



DETECTION OF GENETIC CONTAMINATION IN CULTURES OF *Escherichia coli* by qPCR

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Introduction. Bacterial contamination during culture fermentations is becoming one of the more challenging problems in fermentative process, resulting necessary to find novel sensitive and economic detection methods to detect the contaminant bacteria (even close related bacteria at genetic level), long before they disrupt production (1). Manipulation of cultures can contaminate them. This problem also could happen during the preservation process of pure strains, e. g. preparation of glycerol stocks maintained at $-70\text{ }^{\circ}\text{C}$, resulting in the preservation of contaminated cultures assuming they are pure. In this contribution we evaluate the use of qPCR to detect contamination at genetic level in strains of *Escherichia coli* frozen in glycerol even caused by other *E. coli* strains used in the same laboratory.

Methods. We tested strains stored in glycerol, cells from isolated colonies, chromosomal DNA and cultures artificially contaminated using qPCR technique to identify contamination by other *E. coli* strains or microorganisms with a close similar genome to *E. coli*. Cell samples were used directly (without purification of DNA or RNA) and diluted in TE buffer and used for qPCR analysis (2). For cultured cells, an aliquot with $\text{O.D.}_{600\text{nm}} = 0.4$ was diluted (10^{-1} to 10^{-6}). For glycerol or colony samples it was used a sterile loop to pick up a sample and it was diluted in $20\text{ }\mu\text{L}$ of TE buffer. For chromosomal DNA, they were used $20\text{ ng}/\mu\text{L}$ of extracted DNA for qPCR reaction. For artificial contamination 250 mL flasks with 25 mL of Luria Broth (LB) were inoculated with an *E. coli* strain (SA22) at final cellular density $\text{O.D.}_{600\text{nm}} = 0.4$ and different dilutions of a contaminant *E. coli* strain.

Results. It is important notice that these experiments are not quantitative. They just indicate presence or absence of the selected genes (fig. 1, fig. 2). In this analysis we can detect amplimers of selected genes since $40\text{ cells}/\mu\text{L}$ or $0.0004\text{ OD}_{600\text{nm}}/\mu\text{L}$ as artificial contaminants in a cell cultures with an initial $\text{OD}_{600\text{nm}} = 0.4$, using cells without any purification protocol. Also we could detect amplification directly from frozen glycerol stored strains at $-70\text{ }^{\circ}\text{C}$, a single colony growing on agar plates or chromosomal DNA. Limitation of this protocol is the contaminants that we can detect. We know it is possible detect bacteria with a similar genome to *E. coli*, but we cannot detect other bacteria like e.g. *Bacillus* spp.

Conclusions. We develop a sensitive procedure to verify a specific bacterial genotype in stored *E. coli* strains, screening for mutant strains and contamination in fermentor by qPCR. This method qualitative, but is fast, sensitive and inexpensive for a limited number of samples. We can use chromosomal DNA, cells in culture, from colonies frozen in glycerol at $-70\text{ }^{\circ}\text{C}$.

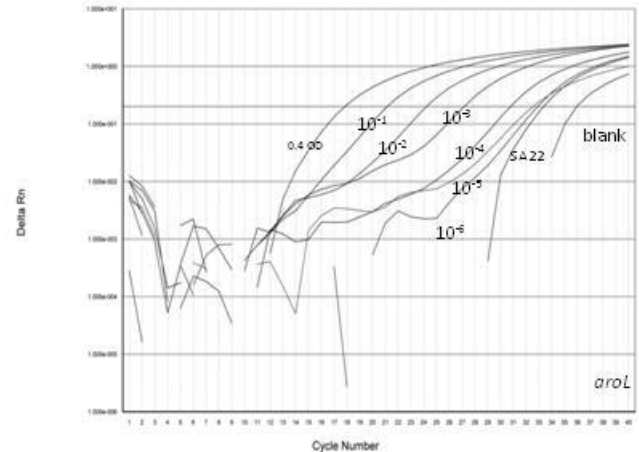


Fig.1 Artificial contamination in SA22 (PB12aroKL) with parental strain JM101. Dilutions were used from 0.4 OD_{600nm} to 10⁻⁶ in TE buffer.

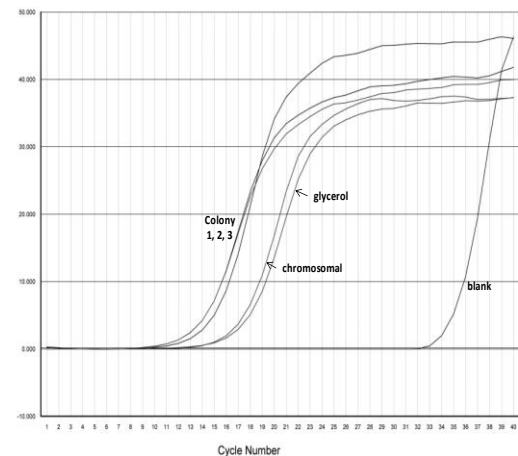


Fig 2. Detection of *ihfB* gene in different samples from glycerol stock, colonies (diluted in $20\text{ }\mu\text{L}$ of TE buffer) and chromosomal DNA ($20\text{ ng}/\mu\text{L}$). In all cases $1\text{ }\mu\text{L}$ was used for the analysis.

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