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. Commercial systems exist that can detect panels of pathogens using both one-step and two-step PCR approaches but utilize enclosed systems (a sealed consumable). While enclosed systems are ideal for point-of-care applications, they are typically expensive, inflexible, and do not utilize existing laboratory equipment. Reagent kits exist that are compatible with standard laboratory equipment, but all use single-step PCR to avoid the added steps and contamination risks associated with two-step PCR. Since the Alluvia system can enable the development of two-step PCR pathogen detection reagent kits for standard laboratory equipment, we investigated if the added PCR step will significantly improve PCR results.

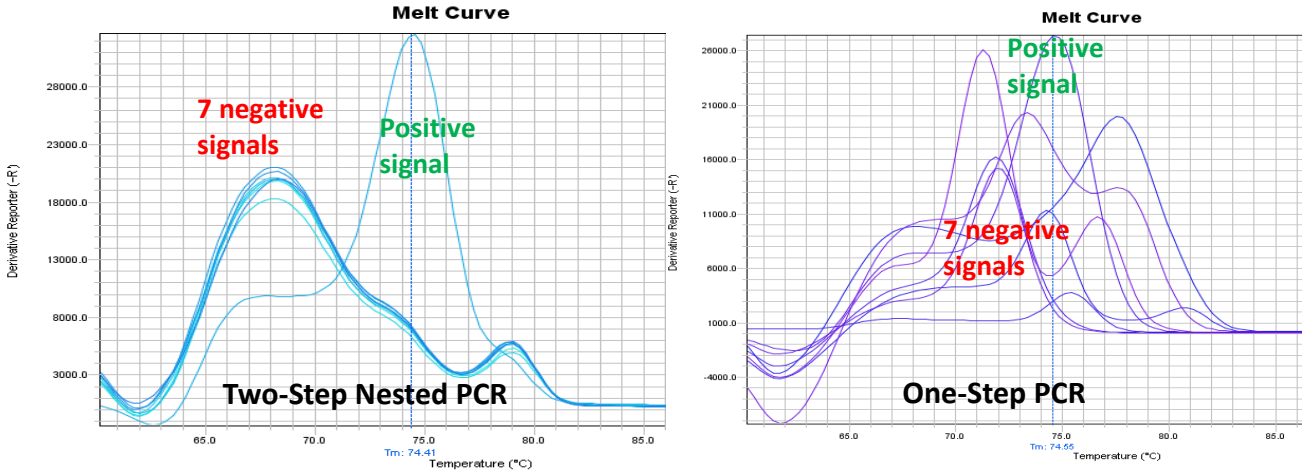
Experimental Design and Methods:

To compare two-step and one-step PCR approaches for pathogen detection, we have developed a two-step PCR panel to differentiate eight major pathogenic species of the yeast *Candida*, a causative agent of serious and often fatal blood infections. Four primers were designed to amplify the 26S ribosomal RNA gene from each strain of *Candida*, two outer primers for the first round of PCR and two nested inner primers for the second round of PCR. Each strain of *Candida* was cultured, and genomic DNA purified to be used as template. The assay was performed as follows.

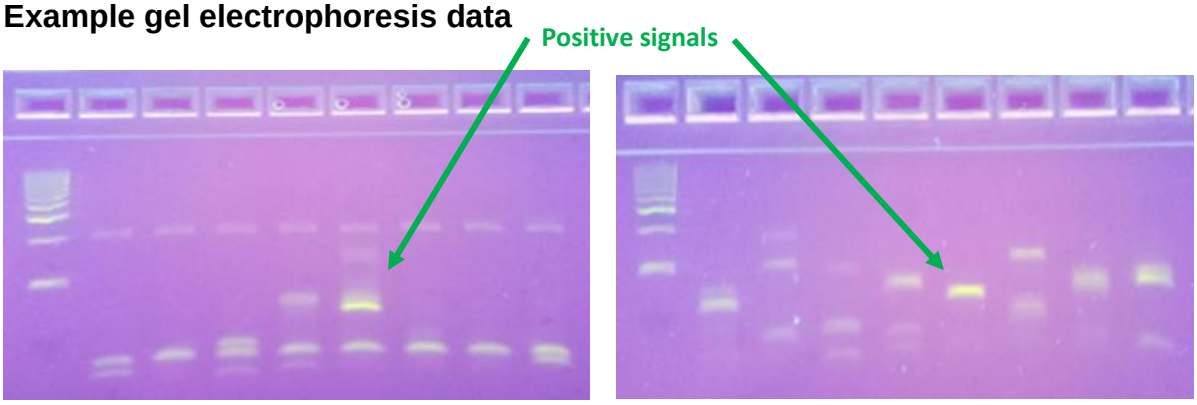
- Applied Biosystems™ (ABI) Power SYBR™ Green PCR master mix (used for all PCR reactions) along with all eight outer primers pairs were added to a single PCR tube, then 1000 copies of genomic DNA from one of the *Candida* species added.
- This primary multiplex PCR reaction was performed for 40 cycles using the ABI StepOne™ instrument (used for all PCR reactions).
- The next round of qPCR was performed by adding master mix and a different pair of nested primers to each well of an 8-well strip.
- To compare one-step to two-step qPCR, either 1000 copies of genomic DNA from one of the *Candida* species or a 100X dilution of the first-round multiplex PCR products were used as templates.
- The second round of qPCR was also performed for 40 cycles and a melting analysis performed.
- PCR products were also visualized on an electrophoresis gel.

Results:

Example qPCR melting curve results (from *C. guilliermondii*)



Example gel electrophoresis data



In this example, background signals exist with both one-step and two-step PCR methods. However, results with two-step PCR are easier to interpret.

Two-step qPCR specificity summary (based on visual analysis)

	Target Template Input (gDNA - 1000 copies for 9-plex preamplification)								Non-Target (gDNA - 100000 copies)		
Nested PCR Result	*C. albicans*	*C. auris*	*C.kruei*	*C. glabrata*	*C. guilliermondii*	*C. lusitanisiae*	*C. parapsilosis*	*C. tropicalis*	*C. dubliniensis*	*S. cerevisiae*	*S. pombe*
C. albicans PM	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C. auris PM	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C. krusei PM	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C. glabrata PM	Neg.	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C. guilliermondii PM	Neg.	Neg.	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
C. lustaniae PM	Neg.	Neg.	Neg.	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.	Neg.
C. parapsilosis PM	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.	Neg.
C. tropicalis PM	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	**Positive**	Neg.	Neg.	Neg.

One-step qPCR specificity summary (based on visual analysis)

	Target Template Input (gDNA - 1000 copies)								Non-Target (gDNA -1000 copies)		
Nested PCR Result	*C. albicans*	*C. auris*	*C.kruei*	*C. glabrata*	*C. guilliermondii*	*C. lusitanisiae*	*C. parapsilosis*	*C. tropicalis*	*C. dubliniensis*	*S. cerevisiae*	*S. pombe*
C. albicans PM	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. auris PM	Cross-talk	**Positive**	Neg.	Neg.	Cross-talk	Neg.	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. krusei PM	Cross-talk	Neg.	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. glabrata PM	Cross-talk	Cross-talk	Cross-talk	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Neg.
C. guilliermondii PM	Cross-talk	Cross-talk	Cross-talk	Cross-talk	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. lustaniae PM	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. parapsilosis PM	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	**Positive**	Cross-talk	Cross-talk	Cross-talk	Cross-talk
C. tropicalis PM	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	Cross-talk	**Positive**	Cross-talk	Cross-talk	Cross-talk

Summary: Two-step PCR significantly improves specificity allowing for more robust distinction of closely-related species of *Candida*.