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 subtyping strategy in epidemiological studies of pathogenic *E. coli* including STEC. Although serotyping is a standard method for subtyping of *E. coli* isolates, agglutination reactions with specific antisera are laborious and time-consuming. To assist conventional *E. coli* serotyping, we developed PCR-based *E. coli* O-genotyping (Og-typing) and H-genotyping (Hg-typing) systems (20 multiplex PCR kits containing 162 Og-typing primer pairs, and 10 multiplex PCR kits containing 51 Hg-typing primer pairs), based on the sequence data sets of O-antigen biosynthesis gene clusters and flagellin-encoding genes. Additionally, we developed 8 PCR primers targeting novel Og-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*.

Relationship between Serotype and Genotype

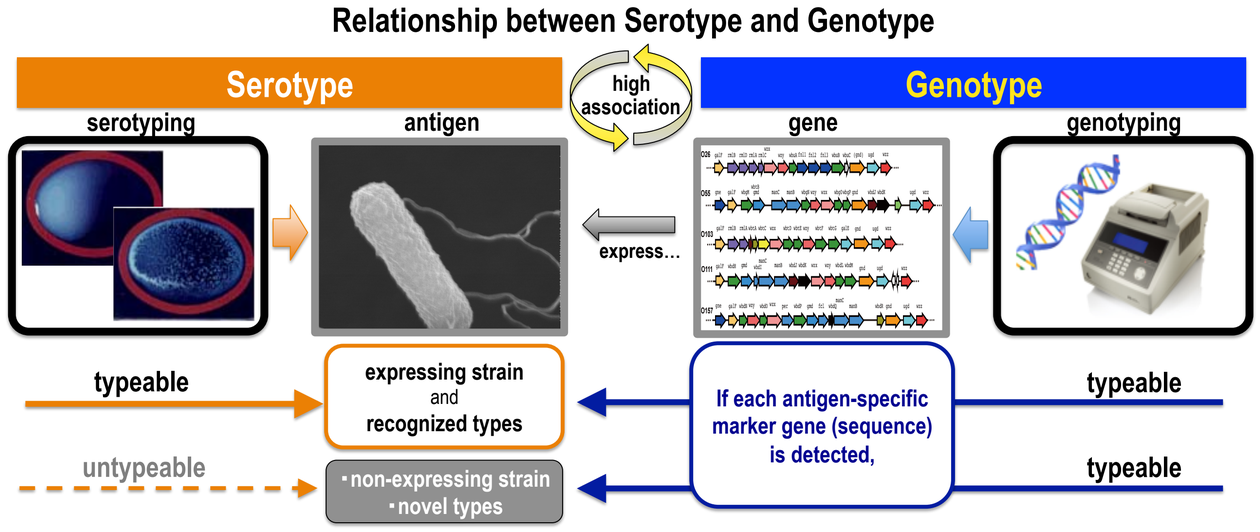


Table 1. *E. coli* serotype, genotype and PCR-based genotyping system

Serotype	Genome/Sequence comparison			Development of PCR-based genotyping method			
Serological type (no. of type)	Sequence source strain	Marker genes	No. of genotype	System	No. of single primer pair	No. of multiplex PCR kit	Reference
***E. coli* O1 – O187 (184 types)**	**O-serogroup reference strains** (Iguchi A et al. DNA Res. 2015)	***wzx/wzy or wzm/wzt***	**162** (see A)	**Og-typing**	**162** (see B)	**20** (see C)	Iguchi A, Iyoda S, Seto K, Morita-Ishihara T, Scheutz F, Ohnishi M; Pathogenic *E. coli* Working Group in Japan. *Escherichia coli* O-Genotyping PCR: a Comprehensive and Practical Platform for Molecular O Serogrouping. ***J Clin Microbiol***. 53(8):2427-32 (2015)
***E. coli* H1 – H56 (53 types)**	**H-type reference strains** (Joensen KG et al. J Clin Microbiol. 2015)	***fliC***	**52** (see D)	**Hg-typing**	**51** (see E)	**10** (see F)	Banjo M, Iguchi A, Seto K, Kikuchi T, Harada T, Scheutz F, Iyoda S; Pathogenic *E. coli* Working Group in Japan. *Escherichia coli* H-Genotyping PCR; a Complete and Practical Platform for Molecular H-typing. ***J Clin Microbiol***. (Accepted)
Additionally, we discovered 15 novel Og-types and developed 8 specific primers.							
?	**Og-type untypeable STEC**	***wzx/wzy***	**6**	**Og-typing**	**6** OgN1, N8, N9, N10, N12, N31	**-**	Iguchi A, Iyoda S, Seto K, Nishii H, Ohnishi M, Mekata H, Ogura Y, Hayashi T. Six Novel O Genotypes from Shiga Toxin-Producing *Escherichia coli*. ***Front Microbiol***. 20;7:765 (2016)
?	**Og-type untypeable ETEC**	***wzx/wzy***	**9**		**2** OgN3, N5	**-**	Iguchi A, von Mentzer A, Kikuchi T, Thomson NR. An untypeable enterotoxigenic *Escherichia coli* represents one of the dominant types causing human disease. ***Microb Genom***. 3;3(9):e000121 (2017)

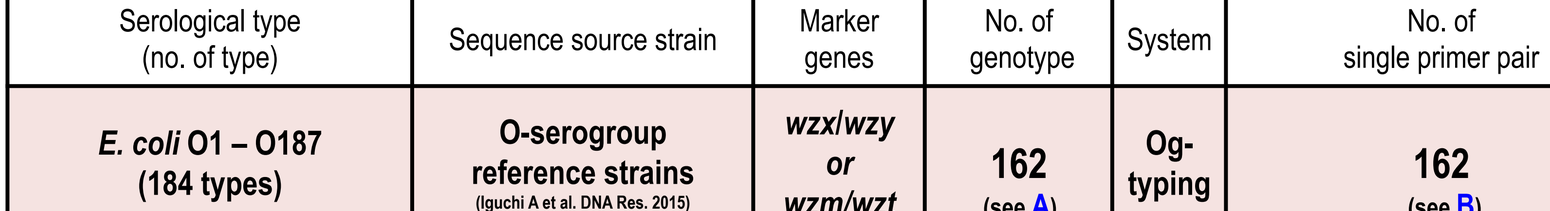
Og-typing PCR

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 cluster.
- 147 O-serogroup strains carried unique marker genes (< 80% DNA sequence identity).
- 35 O-serogroup strains carrying very similar or identical marker genes (≥ 97%) were grouped into 15 Og-types.

OgGp1; O20, O137
OgGp2; O28ac, O42
OgGp3; O118, O151
OgGp4; O90, O127
OgGp5; O123, O186
OgGp6 O46, O134
OgGp7; O2, O50
OgGp8; O107, O117
OgGp9; O17, O44, O73, O77, O106
OgGp10; O13, O129, O135
OgGp11; O153, O178
OgGp12; O18ab, O18ac
OgGp13; O124, O164
OgGp14; O62, O68
OgGp15; O89, O101, O162

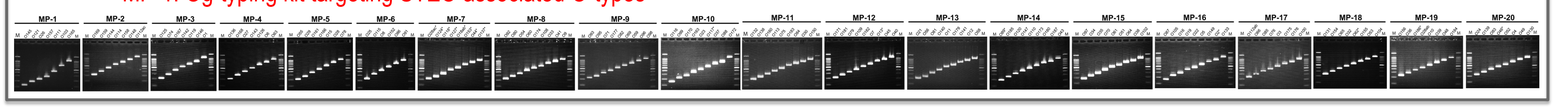
B. 162 single primers for identifying 162 Og-types (product size; 132 – 1,253 bp)



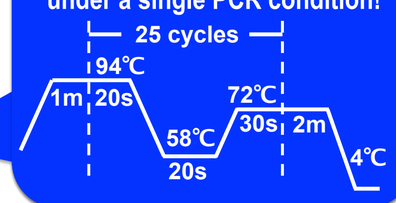
C. 20 multiplex PCR kits

MP 1	MP 2	MP 3	MP 4	MP 5	MP 6	MP 7	MP 8	MP 9	MP 10	MP 11	MP 12	MP 13	MP 14	MP 15	MP 16	MP 17	MP 18	MP 19	MP 20
Og165	Og112ac	Og1	Og63	Og78	Og91	OgGp1	Og9	Og98	Og172	Og150	Og40	Og58	Og43	Og102	Og133	Og100	Og104	Og184	Og130
Og103	Og148	Og146	Og6	Og128	Og86	OgGp9	Og41	Og96	Og88	Og30	Og45	Og12	Og187	Og38	OgGp7	Og176	Og53	Og48	Og49
Og111	Og158	O119	Og126	Og15	Og152	OgGp11	Og33	Og59	Og37	Og84	OgGp10	Og141	Og180	Og64	Og149	Og175	Og155	Og39	Og4
Og157	Og114	Og142	Og143	Og166	Og8	OgGp12	Og108	Og69	OgGp8	Og183	Og7	Og179	Og173	Og51	Og5	OgO3	OgGp14	Og10	Og52
Og26	Og144	Og167	Og27	Og161	Og115	OgGp4	Og174	Og82	Og23	Og75	Og182	Og11	Og110	Og61	Og22	Og76	Og32	Og28ab	OgGp6
Og121	Og159	Og74	Og168	Og29	Og25	OgGp3	Og60	Og177	Og163	Og113	Og109	Og140	Og147	Og70	Og19	Og85	Og65	OgGp5	Og83
Og145	Og169	Og125	Og136	Og55		OgGp13	Og54	Og71	Og170	Og160	Og79	Og81	Og120	Og35	Og16	Og66	Og154	Og36	Og139
						OgGp2	Og80	Og95	Og99	Og138	Og181	Og56	Og185	Og34	Og105	Og112ab	Og131	Og156	Og24
							Og92	Og93	Og116	Og132	Og171	Og21	OgGp15	Og97	Og87				

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



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

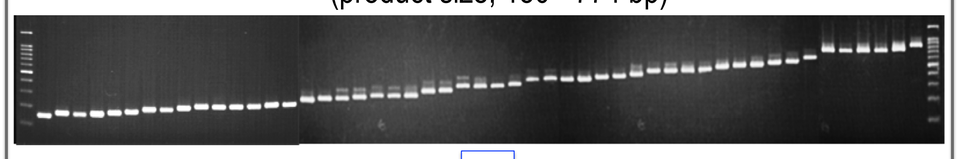


Hg-typing PCR

D. Sequence comparison of flagellin-encoding genes

- 51 H-type strains carried unique marker genes.
- 2 H-type strains H1 and H12 carrying very similar *fliC* genes (98%) and grouped into an Hg-types Hg1/12.
- Because PCR product using the H17 (*flnA*)-targeting primer pair were not obtained from H17 reference strain, the Hg17-typing primer was removed from multiplex PCR kits.

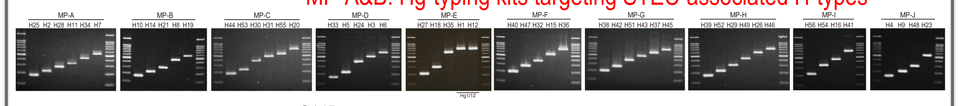
E. 51 single primers for identifying 51 Hg-types (product size; 150 - 774 bp)

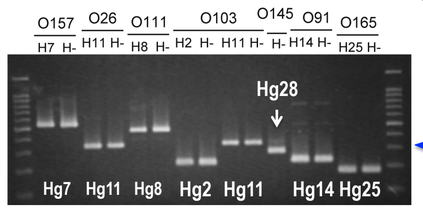


F. 10 multiplex PCR kits

MP A	MP B	MP C	MP D	MP E	MP F	MP G	MP H	MP I	MP J
Hg7	Hg19	Hg20	Hg6	Hg1/12	Hg36	Hg45	Hg46	Hg41	Hg23
Hg34	Hg8	Hg55	Hg3	Hg35	Hg15	Hg26	Hg26	Hg16	Hg48
Hg11	Hg21	Hg31	Hg24	Hg47	Hg32	Hg49	Hg49	Hg54	Hg9
Hg28	Hg14	Hg30	Hg5	Hg40	Hg47	Hg29	Hg29	Hg56	Hg4
Hg2	Hg10	Hg53	Hg33		Hg40	Hg52	Hg52		
Hg25		Hg44				Hg39	Hg39		

*MP-A&B: Hg-typing kits targeting STEC-associated H-types





Non-motile (H-) isolates can also be subtyped into any Hg-type by the Hg-typing PCR.

Table 2. Og:Hg-types of 368 STEC determined by the PCR systems
(STEC were isolated from healthy cattle by using DHL or blood agar plates in Japan, 2010-2016)

Og:Hg*	Og-type: 5	6	8	15	22	23	26	43	45	55	74	82	84	88	91	98	100	103	109	111	113	116	130	139	145	146	156	157	163	168	171	174	175	177	179	181	182	187	Gp2	Gp6	Gp7	Gp8	Gp9	Gp10	Gp11	N1	N3	N5	N8	N10	45+9	116+N31	100+N8	UT	Total
Hg-type 2	0	0	0	0	0	0	0	1	1	0	0	0	5	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	16	0	0	0	1	1	36
7	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10
8	0	0	0	0	8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	22
9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	21
11	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	28
12	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10
16	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	9
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
19	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	10	0	0	0	0	1	0	0	13	58
20	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
21	0	0	3	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	65	4	0	0	0	1	0	0	0	0	0	20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	103
25	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	2	0	0	2	0	5	0	2	0	0	0	0	9	0	0	0	0	0	0	0	1	32
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
28	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
34	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	5
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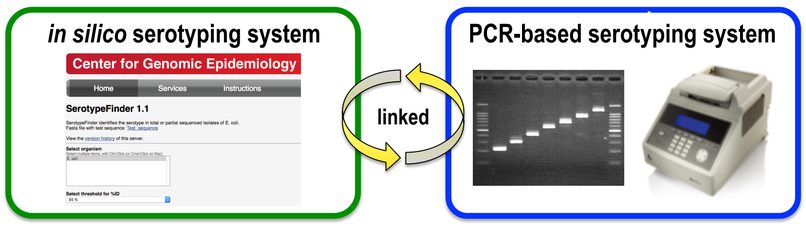
Total	1	8	32	1	8	1	8	1	1	1	2	1	5	1	3	1	1	9	6	4	66	5	15	2	1	1	10	6	6	1	2	20	5	2	7	2	2	3	5	1	4	1	5	1	10	1	10	2	16	1	1	2	1	56	368

eae-positive Og:Hg type UT: untypeable *Red: MP-1 (Og-typing) kit and MP-A&B (Hg typing) kits targeting STEC-associated types

PCR-based serotyping systems were developed based on the reference sequences used in the WGS-based *in silico* serotyping Web-tool, **SerotypeFinder** (Joensen KG et al. J Clin Microbiol. 2015) operated on the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).



The following files can be downloaded from here.

- This poster (PDF)
- Og-typing primer sequences (excel)
- Hg-typing primer sequences (excel)

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