	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*		
Title: invA colony PCR for Salmonella			
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invA colony PCR for Salmonella

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for performing PCR to amplify a 678 bp fragment of the *invA* gene to be used for *Salmonella* spp. confirmation.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases, and the Milk Quality Improvement Program.

1.3 Definitions

dNTPs: deoxyribonucleotide monomers, which are the building blocks of DNA. There are four dNTPs, cytosine, adenine, guanine and thymine.
GoTaq Green: A PCR kit containing thermostable Taq polymerase, $MgCl_2$, and a PCR buffer which contains a green loading dye. This loading dye does not interfere with the PCR and saves the step of loading dye incorporation prior to running gel electrophoresis.
invA: Gene encoding the invasion protein A in *Salmonella* spp.
Master Mix: a mix of all the components required for PCR (taq polymerase if manual hot start, buffer, and $MgCl_2$), except the DNA template.
$MgCl_2$: a cofactor for the polymerase.

1.4 Safety

Be aware when handling BSL-2 pathogens. Appropriate protective measures must be taken. All waste from these experiments needs to be treated as BSL-2 waste.

SECTION 2 MATERIALS

- **Sterile water** - Room 358B and Room 350A
- **Sterile 0.2 mL tubes** or **Sterile 96-well plate** - Room 358B and Room 350A
- **invA-F primer** (5' – GAATCCTCAGTTTTTCAACGTTTC) – 12.5 µM. Room 352C, chest freezer
- **invA-R primer** (5' – TAGCCGTAACAACCAATACAAATG) – 12.5 µM. Room 352C, chest freezer
- **GoTaq DNA Polymerase** - Room 352, "Dumb" freezer
- **GoTaq Flexi 5X PCR buffer** - Room 352, "Dumb" freezer
- GoTaq $MgCl_2$ (25mM) - Room 352, "Dumb" freezer
- **dNTP solution** - consists of 1 mM each dATP, dGTP, dCTP, dTTP dNTP stocks in Room 352, "Dumber" freezer, in covered cryoblock
- **Vortex Mixer** - Room 358B
- **Miniature Centrifuge** - Room 358B
- **Thermocycler** - Room 356
- **Micropipette and sterile filter tips**
- **Crushed Ice**

SECTION 3 PROCEDURES

3.1 Lysate Preparation

3.1.1 Prepare lysates according to the protocol listed in section 3.2 "Microwave Lysis (Dry Colony)" in SOP 8.3.18 "Preparing Cell Lysates for PCR."

3.2 Master Mix Preparation

3.2.1 Gather master mix reagents (see Table 1, below) and place on ice. Go to room 358B for steps 3.2.2 to 3.2.5.

3.2.2 Combine the reagents listed in Table 1, expect the GoTaq DNA polymerase, and store on ice. Make sure to prepare 10% more master mix than will be required to all PCR reactions (including positive and negative controls) to account for pipetting error.

3.2.3 Vortex the master mix.

3.2.4 Add the GoTaq DNA polymerase. Mix well using a pipette. After the GoTaq DNA polymerase is added, the master mix should not be vortexed to avoid denaturing the polymerase.

3.2.5 Briefly spin down the tube containing the master mix to concentrate all components at the bottom on the tube.

3.2.6 Go to a lab bench. Distribute 49µL of master mix into each of the PCR reaction tubes (prepared in step 3.1).

3.2.7 Briefly centrifuge PCR reaction tubes to concentrate all components at the bottom of the tubes.

Table 1. Master mix reagents.

Reagent (starting concentration)	Volume (µL)
dH_2O	31
5x GoTaq Flexi Buffer	10
$MgCl_2$ (25 mM)	3
dNTPs (10 mM, each)	1
invA-F primer (12.5 µM)	2
invA-R primer (12.5 µM)	2
GoTaq DNA Polymerase	0.25
Total	49.25

3.3 Thermocycling Conditions

3.3.1 Place the PCR reaction tubes in a thermocycler and place a compression pad on top of the tubes.

3.3.2 Program and run the thermocycler according to the conditions listed in Table 2.

Table 2. Thermocycler conditions.

Temperature (°C)	Time	No. of Cycles
94	2 min	1
94	30 s	20
60	30 s	
72	30 s	
72	5 min	1
4	∞	

SECTION 4 TROUBLESHOOTING

(1) If colony PCR does not work, try making dirty lysates according to the protocol listed in section 3.3 "Heat Lysis" in SOP 8.3.18 "Preparing Cell Lysates for PCR."

SECTION 5 REFERENCES

Kim, J. S., Lee, G. G., Park, J. S., Jung, Y. H., Kwak, H. S., Kim, S. B., Nam, Y. S., & Kwon, S. T. 2007. A novel multiplex PCR assay for rapid simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. *J Food Prot*. 70(7):1656-1662.

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	08/08/2016		Original SOP
Version 2	01/07/2020	Alexandra Belias	(1) Revised format of original SOP according to the Cornell FSL/MQIP SOP template (2) Added Section 1 Introduction, Section 2 Materials, Section 5 References, and Section 6 Method Version & Changes (3) In Section 3 Procedures, descriptions of the steps to follow for master mix preparation and thermocycling were added