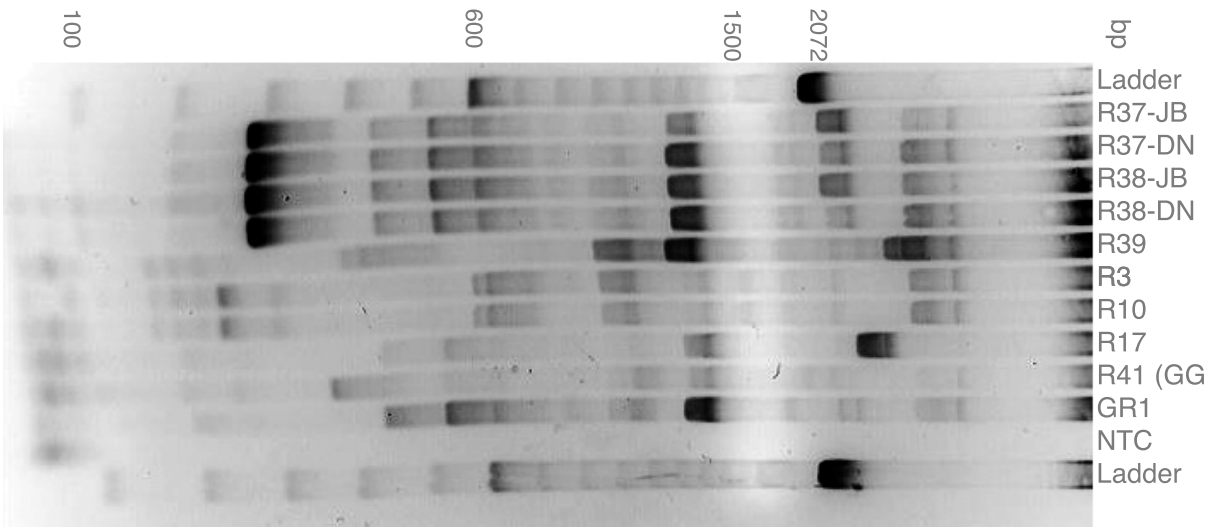

Figure 7.2. ERIC PCR profiled over a 3% gel (~1 foot long) can also be more informative by giving better separation of bands.