

KimanTech Technical Application: 1-Step vs 2-Step PCR for Pathogen DNA Detection from Whole Blood

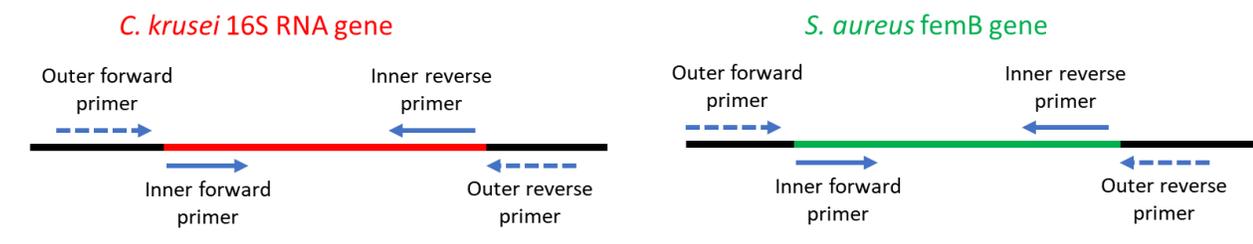
Background

Pathogen detection by PCR analysis has become an area of significant interest and product development. Many commercial systems can detect panels of pathogens but utilize expensive closed systems, preventing the use of existing laboratory equipment or biochemical modifications to suite user needs. Reagent kits exist that are compatible with standard laboratory equipment, but typically require significant upfront sample preparation steps and are prone to errors and PCR product contamination, particularly if two PCR steps are used. We envision the Alluvia system can be used to develop a simple, low cost, and relatively easy to use pathogen detection panel and simplified biochemistry.

To model a very simple sample preparation technique, we investigated if PCR can be performed directly from a blood sample. We utilized KlenTaq (DNA Polymerase Technologies, Inc.) to amplify pathogen DNA mixed with 20% human blood. The results demonstrate it is possible to achieve much clearer gel electrophoresis results and overcome qPCR signal inhibition by performing a second step of nested PCR.

Experimental Design and Methods:

Fig. 1. PCR strategy used in this study.



- Genomic DNA from cultured *C. krusei* and *S. aureus* (purified using a Roche genomic DNA spin column purification kit) was mixed with negative human whole blood (unspun, EDTA, disodium, purchased from Biological Specialty Cooperation).
- PCR using KlenTaq DNA polymerase (DNA Polymerase Technology, Inc.) and Power SYBR Green™ (Applied Biosystems/ThermoFisher) (all steps 40 cycles, anneal at 60C) was performed as follows:

Two-step (nested) PCR:

Step 1: Duplex PCR using KlenTaq and both primer pairs.

Step 2: Dilute PCR 100X in TrisEDTA buffer.

Step 3: Singleplex qPCR using Power SYBR Green and one of the inner primer pairs.

Single-step (direct) PCR:

Step 1: Singleplex PCR using Power SYBR Green and one of the inner primers pairs.

