A PCR-based system for molecular *E. coli* O:H serotyping

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SUMMARY: The serotyping of isolates with O (lipopolysaccharide) and H (flagellar) antigens is used as a conventional method for subtyping of *E. coli* isolates, but agglutination reactions with specific antisera are laborious and time-consuming. To assist conventional *E. coli* serotyping, we developed a PCR-based method for *E. coli* O-gene typing and H-gene typing systems (36 multiplex PCR kits containing 162 *O*-typing primer pairs and 10 multiplex PCR kits containing 51 *H*-typing primer pairs), based on the sequence data of O-antigen biosynthesis gene clusters and flagellin-encoding genes. Additionally, we developed 8 PCR primers targeting novel *O*-types. This optimized and unified PCR-based methodology allows for comprehensive, rapid, and low-cost typing, and is a promising tool for evaluating the routes, sources, and prevalence of isolates in STEC outbreak investigations, as well as in epidemiological studies for monitoring local, national, and international trends in pathogenic *E. coli*.

Table 1. *E. coli* serotype, genotype and PCR-based genotyping system

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Genome/Sequence comparison</th>
<th>Development of PCR-based genotyping method</th>
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<tr>
<td>Serological type (no. of type)</td>
<td>Sequence source strain</td>
<td>Marker genes</td>
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</table>

Additionally, we discovered 15 novel *O*-types and developed 8 specific primers.

A. Sequence comparison of O-antigen biosynthesis gene clusters and marker genes

- Two O-serogroup strains O14 and O57 carried defective O-antigen biosynthesis gene clusters.
- 147 O-serogroup strains carried unique marker genes (>95% DNA sequence identity).
- 35 O-serogroup strains carrying very similar or identical marker genes (95%) were grouped into 15 O-groups.

B. 162 single primers for identifying 162 *O*-types (product size: 132 - 1,253 bp)

C. 20 multiplex PCR kits

- MP-1: O-typing kit targeting STEC-associated *O*-types

D. Sequence comparison of flagellin-encoding genes

- 51 *H*-types carried unique marker genes.
- 2 *H*-type strains H1 and H12 carrying very similar *H*-types (85%) and grouped into an *H*-type H112
- Because PCR product using the H17 (rfa) targeting primer pair were not obtained from H17 reference strain, the H17-typing primer was removed from multiplex PCR kits.

E. 51 single primers for identifying 51 *H*-types (product size: 150 - 774 bp)

F. 10 multiplex PCR kits

- MP-1: O-typing kit targeting STEC-associated *O*-types

All results can be well obtained under a single PCR condition!

The following files can be downloaded from here.

- This paper (PDF)
- *O*-typing primer sequences (excel)
- *H*-typing primer sequences (excel)

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