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Standard Uniplex PCR SOP for *Escherichia coli* and *Salmonella*

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Justine Condon[1], Lisa Durso[2]
[1]USDA-ARS; [2]USDA - ARS

Justine C. Condon
USDA-ARS

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We use this protocol and it's working

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Disclaimer

Abstract

To describe the procedures on how to set-up polymerase chain reaction (PCR) and process gel electrophoresis for the detection of *Escherichia coli* (*E. coli*) and *Salmonella*.

Guidelines

Scope

E. coli is a gram-negative rods genus belonging to the Enterobacteriaceae family. They are commonly found in the lower intestinal regions of humans and animals. Most are part of the normal microbiota and can be harmless or beneficial. Some serotypes are pathogenic and can cause severe gastrointestinal illness as a food-borne disease. (WHO, 2018). The *uidA* gene, which encodes the beta-glucuronidase enzyme, was detected in 97.7% of 435 *Escherichia coli* isolates (Martins, 1993) and is used as an indicator of *E. coli*.

Salmonella is a gram-negative rods genus belonging to the Enterobacteriaceae family. *Salmonella* is a ubiquitous and hardy bacterium that can survive several weeks in a dry environment and several months in water. (WHO, 2018). Many *Salmonella* serovars have a broad host range and can infect a wide variety of animals, including mammals, birds, reptiles, amphibians, and insects. In addition, *Salmonella* can grow in plants and can survive in protozoa, soil, and water. (Silva, 2014). They are a group of bacteria that can cause gastrointestinal illness and fever called salmonellosis. It's a common food-borne illness often spread through contaminated food items, or direct contact with infected animals and feces. *invA* is a common molecular target for Salmonella-specific detection methods and is recommended by the U.S. Food and Drug Administration Bacteriological Analytical Manual as a target for PCR confirmation of putative *Salmonella* isolates (Buehler, 2019).

Pathogens and antimicrobial resistance are a global public health concern, and tracking indicators assist in surveillance and mitigation efforts. This protocol describes the procedures used to detect the presence of the *uid*A gene from *E. coli* and *invA* gene from *Salmonella* using standard PCR, with detection via gel electrophoresis.

Responsibility

This SOP applies to all staff members and students. These individuals must be knowledgeable about the requirements set forth within this document. The lab manager or designee shall ensure that all staff and students know the proper techniques.

Materials

PCR

- Forward and Reverse Primers (IDT, Coralville, IA) for:

1. ***invA* - *Salmonella*** (Sequences in **Table 2**)
2. ***uidA* – *E. coli*** (Sequences in **Table 4**)

- JumpStart™ REDTaq® ReadyMix™ Reaction Mix **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P0982-800RXN , St. Louis, MO)
- Molecular Biology Grade Water **HyClone Catalog #**SH30538.01 , St. Louis, MO)
- Positive Control – Salmonella typhimurium (for *invA*)
- Positive Control – STEC O157 (for *uidA*)
- PCR Prep Cabinet (3620804 Labconco, Kansas City, MO)
- 0.2mL x 8 PCR reaction strips (T320-2N, Simport, Quebec, Canada)
- 2mL sterile microcentrifuge tube (02.681.299 Fisher Scientific, Pittsburg, PA)
- Aerosol-barrier, no-retention pipette tips for various volumes from 1-1000µL
- Pipettors for various volumes from 1-1000µL
- Thermal cycler (T100, BioRad, Hercules, CA)
- Mini Centrifuge (C1301P, Labnet International, Big Flats, NY)

Gel Electrophoresis

1. Agarose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9539 500g, St. Louis, MO)
2. 1X Sodium Borate (SB) Buffer (SB20-4 20X SB Buffer FasterBetter Media, Hunt Valley, MD) (Recipe **Appendix A - Step 34**)
3. Microwave
4. Silicone Hot-Pad gloves
5. SYBR™ Safe DNA Gel Stain **Thermo Fisher Scientific Catalog #**S33102 , Carlsbad, CA)
6. PCR amplicons
7. DirectLoad™ PCR 100 bp Low Ladder **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D3687-1VL , St. Louis, MO)
8. 500mL or 1-Liter sterile Pyrex glass bottle
9. Gel casting tray, gel combs, and gel boat
10. Electrophoresis machine (PowerEase 300W, Life Technologies, Carlsbad, CA)
11. Micro-Pipettors and appropriate tips (10-20µL)
12. Gel imaging apparatus (Gel Doc XR+, BioRad, Hercules, CA)
13. Lighter

Procedure - *invA* for *Salmonella* PCR

15m 20s

1 If frozen, thaw all reagents and samples and keep on an ice block or ice for stability. If kept in the refrigerator, keep there until ready for use, and keep on an ice block or ice for stability when in use.

2 Label tubes for the samples and controls.

3 Prepare the PCR Prep area for setting up a PCR reaction to eliminate contamination – UV PCR tubes, PCR water, and centrifuge tube for at least 00:15:00 .

15m

4 Combine the Master Mix components for the number of reactions (allow for 10% extra Master Mix) using the recipe from **Table 1** with the correct primer sets in **Table 2** for 25 µL reactions.

20s

- Before adding to the Master Mix - mix the primers by aspirating and expelling with the pipette tip.
- Vortex the completed Master Mix for at least 00:00:20 . Centrifuge briefly.

	A	B	C	D
	Component	**Description**	**Volume/RXN (µL)**	**Final Concentration**
	TAQ	JumpStart RedTaq ReadyMix Reaction Mix (P0982)	12.5	1X
	PCR Water	PCR Water (HyClone SH30538.01)	7.3	N/A
	Primer 1	Forward Primer (100µM)	0.1	2µM
	Primer 2	Reverse Primer (100µM)	0.1	2µM
	Sample	Template	5	1µL

Table 1. PCR Master Mix recipe. 25µl total reaction volume.

	A	B	C	D	E	F	G
	Gene	**Type**	**Sequence 5' to 3'**	**TM (°C)**	**TC Conditions**	**Amplicon Size (bp)**	**Sequence Reference**
	invA	Forward (invA139) Reverse (invA141)	GTGAAATTATCGCCACGTTCGGGCAATCATCGCACCGTCAAAGGAACC	64	1 cycle at 94°C for 10 min; 30 cycles at 94°C for 30 sec, 64°C for 30 sec, 72°C for 30 sec; 1 cycle at 72°C for 5 min.	284	Rahn et al., 1992

Table2. Primer sequences, melting temperature, and thermal cycling conditions.

5

Note

Check the pipette's volume as you go along, making sure it is exactly at the correct volume.

6 Carefully pipette 5 µL of PCR Water to the non-template control (NTC) tube.

7 Add 20 µL of Master Mix to each labeled PCR tube.

8 Next, gently vortex to mix then pipette 5 µL of sample (5 µL of working stock; or 1 µL straight DNA + 4 µL PCR water) into its same labeled tube.

Note

- **Use a new pipette tip for each sample**
- To make a working stock dilution, see **SOP: IDT Primer and gBlock Hydration and Aliquots**

9 Add 1 µL of positive control (mix first by aspirating and expelling with the pipette tip) to its same labeled tube.

10 Centrifuge the strip tubes so that the entire volume is at the bottom.

Note

Make sure the centrifuge is balanced with both sides containing tubes.

11 Load the tubes into the Thermocycler, keeping them in order.

12 Find the programmed gene conditions and double check that they match the conditions listed in **Table 2**.

	A	B	C	D	E	F	G
	Gene	**Type**	**Sequence 5' to 3'**	**TM (°C)**	**TC Conditions**	**Amplicon Size (bp)**	**Sequence Reference**
	invA	Forward (invA139) Reverse (invA141)	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	64	1 cycle at 94°C for 10 min; 30 cycles at 94°C for 30 sec, 64°C for 30 sec, 72°C for 30 sec; 1 cycle at 72°C for 5 min.	284	Rahn et al., 1992

Table2. Primer sequences, melting temperature, and thermal cycling conditions.

13 Run PCR

13.1 When cycle is complete:

- Gel electrophoresis (See Section **Procedure Gel Electrophoresis** go to step #17)
  OR
- Store the tubes in a labeled rack at -20 °C until ready to run a gel.
- **Turn off the Thermocycler.**

Procedure - *uid*A for *E. coli* PCR

20s

14 Follow **Steps 1 – 3** go to step #1 to prep for PCR.

15 Combine the Master Mix components for the number of reactions (allow for 10% extra Master Mix) using the recipe from **Table 3** with the correct primer sets in **Table 4** for 25 µL reactions. 20s

- Before adding to the Master Mix - mix the primers by aspirating and expelling with the pipette tip.
- Vortex the completed Master Mix for at least 00:00:20 .

	A	B	C	D
	Component	**Description**	**Volume/RXN (µL)**	**Final Concentration**
	TAQ	JumpStart RedTaq ReadyMix Reaction Mix (P0982)	12.5	1X
	PCR Water	PCR Water (HyClone SH30538.01)	7.3	N/A
	Primer 1	Forward Primer (100µM)	0.1	2µM
	Primer 2	Reverse Primer (100µM)	0.1	2µM
	Sample	Template	5	1µL

Table 3. PCR Master Mix recipe. 25µl total reaction volume

	A	B	C	D	E	F	G
	Gene	**Type**	**Sequence 5′ to 3′**	**TM (°C)**	**TC Conditions**	**Amplicon Size (bp)**	**Sequence Reference**
	uidA	Forward (uidA241) Reverse (uidA383)	CAGTCTGGATCGCGAAAACTG ACCAGACGTTGCCCACATAATT	63	1 cycle at 95°C for 1 min; 40 cycles at 94°C for 10 sec, 63°C for 40 sec, 72°C for 30 sec; 1 cycle at 72°C for 5 min.	142	USDA SOP: MDP-MTH-11

Table 4. Primer sequences, melting temperature, and thermal cycling conditions.

16 Follow **Steps 5 – 13** go to step #5 using **Table 4** to run the PCR.

- Since the total reaction volume is 25 µL, pipette 20 µL of Master mix into each tube then 5 µL of sample or 1 µL control.

Procedure - Gel Electrophoresis

2h 11m 30s

17 Using a spoon, measure out 2.6 g of powdered agarose into a weigh boat.

18 Measure 260 mL of **1X SB** buffer into a graduated cylinder.

19 Add powdered agarose and **1X SB** into 1L bottle and swirl to mix.

20 Microwave **SB** and agarose mixture for 00:01:00 with bottle lid LOOSE. 1m

21 Remove bottle from microwave (**using hot pad gloves**), tighten the lid and gently swirl.

22 LOOSEN the lid and put in microwave for another minute.

23 Check mixture visually, if flecks are present repeat go to step #21, loosen lid and microwave again for 00:00:30. 30s

24 Add 26 µL of SYBR safe to the molten gel.

25 Swirl gently with a loose lid to distribute the SYBR.

26 Place bottle in 55°C water bath for 00:10:00 to temper. 10m

27 While the mixture is tempering, set up gel cast by putting two plastic stoppers into the ends of the cast, with the orange rubber side of the stopper facing out.

28 Put two (or three) 36-well combs into the first and fourth notches of the gel cast (gel cast is right side up if first notch is close to the top of the cast).

29 After the gel has tempered, slowly pour it (**using hot pad gloves**) into the gel cast.

30 If there are any noticeable bubbles, take a lighter and hold it over the bubble, which should make it pop.

31 Cover gel loosely with foil and allow it to set (up to an hour) before use.

32 Make sure **SB gel** goes into a gel boat with **SB buffer**.

33 **Electrophoresis Conditions**

33.1 10 µL of PCR amplicon will go into a well, flanked by 5 µL of ladder.

33.2 Run at 120 V for 02:00:00 in 1X SB buffer. 2h

33.3 Remove processed gel and analyze using a gel doc (BioRad Gel Doc).

33.4 Samples with a positive band will be confirmed on qPCR (See **SOP: 5-Gene Uniplex Probe-Based qPCR SOP**).

Appendix A – Recipes

34 **1x SB Buffer Stock – 10 liters**

34.1 **Directions to make 10L:**

Prepare a clean, 10 L carboy.

34.2 In a graduated cylinder, measure out 500 mL of 20x SB, and pour into the carboy.

34.3 In a graduated cylinder, measure out 9500 mL of dH_2O, and pour into the carboy.

34.4 Put the cap on tight, and swirl gently to mix.

34.5 Autoclave the carboy.

- Place autoclave tape on the cap, and write SB on the tape
- Put carboy in a large autoclave bin
- Loosen the cap
- Autoclave at 121 °C
- Label and store at Room temperature.

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