Fact Sheet on ActA Differentiation PCR & Interpretation of Results

What is actA? actA is a surface protein that induces accumulation of host-cell actin, which aides in motility and cell-to-cell spread. The protein is encoded by the actA gene, which resides in a virulence gene cluster on the bacterial chromosome of pathogenic *Listeria*.

Which Listeria species contains actA? Only 2 out of the 5 *Listeria* spp. are pathogenic and contain functional actA genes, *Listeria monocytogenes* and *Listeria ivanovii*. *L. ivanovii's* actA gene is ~3242 base pairs long and encodes for the iactA protein, whereas *L. monocytogenes* actA gene is ~1919 base pairs long. It should be noted that *L. ivanovii* is almost exclusively an animal pathogen, but *L. monocytogenes* is pathogenic to both animals and humans. *L. seeligeri* is non-pathogenic and contains a non-functional actA gene.

What is FSL R3-001? R3-001 is a genetically modified strain of wild-type *Listeria monocytogenes* created by the Food Safety Laboratory at Cornell University. R3-001 does not contain a functional actA gene. In fact, the majority of the actA gene has been removed from this strain.

What is the actA differentiation PCR? The actA differentiation PCR is a standardized PCR method that allows differentiation of the *L. monocytogenes* control strain FSL R3-001 from wild-type *L. monocytogenes* strains. FSL R3-001 is currently used as a genetically unique control strain by Silliker Laboratories and this procedure allows the user to determine whether *L. monocytogenes* isolates from test samples represent wild-type isolates or contamination with FSL R3-001.

How can we be sure this differentiation method works? Using the genome of *Listeria monocytogenes* as a template, primers (2 mpl-xba-F and actAmass-R) were designed to bind to the genes flanking the actA gene. Under specific PCR conditions this primer pair will generate a fragment of ~2400 base pairs from wild-type *Listeria monocytogenes*, but a fragment of only ~600 base pairs will be produced from genetically modified FSL R3-001. This primer pair does not amplify iactA from *L. ivanovii* or the non-functional actA from *L. seeligeri*.