

Multiplexed Differential Identification of Enteric Bacteria by Bacteriophage Amplification Coupled to MALDI-TOF-MS

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Abstract

Background: Bacteriophage amplification detection coupled with modern protein assays has been shown to be efficacious for the detection of bacteria. This study demonstrates the ability of a modern protein detection device, Matrix-Assisted Laser Desorption-Ionization mass spectrometry (MALDI-MS), to simultaneously detect the presence of two bacteria in the same sample by monitoring mass spectrally the amplification of phages specific for each bacterium. **Methods:** *E. coli*, *Salmonella* spp, and coliphage MS2 were acquired from the ATCC (Manassas, VA), and the bacteriophage SS1, a virus specific for *Salmonella* spp, was developed in-house. All bacteria were cultured in tryptic soy broth, and phage were propagated and enumerated according to well-established protocols. Phage titers were added to bacterial suspensions at phage concentrations below the detection limit of the MALDI instrument, and allowed to incubate for 3 hours. All mass spectra were collected on an AB Biosystems Voyager MALDI mass spectrometer. **Results:** A control spectrum of the MS2 coliphage shows a peak at approximately 13.75 kDa, while a control spectrum of SS1 shows a peak at approximately 13.55 kDa. When MS2 and SS1 are simultaneously added to suspension of *E. coli*, only the peak consistent with the MS2 phage can be detected in the resultant mass spectrum after incubation. Additionally, when titers of MS2 and SS1 are added to a suspension of *Salmonella*, only a peak consistent with that of SS1 can be seen in the mass spectrum. Furthermore, when both phages are added to a suspension of both *E. coli* and *Salmonella*, a peak consistent with SS1 and MS2 can be detected in the same mass spectrum after suitable incubation. **Conclusions:** Multiple bacteria can be detected in the same suspension using bacteriophage amplification coupled with MALDI-MS.

Introduction

The use of bacteriophages for diagnostic purposes has received renewed interest. The exquisite specificity of phages for their bacterial hosts can be exploited to imply the presence of bacterial strains in a given sample. The bacteriophage replication cycle can further be utilized to amplify a signal generated from a phage infectious event. For example, one phage infecting one bacterium can produce hundreds to thousands of progeny from a single phage infection. Thus if one has the capability of detecting an increase in a specific phage's concentration in a sample, clearly the presence of a specific bacterium can be implied.

Modern protein detection methods, such as Enzyme Linked Immunosorbent Assays, Lateral Flow Immunochromatography, and mass spectrometry have the potential for replacing the traditional plaque assay as a means of detecting the presence of phage. Of pertinence to this study, matrix assisted laser desorption-ionization mass spectrometry (MALDI-MS) can readily visualize proteinaceous components of the phage, in particular the oft-times repeating motif of the capsid protein. Therefore, MALDI-MS can be used to monitor the increase in phage concentration by detecting the appearance of a biomarker as a function of time.

In this study, MALDI-MS is used to simultaneously monitor the increase in concentration of two phages when both hosts are present in a single sample. The results of this study clearly demonstrate the ability of multiple phages revealing the presence of multiple genera of bacteria in an individual sample.

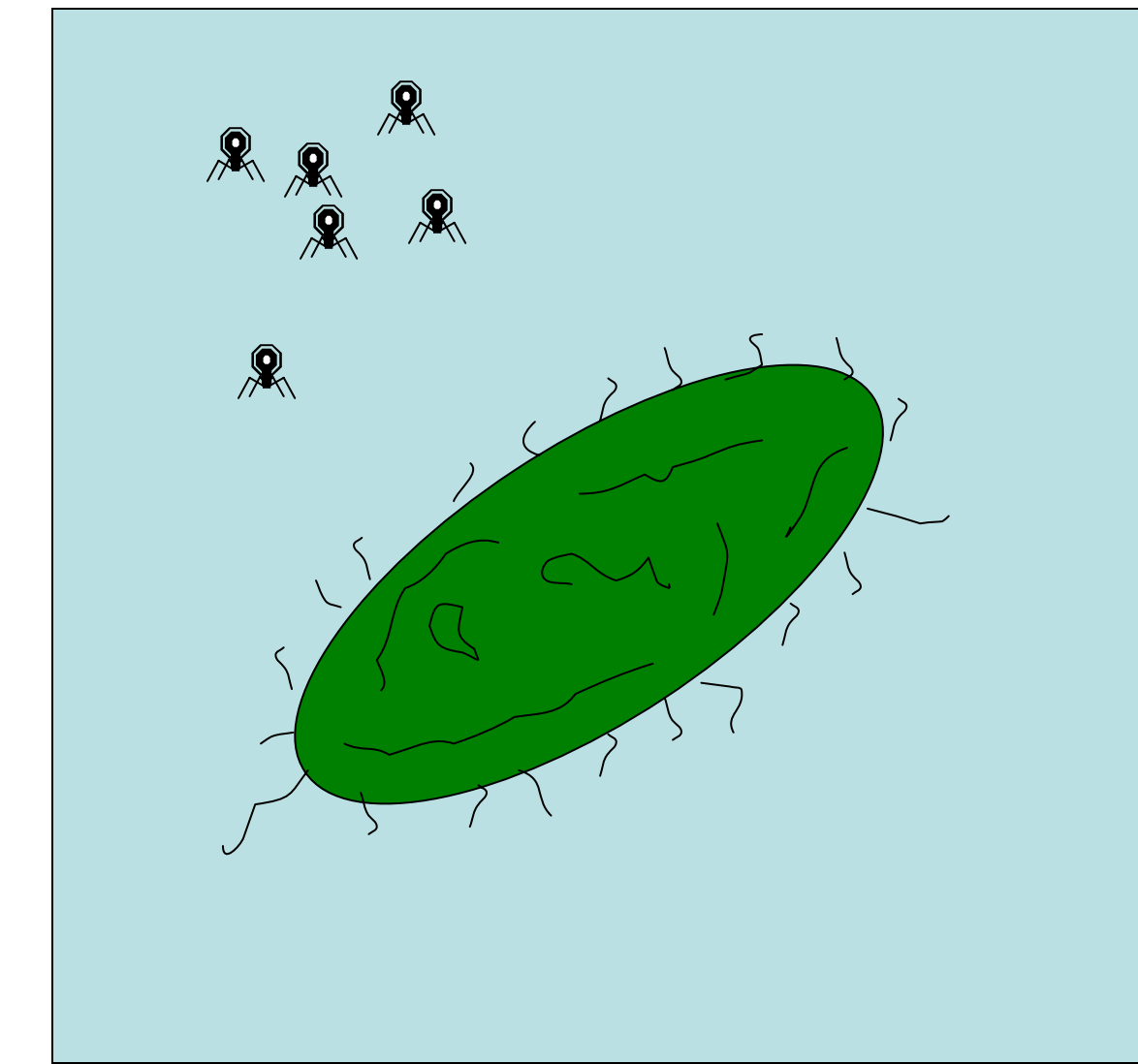
Methods

E. coli (ATCC 11755), MS2 coliphage and *Salmonella* spp. (ATCC 13311) were obtained from the American Type Culture Collection (Manassas, VA USA). SS1 phage was developed at MicroPhage, Inc. (Longmont, CO). Bacterial samples were cultured in tryptic soy broth, (Difco), and bacterial cell concentrations were estimated using standard optical density measurements. Phage concentrations and phage cross-reactivity were determined using the standard plaque assay.

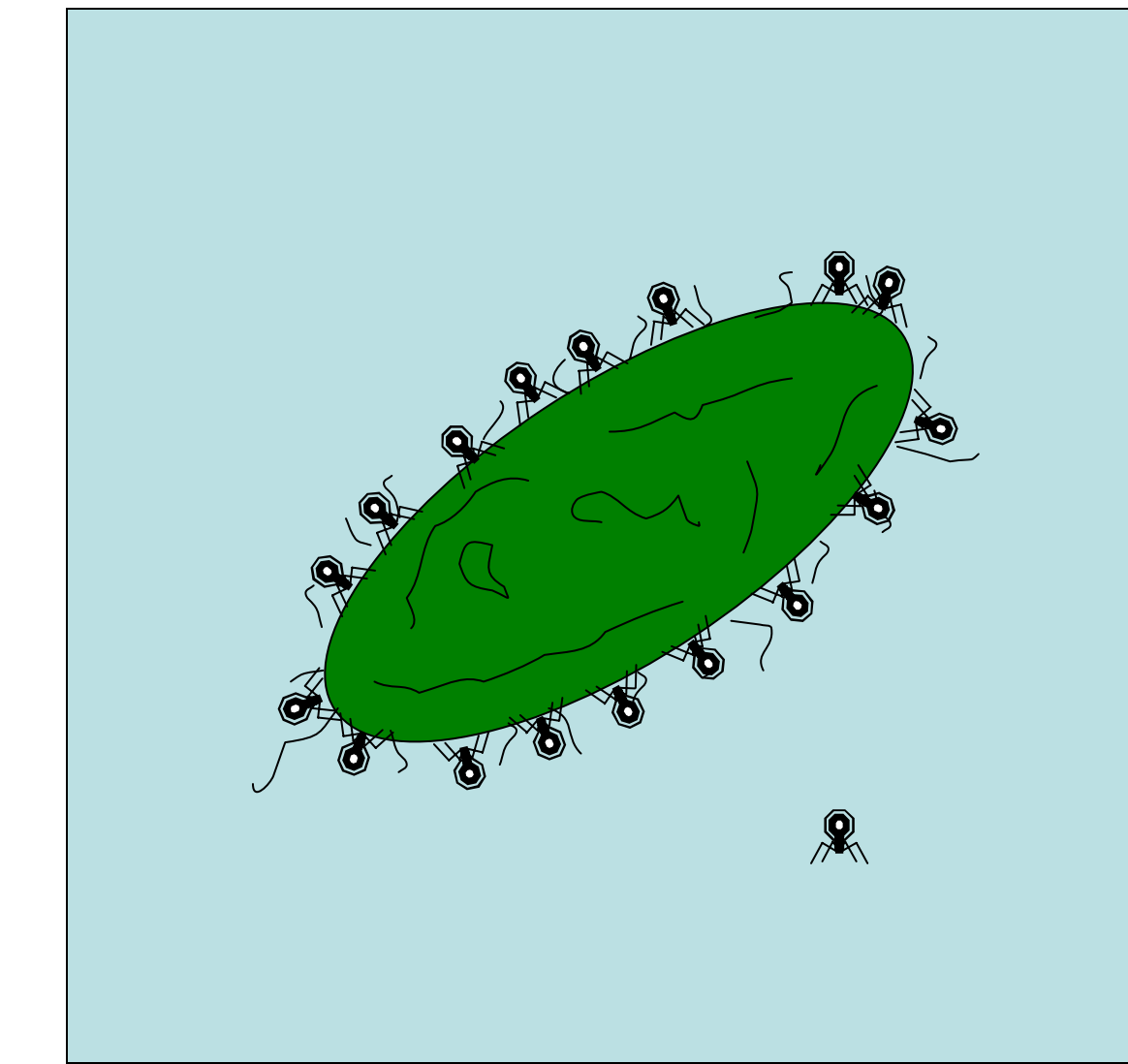
800 µl of each bacterium at a concentration of approximately 10⁵ cells/ml was inoculated with 100 µl of each respective phage solution below the detection limit of MALDI, so that the final volume of each sample was 1000 µl. Additionally, a single sample containing both *Salmonella* spp. and *E. coli* at a concentration of 10⁵ cells/ml was inoculated with a suspension containing both SS1 and MS2 at titers below the detection limit of MALDI. All samples were examined for phage amplification by MALDI-MS after 3 hours of incubation at 37° C.

All mass spectra were collected on a Voyager DE-STR+ (Applied Biosystems, Framingham, MA USA) MALDI-TOF instrument operating in positive, linear mode. Samples were prepared for MALDI using a sandwich technique by placing 1µl of matrix on a sample plate and allowing it to dry, followed by 1µl of sample, and finally another 1µl aliquot of matrix was added to the sample as it was drying. All samples utilized a matrix of 12.5 mg of ferulic acid in a solvent system of 17% formic acid, 33% acetonitrile, and 50% water.

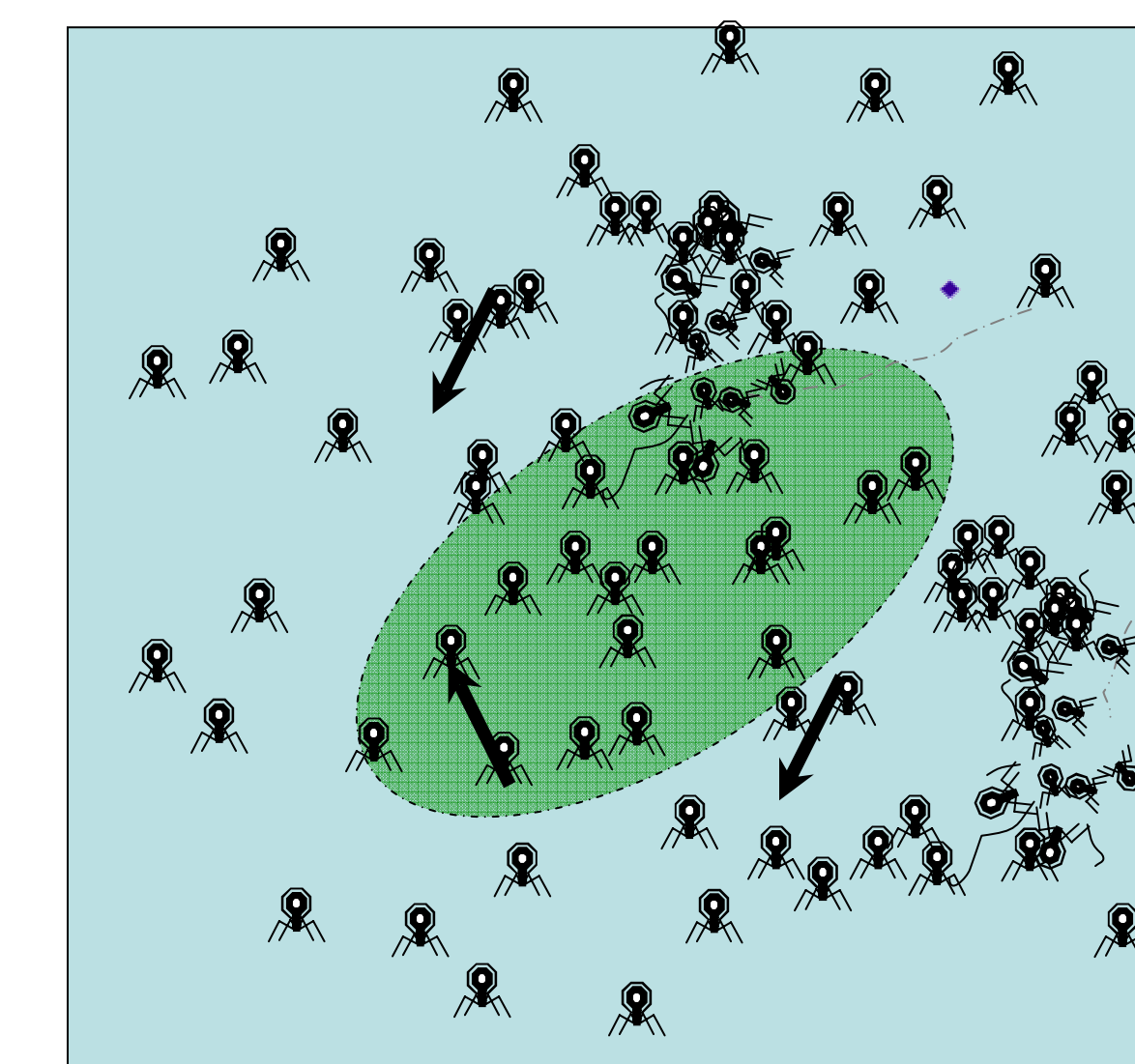
Phage Amplification



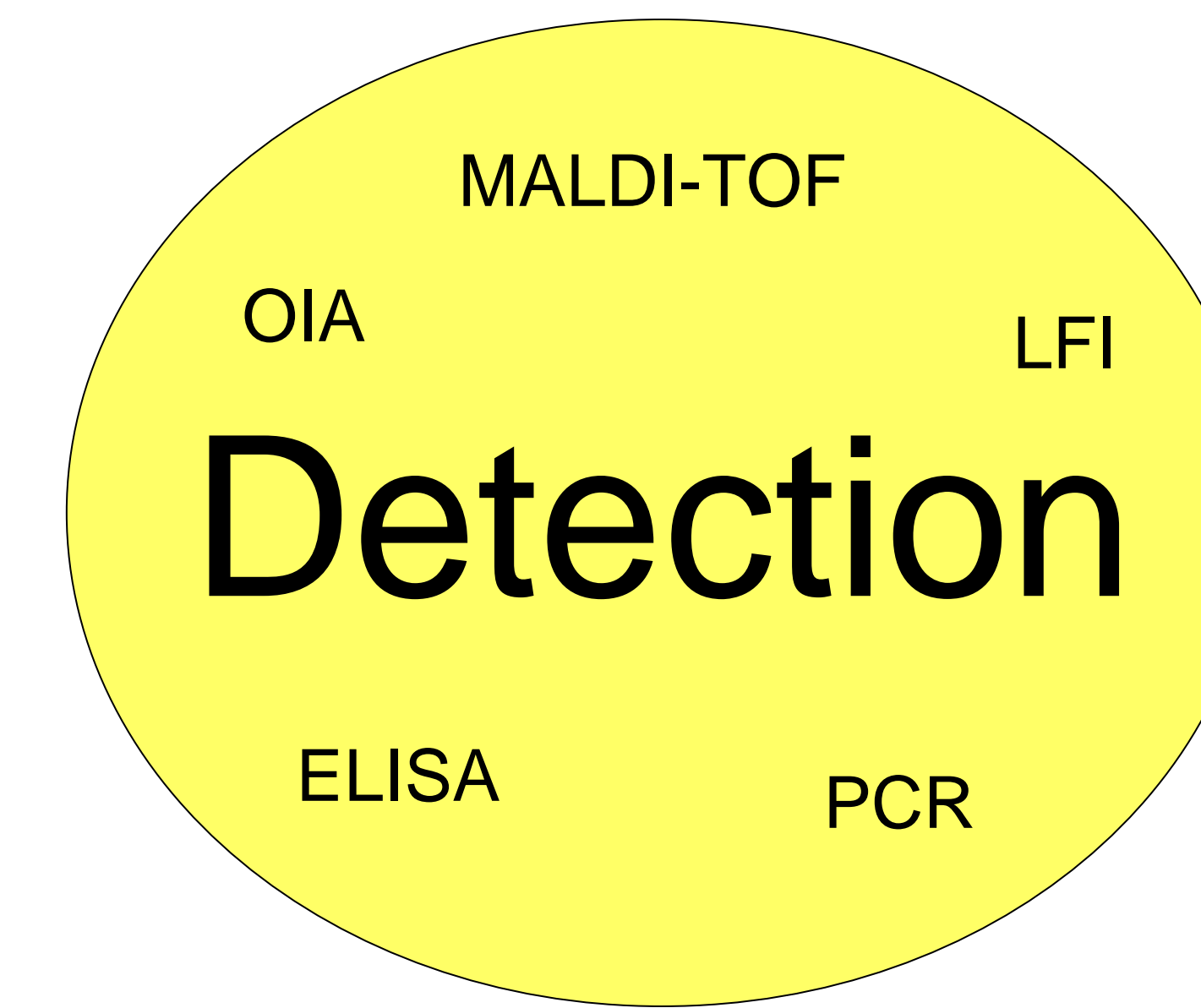
Step 1 Incubate phage with bacteria



Step 2 – Allow time for phage to bind and infect

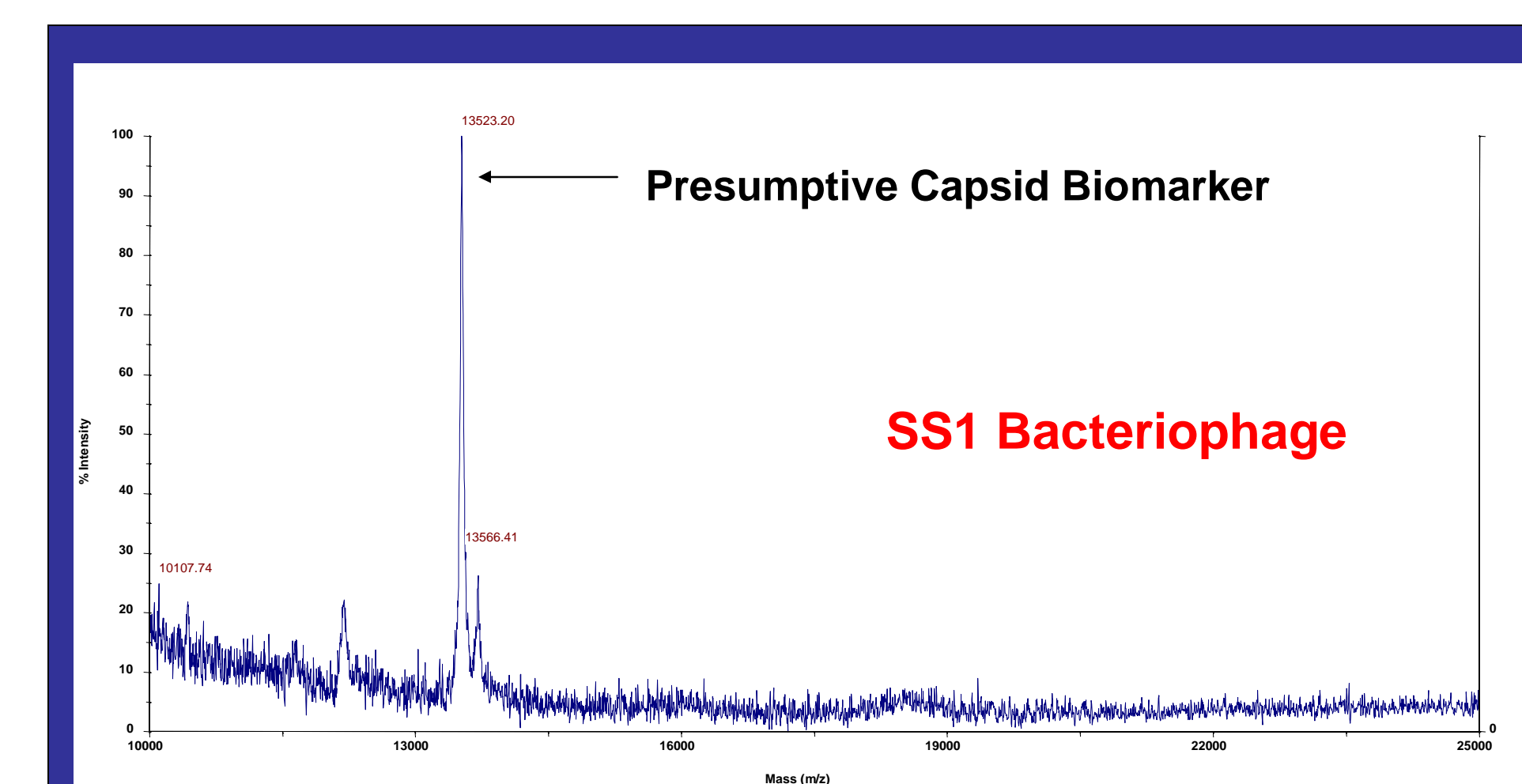
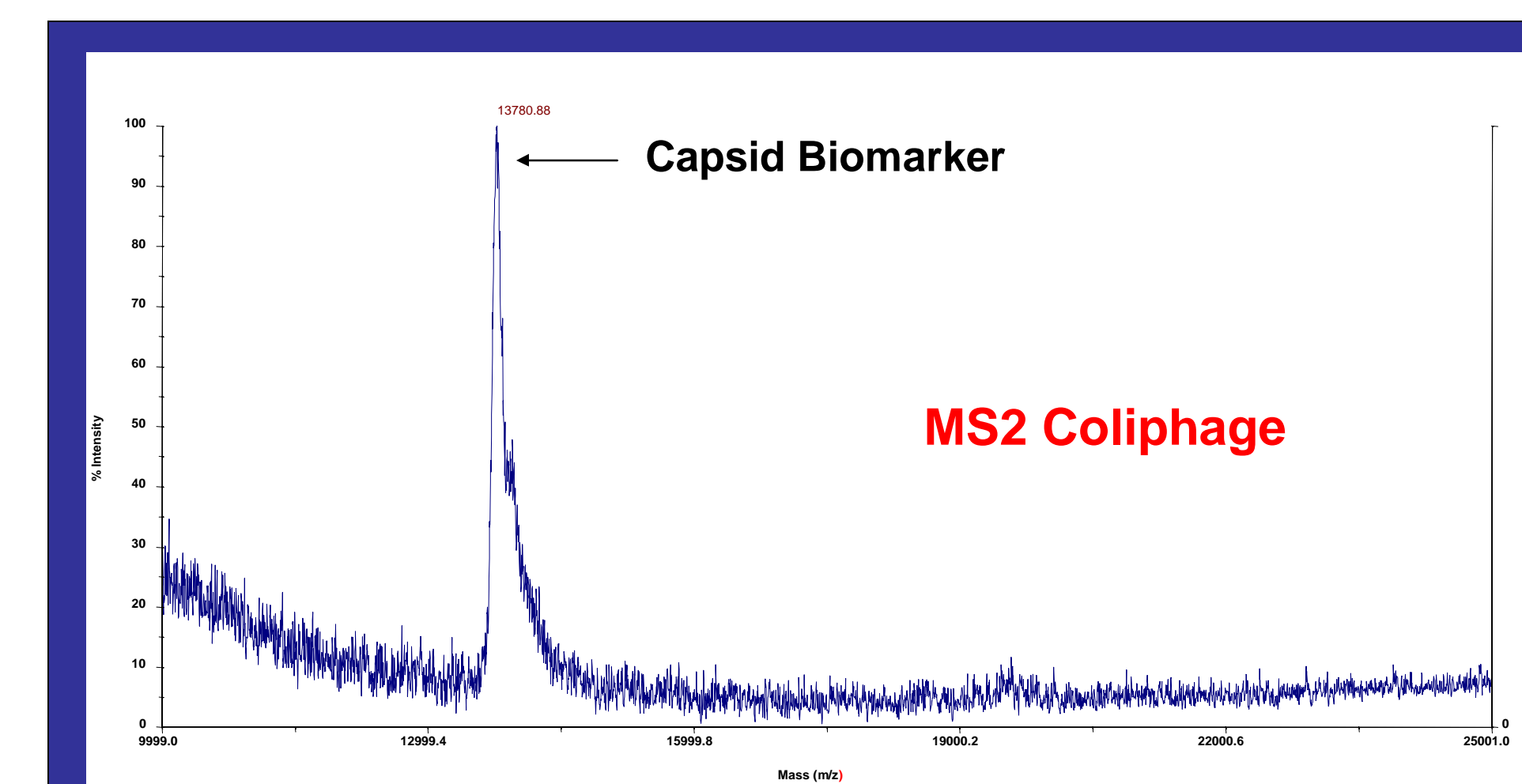


Step 3 – Incubate samples until Phage lyse the bacteria, releasing progeny



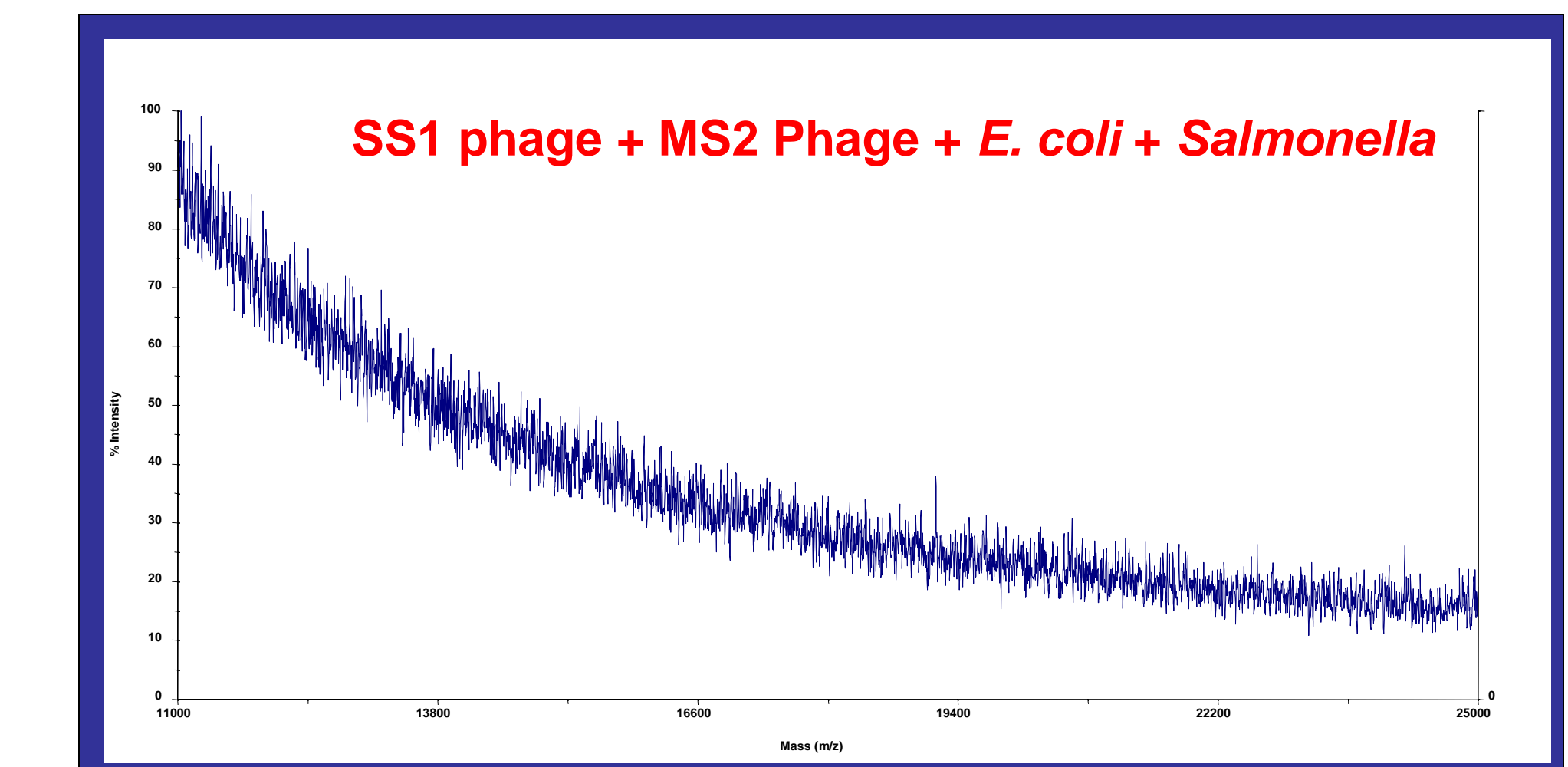
Step 4 – Detect the results of phage amplification with modern methods

Results



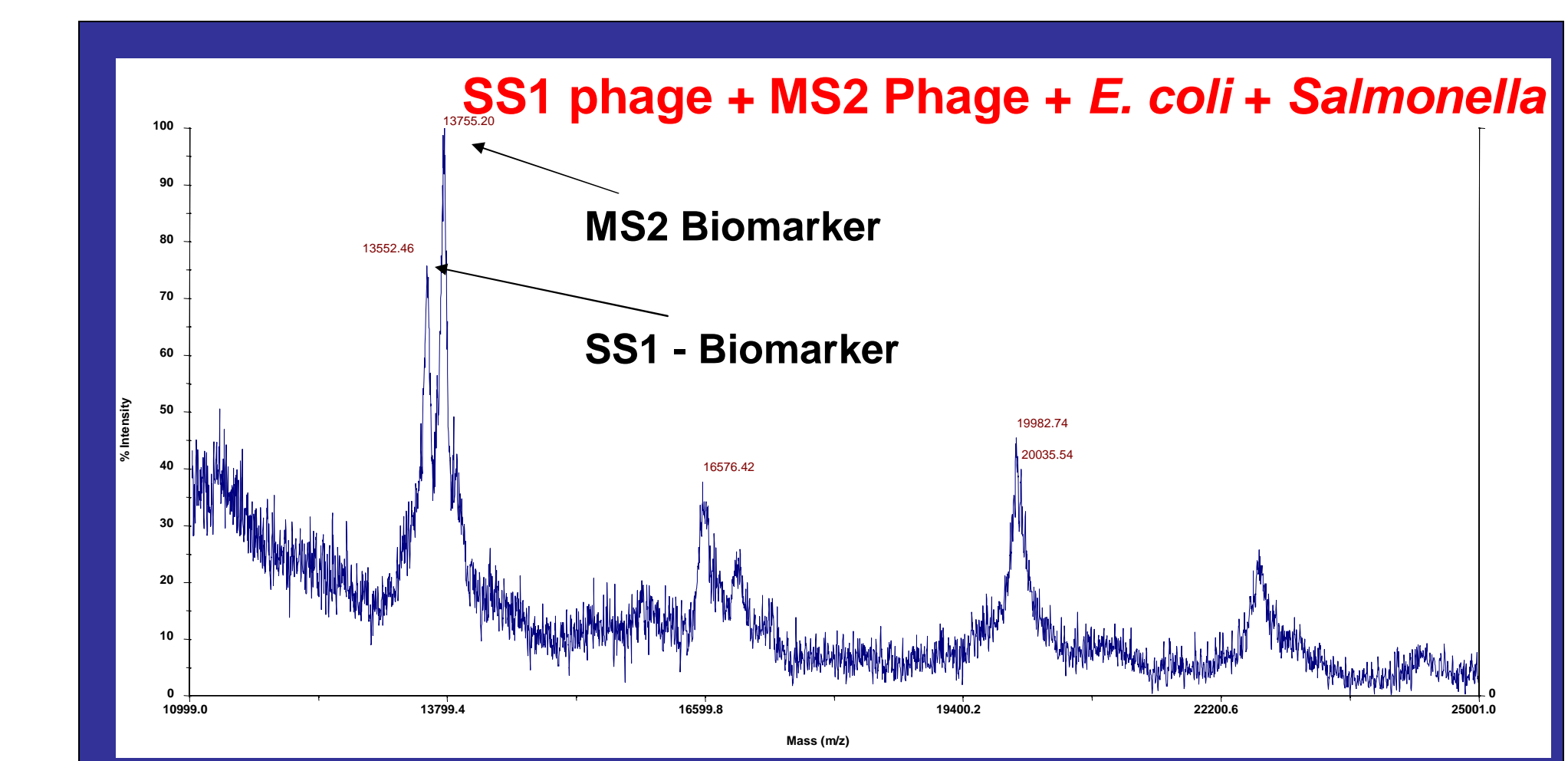
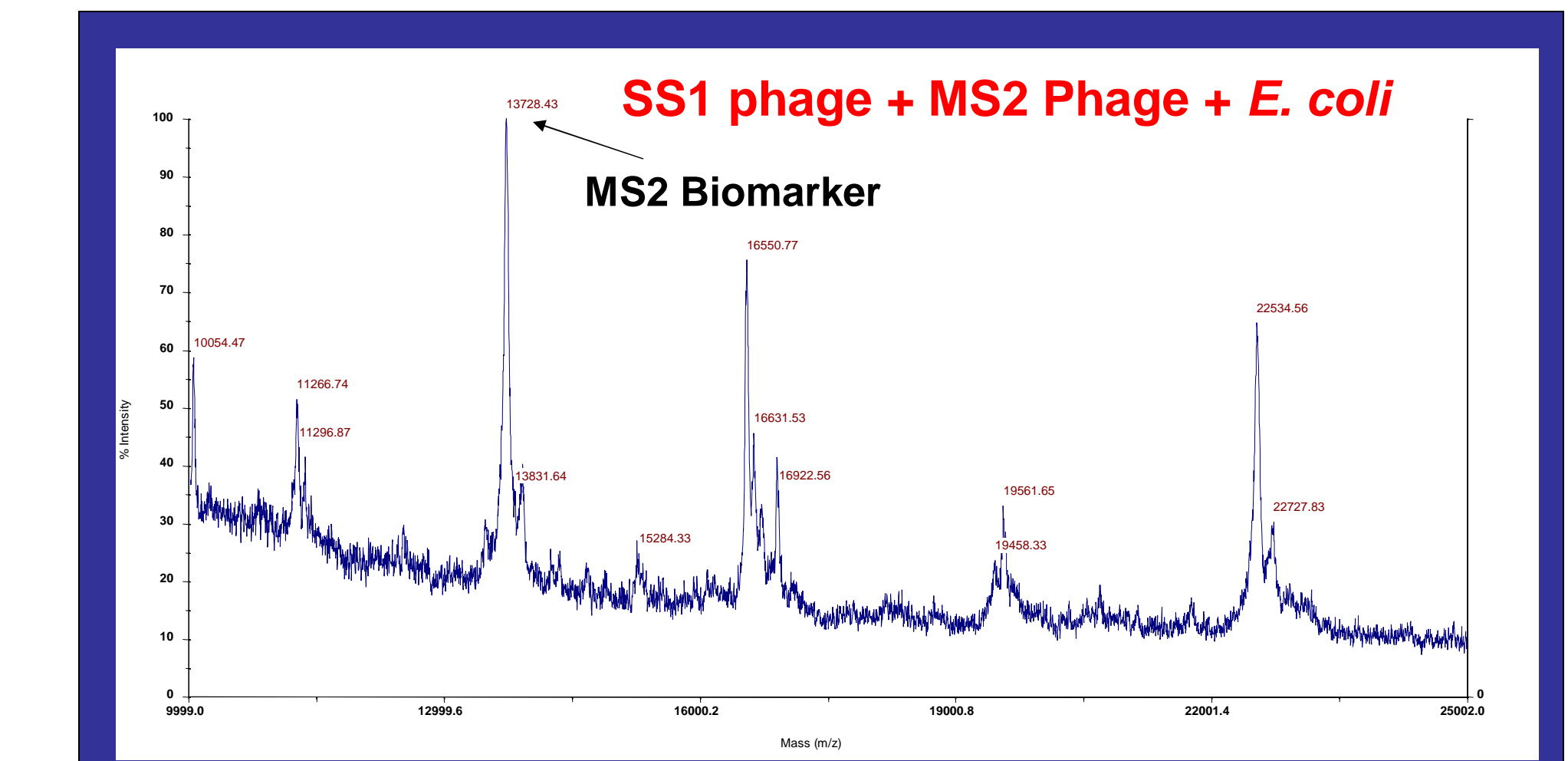
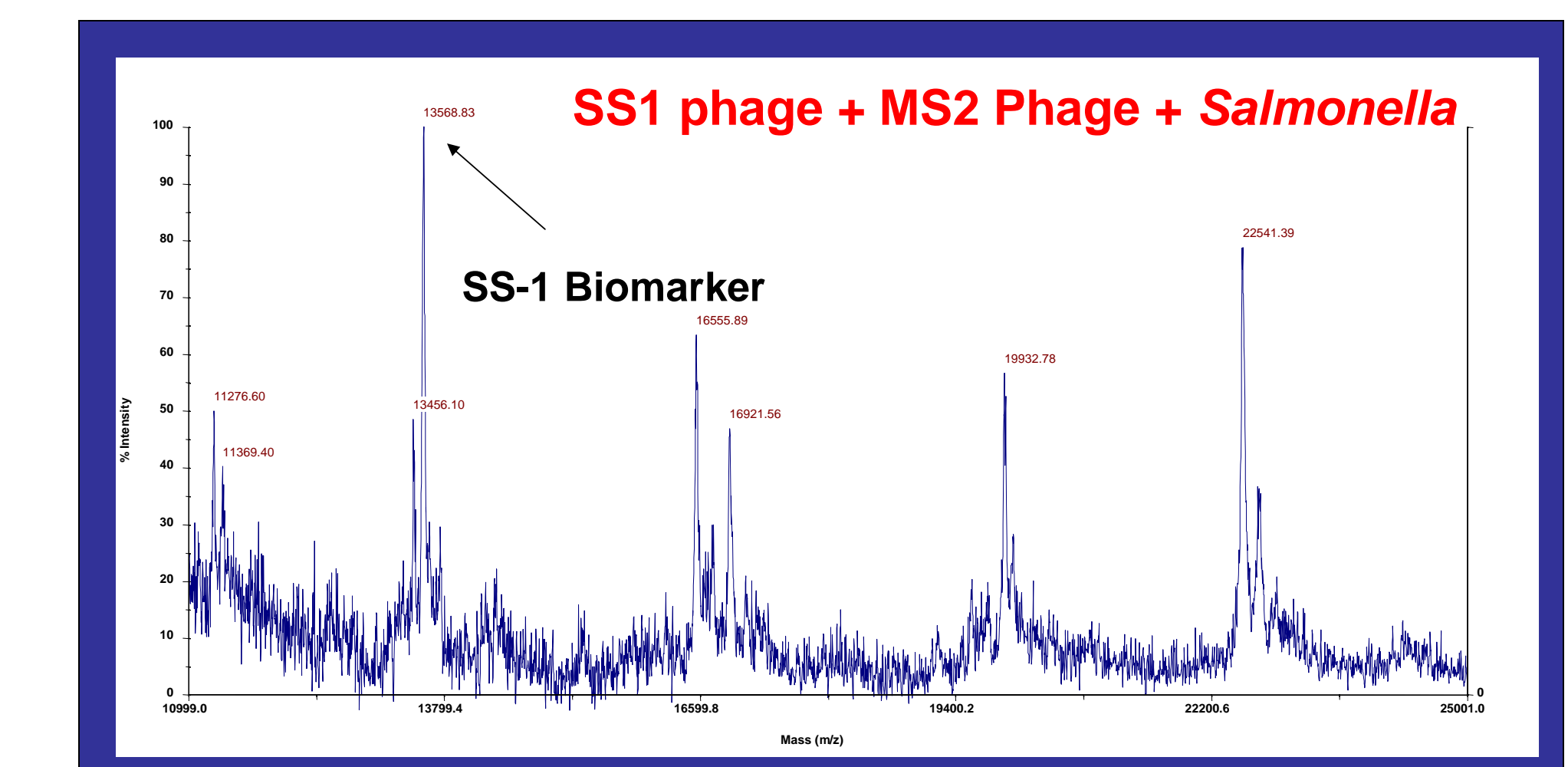
Reference spectra of high concentrations of phage show consistent biomarkers indicative of the respective phage, approximately 13.7 kDa for MS2, and 13.5 kDa for SS1

Reference Spectra



Immediately after combining low concentrations of phage with low concentrations of bacteria, no peaks consistent with the reference spectra can be seen

Time = 0



When both MS2 and SS1 are incubated with *E. coli*, only the peak consistent with MS2 can be seen in the resultant spectrum, verifying the presence of *E. coli*. When both phages are incubated with *Salmonella*, only the peak consistent with SS1 can be seen, indicating the presence of the *Salmonella*. When both phages are added to samples containing both *E. coli* and *Salmonella*, peaks consistent with both phages can be seen, indicating the presence of both bacteria in the sample

Time = 3 hours of incubation