Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

We revised the Short Abstract as suggested.

Please revise and expand the Introduction to include all of the following:

a) A clear statement of the overall goal of this method

b) The rationale behind the development and/or use of this technique

c) The advantages over alternative techniques with applicable references to previous studies

d) A description of the context of the technique in the wider body of literature

e) Information to help readers to determine whether the method is appropriate for their application.

We believe items a) and b) have been adequately covered:

a) A clear statement of the overall goal of this method  
“The overall goal of this method is to prepare quasi-metagenomic DNA from food samples to allow targeted concentration of *Salmonella* genomic DNA and subsequent detection and subtyping of the *Salmonella* contaminant by sequencing.”

b) The rationale behind the development and/or use of this technique.  
“Metagenomics sequencing theoretically allows concerted detection and subtyping of foodborne pathogens. However, food samples present challenges to the pathogen analysis by direct sequencing of the food microbiome. First, foodborne pathogens are often present at low levels in food samples. Most of the commercially available rapid detection methods still require 8 - 48 h culturing to enrich pathogen cells to a detectable level1. Second, many foods contain abundant microflora cells and/or food DNA, making foodborne pathogen DNA a small fraction of food metagenome and an elusive target for detection and subtyping by direct metagenomic sequencing. ”

We revised the INTRODUCTION to address c) and e)

c) The advantages over alternative techniques with applicable references to previous studies:  
“Compared to standard methods for *Salmonella* detection8,9 and subtyping10, the quasimetagenomic approach can substantially shorten the turnaround time from contaminated food and environmental samples to molecular subtypes of the pathogen by unifying the two typically separated analyses into a single workflow.”

e) Information to help readers to determine whether the method is appropriate for their application.

“This method is particularly useful for applications such as foodborne outbreak response and other trace back investigations where robust pathogen subtyping is required in addition to pathogen detection and rapid analytical turnaround is important.”

We don’t understand item d) “d) A description of the context of the technique in the wider body of literature”. Please explain and be specific. Do mean by some sort of primacy claim (e.g., this method being the first of its kind)?

Please move the weblinks to the Reference section and cite them here using intext citations.

We revised as suggested.

Citation?

Please note we pointed out “theoretically”. To our knowledge, there is no paper that reports concerted detection and subtyping of foodborne pathogens using metagenomics sequencing, which is only theoretically possible but highly unlikely. That’s why we developed the quasi-metagenomics approach as an alternative. We don’t think a citation here is available.

What food sample is used here? How do you make environmental swabs? Please write the protocol being as specific as you can with respect to your experiment using imperative tense throughout.

We added representative food samples used in this paper. We added how to prepare environmental swabs.

This is commercial. Please use generic term instead. Maybe sterile lab blender bags. Also Please refer to the commercial terms in the table of materials.

We changed the term as suggested. We added the commercial term to the table of materials.

This is commercial. Please use generic term instead. Maybe lab blender.

Also Please refer to the commercial terms in the table of materials.

We changed the term as suggested. We added the commercial term to the table of materials.

Please expand. Also if this is commercial please use generic terms.

MLG and BAM have previously been defined and cited (see below). We don’t think we need to expand again.

“Note: Food samples are prepared for pre-enrichment according to Microbiology Laboratory Guidebook (MLG) of U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) and Bacteriological analytical manual (BAM) of U.S. Food and Drug Administration (FDA)”

What and how? In which container. Please provide details.

We added details as suggested.

Tube or tubes ? You always get 50 ml samples or more?

We changed “tubes” to “tube”. Yes, as stated in the protocol, always take a 50 mL sample.

Place tube close together or do not place it close together?   
We added “do not”.

The note fits here better. Moved under this step. Please check.  
We agree and confirm.

Please use generic term

We changed it to polysobate 20.

So, after repeating the steps 2.3 – 2.5 you again repeat 2.3 to 2.4? This clarity needs to be brought out

That’s correct. 2.3 and 2.4 together allow magnetic separation of beads. After the third wash, this is needed. We clarified this step as flollows.

After the 3rd wash, perform **the final magnetic separation of beads** by repeating steps 2.3. - 2.4.

Please provide the speed in x g

We changed the unit.

How do you avoid the non- specific binding?

Non-specific binding is not avoidable and does not affect downstream sequencing and analysis. Non-specific binding is the reason why this method is called quasimetagenomics. We added a note to clarify.

Please provide the primer sequence as well.

We added primer sequences.

Along with the beads? Please provide the step number.

Yes. We clarified this step as follows.

“3.2. Mix the MDA product from 2.10 by gently pipetting up and down bead-*Salmonella* complexes along with liquid to create a suspension. Add 2 μL of the suspension into 18 μL of PCR mixture.“

We cannot have three instances of note together. Please consider moving some details to the discussion section. Please have one note at one place.

We combined two notes and changed a note to main text.

Fluorospectrometer or spectrofluorometer ?

Flurospectormeter is the generic name for Nanodrop. <https://www.thermofisher.com/order/catalog/product/ND-3300>

As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
“Because of the often-low abundance and in-homogenous presence of Salmonella in food and environmental samples, culture enrichment before IMS-MDA is still necessary for Salmonella detection and subptyping; **it is therefore a critical step of the protocol.** ”

b) Any modifications and troubleshooting of the technique  
Paragraph 4 of DISCUSSION is dedicated to troubleshooting, including the discussion of a specific example. We revised this part to make it more explicit.

“Higher levels of Salmonella contamination and/or longer culture enrichment of contaminated food samples can result in higher concentrations of Salmonella genomic DNA in modified food microbiomes, which lead to lower Ct values from the real-time PCR analysis. Ct values display a negative correlation with sequencing coverage of the Salmonella contaminant genome4, **and therefore can be used for optimization and modification of the workflow**. According to our experience, culture enrichment, especially the length of it, **is often the focus of troubleshooting efforts.** **For example,** the Ct value of the IMS-MDA product from a raw chicken breast sample was relatively high at 35.94 (Figure 1). This sample was inoculated with 2.5 CFU/g of SE cells and incubated in BPW at 37 °C for 2 h. Sequencing of this sample did not provide sufficient coverage of the SE genome to allow serotype prediction. In comparison, all the other samples with 4 h or longer enrichment were successfully serotyped from quasimetagenomic sequencing data (data not shown).”

c) Any limitations of the technique

“**The quasimetagenomic technique has several limitations.** First, DNA concentration and purity of IMS-MDA products measured by fluorospectrometer should be used only as a preliminary indicator of proper sample preparation. Due to the high efficiency of MDA, trace amounts of input DNA can be amplified to suffice whole genome sequencing17. Therefore, high quality and quantity DNA samples can still be generated even if culture enrichment or IMS fails to effectively concentrate Salmonella cells. The optional real-time PCR analysis provides a more targeted and informative assessment of the entire sample preparation workflow. Second, difficult-to-culture Salmonella cells such as so called viable but nonculturable (VBNC) bacteria18 may not grow during culture enrichment. Therefore, these cells may not be detected by our method.”

d) The significance with respect to existing methods

“In summary, **compared to traditional methods**, our sample preparation method and quasi-metogenomic sequencing approach can allow substantial reduction of the analytical turnaround from Salmonella-contaminated food and environmental samples to robust subtyping of the Salmonella contaminants.”

e) Any future applications of the technique

“In addition to Salmonella, this technique has the potential be applied to rapid and concerted detection and subtyping other foodborne pathogens.”

Is this open access?

Yes.

Are this open access?

Yes.