End point PCR† for MRSA from broth with TaqMan® probes

A. Introduction

This protocol uses end point PCR† with Minor Groove Binder™ (MGB) TaqMan® probes to test for Methicillin-resistant *Staphylococcus aureus* (MRSA) from broth cultures. There are two target sequences in this assay. The *mecA* gene codes for methicillin resistance and the *nuc* gene codes for a nuclease specific to *S. aureus*.

The Spartan DX™ instrument has a single channel for fluorescence detection. The presence of each gene (*mecA* and *nuc*), and their corresponding negative controls, are tested separately as singleplex reactions.

DNA extraction from broth is about 20 min. PCR run time is about 34 min.

B. Equipment

- Spartan DX instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

C. Materials

- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Cat. No. 11730-017)
- Sterile water (DNase-free)
- TE Buffer (50 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, pH 8)
- Achromopeptidase (40 U/µl in TE buffer, stored at -20°C) (Sigma-Aldrich, Cat. No. A3547)
- 1.5 ml microcentrifuge tubes
- Primers which recognize *mecA* and *nuc* genes (Sigma-Genosys)(see Table 1)
- MGB TaqMan probes which recognize *mecA* and *nuc* genes (Applied Biosystems)(see Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
mecA primers	TCC Agg AAT gCA gAA AgA CCA A	ggC CAA TTC CAC ATT gTT TCg	92
mecA probe	6-FAM-CAT ATT gAA AAT TTA AAA TCA gAA CgT g-MGBNFQ		
nuc primers	gCg ATT gAT ggT gAT ACg gTT	AgC CAA gCC TTg ACg AAC TAA AgC	279
nuc probe	6-FAM CTg AAg CAA gTg CAT TTA-MGBNFQ		

6-FAM = 6-carboxy-fluorescein, MGB = minor groove binder, NFQ = non-fluorescent quencher

Table 1. Primer/probe sequences and amplicon sizes.

D. Preparation

1. Prepare fresh ATE buffer (195 µl TE buffer + 5 µl of 40 U/µl achromopeptidase)
2. Transfer a 50 µl aliquot of overnight broth culture into 200 µl of freshly prepared ATE buffer
3. Incubate at 30°C for 10 min
4. Boil samples for 10 min to inactivate achromopeptidase
5. Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
6. Set up cycling program as per Table 2

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Step	Temperature	Time	Cycles
Initial denaturation	95°C	44 s	1
Denaturation	95°C	16 s	40
Annealing/extension	57°C	26 s	40

Table 2. Cycling parameters.

E. Protocol

1. Prepare two separate *mecA* and *nuc* master mixes, as per Table 3
2. Since the Spartan DX instrument holds 4 tubes, we recommend setting up reactions as follows:
   - Tube 1: 18 µl of *mecA* master mix
   - Tube 2: 18 µl of *nuc* master mix
   - Tube 3: 18 µl of *mecA* master mix
   - Tube 4: 18 µl of *nuc* master mix
3. In a separate lab area, add the following to each tube:
   - Tube 1: 2 µl of extracted DNA (sample in question)
   - Tube 2: 2 µl of extracted DNA (sample in question)
   - Tube 3: 2 µl of sterile water (*mecA* negative control)
   - Tube 4: 2 µl of sterile water (*nuc* negative control)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reactions again
7. Insert tubes into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater that 5
9. Table 4 lists possible genotypes.

Reagent	***mecA* Master Mix**		***nuc* Master Mix**	
	Reaction Formulation	**Volume**	**Reaction Formulation**	**Volume**
QUANTIPROBES Reaction Mix (2X)	10 µl x (______+0.5*) Sample #		10 µl x (______+0.5*) Sample #	
Forward primer (10 µM)	2 µl x (______+0.5*) Sample #		2 µl x (______+0.5*) Sample #	
Reverse primer (10 µM)	2 µl x (______+0.5*) Sample #		2 µl x (______+0.5*) Sample #	
Probe (0.5 µM)	2 µl x (______+0.5*) Sample #		2 µl x (______+0.5*) Sample #	
Sterile water	2 µl x (______+0.5*) Sample #		2 µl x (______+0.5*) Sample #	
Total volume of master mix	**18 µl/reaction**	**____ µl**	**18 µl/reaction**	**____ µl**

* Recommended volume correction factor for pipetting error.

Table 3. Primer/probe mixes for *mecA* and *nuc*.

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Genotype	*mecA*	*nuc*
MRSA (Methicillin-resistant *Staphylococcus aureus*)	+	+
MSSA (Methicillin-sensitive *Staphylococcus aureus*)	-	+
MR-CNS (Methicillin-resistant coagulase-negative *Staphylococci*)	+	-
MS-CNS (Methicillin-sensitive coagulase-negative *Staphylococci*)	-	-

Table 4. Possible genotypes.

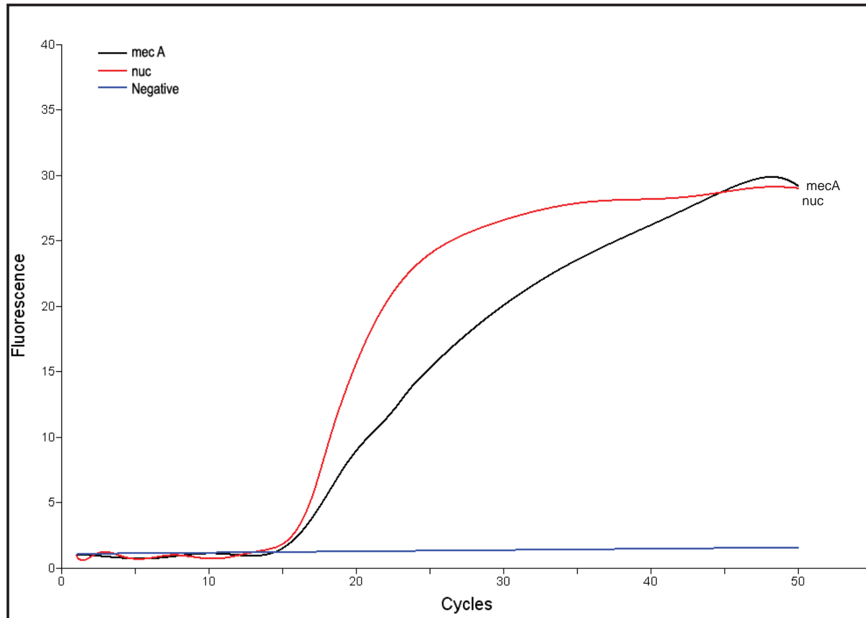

Figure 1. Real-time graph of *MRSA* results.

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.

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