Designing Synthetic Positive Controls for Highly Multiplexed Amplicon Sequencing Panels

Primer

1. Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA; 2. ASRT, Inc., Atlanta, GA; 3. WDS, Inc., Suwanee, GA; 4. Oak JR Hensley1,2, R Jin1,3, GK Vestal1,4, JL Rowell1,3, ST Lucking1,3, A Khan1,3, AD Huang1, HA Carleton1, AJ

Updated Abstract

Background: For characterization of antimicrobial resistance determinants, various sequencer-based approaches for analyzing complex samples are used. The high throughput nature of these technologies, however, requires lengthy intervals between the completion of sample runs and the availability of results. In order to achieve maximum cost-effectiveness in this workflow, synthetic controls are widely used in the form of PhiX, 51x and 100x, 35% were between 100x

Methods: Traditional plasmid-based methods creates substantial biosafety concerns. Therefore, we designed a pool of synthetic sequences for PhiX, mappings. Mapping results were filtered using in-house R scripts. Updated Abstract

Conclusion

Without requiring isolation of host microbes. However, validation of every primer pair on a HMAS panel is more challenging than validating individual targets. Using Plasmid-based methods, it would only require a single sample's space on the

Acknowledgments

Methods: Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, which includes expanding the primer panel to include additional targets, evaluating routes of potential contamination, and designing targets for each primer pair. These targets were designed using methods to evaluate the performance of the HMAS platform in detection of antimicrobial resistance determinants.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Table: Synthetic Positive Controls

<table>
<thead>
<tr>
<th>Antimicrobial Class</th>
<th>Number of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>5</td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>2</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>1</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>2</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>2</td>
</tr>
<tr>
<td>Penicillin</td>
<td>3</td>
</tr>
<tr>
<td>Other Anti-infectives</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>118</td>
</tr>
</tbody>
</table>

Conclusions:

The synthetic positive controls were an efficient method to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

The synthetic positive control for use in a network of laboratories. Future studies will

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Figure 1: Method Workflows

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

Figure 1: Method Workflows

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.

The synthetic positive controls were an efficient way to validate a HMAS panel targeting sequences from photobacterium. The HMAS platform provides rapid detection of thousands of antimicrobial resistance determinants (ARDs) directly from clinical samples with the

Amplicons were successfully detected for all synthetic positive control sequences. However, amplification efficiencies varied across targets. Evaluating routes of potential contamination, and designing targets for each primer pair is expensive and time-consuming. Therefore, we designed a pool of synthetic sequences for PhiX, Wolbachia pipientis, and Streptomyces coelicolor.