Design, Development and Applications of the MycoSEQ™ Mycoplasma Scan (Myco Scan) Assay

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Introduction

Mycoplasma contamination remains a major problem for researchers using mammalian cell culture. Many studies have underlined the need for routine screening of cell lines since Mycoplasma infection is common in laboratories and can affect virtually every cell parameter, subsequently impacting the results of studies performed on those cell lines. Mycoplasma infection have been shown to deplete nutrients, promote metabolic accumulation resulting in pH shifts, induce or suppress cytokine expression, alter metabolism, proliferation characteristics and the morphology of cultured cells[1].

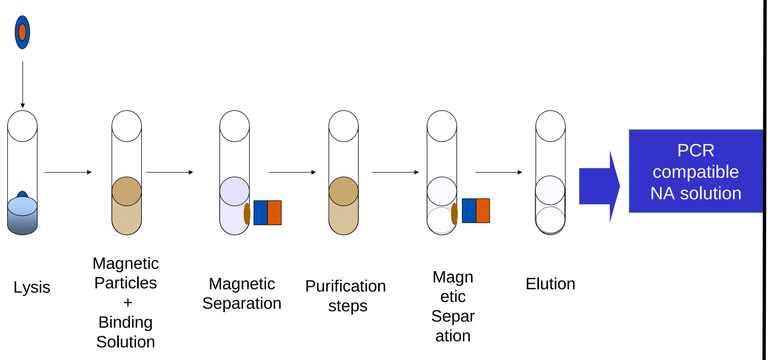
Mycoplasmas pose a challenge to detect and eliminate because of their small cell size, wide diversity of species, limited turbidity produced in culture, requirements for enriched culture media and some species/strains may be cell-invasive. All of these properties have posed a challenge to the development of a reliable rapid molecular test for Mycoplasma detection.

In this poster we present results from the Myco Scan Kit from Applied Biosystems that can be used for rapid detection Mycoplasma contamination using Real-Time PCR. Through intensive bioinformatics and highly optimized multiplexed primer design, the assay allows for highly sensitive, specific and comprehensive Mycoplasma species detection and prevents the detection of other closely related species. Providing results in less than five hours, the kit is a critical tool in testing the authenticity of valuable cell lines used in basic research.

Rapid 4 hour protocol from DNA extraction to presence absence call

PrepSEQ™ 1-2-3 Sample Prep (1 hour)

RNase/Proteinase treatment of sample



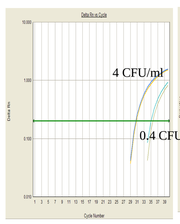
Real-Time PCR Assay Set Up (30 minutes)

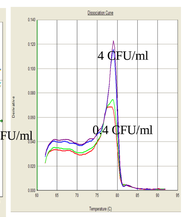
Component for premix solution	Volume for one 30-µL reaction (µL)
Power SYBR® Green PCR Master Mix (2X)	15.0
Mycoplasma Real-Time PCR Primer Mix (10X)	3.0
Total premix solution volume	18.0

To prepare...	Combine in each tube or well...
Negative-control reaction	• 18 µL of Premix Solution • 12 µL of Negative Control (water)
Your unknown sample reaction	• 18 µL of Premix Solution • 2 to 10 µL of unknown sample • Adjust the final reaction volume to 30 µL with Negative Control (water)
Inhibition-control reaction	• 18 µL of Premix Solution • 2 to 10 µL of unknown sample • 3 µL of Mycoplasma Real-Time PCR DNA Control (positive control) • Adjust the final reaction volume to 30 µL with Negative Control (water)
Positive-control reaction	• 18 µL of Premix Solution • 3 µL of Mycoplasma Real-Time PCR DNA Control (positive control) • 10 µL of Negative Control (water)

Real-Time PCR Run and Data Review (2.5 hours)

Step	AmpliTaq Gold® enzyme activation	PCR			Dissociation†	
	HOLD	Cycle (40 cycles)			Melt	
		Denature	Anneal/extend			
Temp	95 °C	95 °C	60 °C		95 °C	60 °C
Time	10 min	15 sec	1 min		15 sec	1 min



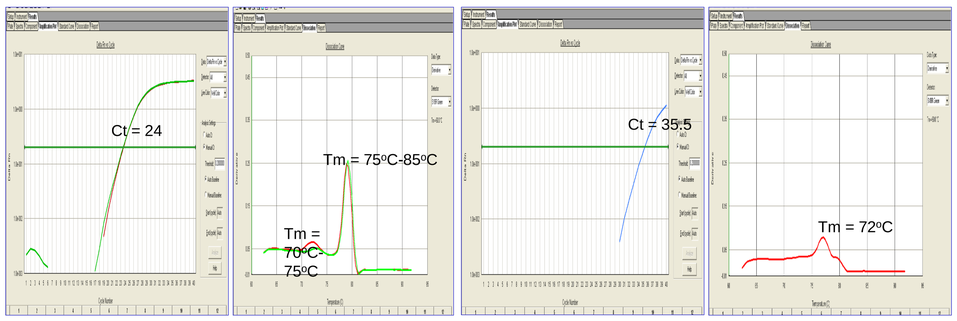


[1] Kagemann *et al.*, Impact of Mycoplasma hyorhinis infection on L-arginine metabolism: differential regulation of the human and murine iNOS gene. Biol. Chem. 2005 Oct; 386(10):1055-63.

Results from Mycoplasma positive and negative samples

Positive

Negative

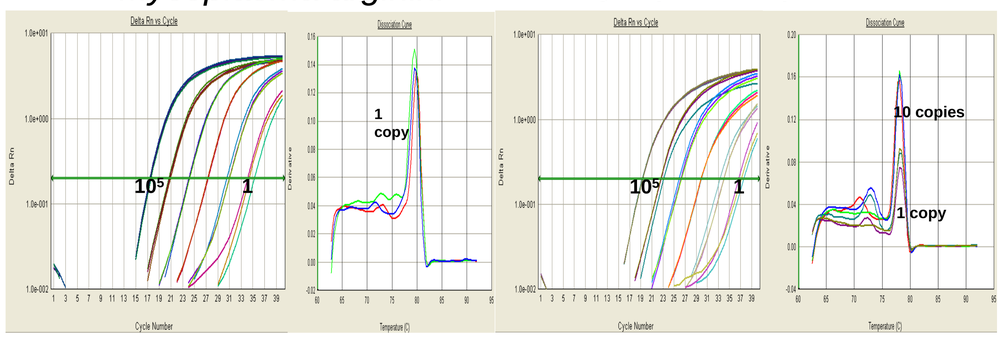


Highly optimized Power-SYBR® Green detection technology allows analysis of multiple parameters (Ct and Tm) for results interpretation

Sensitivity down to 1-10 copies/reaction

Mycoplasma arginini

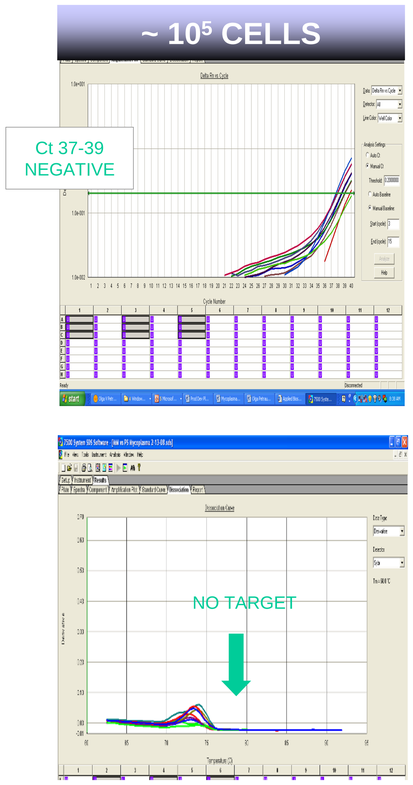
Mycoplasma pneumoniae



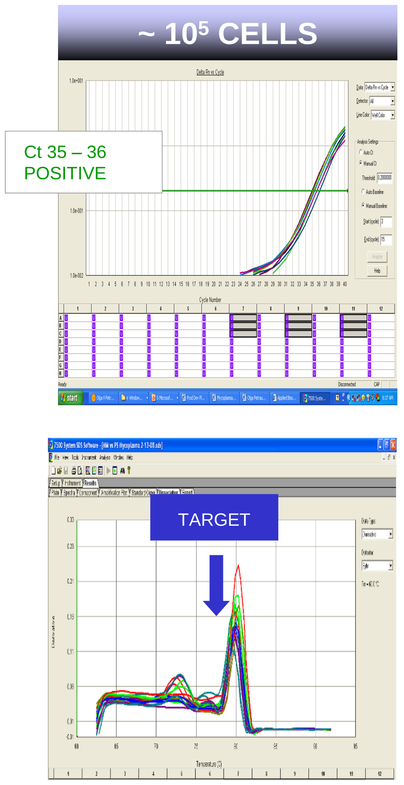
10-fold serial dilution of purified genomic DNA was tested using the assay

High sensitivity extraction of *M. bovoculi* gDNA using PrepSEQ™ 1-2-3 Kit

Commercial Sample Preparation



PrepSEQ™ Sample Preparation



M. Bovoculi DNA was spiked in cells and extracted using the PrepSEQ kit

Broad species detection

Detection of over 90 species

Acholeplasma granularum	*Mycoplasma genitalium*	*Mycoplasma synoviae*
Acholeplasma laidlawii	*Mycoplasma gypis*	*Mycoplasma testudinis*
Acholeplasma pleciae	*Mycoplasma hominis*	*Mycoplasma timone*
Mycoplasma alkalescens	*Mycoplasma hyorhinis*	*Spiroplasma citri*
Mycoplasma alvi	*Mycoplasma imitans*	*Spiroplasma endosymbiont*
Mycoplasma anseris	*Mycoplasma indiense*	*Spiroplasma insolitum*
Mycoplasma arginini	*Mycoplasma lagogenitalium*	*Spiroplasma kunkelii*
Mycoplasma auris	*Mycoplasma lipofaciens*	*Spiroplasma melliferum*
Mycoplasma buccale	*Mycoplasma mobile*	*Spiroplasma mirum*
Mycoplasma californicum	*Mycoplasma molare*	*Spiroplasma phoeniceum*
Mycoplasma canadense	*Mycoplasma mycoides*	*Spiroplasma poulsonii*
Mycoplasma capricolum	*Mycoplasma neurolyticum*	*Spiroplasma sp.*
Mycoplasma caviae	*Mycoplasma orale*	*Mycoplasma bovirhinis*
Mycoplasma collis	*Mycoplasma phocidae*	*Mycoplasma bovis*
Mycoplasma cricetuli	*Mycoplasma pirum*	*Mycoplasma bovigenitalium*
Mycoplasma equirhinis	*Mycoplasma pneumoniae*	*Mycoplasma canis*
Mycoplasma fermentans	*Mycoplasma salivarium*	*Mycoplasma felis*
Mycoplasma gallinaceum	*Mycoplasma simbae*	*Mycoplasma fastidiosum*
Mycoplasma gallisepticum	*Mycoplasma sp.*	*Mycoplasma muris*
Mycoplasma gateae	*Mycoplasma spumans*	*Mycoplasma pulmonis*

*No cross reactivity with Human, CHO, mouse and different microorganisms such as *E.coli*, *Bacilus cereus*, *Candida albicans*, *Staphylococcus aureus*, *Micrococcus luteus*, *Clostridium perfringens*

Summary

The Applied Biosystems Myco Scan Kit can be used for rapid and reliable detection of Mycoplasma contamination in mammalian cell culture:

→Designed for routine screening of high value cell lines for Mycoplasma contamination using highly sensitive Real-time PCR
→Rapid Time to Results in under five hours
→Detection of greater than 90 species
→Demonstrated sensitivity and specificity
→High DNA recovery sample preparation