A Novel Lysis Method for the Purification (Gram +) and (Gram –) Bacterial gDNA from Food Samples

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1. Introduction

Pathogen detection from raw and processed food samples requires specialized testing to identify and quantify the presence of harmful bacteria. Testing is performed by a variety of organizations including contract food testing labs, food manufacturers, academic researchers, and consumer protection agency labs. Most of the contract food testing labs test for pathogens using direct amplification methods whereas academic researchers and government agencies preferred qPCR based methods that start with purified bacterial gDNA.

Direct amplification methods do reduce some of the pre-processing steps however, we have learned that in many cases the presence of sugars, fats, and oils, inhibit direct amplification assays.

Here we present both customer evaluation data and Promega testing and provide performance and preference feedback.

Here we present two Maxwell RSC protocols (Method 1 and Method 2) designed for the efficient purification of Gram - and Gram + bacterial amplifiable DNA from raw and processed food samples.

The goal was to have customers evaluate and compare the two methods and provide performance and preference feedback.

Here we present both customer evaluation data and Promega testing data demonstrating a preference for Method 1.

2. Reagents and Instrument

The PureFood Pathogen kit (Catalog # AS1660) includes:

- 50 Elution Tubes (0.5ml)
- 50 Maxwell Plungers
- 48 Maxwell® RSC Cartridges (RSCJ)
- 25ml Lysis Buffer B

The PureFood Pathogen kit (Catalog # AS1660) includes:

- Maxwell® RSC Instrument, (Cat. #AS4500)

Instrument:

Maxwell® RSC instrument, (Cat. #AS4500)

- Purifies 1 to 16 samples of DNA or RNA in less than 40 minutes.
- Controlled by software user interface on a Windows 8 Surface Pro tablet
- Integrated quantitation with Quanta™ Fluorometer

3. Sample Enrichment

During development collaborating customers were provided early access kits along with two protocol versions to evaluate. Both protocols were summarily designed to:

- Disrupt the cell walls of Gram + bacteria and the cell envelopes of Gram – bacteria without the need for multiple protocols or separate reagents. Salmonella, E. coli, and Listeria
- Minimize the carry-over of potential PCR inhibitors from the food matrices, i.e. fats and oils

The two methods evaluated differed by sample input volume and the addition of a centrifugation step:

Method 1: 800µl of media/food sample input volume
Method 2: 1000 µl of media/food sample input volume and a centrifugation step

Method 1 - Current PureFood Pathogen Protocol
1. Add 800µl of Media/food sample to 1.5ml tube
2. Add 200µl of Lysis Buffer A vortex to mix
3. Incubate at 56°C, 4-6min on Thermomixer shaking at 1000-1200 rpm
4. Add 300µl of Lysis Buffer B vortex to mix
5. Add to well-1 of Maxwell FFS cartridge
6. Elute with 50ul of Elution Buffer

Method 2 - Alternative Method Tested
1. Add 960µl of Media/food sample to 1.5ml tube
2. Centrifuge 1ml of culture 10,000g in microfuge, 5min to pellet (remove supernatants via pipet)
3. Add 200µl of Lysis Buffer A vortex to mix
4. Incubate at 56°C, 3-4min on Thermomixer shaking at 1000-1200 rpm
5. Add 300ul of Lysis Buffer B vortex to mix
6. Add to well-1 of Maxwell FFS cartridge
7. Elute with 50ul of Elution Buffer

4. Analysis and Results

5. Competitive Benchmarking

Benchmarking was conducted comparing the Promega PureFood Pathogen kit to CONGEN SureFast Prep and Qiagen mericon kits. The PureFood Pathogen kit samples were processed using Method 1 and all other samples were processed using the manufacturers recommended protocols.

Comparison of Promega's Direct lysis and Pellet methods for the purification and identification of Listeria innocua in beef samples selected at 19 CFU.

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6. Conclusion

We have developed a magnetic particle-based method for the efficient purification of Gram - and Gram + bacterial amplifiable DNA from raw and processed food samples.

- Internal testing and customer alpha testing demonstrated a preference for Method 1 which is now the current PureFood Pathogen Prototype Protocol
- The system can process 1 to 16 on the Maxwell® RSC
- The chemistry gives equivalent to superior yields to silica-based system methods as determined by qPCR.
- The chemistry is also robust to handle fungal samples and challenging Gram + such as Micrococcus luteus.
- This chemistry will continue through development process with a target release of September 2017.

Figure 1. Maxwell RSC and Quanta

Table 1. Customer Data from Alpha Testing

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Protocol</th>
<th>Pre-enrichment</th>
<th>Direct Lysis</th>
<th>Pellet (Std)</th>
<th>Direct Lysis</th>
<th>Pellet (Std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria innocua</td>
<td>Method 1</td>
<td>19 CFU</td>
<td>19 CFU</td>
<td>19 CFU</td>
<td>19 CFU</td>
<td>19 CFU</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>Method 1</td>
<td>100 CFU</td>
<td>100 CFU</td>
<td>100 CFU</td>
<td>100 CFU</td>
<td>100 CFU</td>
</tr>
</tbody>
</table>

Sample spiked with Listeria innocua at 19 CFU per inoculant

7. Competitive Benchmarking

Prototype Method comparison using ground beef

Figure 4. qPCR amplification of Listeria innocua (lin02483 target): 34 CFU/Whole Milk

Figure 5. qPCR amplification of Listeria innocua (lin02483): 19 CFU HVAC/ground beef