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Rapid and Accurate Identification of *Escherichia coli* K-12 Strains

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A specific PCR for the identification of K-12 strains, based on the genetic structure of the O-antigen gene cluster (*rfb*) of *Escherichia coli* K-12, is described. The assay clearly differentiates *E. coli* K-12-derived strains from other *E. coli* strains used in the laboratory or isolated from human and animal clinical specimens, from food, or from environmental samples. Moreover, lineages of K-12 strains can be distinguished with a second PCR based on the same gene cluster. The method presents a useful tool in identifying K-12 for monitoring strains which are used as biologically safe vehicles in biotechnological research, development, and production processes.

Escherichia coli K-12 strains are by far the most frequently used host strains in gene cloning experiments, since they have the following advantages: (i) they represent the genetically best understood living organism, (ii) they are easily modified by many genetic methods, and (iii) they are classified as biologically safe vehicles for the propagation of many efficient gene cloning and expression vectors in all major national and international guidelines on biological safety for work with recombinant DNA technology. An increasing number of different strains, both K-12 derivatives and other *E. coli* strains, are ever emerging for use in biotechnological research and development. The broad genetic and phenotypic ranges of the varieties deduced from K-12 and other *E. coli* strains cause major problems in identifying their correct origins. Entire pedigrees (1) would therefore be required for clear identification of strains. A complete description of bacterial lineages, however, cannot be given in many cases. This can cause severe problems in the interpretation of experimental data and in particular in biosafety assessments, especially since no character common to *E. coli* K-12 strains is known to clearly differentiate them from other, apparently very similar, *E. coli* strains.

The wild-type strain of *E. coli* K-12 was isolated from the feces of a convalescent diphtheria patient in 1922 at Stanford University. Subcultures and derivatives of this strain were first reported in 1944 (3). Since then, the strain has been intensively used and mutagenized in many laboratories, and no case of disease has ever been reported to be caused by it. It was also shown that K-12 strains are unable to colonize the human gut (9). Moreover, K-12 strains are devoid of all known *E. coli* virulence genes (6). The K-12 lineage is therefore considered to be a prototype of safe and nonpathogenic bacterial strains. Hence, an accurate and rapid method to discriminate *E. coli* K-12 from other *E. coli* strains is needed as a standard tool in biological safety procedures.

E. coli K-12 strains are rough, apparently lacking the O antigen which is part of the lipopolysaccharide and which is encoded by the *rfb* gene cluster. Liu and Reeves (5) recently showed that the lack of O antigen in some K-12 derivative strains is due to a mutation (*rfb-50*) within the *rfb* cluster which inactivates the rhamnose transferase, a key enzyme in the O antigen biosynthesis. The *rfb-50* mutation is characterized by an IS*5* insertion that is located within the last gene (*orf264*, also named *orf11* or *orf5*) of the *rfb* cluster encoding the rhamnose transferase (5, 10) (Fig. 1). This mutation can be complemented with the wild-type sequences from the ancestral K-12 strain WG1 as well as from *Shigella flexneri* (5, 11). While most K-12 derivatives seem to carry the *rfb-50* mutation, the ancestral strain WG1, which is assumed to be the K-12 wild-type strain, contains a functional *orf264* (5). However, WG1 carries a mutation (*rfb-51*) in another gene of the *rfb* cluster. This *rfb-51* mutation is not present within most K-12 derivatives and might have occurred as an independent event (5).

A variation of the *rfb-50* mutation characteristic for K-12 derivatives is described for K-12 strain AB311. This strain contains a deletion of part of *orf264* upstream IS*5* and a substitution by a sequence (*sqx*) of unknown function (5). We developed two PCRs based on the DNA sequences of *orf264* and IS*5*. With these reactions, we detected the presence of *orf264*; the IS*5*-induced mutation *rfb-50*, which is characteristic of K-12 derivatives; and the variation of *rfb-50* which distinguishes the AB311 side lineage of K-12. We present data from screening of a large variety of *E. coli* K-12 strains as well as other *E. coli* strains used in laboratories or isolated from humans, animals, or the environment.

Sample preparation for PCR. The bacterial strains were obtained from different origins including the *E. coli* Genetic Stock Center, Yale University (kindly provided by B. Bachmann), the collection of L. Caro (University of Geneva, Geneva, Switzerland), commercial suppliers, and our own collection. For the human pathogens, genomic DNA from strains of the *Salmonella* Reference Bank and from the collection of the Swiss Reference Laboratory for Foodborne Diseases, Bern, Switzerland, was used (kindly provided by A. Burnens). Environmental *E. coli* isolates were obtained from the Group for Environmental Hygiene, University of Zurich (kindly provided by A. Metzler), and from the Institute for Medical Microbiology, University of Hannover (kindly provided by M. Frosch).

All *E. coli* strains were grown overnight on Luria-Bertani plates containing, per liter, 10 g of Bacto Tryptone (Difco), 5 g of yeast extract (Difco), 5 g of NaCl, 2 ml of 1 M NaOH, and 15 g of agar (Difco). Three to five colonies were lysed in 450 μl of lysis buffer (100 mM Tris-HCl [pH 8.5], 0.05% Tween 20, and 240 μg of proteinase K per ml). Samples were incubated for 1 h at 60°C and then heated to 97°C for 15 min in order to inactivate proteinase K.

Oligonucleotide primers and PCR assay. On the basis of the report of Liu and Reeves (5) describing the *rfb-50* mutation, we

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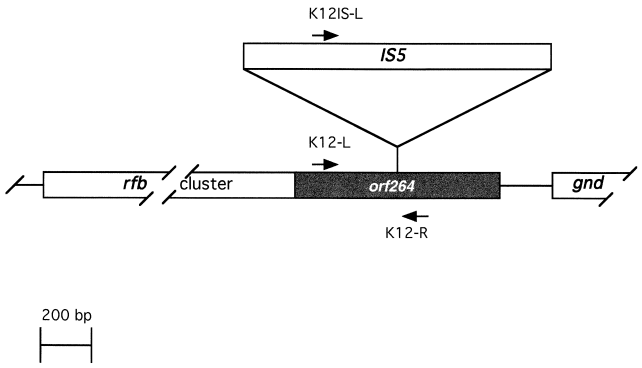

FIG. 1. Map of the *rfb* cluster and region analyzed in this study. The *rfb* cluster is located 5′ to the 6-phosphogluconate dehydrogenase gene (*gnd*). Boxes represent genes. The entire *rfb* cluster extends over more than 10 kb and contains 11 genes. The gene located at the 3′ end of the cluster, *orf264* (shaded box), encodes the rhamnose transferase. It harbors an IS*5* sequence in most K-12 strains specifying the *rfb-50* mutation. Arrows indicate the locations of primers used for PCR.

selected two oligonucleotide primer sets to amplify a region of *orf264*. One pair of primers is dependent on the complete gene *orf264* and consists of primers K12-R (5′-ATCCTGCGCACC AATCAACAA-3′) (nucleotides [nt] 508 to 488 on *orf264*) and K12-L (5′-TTCCCACGGACATGAAGACTACA-3′) (nt 21 to 43 on *orf264*). The second pair includes primers K12-R and K12IS-L (5′-CGCGATGGAAGATGCTCTGTA-3′) (nt 293 to 313 on IS*5*) and is dependent on the 3′ end of *orf264* and the presence of IS*5* (Fig. 1).

In order to have an internal technical control for the PCRs, we synthesized primers that amplify a segment of the *pal* gene encoding the peptidoglycan-associated lipoprotein (2), which is conserved in *E. coli* and closely related bacteria. The primers are ECPAL-L (5′-GGCAATTGCGGCATGTTCTTCC-3′) (nt 50 to 71 on *pal*) and ECPAL-R (5′-CCGCGTGACCTTCT ACGGTGAC-3′) (nt 328 to 307 on *pal*). Primer synthesis was done at Microsynth, Balgach, Switzerland.

PCR was performed with either a PE9600 or PE2400 automated thermocycler with MicroAmp tubes (Perkin-Elmer Cetus, Norwalk, Conn.). The reaction was carried out in a 50-μl volume containing 5 μl of 10× PCR buffer (supplied with *Taq* DNA polymerase), 20 pmol of primer (each), 1 mM deoxynucleoside triphosphate, 2.5 U of *Taq* DNA polymerase (Boehringer, Mannheim, Germany), and 1 μl of bacterial lysate or DNA. PCR conditions were as follows: 3 min at 94°C followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1.5 min. PCR products were analyzed on a 1% agarose gel (Sigma). Results from a few strains giving the typical patterns are shown in Fig. 2.

Sampling of *E. coli* strains and related enteric bacteria. Three major groups of *E. coli* strains were analyzed: (i) K-12 derivatives, (ii) other *E. coli* laboratory strains not derived from K-12, and (iii) *E. coli* strains isolated from patients, food, and the environment. Since *E. coli* is phylogenetically closely related to *Shigella* and *Salmonella* spp. and since the genes for the biosynthesis of the O antigens of certain *Shigella* and *Salmonella* strains are closely related to the *rfb* genes found in K-12 (11), we also included *Shigella* and *Salmonella* strains in our study. Tables 1 and 2 give the list of all strains investigated and the data obtained from their analysis.

All *E. coli*, *Shigella*, and *Salmonella* strains amplified a 0.28-kb fragment of the *pal* gene with the primer pair ECPAL-L–ECPAL-R, which we included in our analysis as control, indicating that the *pal* gene is conserved among enteric bacteria. Nonenteric, gram-negative, and gram-positive bacteria showed no signal in this assay.

Identification of *E. coli* K-12 derivatives. WG1 is assumed to be the wild-type strain of *E. coli* K-12, or at least the nearest known descendant from it. Strains 58 and 679, both used by Gray and Tatum in the 1940s (3), are the most antecedent-documented mutants derived from *E. coli* K-12 (1). Virtually all K-12 derivative strains which are currently used as laboratory strains for gene cloning and expression are supposed to originate from these two strains, although the pedigree (1) does not enumerate many strains which are frequently used in gene cloning. In our K-12-specific PCR assay with oligonucleotide primers K12IS-L and K12-R (Fig. 1), all 39 analyzed K-12 derivatives including strains 58 and 679 revealed the 0.97-kb band from amplification of the *orf264*-IS*5* junction, showing that they all contain the *rfb-50* mutation (Table 1). This set of strains contained known K-12 derivatives described in the pedigree (1) and commonly used hosts for gene cloning (7). This supports the data of the pedigree presented by Bachmann (1), indicating that all currently used *E. coli* K-12 strains are descendants from strain 58 or 679 or a very close relative of them. The PCR clearly distinguished the K-12 derivative strains from the supposed wild-type strain WG1, as well as from the related *E. coli* O16:K1:H and from all other strains of *E. coli*, *Shigella*, and *Salmonella* used in this study of different origins which lack the 0.97-kb fragment of the IS*5*-*orf264* junction (Tables 1 and 2).

Phylogenetic relationship of K-12 strains. A complementary PCR with the oligonucleotide primer pair K12-L–K12-R was designed to amplify the segment of *orf264* flanking the insertion locus of IS*5* in K-12 derivative strains (Fig. 1). This PCR resulted in a 1.69-kb fragment from amplification of part of *orf264* and IS*5* in all K-12 derivatives except in strains AB311

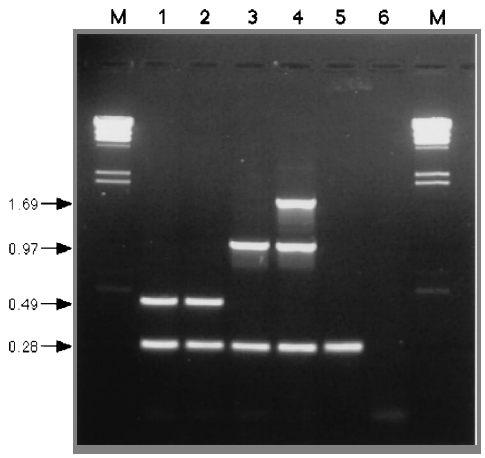

FIG. 2. PCR results for selected strains showing all observed patterns. Lanes: 1, *E. coli* O16:K1:H; 2, WG1 (assumed wild type); 3, AB311 (side lineage of K-12 derivatives); 4, C600 (representing the majority of K-12 derivatives); 5, *S. flexneri* (able to complement *rfb-50*); 6, *C. perfringens* (gram-positive control); M, λ-*Hin*dIII marker with 23.1-, 9.4-, 6.6-, 4.4-, 2.3-, 2.0-, and 0.6-kb bands. Lanes 1 to 6 of the gel contain the amplification products of each of the three separate PCRs: specific 0.97-kb fragment of K-12 derivatives, resulting from amplification with primer pair K12IS-L–K12-R; amplification with primer pair K12-L–K12-R based on the *orf264* sequence, resulting in the 1.69-kb band of practically all K-12 derivatives with the exception of strains AB311 and YN2980, or the 0.49-kb band present in *E. coli* serovar O16 and in strain WG1; and control amplification with primer pair ECPAL-L–ECPAL-R, resulting in a 280-bp band with any *E. coli* or related enteric species. Aliquots of 2.5 μl of the *orf264*-specific reaction mixtures (the first two above) and 5 μl of the *pal*-specific reaction mixture (the last one above) were loaded on the same slot on a 1% agarose gel.

TABLE 1. Laboratory bacterial strains used in this study and PCR results with the primer sets K12IS-L–K12-R, K12-L–K12-R, and ECPAL-L–ECPAL-R

Strain no.	Strain designation	Name	Origin or collection	K12IS-L–K12-R, 969 bp	K12-L–K12-R		ECPAL-L–ECPAL-R, 278 bp
					1,687 bp	488 bp	
Ancestral *E. coli* K-12 strains							
CGSC 5073	WG1	K-12	Wisconsin strain (wt)[a,b]	−	−	+	+
CGSC 5587	58	K-12	Stanford strain (*bio-1*)[b]	+	+	−	+
CGSC 5588	679	K-12	Stanford strain (*thr-1*)[b]	+	+	−	+
E. coli K-12 derivatives							
JF1512	MB408	K-12	Laboratory strain	+	+	−	+
JF1513	AG1	K-12	Laboratory strain	+	+	−	+
JF1514	JM101	K-12	Laboratory strain	+	+	−	+
JF1515	DH5α	K-12	Laboratory strain	+	+	−	+
JF1412	DH5α	K-12	Laboratory strain	+	+	−	+
JF49	C600	K-12	Laboratory strain[b]	+	+	−	+
JF129	W3110	K-12	Laboratory strain[b]	+	+	−	+
JF172	Hfr3000	K-12	Laboratory strain[b]	+	+	−	+
JF273	JM83	K-12	Laboratory strain	+	+	−	+
JF520	5K	K-12	Laboratory strain	+	+	−	+
JF553	DH1	K-12	Laboratory strain[b]	+	+	−	+
JF1464	DH1	K-12	Laboratory strain[b]	+	+	−	+
JF1078	H1443	K-12	Laboratory strain	+	+	−	+
JF1208	TG1	K-12	Laboratory strain	+	+	−	+
JF1224	DP50	K-12	Laboratory strain	+	+	−	+
JF1427	?	K-12	Laboratory strain	+	+	−	+
JF1501	W945	K-12	Laboratory strain[b]	+	+	−	+
JF1502	PA309	K-12	Laboratory strain[b]	+	+	−	+
JF1503	58-161	K-12	Laboratory strain[b]	+	+	−	+
JF1507	P678	K-12	Laboratory strain[b]	+	+	−	+
HB101	HB101	K-12/B hybrid	Laboratory strain	+	+	−	+
JF554	XL1-Blue	K-12	Stratagene[c]	+	+	−	+
JF1223	XLOLR	K-12	Stratagene	+	+	−	+
JF980	SURE	K-12	Stratagene	+	+	−	+
JF1066	HMS 174	K-12	Novagen[d]	+	+	−	+
JF1069	HMS 174	K-12	Novagen	+	+	−	+
JF1392	YN2980	K-12	Laboratory strain	+	−	−	+
CGSC 311	AB311	K-12	Laboratory strain[b]	+	−	−	+
CGSC 5357	Hfr 3000 X74	K-12	Laboratory strain[b]	+	+	−	+
CGSC 4488	Cavalli Hfr	K-12	Laboratory strain[b]	+	+	−	+
CGSC 253	W208	K-12	Laboratory strain[b]	+	+	−	+
CGSC 284	AB284	K-12	Laboratory strain[b]	+	+	−	+
CGSC 4401	EMG2	K-12	Laboratory strain	+	+	−	+
CGSC 5024	W1485	K-12	Laboratory strain[b]	+	+	−	+
CGSC 5037	Y10	K-12	Laboratory strain[b]	+	+	−	+
CGSC 5608	WA704	K-12	Laboratory strain[b]	+	+	−	+
CGSC 6613	JC9387	K-12	Laboratory strain[b]	+	+	−	+
E. coli laboratory strains not derived from K-12							
JF1504	B	B	Laboratory strain	−	−	−	+
JF1505	B-3	B	Laboratory strain	−	−	−	+
JF1506	B/R	B	Laboratory strain	−	−	−	+
JF1508	C	C	Laboratory strain	−	−	−	+
JF1509	C	C	Laboratory strain	−	−	−	+
JF1510	C	C	Laboratory strain	−	−	−	+
JF1500	TOPP	ND[e]	Stratagene	−	−	−	+
JF702	BL21	B	Novagen	−	−	−	+
JF703	BL21	B	Novagen	−	−	−	+

[a] wt, wild type.
[b] Reference 1.
[c] Stratagene, La Jolla, Calif.
[d] Novagen, Madison, Wis.
[e] ND, not determined.

and YN2980 (8) (Table 1), which showed no reaction product. Only the wild-type strain WG1 and *E. coli* serovar O16:K1:H showed a fragment of 0.49 kb in this PCR, which indicates that these two strains possess the original *orf264* gene without IS*5* insertion. Previous results (5) showed that the *E. coli* derivatives EMG2 and W1485 (Table 1) could be complemented with the wild-type gene for rhamnose transferase (*orf264*) from WG1 for the expression of an intact O antigen which strongly reacted with anti-O16 antibodies but showed little cross-reaction with anti-O17 antibodies. While in our PCR *E. coli* serovar

TABLE 2. Diagnostic and environmental isolates used in this study and PCR results[a]

Strain no.	Sp.	Serovar	Origin or collection	K12IS-L–K12-R, 969 bp	K12-L–K12-R		ECPAL-L–ECPAL-R, 278 bp
					1,687 bp	488 bp	
Gram-negative							
NCTC 9016	*E. coli*	O16:K1:H	Reference strain	−	−	+	+
NCTC 9017	*E. coli*	O17:K16:H18	Reference strain	−	−	−	+
JF1556	*E. coli*	ND	Dairy product	−	−	−	+
JF1557	*E. coli*	ND	Dairy product	−	−	−	+
JF1558	*E. coli*	ND	Dairy product	−	−	−	+
JF1559	*E. coli*	ND	Dairy product	−	−	−	+
JF1560	*E. coli*	ND	Dairy product	−	−	−	+
JF1561	*E. coli*	ND	Dairy product	−	−	−	+
JF1564	*E. coli*	ND	Environment (water)	−	−	−	+
JF1584	*E. coli*	ND	Environment (water)	−	−	−	+
JF1585	*E. coli*	ND	Environment (water)	−	−	−	+
JF1586	*E. coli*	ND	Environment (water)	−	−	−	+
JF1587	*E. coli*	ND	Environment (water)	−	−	−	+
JF1565	*E. coli*	O128:K67	Human, dysenteric stool	−	−	−	+
JF1566	*E. coli*	O128:K67	Human, dysenteric stool	−	−	−	+
JF1567	*E. coli*	O128:K67	Human, dysenteric stool	−	−	−	+
JF1568	*E. coli*	O119:K69	Human, dysenteric stool	−	−	−	+
JF1569	*E. coli*	O127:K63	Human, dysenteric stool	−	−	−	+
JF1570	*E. coli*	O125:K70	Human, dysenteric stool	−	−	−	+
JF1572	*E. coli*	O111:K58	Human, dysenteric stool	−	−	−	+
JF1573	*E. coli*	O125:K70	Human, dysenteric stool	−	−	−	+
JF1574	*E. coli*	O55:K59	Human, dysenteric stool	−	−	−	+
JF1260	*E. coli*	ND	Bovine mastitis	−	−	−	+
JF1278	*E. coli*	ND	Bovine mastitis	−	−	−	+
JF1266	*E. coli*	ND	Bovine mastitis	−	−	−	+
JF1300	*E. coli*	ND	Bovine septicemia	−	−	−	+
JF1410	*E. coli*	K99	Bovine diarrhea	−	−	−	+
JF1275	*E. coli*	ND	Red deer, septicemia	−	−	−	+
JF1295	*E. coli*	ND	Ape, septicemia	−	−	−	+
JF1265	*E. coli* (ETEEC)	O139:K82	Porcine diarrhea	−	−	−	+
JF1321	*E. coli* (ETEEC)	O139:K82	Porcine diarrhea	−	−	−	+
JF1305	*E. coli* (ETEEC)	O141:(H4)	Porcine diarrhea	−	−	−	+
JF1369	*E. coli* (ETEEC)	O141:(H4)	Porcine diarrhea	−	−	−	+
JF1381	*E. coli* (ETEEC)	O141:(H4)	Porcine diarrhea	−	−	−	+
JF1276	*E. coli* (ETEEC)	O147:H19:K88	Porcine diarrhea	−	−	−	+
NZ2956	*E. coli* (VTEC)	O157	Human, hemorrhagic colitis	−	−	−	+
NZ4253	*E. coli* (VTEC)	O157	Human, hemorrhagic colitis	−	−	−	+
E1630-91	*E. coli* (EPEC)	ND	Human, infant diarrhea	−	−	−	+
E. coli O111	*E. coli* (EPEC)	ND	Human, infant diarrhea	−	−	−	+
NZ3211-94	*E. coli* (ETEC)	ND	Human, traveller's diarrhea	−	−	−	+
NZ3213-94	*E. coli* (ETEC)	ND	Human, traveller's diarrhea	−	−	−	+
NZ1631-94	*Shigella sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ666-94	*S. sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ1475-94	*S. sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ425-94	*S. sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ879-95	*S. sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ1360-95	*S. sonnei*	ND	Human, dysenteric stool	−	−	−	+
NZ1403-94	*Shigella dysenteriae*	ND	Human, dysenteric stool	−	−	−	+
NZ4142-90	*S. dysenteriae* 1	ND	Human, dysenteric stool	−	−	−	+
NZ4800-92	*S. dysenteriae* 1	ND	Human, dysenteric stool	−	−	−	+
NZ4894-94	*S. dysenteriae* 1	ND	Human, dysenteric stool	−	−	−	+
NZ329-94	*S. dysenteriae* 2	ND	Human, dysenteric stool	−	−	−	+
NZ359-94	*S. dysenteriae* 2	ND	Human, dysenteric stool	−	−	−	+
NZ1208-94	*S. flexneri*	ND	Human, dysenteric stool	−	−	−	+
NZ1679-94	*S. flexneri*	ND	Human, dysenteric stool	−	−	−	+
NZ233-94	*S. flexneri*	ND	Human, dysenteric stool	−	−	−	+
NZ936-94	*S. flexneri*	ND	Human, dysenteric stool	−	−	−	+
NZ816-94	*S. flexneri*	ND	Human, dysenteric stool	−	−	−	+
NZ6733-93	*Shigella boydii*	ND	Human, dysenteric stool	−	−	−	+
NZ2142-93	*S. boydii*	ND	Human, dysenteric stool	−	−	−	+
SARB 17	*Salmonella enterica*	Enteritidis	Reference strain	−	−	−	+
SARB 18	*S. enterica*	Enteritidis	Reference strain	−	−	−	+
ATCC 23564	*S. enterica* LT2	Typhimurium	Reference strain	−	−	−	+
SARB 66	*S. enterica*	Typhimurium	Reference strain	−	−	−	+
SARB 65	*S. enterica*	Typhimurium	Reference strain	−	−	−	+
SARB 27	*S. enterica*	Infantis	Reference strain	−	−	−	+
SARB 56	*S. enterica*	Saintpaul	Reference strain	−	−	−	+
SARB 63	*S. enterica*	Typhi	Reference strain	−	−	−	+
SARB 5	*S. enterica*	Choleraesuis	Reference strain	−	−	−	+
SARB 22	*S. enterica*	Haifa	Reference strain	−	−	−	+
Gram-positive control							
NCTC 10239	*Clostridium perfringens*	Gram positive	Reference strain	−	−	−	−

[a] ND, not determined; ETEEC, enterotoxemic *E. coli*; VTEC, verocytotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli.*

O16:K1:H showed the same pattern as WG1, *E. coli* serovar O17:K16:H18 and all other non-K-12 strains showed no bands with the primers derived from *orf264*. This confirms the close relationship of K-12 *rfb* wild-type genes with those of *E. coli* serovar O16. It implies that the rhamnose transferase is correlated with the expression of a specific O antigen. In addition, our results show that the gene *orf264* is present only in *E. coli* K-12 and its derivatives and in serovar O16:K1:H. It is not detected in other *E. coli*, *Salmonella*, and *Shigella* strains isolated from a broad range of origins including humans, animals, and the environment.

Differentiation of a side lineage of K-12 derivatives. Interestingly, two K-12 derivative strains, AB311 and YN2980, showed no amplification product in the PCR with the primers K12-L–K12-R flanking the IS*5* insertion in *orf264*. Since both AB311 and YN2980 did amplify the right-handed junction of IS*5* and *orf264* in the specific reaction for K-12 derivatives with the primer pair K12IS-L–K12-R, it seems that these two K-12 derivative strains have lost the segment of *orf264* upstream of the site of integration of IS*5* and therefore present a particular side lineage of K-12 derivatives which must have developed during successive mutagenesis. Moreover, we speculate that strain YN2980, which is not in the pedigree, is a descendant of AB311. Strains AB284 and W208, which are ancestors of AB311 (1), do not contain this deletion.

In summary, we have developed a PCR-based method which allows a rapid and accurate identification of *E. coli* K-12 derivative strains. A single PCR with the primer pair K12IS-L–K12-R amplifying the junction of the IS*5* insertion in the rhamnose transferase gene (*orf264*) is sufficient for their positive identification. This insertion is expected to be highly specific to K-12 derivative strains, since the *orf264* gene was found only in K-12 strains and serovar O16, and IS*5* was shown to be infrequent in natural *E. coli* isolates, to transpose rarely, and to integrate in various chromosomal loci (4).

Our analysis of 90 different *E. coli* strains from most different sources and 29 *Salmonella* and *Shigella* isolates showed that the method reliably identified all 39 K-12 derivative strains and excluded all other *E. coli*, *Salmonella*, and *Shigella* strains, in spite of the fact that some of them are apparently very similar to the K-12 derivatives.

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