REFERENCE
S9/P3

Development of novel peptide nucleic acid (PNA) probes for the rapid identification of pathogens by fluorescence *in situ* hybridization (FISH)

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Peptide nucleic acid (PNA) molecules are DNA mimics, where the negatively charged sugar-phosphate backbone is replaced by an achiral, neutral polyamide backbone formed by repetitive units of N – (2-aminoethyl) glycine[2,3]. Due to their superior hybridization properties, PNA probes to detect pathogens by fluorescent *in situ* hybridization (FISH) have been challenging DNA probes over the last few years (e.g. 1). In this work, we have designed and developed three new probes for the specific detection of *Enterobacter sakazakii, Staphylococcus epidermidis* and *Salmonella* spp. Probes were tested against several related species, and were shown to be specific for the microorganisms of interest. All three techniques were optimized in slides and then adapted for different types of samples, depending on the microorganism: *E. sakazakii* is a major contaminant of milk-based powdered infant formulas detection, and as such a membrane-based method to detect the pathogen after filtration of contaminated milk was devised; *Staphylococcus epidermidis*, which is frequently present on the skin of humans, had methods developed for its identification in contact lenses and catheters; and locations of interest for *Salmonella* spp. included pipes of drinking water distribution systems. Future work with PNA probes will involve multiplexing experiments (i.e. simultaneous detection of several species in a single sample) and application of flow citometry to compare fluorescent intensities.

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