U.S. FOOD & DRUG ADMINISTRATION

Protocol for Screening of *Salmonella* in Foods and on Environmental Surfaces by Real-Time Quantitative PCR (qPCR)

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Tables and Figures

Introduction

This FDA developed qPCR method is applicable for the rapid screening of foods and environmental surfaces for *Salmonella*, using Applied Biosystems ™ (ABI) 7500 Fast Real-time PCR system. The method targets the *Salmonella* invasion gene (*invA*) which has been shown to be involved in internalization of *S*. Typhimurium in mammalian epithelial cells (*1-4*). This gene is found to be unique to *Salmonella* (*3,5*) and its DNA sequence is highly conserved among *Salmonella* spp. (*2,4,6*). The method uses custom-designed primers and a TaqMan probe to amplify a 262-bp fragment of *Salmonella* specific *invA* gene with stringent specificity (*7,8,9*) and includes a custom-designed internal amplification control (*9*), which is needed to identify false-negative results caused by PCR inhibitors often found in foods.

The protocol for using this qPCR method as a confirmatory assay for *Salmonella* isolates can be found in the BAM Chapter 5 E9 (https://www.fda.gov/media/107724/download).

This qPCR method has been shown to be an effective and rapid screening tool for a broad range of foods that includes fruits, fresh leafy green vegetables and herbs (blackberry, blueberry, raspberry, strawberry, baby spinach, cabbage, iceberg lettuce, romaine lettuce, spring mix, basil, cilantro, parsley, dill, oregano, watercress), low-moisture foods (almond, almond butter, chia seed powder, dried cereal, dried egg noodle, infant formula, peanut butter, pine nuts, soy formula, walnuts), seafoods (fish, shrimp, raw oyster), whole shell eggs, spices (crushed red pepper, ground basil, ground black pepper, ground cumin, ground white pepper, paprika, red chili powder), and environmental surfaces (plastic, stainless steel, ceramic tile, rubber, and cast iron), and multiple animal feeds (chick feed, premium alfalfa pellets, wheat bran, whole oats), which were prepared with either a soak or blend procedure in a series of SLV studies (*7,8,9,10*). The qPCR method was shown to be a reproducible, sensitive, and specific rapid screening method in our two MLV studies (*11*). The *Limit of Detection*$_{50}$ (*LOD*$_{50}$) is 0.811 CFU/25g for qPCR method and 0.837 CFU/ 25g for BAM culture method in the MLV baby spinach study (*13*). The *LOD*$_{50}$ is 0.75 CFU/25g for both qPCR and culture methods in the MLV frozen fish study.

The protocol, using qPCR method as a rapid screening tool, is described at below. The qPCR assembly and data analysis in the protocol are for ABI 7500 Fast Real-time PCR system. Use of other platforms and protocols must first be validated per FDA microbiological methods validation guidelines (https://www.fda.gov/media/83812/download) or other internationally recognized validation guidelines such as AOAC International's Appendix J (http://www.eoma.aoac.org/app_j.pdf) or the International Organization for Standardization's 16140:2 2016 (www.iso.org).

A. Equipment and Materials

1. ABI 7500 Fast Real-time PCR System (Software version 1.4, 2.04. 2.3) (ThermoFisher Scientific, Waltham, MA)
2. MicroAmp™ Fast Optical 96-Well Reaction Plates (ThermoFisher Scientific Cat# 4346906)
3. MicroAmp™ Optical Adhesive Film for 7500 Fast Plates (ThermoFisher Scientific Cat# 4311971)
4. Appropriate ABI 7500 Fast Plate Holder (specific for 96-well tray or 8-strip well tubes)
5. Microcentrifuge tubes (0.5 to 2.0 mL)
6. Sterile black microcentrifuge tubes (1.5 mL)

7. Aluminum foil pouch
8. Silica gel packets 10 gram (Fisher Scientific Cat# 50-930-892)
9. Pipettes (1-1000 µL volume)
10. Pipette tips (0.2 to 1000 µL volume) (aerosol resistant tips)
11. Serological pipettor (1-1000 mL)
12. QIAprep Spin Miniprep Kit (QIAgen Cat# 27104)
13. Qubit dsDNA BR Assay Kit (ThermoFisher Scientific Cat# 32850 or 32853)
14. Vortex Mixer
15. Water bath or heat block capable of maintaining 100°C

B. Media and Reagents

1. Sterile molecular grade water
2. InstaGene™ matrix (Bio-Rad Cat# 7326030)
3. Applied Biosystems™ TaqMan Fast Advance Master Mix (ThermoFisher Scientific Cat# 4444556)
4. *Salmonella* primers and probes listed in **Table 1** are specific to real-time PCR platforms being used.
   a. Primers – 10 µM working solution of each primer listed in Table 1. Stock (1000 µM) and working solutions can be prepared from commercially synthesized primers with basic desalt purification (Biosearch Technologies, Integrated DNA Technologies IDT or equivalent) by rehydrating with sterile molecular biology grade water to appropriate concentrations. Store at -20°C to -70°C non-frost-free freezer (frost-free freezers cycle warm/cold which will damage the primer solution).
   b. Probes – 10 µM working solution of each probe listed in Table 1. Dual hybridization probes should be purchased as Dual HPLC-purified and labeled as indicated in Table 1. Stock (100 µM) and working solutions can be prepared from commercially synthesized probes (Biosearch Technologies, Integrated DNA Technologies IDT or equivalent) by rehydrating with sterile molecular biology grade water to appropriate concentrations. Working solutions should be aliquoted in small amounts and stored frozen (-20 to -70°C) and away from light until use to avoid fluorophore degradation.
   c. Exogenous Internal Amplification Control (IAC)
   IAC is a synthetic 100 bp sequence: 5'-
   AGTTGCAGTGTAACCGTCATGTACCAGTAATCTGCGTCGCACGTGTGCACCTAGT
   CTAATCACTTATGACTCAGATAACTTAACAGCAGAGTCTCGTCGA.
   IAC plasmid pCR2.1-InC (Plasmid #83959) is available through Addgene at https://www.addgene.org/83959/. Host strain containing pCR2.1-InC plasmid (4029 bp) can be cultured in LB (Lysogeny broth) or BHI (Brain Heart Infusion) broth containing either kanamycin (50 µg/mL) or ampicillin (100 µg/mL) at 37°C for 16 hours.
   The pCR2.1-InC plasmid can be extracted from overnight culture by using QIAprep Spin Miniprep Kit. The plasmid concentration can be quantitated with Qubit dsDNA BR Assay Kit. The yield of plasmid preparation using QIAprep Spin Miniprep Kit is about 5 to 10 µg per preparation at a final concentration of 100-200 ng/µl in 50 µl eluent.

Table 1. Primer/probe sequences for ABI 7500 Fast platforms

Primers[1]	**Gen Bank #**	**Bases**	**5' → 3' Sequence**
*Sal*1598 F	U43273	20	AACGTGTTTCCGTGCGTAAT
*Sal*1859 R	U43273	20	TCCATCAAATTAGCGGAGGC
IAC F		22	AGTTGCAGTGTAACCGTCATGT
IAC R		22	TCGACGAGACTCTGCTGTTAAG
Probes[1]			
*Sal*1631PFAM		20	FAM-TGGAAGCGCTCGCATTGTGG-BHQ
IAC30PCy5		20	Cy5-ATCTGCGTCGCACGTGTGCA-BHQ

[1]Primer/Probe name composed of target (*Sal* = *Salmonella* species targeting *invA* gene, IAC = Internal Amplification Control), 5' base position of oligonucleotide in the respective gene sequence specified in column 3 and forward primer (F), reverse primer (R) or probe (P).

C. PCR Controls

1. For a positive PCR control, use a template prepared from *Salmonella* Typhimurium or any other serovar from *Salmonella enterica subsp. enterica*.
2. For a negative control, always include a no template (water) control in every run.

D. Sample Preparation and Pre-enrichment

Follow the BAM Chapter 5: *Salmonella* | FDA for sample preparation and pre-enrichment. Whirl-Pak bag with filter is recommended for sample pre-enrichment. **Do not shake the food sample bag after 24 h pre-enrichment**. Use a serological pipettor to transfer a 1 mL aliquot of pre-enriched cultures from upper part of the container into a 1.5 mL safe-lock microcentrifuge tube for DNA extraction. **Do not use non-serological pipettors.**

E. DNA Template Preparation from 24-hour Pre-enriched Culture

(1) Manual DNA extraction for <u>soaked</u> sample preparation (boiling method):

For the foods using soak sample preparation, but with high fat content like whole milk, powdered infant formula or nuts etc., use the modified boiling method at E (2) or automatic DNA extraction methods at E (3).

1. Do not shake the sample bag after 24-hour pre-enrichment. Transfer a 1 mL aliquot of pre-enriched cultures from upper part of the filtered side of the sample bag into a 1.5 mL safe-lock microcentrifuge tube.
2. Centrifuge 15,000 × g (RCF) for 5 min.
3. Carefully remove the supernatant from the pellet.

4. Re-suspend the pellet in 200 µL sterile DNase-free and RNase-free water by vortexing (ensure that all of the pellet is suspended in the water).
5. Heat the suspension to 100°C using a heating block or boiling water bath for 10 min and cool on ice for 5 min.
6. Centrifuge at 15,000 × g (RCF) for 5 min.
7. Remove and save the supernatant as DNA template (This may be frozen, minimum -20°C, for future PCR tests)

Note: Pre-enrichment broth residues in the DNA extract may cause qPCR IAC (Internal Amplification Control) to fail with a late C_T value or an undetermined result. If qPCR fails due to inhibition, you may re-run the qPCR using one of the following:

- Take 10 µL of DNA extract to make 1/5 dilution in 40 µL DNase/RNase-free water in a new microfuge tube, for qPCR.
- You may also use any DNA purification kit to purify the DNA extract by following the kit manufacturer's instruction.

(2) Manual DNA extraction for <u>blended</u> sample preparation (modified boiling method):

1. Do not shake the sample bag after 24-hour pre-enrichment. Transfer a 1 mL aliquot of pre-enriched cultures from upper part of the filtered side of the sample bag into a 1.5 mL safe-lock microcentrifuge tube.
2. Centrifuge at 900 × g (RCF) for 1 min to remove large particles and then transfer 500 µL supernatant to a new microfuge tube for each sample.
3. Centrifuge 15,000 × g (RCF) for 5 min.
4. Carefully remove the supernatant from the pellet.
5. Re-suspend the pellet in InstaGene™ matrix (Bio-Rad Cat# 7326030). Thoroughly vortex InstaGene matrix and use a 1,000 µl pipet tip to add 100 µL of InstaGene matrix to the pellet and vortex to mix well to ensure all of the pellet is suspended in the InstaGene matrix.
6. Heat the suspension to 100°C using a heating block or boiling water bath for 10 min and then vortex for 10 sec followed by centrifugation at 15,000 x g (RCF) for 5 min.
7. Transfer 50 µL supernatant as DNA template to a new microfuge tube (This may be frozen, minimum -20°C, for future PCR tests).
8. Take 10 µL of each sample to make 1/5 dilution in 40 µL DNase/RNase-free water in a new microfuge tube, mix well for qPCR.

Note: Pre-enrichment broth residues in the DNA extract may cause qPCR IAC (Internal Amplification Control) to fail with a late C_T value or an undetermined result. If qPCR fails due to inhibition, you may re-run the qPCR using one of the following:

- Take 10 µL of DNA extract to make 1/5 dilution in 40 µL DNase/RNase-free water in a new microfuge tube, for qPCR.
- You may also use any DNA purification kit to purify the DNA extract by following the kit manufacturer's instruction.

(3) Automatic DNA extraction for all food sample preparations:

The following instruments and commercial kits have been validated to be used for a high through-put DNA extraction from 24-hour pre-enriched food samples:

1. Maxwell® RSC 48 System with Maxwell® RSC Cultured Cells DNA kit (48 Preps, Promega Cat# AS1620): See Appendix 1.
2. KingFisher Apex system / KingFisher Flex system / MagMax™ Express-96 Magnetic Particle Processor with BioSprint® 96 One-For-All Vet Kit (384 reactions, INDICAL BIOSCIENCE Cat# SP947057): See Appendix 2.
3. KingFisher Flex system / MagMax™ Express-96 Magnetic Particle Processor with PrepSEQ™ Nucleic Acid Extraction Kit for Food and Environmental Testing (ThermoFisher Scientific Cat# 4428176): See Appendix 3.

F. Preparation of qPCR *Salmonella* Master Mix

(1) Preparation of Dehydrated qPCR *Salmonella* Master Mix

1. Mix all components in **Table 2** in 1.5 mL microcentrifuge tube by vortex at top speed and centrifuge briefly.

Table 2. Recipe for the preparation of 100 tubes of dehydrated qPCR *Salmonella* Master Mix

Component	Volume (µL)
Primer *Sal*1598 F (1000 µM Solution)	16.0
Primer *Sal*1859 R (1000 µM Solution)	16.0
Primer IAC F (1000 µM Solution)	6.7
Primer IAC R (1000 µM Solution)	6.7
Probe *Sal*1631PFAM (100 µM Solution)	200.0
Probe IAC30PCy5 (100 µM Solution)	250.0
IAC DNA template (0.75 pg/µL)*	25.0
20% sucrose (0.22 µM filter-sterilized)	130.0
PCR grade water	349.6
Total	1000.0

***Make fresh IAC DNA template working solution from concentrated stock solution**

2. Aliquot 5 µL Master Mix solution (with 3 µL PCR grade water, 10 µL TaqMan Fast Advance Master Mix, and 2 µL of DNA template from *Salmonella* and negative controls) for a quality assurance (QA) on ABI 7500 Fast, respectively.

3. When both evaluation runs were satisfactory as shown in Figure 1 (IAC C_T value should be 28 ± 1), dispense 10 µL Master Mix solution per tube to sterile 1.5 mL black microcentrifuge tubes.
4. Dry the qPCR *Salmonella* Master Mix solution with tube lids open in a vacuum chamber for 2 to 3 days and shield the vacuum chamber completely from light.

5. Take a vacuum dried qPCR *Salmonella* Master mix from top and middle shelves each for a QA run with *Salmonella* and negative controls on ABI 7500 Fast (see G for reaction assembly).
6. When both evaluation runs were satisfactory, the vacuum dried qPCR *Salmonella* Master Mix can be stored in aluminum foil porch with silica gel packet at room temperature in a desiccator for 2 years. The dehydrated qPCR master mix can also be stored at -20ºC in a freezer or refrigerator for a up to 2 years. After 2-year storage, a quality assurance (QA) must be performed to ensure that the dehydrated master mix will work properly to have a normal amplification curve as shown in Figure 1 before use.

7. Each tube of qPCR *Salmonella* Master Mix can carry out fifty 20 µL reactions on ABI 7500 Fast systems.

(2) Preparation of Wet qPCR *Salmonella* Master Mix

1. Mix all components in **Table 3** in a 1.5 mL black microcentrifuge tube, vortex at top speed, and centrifuge briefly.
2. Dilute to 1X or 2.5X for reaction set up (see G for reaction assembly).
3. Perform a QA run for every batch of wet master mix on the ABI 7500 Fast to ensure that the IAC C_T value is 28 ± 1.
4. Shield the wet qPCR *Salmonella* master mix from light and store for up to one week at 4°C for immediate use or store at -20 °C for up to 2 years. After 2-year storage, quality assurance (QA) must be performed to ensure the master mix will work properly to have a normal amplification curve as shown in Figure 1 before use.

Table 3. Recipe for the preparation of 1 tube of wet qPCR *Salmonella* Master Mix (10X)

Component	Volume (µL)
Primer *Sal*1598 F (10 µM Solution)*	16.0
Primer *Sal*1859 R (10 µM Solution)*	16.0
Primer IAC F (10 µM Solution)*	6.7
Primer IAC R (10 µM Solution)*	6.7
Probe *Sal*1631PFAM (10 µM Solution)**	20.0
Probe IAC30PCy5 (10 µM Solution)**	25.0
IAC plasmid DNA template (0.075 pg/µl)***	2.5
20% sucrose (0.22 µM filter-sterilized)	1.3
PCR grade water	5.8
Total	100.0

* 10 µM primer working solutions were used.
** 10 µM probe working solutions were used.
*** 10-fold diluted IAC template was used.

G. Real-time PCR Assembly for ABI 7500 Fast

(1) qPCR Amplification Reaction Components

Table 4. qPCR Amplification Reaction Components

Volume (µL) /reaction	Component
8.0	2.5X working solution of qPCR *Salmonella* Master Mix (2.5X MM) [1]
10.0	TaqMan Fast Advance Master Mix
2.0	Template (Sample or control)

[1] Resuspend one tube of dehydrated *Salmonella* Master Mix in 400 µl of PCR grade water to make 2.5X MM.
[1] Add 300 µl of PCR grade water to 1 tube of wet qPCR *Salmonella* master mix (10X) to make 2.5X MM.
Note: Each 400 µl of 2.5X qPCR *Salmonella* master mix tube will need 500 µl TaqMan Fast Advance Master Mix for total 50 reactions.

(2) Instructions for preparing qPCR Reaction Mix from dehydrated *Salmonella* master mix

The dehydrated *Salmonella* Master Mix must be kept in the sealed aluminum foil pouch at room temperature in a desiccator until used. The *Salmonella* Master Mix **DOES NOT** contain Taq® polymerase, dNTPs, $MgCl_2$ and buffer. **The addition of the Applied Biosystems™ TaqMan Fast Advance Master Mix is essential.**

Table 5. Reaction Master Mix (RMM) from dehydrated *Salmonella* master mix

Reagents	Amount
Dehydratred qPCR *Salmonella* Master Mix	1 tube (50 Reactions)
DNase-free, RNase-free Water	400 µL
ABI TaqMan Fast Advanced qPCR Master Mix	500 µL
Total	900 µL

On the day of PCR run:

1. Take one dehydrated qPCR *Salmonella* Master Mix black tube.
2. Add 400 µL DNase& RNase-free water, vortex, hold for 10 min for complete dissolution.
3. Add 500 µL of TaqMan Fast Advanced qPCR Master Mix. Vortex briefly and spin down**.**
4. Label Tube as "ABI RMM" (Reaction Master Mix) and date to avoid any mistakes.
5. One prepared "ABI RMM" with TaqMan Fast Advanced Master Mix can run 50 reactions.
6. The prepared ABI RMM can be stored refrigerated at 4ºC for up to 1 week.
7. Aliquot 18 µL of the qPCR Reaction Mix into each well of 96-well plate, duplicate wells for each sample if necessary, and add 2 µL of sample or control DNA templates into each reaction (well).

(3) Instructions for preparing qPCR reaction mix from wet qPCR *Salmonella* master mix

1. The wet *Salmonella* Master Mix must be stored in dark at 4°C for up to one week for immediate use or store at -20 °C for up to 2 years. After 2-year storage, a quality assurance (QA) must be performed to ensure that the master mix will work properly to have a normal amplification curve as shown in Figure 1 before use.

The *Salmonella* Master Mix **DOES NOT** contain Taq® polymerase, dNTPs, $MgCl_2$ and buffer. The addition of the Applied Biosystems™ TaqMan Fast Advance Master Mix is essential.

Table 6. Reaction Master Mix (RMM) for wet *Salmonella* master mix

Reagents	Amount
Wet qPCR *Salmonella* Master Mix	1 tube (50 Reactions)
DNase-free, RNase-free Water	300 µL
ABI TaqMan Fast Advanced qPCR Master Mix	500 µL
Total	900 µL

On the day of PCR run:

1. Take one wet qPCR *Salmonella* Master Mix black tube.
2. Add 300 µL DNase & RNase-free water, vortex, hold for 10 min for complete dissolution.
3. Add 500 µL of TaqMan Fast Advanced qPCR Master Mix. Vortex briefly and spin down**.**
4. Label Tube as "ABI RMM" (Reaction Master Mix) and date to avoid any mistakes.
5. One prepared "ABI RMM" with TaqMan Fast Advanced Master Mix can run 50 reactions.
6. The prepared ABI RMM can be stored refrigerated at 4°C for up to 1 week.
7. Aliquot 18 µL of the qPCR Reaction Mix into each well of 96-well plate, duplicate wells for each sample if necessary, and add 2 µL of sample or control DNA templates into each reaction (well).

H. Real-Time PCR run set up on ABI 7500 Fast

(1) qPCR running protocol

Table 7. PCR running protocol – 2 steps

First Hold stage Activation of UDG	Second Hold stage Initial Activation	Cycling stage Each of 50 Cycles	
50°C, 2 min	95°C, 5 min	95°C, 3 sec	60°C, 30 sec

(2) qPCR result analysis set up after the reaction completed successfully

Table 8. qPCR result analysis set up

Amplification Plot Setting	Analysis Setting
1. Data: Delta Rn vs. Cycle 2. Detector: All 3. Line Color: "Well color" or "Detector Color"	1. Manual C_T 2. Threshold: 0.05 (w/ROX) 3. Manual Baseline Start (cycle): 3 End (cycle): 15

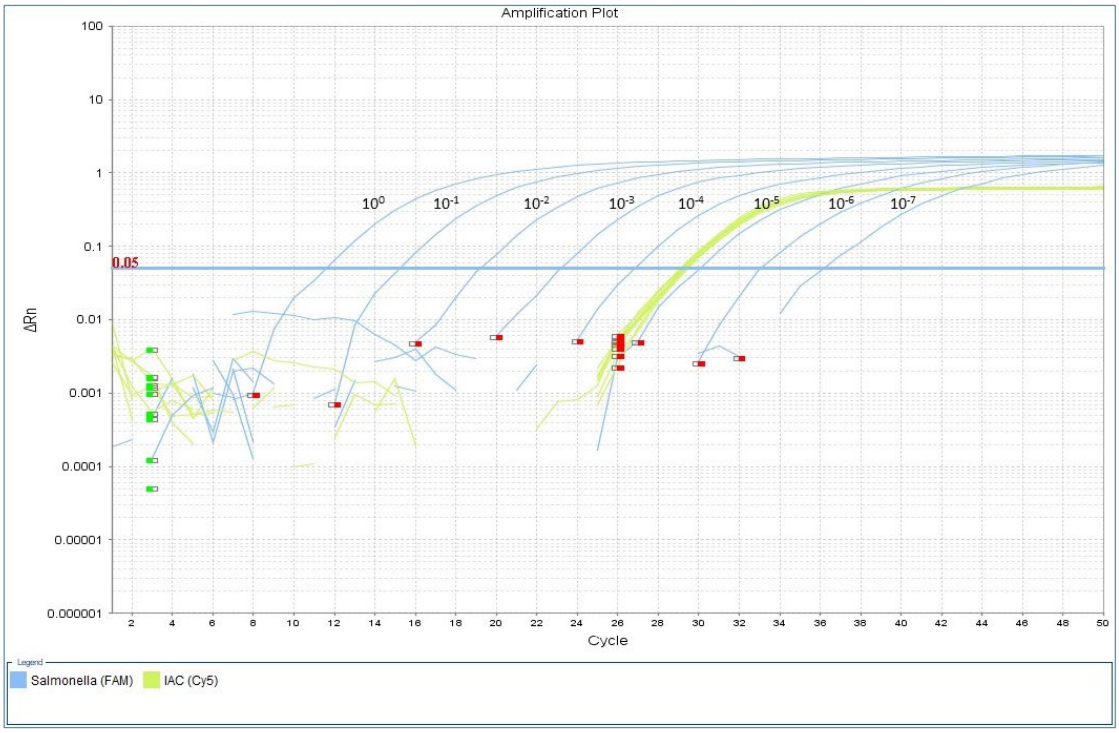
(3) qPCR result interpretation

PCR results are only considered valid for each sample if the IAC amplification is not inhibited and the IAC has a normal amplification curve. The fluorescence signal is analyzed using ABI 7500 software versions1.4, 1.5 or 2.3 by manually setting the threshold line at 0.05, and baseline starting at cycle 3 and ending at cycle 15. Threshold cycle (C_T value) is recorded for each PCR reaction for the target and IAC. A C_T value less than 45 cycles is considered *Salmonella* positive. If IAC inhibition is observed, but the tested sample is *Salmonella* positive, the PCR amplification curve should be examined. An amplification curve that is the same as the amplification curve of the positive control with baseline, exponential region and plateau phases,

as shown in **Figure 1**, should be observed to consider the sample as being *Salmonella* positive. If the IAC is inhibited, then the qPCR reaction should be repeated with diluted DNA to reduce inhibition from the DNA extract.

Figure 1. View of *Salmonella* amplification plot **(Log type) from a 10-fold serial dilutions of *Salmonella* pure culture from ABI 7500 Fast**

The PCR cycle number is shown on the x-axis, and the magnitude of normalized fluorescence (ΔRn) generated at each cycle is shown on the y-axis.



(4) qPCR run set up on ABI 7500 Fast system (V.1.4, 1.5, and 2.3)

Version 1.4 and 1.5

1. Turn on the computer and ABI 7500 Fast.
2. Launch ABI 7500 Fast System Software
3. Create run by using New Document Wizard.
4. Highlight all wells and select "Detector Manager" under "Tool".
5. Shift select ***Salmonella* (FAM) and IAC (Cy5)** and click "Add to Plate Document.
6. Under "Well Inspector" in "View", mark both "*Salmonella*" and "IAC", and select "**ROX**" for **Passive Reference.**
7. Under "Instrument", select 2-step PCR protocol as described at H (1)
8. Save the newly created file as template for *Salmonella* in **.sdt** format. The *Salmonella*_template.**sdt** file can be used as template for future .sds run file with all preset parameters.
9. Save the file again in.sds format as a run file with a different name such as "*Salmonella*_Test.**sds**".

10. Assign appropriate sites with sample names on corresponding wells and save the file.
11. Load the samples and start the run under "Instrument". If the "Start" button under the "Instrument" is not highlighted, close and re-open the "*Salmonella*_Test.sds" file to initialize ABI 7500 Fast System. Start the run by clicking the "Start" button.
12. After the reaction completed successfully, the results can be analyzed, viewed and reported as described at H (2).
13. Interpretation of result as described at H (3).

Version 2.3

1. Turn on the computer and ABI 7500 Fast.
2. Launch ABI 7500 Fast System Software v 2.3.
3. Click New Experiment to create and set up a new experiment.
4. Click Experiment Properties
   White down Experiment name
   Click to choose 7500 Fast (96 wells)
   Click to choose Fast (~40 minutes to complete run)
5. Click Plate Setup
   Click tab Define Targets and Samples
   Click Define Targets
   Define first target as ***Salmonella*** (Choose **FAM** for reporter; Choose None for Quencher)
   Add second target as **IAC** (Choose **Cy5** for reporter; Choose None for Quencher)
   Click Define Samples
   Add new samples and sample name
   Click tab Assign Targets and Samples
   i. Under View Plate Layout
   Select wells for samples and controls
   Highlight these wells
   Under Assign target (s) to selected well
   Check assign to both *Salmonella* and IAC
   ii. Highlight first well
   Under Assign sample(s) to selected well
   Assign sample 1 to the first well
   Following same procedue to assign samples to the rest of wells
   iii. Under Select the dye to use as the **passive reference**
   Choose **ROX**
6. Click Run Method
   Under Graphical View,
   Set reaction volume per well 20 µL
   Set up run method as described at H (3)
7. Click Save icon
   Choose Save as Templet (.edt) file with a name such as "*Salmonella*_Template.edt" to be used as template for future.eds run file with all preset parameters.
8. Click Save icon
   Choose Save as (.eds) as a run file with a different name such as "*Salmonella*_Test.eds" .
9. Load the samples in plates into the ABI 7500 Fast system

10. Click START RUN green button on the upper right corner.
11. After the reaction completed successfully, the results can be analyzed, viewed and reported as described at H (2).
12. Interpretation of result as described at H (3).

I. Confirmation

Presumptive positive results should be confirmed by following BAM Chapter 5 Section D-E for the isolation, identification, and confirmation (https://www.fda.gov/media/172194/download?attachment).

J. References

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Appendix: Automatic DNA Template Preparation Protocols

Appendix 1. DNA Extraction from Food Preenrichment Culture Using Maxwell® RSC Instrument

Purpose/Objective

This protocol describes the procedure for obtaining purified DNA from food preenrichment culture for up to 48 samples at a time using the Promega Maxwell® RSC (Rapid Sample Concentrator) automated instrument. The Maxwell® RSC DNA or RNA extraction methods start with cartridges prefilled with purification reagents and paramagnetic particles, ready for your samples. After sample addition, the Maxwell® RSC moves the paramagnetic particles and associated nucleic acids through multiple steps ultimately yielding highly pure RNA or DNA in 30–100µl. Because the Maxwell® RSC is a magnetic particle mover, not a liquid handler, it offers advantages over other automated systems. There is minimal risk of cross-contamination because no liquid handling or splashing happens during sample processing. With no clogs and fewer breakdowns, there are fewer disruptions to the downstream workflows. We have validated this Maxwell® RSC automated instrument with Maxwell® RSC Cultured Cells DNA kit for rapid automated purification of bacterial DNA from a broad range of food samples to provide a fast high throughput DNA extraction method for our FDA qPCR method.

Materials

1. Maxwell® RSC automated instrument
2. Maxwell® RSC Cultured Cells DNA kit (Promega Cat# AS1620). The kit includes Prefilled Reagent Cartridge (48 Preps), Plunger Pack (48 Plungers), 50 Elution Tubes (0.5ml), Elution Buffer (20 mL)
3. Pipettors and pipette tips for sample transfer into prefilled reagent cartridges
4. Microcentrifuge tubes (1.5- or 2.0-mL)
5. Serological pipettor
6. Pipettor aid

Sample Collection

1. Do not shake the food sample bag after 24-hour preenrichment. Use a serological pipettor to transfer a 1 mL aliquot of preenrichment cultures from upper part of the filtered side of the sample bag into a 1.5 mL microcentrifuge tube.
2. Store the collected samples tubes at 4˚C until prefilled reagent cartridge set up in cartridge rack.
3. Transfer 400 µL of preenrichment cultures from 1.5 mL microcentrifuge tube to well #1 in the cartridge.

Instrument Operation

1. The Maxwell RSC-16 or -48 instrument and the attached tablet should be on. If not, turn both on and wait for instrument to initialize. The RSC 48 has a rocker switch in the back left near cord (first) and a push button (second) on the front right side. The RSC 16 only has a rocker switch in

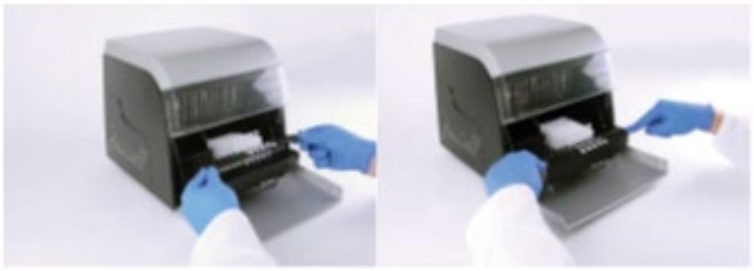
the back left near cord. The power button for the tablet is the top left push button. Once the tablet is on, select the Maxwell RSC software and wait for the instrument self-test to finish.

2. From the 'Home' screen select the Start button to begin the process of running an extraction method.
3. On the 'Methods' screen, select the method from the list that matches the kit name or catalog number. Alternatively, you can scan the bar code on the Maxwell® RSC reagent kit box indicated by the Scan Here label.
4. On the 'Cartridge Setup' (if shown – RSC-16 only) screen, touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information (default is no information including sample ID).
5. After all required information has been entered, touch the Proceed button to display the 'Door' prompt. Press the OK button to open the Maxwell® RSC Instrument door.
6. For 1-24 samples, remove the front tray. For more, remove both trays for the instrument.
7. Ensure sample preprocessing is complete. Sample preprocessing, if required, is described in the Technical Manual for the specific Maxwell® RSC reagent kit.
8. Change gloves before handling Maxwell® RSC Cartridges, Plungers and Elution Tubes.
9. Place each cartridge (1 per sample) in the deck tray(s) with the printed plastic side facing away from the elution position, which is the numbered side of the tray.
10. Press down firmly on the cartridge to snap it in place at both ends. There should be an audible click.

Note: If you are processing fewer than 24 (RSC 48) or 16 (RSC 16) samples, center the cartridges on the deck tray.

11. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
12. Transfer cultured cell sample to well #1 of each cartridge and thoroughly mix the cultured cell samples into the lysis buffer by pipetting at least 10 times. (Well #1 is the well closest to the printed side and furthest from the elution tube.) Change pipette tips between samples.
13. Place one plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
14. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s). You may label the elution tubes now or upon completion of the extraction.
15. Add 100µl of Elution Buffer to the bottom of each elution tube. Final volume will be less than 100µl. **Note:** Use only the Elution Tubes (0.5ml) provided with the kit; other tubes may be incompatible with the Maxwell® RSC Instrument. Leave the elution tube lids open.

16. To place the deck trays in the instrument, hold the deck tray by the sides to avoid dislodging cartridges and ensure that the deck tray is placed in the Maxwell® instrument with the elution tubes closest to the door.



17. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform.
18. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation.
19. Ensure the deck tray is level on the instrument platform and fully seated.
20. On the screen, you will be presented with an 'Extraction Checklist' screen to confirm that the deck tray and instrument have been prepared as indicated on the 'Extraction Checklist' screen (steps 5 – 18).
21. After all of these steps have been performed and confirmed, touch the Start button to begin the purification.
22. When the method completes successfully the method 'Running' screen will indicate that the extraction method has been completed. After a method is complete the Current Step will be listed as Completed.
23. Touch the Open Door button to open the door of the Maxwell® RSC Instrument.
24. Close the caps of the elution tubes.
25. Verify that all the cartridges have a plunger in well #8. Remove the deck tray by gripping firmly by the elution tube position, lifting up and pulling the tray out. The deck tray may be warm to the touch after a run is completed. Exercise caution while removing the deck tray. If there were cartridges missing plungers, remove them by running the Clean Up method.
26. The extracted material is present in the elution. Ensure that each elution tube is labeled accordingly or do so now. After purification, the elution tubes may have resin that adheres to the side of the tube. This is normal and will not affect downstream assay performance. Residual particles may be removed by centrifuging the elution tube or placing on a magnet and transferring the supernatant to a clean tube (not provided).
27. Remove the cartridges and plungers from the Maxwell® RSC deck tray and dispose of in a sharp's biohazard container.
28. Sanitize the deck tray with 70% Ethanol and return the deck tray to the instrument and sanitize the instrument following the Sanitization button on Home screen.

Appendix 2. DNA Extraction from Food Preenrichment Culture Using KingFisher Apex, KingFisher Flex with BioSprint® 96 One-For-All Vet Kit

Purpose/Objective

This protocol describes the procedure for obtaining purified DNA from food preenrichment culture for up to 96 samples at a time using the KingFisher Apex system, KingFisher Flex system, or MagMaxTM Express-96 Magnetic Particle Processor and BioSprint® 96 One-For-All Vet kit. The BioSprint® 96 One-For-All Vet kit uses magnetic-particle technology for rapid purification of nucleic acids. We have validated this kit for rapid automated purification of bacterial DNA from a broad range of food samples on the KingFisher Apex, KingFisher Flex and MagMax automated instruments to provide a fast high throughput DNA extraction method for our FDA qPCR method.

Materials and Reagent

1. KingFisher Flex system or KingFisher Apex system,
2. Plastics for KingFisher **Apex** system:
    a. 96-well Deep-well (DW) Plate (Fisher Scientific Cat# 14387349);
    b. 96-well DW Tip Comb (Fisher Scientific Cat# 14387354);
    c. 96-well DW Tip Comb& Plate (Fisher Scientific Cat# 14387358)
3. Plastics for KingFisher **Flex** system and MagMax:
    a. 96-well Deep-well (DW) Plate (Fisher Scientific Cat# 22387031).
    b. 96-well DW Tip Comb (Fisher Scientific Cat# 22387029);
    c. 96-well DW Tip Comb& Plate (Fisher Scientific Cat# FER97002820)
4. BioSprint 96 One-For-All Vet Kit (INDICAL BIOSCIENCE Cat# SP947057).
    a. The kit includes plasticware and all reagents except Ethanol and Isopropanol which are needed for the DNA extraction.
    b. **Do not use** plasticware provided in the kit on KingFisher Apex and KingFisher Flex.
5. Pipettors and pipette tips
6. Microcentrifuge tubes (1.5- or 2.0-mL)
7. Serological pipettor (1-1000 mL)
8. Vortex mixer
9. Graduated cylinder
10. Ethanol (200 proof)
11. Isopropanol

Notes

- Using the ThermoFisher 96-well DW plates and tip comb for the DNA extraction on KingFisher Apex and Flex.
- Ethanol and isopropanol are not included in the kit and are needed for the protocol.

Installation of the automated protocol "Combined Boil 3 Wash" on KingFisher Flex/MagMax:

1. Install Bindlt 4.1Software in your laptop: https://www.thermofisher.com/us/en/home/global/forms/life-science/download-bindit-software-kingfisher-instruments.html

2. Save the protocol "Combined Boil 3 Wash" in your laptop:
3. Connect your PC with USB cable to KingFisher instrument and select ***Home > Connect*** in Bindlt Software.
4. You can start program protocol (See below) from the Bindlt or transfer it into instrument. If you transfer the protocol select ***Home > Transfer*** in Bindlt Software.
5. Select Upload and find the saved protocol. Click open.
6. Select the folder (category) you want to store the protocol in the instrument.
7. Your protocol can be now found in the instrument memory.
8. Disconnect your instrument from Bindlt and check using keypad that your protocol is in the desired folder

Note: If the protocol "Combined Boil 3 Wash" cannot be transferred to the instrument, you can create the protocol following the protocol steps at below in the Bindlt 4.1 Software and then transfer the protocol to the instrument as above-described procedures.

Program the following steps in the Bindlx 4.1 Software specific to the instrument.

a. The protocol contains 6 plates:
    i. Lysis – position 1
    ii. Wash 1 – position 2
    iii. Wash 2 – position 3
    iv. Wash 3 – position 4
    v. Elution – position 5
    vi. Rod Cover – position 6.
b. The Lysis Plate will have three action steps:
    i. Heat: 15 minutes at 96ºC with mixing at medium speed
    ii. Pause: Add Buffer RLT
    iii. Lysis/Bind: 5-minute bind with mixing at medium speed
c. The Wash 1 Plate will have 1 action step: 2-minute wash with mixing at medium speed
d. The Wash 2 Plate will have 1 action step: 2-minute wash with mixing at medium speed
e. The Wash 3 Plate will have 1 action step: 2-minute wash with mixing at medium speed
f. There will be an Air-Dry step outside of Wash Plate 3 with a 9-minute drying time.
g. For the Elution Plate, the beads will be released very slowly with a release time of 5 seconds. Elute for 5 minutes with a grind mix.
h. The magnets and rod cover should be returned to the Wash Plate 3 when finished.

Installation of the automated protocol "Combined Boil 3 Wash" on KingFisher Apex

1. Create the protocol "Combined Boil 3 Wash" using Bindlx software on the instrument.
2. Here is the link to download the KingFisher Apex (and Bindlx software- pages 40-55) **User Guide**: PDF.
3. Follow the User Guide page 40-55 to create the protocol according to protocol steps: PDF

Chemical lysis protocol for the isolation of DNA from Gram-negative bacteria

1. Open BioSpint 96 One-For-All Vet Kit and remove the following items:
    a. 1 Bottle of Buffer AVE (Elution Buffer)
    b. 1 Bottle of Buffer AW1 (Wash Buffer 1)

c. 1 Bottle of Buffer RPE (Wash Buffer 2 &3)
d. 1 Bottle of Buffer RLT (Lysis Buffer)
e. 1 Bottle of MagAttract® Suspension G

2. Prepare Buffer AW1 by adding 160 mL ethanol to the bottle and mixing.
3. Prepare Buffer RPE by adding 220 mL ethanol to the bottle and mixing.
4. Prepare Buffer RLT by adding 35 mL isopropanol and 3 mL of MagAttract® Suspension G (Vortex the Magnetic Particles for 5 seconds until resuspension is complete) and mixing.
5. Use ThermoFisher 96-well DW plates for KingFisher Apex/Flex and label the plates as following:
    a. Lysis
    b. Wash 1
    c. Wash 2
    d. Wash 3
    e. Elution
    f. Tip Comb in microtiter 96-well DW plate (label not needed)
6. Sample collection
    a. Do not shake the food sample bag after 24-hour preenrichment. Use a serological pipettor to transfer a 1 mL aliquot of preenrichment cultures from upper part at filter side of the sample bag into a 1.5 mL microcentrifuge tube.
    b. Store the collected samples tubes at 4˚C while 96 deep-well plates set up.
    Note: If your sample produces a large pellet at this step, such as found with chocolate food samples, the following preclarification protocol is recommended:
        - Transfer 1 mL of sample into a 1.5-mL microcentrifuge tube.
        - Centrifuge the tube containing your sample for 1 minute at 900 x g.
        - Transfer supernatant to a new 1.5-mL microcentrifuge tube, without disturbing the pellet
        - Proceed to step 7.
7. Transfer 500 µL of preenrichment cultures from 1.5 mL microcentrifuge tube to a KingFisher microtiter 96-well deep well plate.
8. Prepare the plates:
    a. To prepare the elution plate, add 100 µL of Elution Buffer (AVE) to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
    b. To prepare wash plates 1, add 700 µL of Wash Buffer 1 (AW1) to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
    c. To prepare wash plates 2, and 3, add 500 µL of Wash Buffer 2&3 (RPE) to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
9. Select run protocol **"Combined Boil 3 Wash"** from the KingFisher Apex/ Flex/MagMax Express magnetic particle processor. Press **Start.**
10. Load the plates according to the read out. Verify orientation A1 to A1.
    a. Tip combs – in microtiter 96-well DW plate; press Start.
    b. Elution plate (100 µL of Elution Buffer AVE) – In microtiter 96-well plate DW plate; press Start.
    c. Wash plate 3 (500 µL of Wash Buffer RPE) - In microtiter 96-well plate DW plate; press

Start.

d. Wash plate 2 (500 µL of Wash Buffer RPE) - In microtiter 96-well plate DW plate; press Start.
e. Wash plate 1 (700 µL of Wash Buffer AW1) - In microtiter 96-well plate DW plate; press Start.
f. Lysis plate (500 µL sample) - In microtiter 96-well plate DW plate; press Start.

11. After 15-20 minutes, the KingFisher Apex/ Flex/MagMax Express magnetic particle processor prompts you to add 500 µL of the **Binding Mix** Buffer RLT mixed with magnetic beads to each well of the lysis plate (Vortex RLT well before adding).
12. Load the plate back into the instrument. Press **Start.**
13. When prompted (about 30 minutes later), remove plates from instrument. Retain elution plate (containing template); discard other plates.
14. Transfer eluate to sterile microcentrifuge tubes or seal 96 well elution plate. Label as KF with appropriate sample information.
15. Freeze templates at -20ºC for future PCR testing.

Appendix 3. DNA Extraction from Food Preenrichment Culture Using KingFisher Flex with PrepSEQ™ Nucleic Acid Extraction Kit

Purpose/Objective

This protocol describes the procedure for obtaining purified DNA from food preenrichment culture for up to 96 samples at a time using the KingFisher Flex system, or MagMaxTM Express-96 Magnetic Particle Processor and PrepSEQTM Nucleic Acid Extraction Kit for food and environmental testing. The kit uses magnetic-particle technology for rapid purification of nucleic acids. We have validated this kit for rapid automated purification of bacterial DNA from a broad range of food samples on the KingFisher Flex and MagMax automated instruments to provide a fast high throughput DNA extraction method for our FDA qPCR method.

Materials and Reagent

1. KingFisher Flex system,
2. Plastics for KingFisher **Flex** system:
    a. 96 Deep-well (DW) Plate (Fisher Scientific Cat# 22387031).
    b. 96 Deep-well Tip Comb (Fisher Scientific Cat# 22387029);
    c. 96 Deep-well Tip Comb& Plate (Fisher Scientific Cat# FER97002820)
3. PrepSEQ™ Nucleic Acid Extraction Kit for food and environmental testing (300 Preps, ThermoFisher Cat# 4428176). The kit provides all reagents except Ethanol and Isopropanol which are needed for the DNA extraction.
4. Pipettors and pipette tips for sample transfer into 96-well DW plate
5. Microcentrifuge tubes (1.5 or 2.0 mL)
6. Serological pipettor (1-1000 mL)
7. Vortex mixer
8. Graduated cylinder
9. Ethanol (95%)
10. Isopropanol

Installation of the automated protocol "4412639PrepSEQ_Sal" on KingFisher Flex/MagMax:

1. Install Bindlt 4.1Software in your laptop: https://www.thermofisher.com/us/en/home/global/forms/life-science/download-bindit-software-kingfisher-instruments.html
2. Download and save the protocol " 4412639PrepSEQ_Sal" in your laptop: https://www.thermofisher.com/order/catalog/product/4412639?SID=srch-hj-4412639
3. Connect your PC with USB cable to KingFisher instrument and select ***Home > Connect*** in Bindlt Software.
4. You can start program protocol (See below) from the Bindlt or transfer it into instrument. If you transfer the protocol select ***Home > Transfer*** in Bindlt Software.
5. Select Upload and find the saved protocol. Click open.
6. Select the folder (category) you want to store the protocol in the instrument.
7. Your protocol can be now found in the instrument memory.

8. Disconnect your instrument from Bindlt and check using keypad that your protocol is in the desired folder
   Note: If the protocol "4412639PrepSEQ_Sal" cannot be transferred to the instrument, you can create the protocol following the Protocol steps (page 5-6) in the Bindlt 4.1 Software and then transfer the protocol to the instrument as above-described procedures.

Chemical lysis protocol for the isolation of DNA from Gram-negative bacteria

1. Open PrepSEQ™ Nucleic Acid Extraction Kit and remove the following items:

   a. 1 Bottle of Elution Buffer (25 mL)
   b. 2 Bottles of Wash Buffer Concentrate (26 mL/bottle)
   c. 1 Empty Bottle for Binding Solution (Isopropanol, not provided in the kit)
   d. 2 Bottles of Lysis Buffer (50 mL/bottle)
   e. 2 Tubes of Magnetic Particles (1.5 mL/tube)
   f. 1 Bottle of Proteinase K (PK) Buffer (50 mL)
   g. 1 Tube of Proteinase K (1.25 mL)

   Note: for information on storage of kit components, refer to the "Storage" section in the packaging insert.
2. Prepare Binding Solution by adding 30 mL of 100% isopropanol the empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
3. Prepare Wash Buffer RPE by adding 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, mix well, then label the bottle to indicate that ethanol is added.
4. label KingFisher microtiter 96-well DW plates and the plates with the following:
   a. Lysis
   b. Wash 1
   c. Wash 2
   d. Elution
   e. Tip Comb in microtiter 96-well DW plate (label not needed)
5. Sample collection
   a. Do not shake the food sample bag after 24-hour preenrichment. Use a serological pipettor to transfer a 1 mL aliquot of preenrichment cultures from upper part at filter side of the sample bag into a 1.5 mL microcentrifuge tube.
   b. Centrifuge the tube for 3 minutes at 16000 x g (Discard the supernatant without disturbing the pellet. Remove liquid as quickly as possible to prevent dissipation of pellets).

      If your sample produces a large pellet at this step, such as found with chocolate food samples, the following PrepSEQ™ preclarification protocol is recommended:
      - Transfer 1 mL of sample into a 1.5-mL microcentrifuge tube.
      - Centrifuge the tube containing your sample for 1 minute at 4000 x g.
      - Transfer supernatant to a new 1.5-mL microcentrifuge tube, without disturbing the pellet
      - Proceed to step 5b.
6. Add 300 µL of the Lysis Buffer to the tube. Resuspend by pipetting up and down, or vortex until the pellet is resuspended. Quick spin for 5 seconds to remove the Lysis Buffer form the tube lid.
7. Transfer the sample to a KingFisher microtiter 96-well deep well plate.

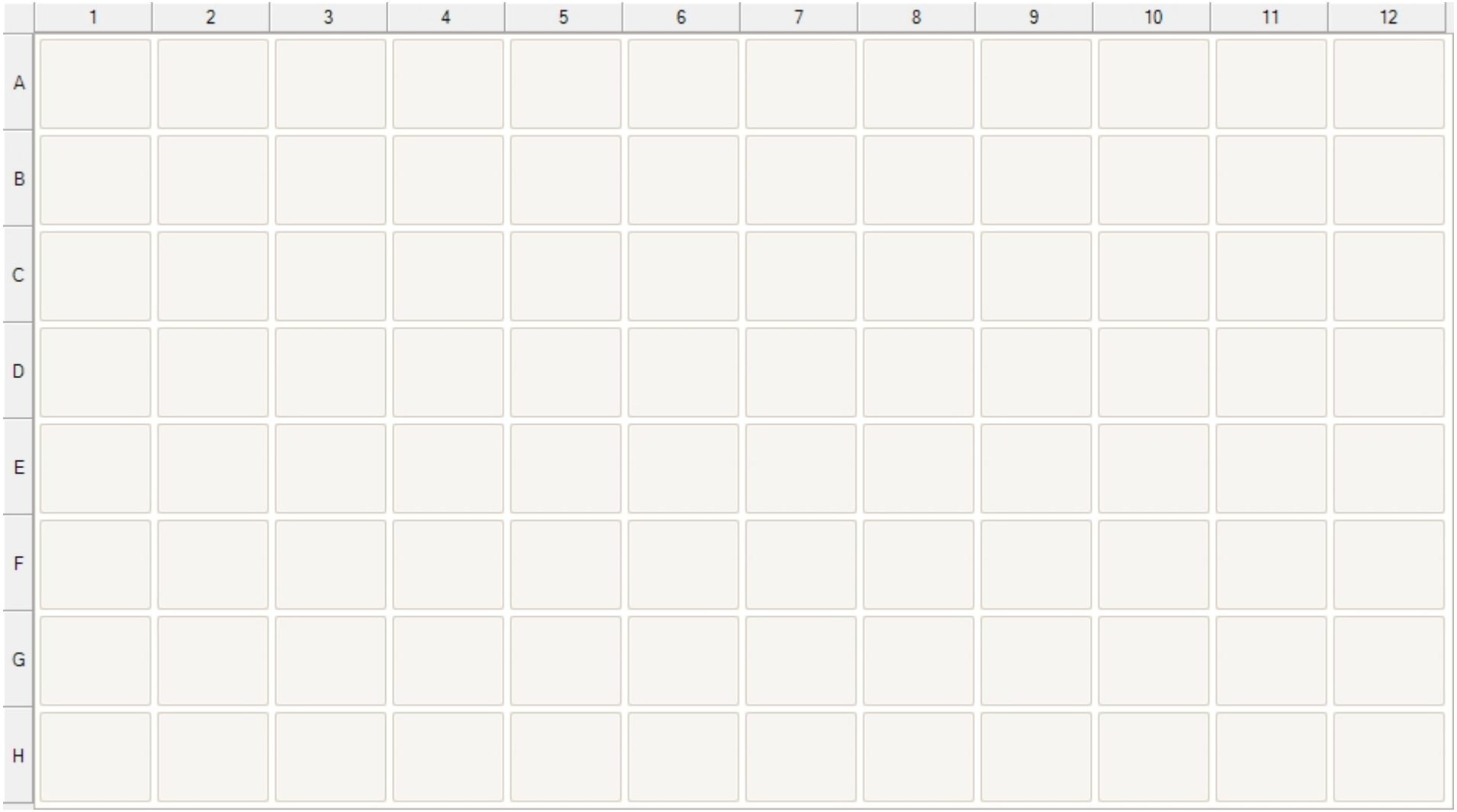
8. Prepare the plates:
    a. To prepare the elution plate, add 140 µL of Elution Buffer to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
    b. To prepare wash plates 1 and 2, add 300 µL of Wash Buffer to those wells of the microtiter 96-well plate that correspond to the microtiter 96-well DW plate containing sample.
9. Select run protocol "**4412639PrepSEQ_Sal**" from the KingFisher Flex/MagMax Express magnetic particle processor. Press **Start.**
10. Load the plates according to the read out. Verify orientation A1 to A1.
    a. Tip combs – in microtiter 96-well DW plate; press Start.
    b. Elution plate (140 µL of Elution Buffer) – In microtiter 96-well plate DW plate; press Start.
    c. Wash plate 2 (300 µL of Wash Buffer) - In microtiter 96-well plate DW plate; press Start.
    d. Wash plate 1 (300 µL of Wash Buffer) - In microtiter 96-well plate DW plate; press Start.
    e. Lysis plate (sample in Lysis Buffer) - In microtiter 96-well plate DW plate; press Start.
11. After 18 minutes, the KingFisher Flex/MagMax Express magnetic particle processor prompts you to dispense the **Binding Mix**.
    a. Vortex the Magnetic Particles for 5 seconds until resuspension is complete.
    b. Add 30 µL of Magnetic Particles to each well. Swirl the plate.
    c. Add 180 µL of Binding Solution to each well. Swirl the palte.
    d. Load the plate back into the instrument. Press **Start.**
12. When sample preparation is complete, the message "Enjoy your DNA" is displayed on the screen. Remove the elution plate (containing templates). Discard other plates. Seal and store elution plate at -20˚C for future PCR testing.

General info

Protocol information

Protocol name	4412639PrepSEQ_Sal
Modified by	admin
Kit name	-
Description	-

Sample layout



Reagent info

Plate_1		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Plate_2		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis Buffer	300	-	Reagent
Binding Solution	200	-	Reagent
Magnetic Particles	15	-	Reagent

Plate_3		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Solution	300	-	Reagent

Plate_4		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Solution	300	-	Reagent

Plate_5		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Solution	300	-	Reagent

Plate_6		**96 standard plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	100	-	Reagent

Plate_7		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Plate_8		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Lysis Binding Beads		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis Buffer	700	-	Reagent
Binding	500	-	Reagent
Beads	30	-	Reagent

Wash Plate_1		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer	300	-	Reagent

Wash Plate_2		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**

Wash Solution	300	-	Reagent

Elution Buffer		**96 standard plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	75	-	Reagent

Tips		**96 standard plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Mix Plate		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Lysis Plate		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis content	300	-	Reagent
Isopropanol	210	-	Reagent
Magnetic Particles	30	-	Reagent

Lysis PrepSeq Plate		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis Buffer	300	-	Reagent

Lysis and Binding		**96 DW plate**	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Lysis content	300	-	Reagent
Mgnetic Beads	30	-	Reagent
Binding Buffer (Isopropanol)	210	-	Reagent

Dispensed reagents

Lysis PrepSeq Plate		**96 DW plate**	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Binding Mix	Pause	240	-

Steps data

Tip		96 DW tip comb	
Pick-Up		Tips	
Heating		Lysis PrepSeq Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:07:00, Medium
		Heating temperature [°C]	83
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
Heating 2		Lysis PrepSeq Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:10:00, Medium
		Heating temperature [°C]	37
		Preheat	No
	End of step	Postmix	No
		Collect beads	No
Pause		Lysis PrepSeq Plate	
		Message	Add Binding Mix
		Dispensing volume [µl]	240
	Reagent(s)	Name	Binding Mix
		Volume [µl]	240
Binding of DNA		Lysis PrepSeq Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:07:00, Slow
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	1.5
Wash		Wash Plate_1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	1.5

	Wash 2	Wash Plate_2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	1.5
	Dry	Wash Plate_2	
		Dry time	00:03:00
		Tip position	Outside well / tube
	Elution	Elution Buffer	
	Beginning of step	Precollect	No
		Release time, speed	00:01:00, Slow
	Mixing / heating:	Mixing time, speed	00:01:00, Slow
		Heating during mixing	No
	End of step	Postmix	No
		Collect beads	No
	Elution 2	Elution Buffer	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:04:00, Slow
		Heating temperature [°C]	83
		Preheat	Yes
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	1.5
	Elution 3	Wash Plate_2	
		Release time, speed	00:00:20, Fast
	Leave	Tips	

Lot info

No lot numbers have been defined.