

# Differential gene expression in meat borne pathogens under cold stress conditions: random arbitrarily primed PCR in prokaryotic systems

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## Background

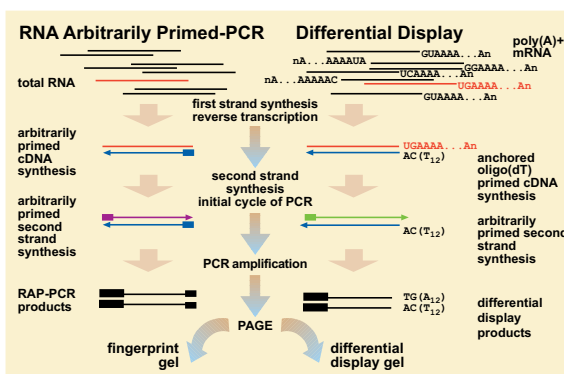
- The occurrence of psychrotolerant meat-borne microorganisms during meat processing and cold storage reduces the meat quality and shelf life, and may represent a threat to public health.
- One approach of resolving this problem is to detect and characterise genes that enable the bacteria to survive under cold stress.
- Random arbitrarily primed PCR (RAP-PCR) was applied in this study to identify differentially expressed genes in meat-borne pathogens in response to cold stress.

## Aims

- To understand the mechanisms of bacterial survival under cold stress conditions.
- To suggest strategies to eliminate or reduce the pathogens found in meat products during meat processing and cold storage by control of gene expression.

## RAP-PCR in prokaryotic systems

- RAP-PCR used in prokaryotic systems is technically challenging due to:
  - no equivalent to the polyadenylate tail in eukaryotic mRNA;
  - different GC content between different bacterial genomes.



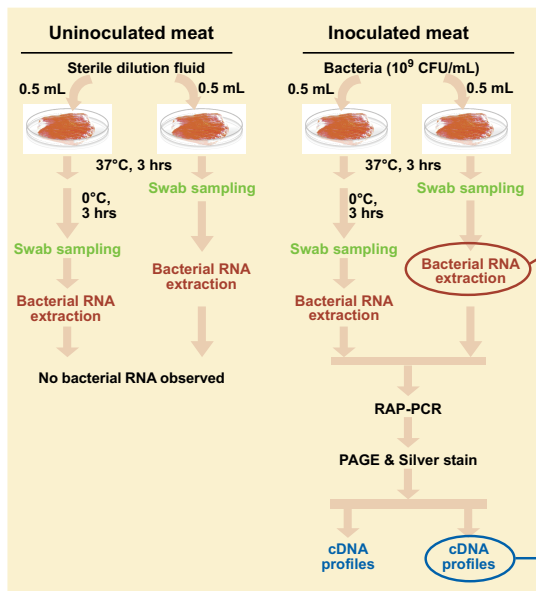
Modified from Bruce, 2002

## Primer design

- A computer program was written in Python language to calculate 6 mer, 8 mer and 10 mer frequency distribution using the nucleotide sequences of total protein coding genes from each strain.
- The frequency distribution was loaded in AgR Oracle based sequence database. Most commonly found 8 mers were found using database query language (SQL).

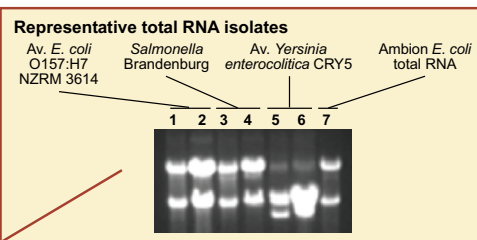
- For each 10 mer primer, the first 8 bases were derived from an 8 mer frequency distribution, and then further SQL queries were run to find out another most commonly found 8 mer harbouring last (or 3') six bases of the initial 8 mer. The two 3' bases from the latter 8 mer were added to the original 8 mer to make up the 10 mer.
- Another computer program was written in Python to find the location of each oligo in the protein coding genes and to find out the coverage of these oligos.

## Meat storage trial

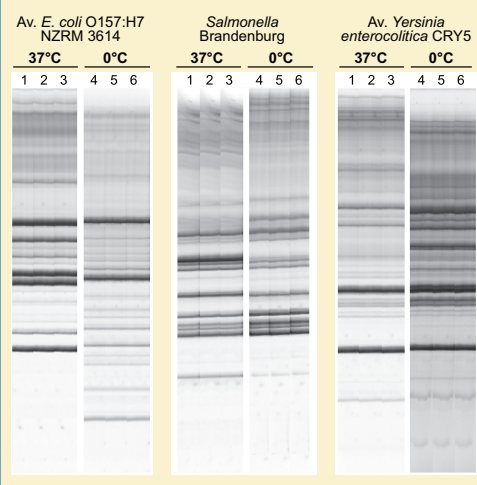


Reference  
Bruce, I. J. (2002) Bacterial differential gene expression (BDD-RTPCR) in synchronous cell cultures.  
[On-line] [www.gre.ac.uk/~i.j.bruce/present\\_2002.ppt](http://www.gre.ac.uk/~i.j.bruce/present_2002.ppt)

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Gene expression profiles generated from bacteria which were isolated from pre-inoculated meat surfaces before and after cold storage. A 10-bp in-house designed primer Salm 8 was used for RAP-PCR.



## Future work

- Isolating, cloning, and sequencing the differentially expressed genes in bacterial isolates on meat.
- Exploring the molecular mechanisms of bacterial survival under cold stress conditions.
- Eliminating or reducing the pathogens found in meat products during meat processing and cold storage by control of gene expression.