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Purification of *Escherichia coli* Chromosomal Segments without Cloning

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Pairs of genomic insertions made with elements carrying any one of several frequently used rare restriction sites allow physical purification of insertion delimited genes. However, native rare restriction sites can, either by causing (*i*) fragmentation of targeted intervals or (*ii*) generation of additional fragments that overlap electrophoretically with targeted ones, place severe limitations on this approach. We present a series of *Escherichia coli* mini-Tn*10* insertions containing the rare-cutting polylinker 2 (RCP2) of rare restriction sites, which includes the 18-base-pair I-*Sce*I site (absent from native *E. coli* sequences). Pulsed-field gel purification from RCP2 double insertion mutants of both an I-*Sce*I fragment from strain K-12 (containing ~90–95 min) and an allelic I-*Sce*I fragment from a pathogenic strain is demonstrated. The complete series of RCP2 insertions, containing different antibiotic resistances at intervals of ~35 kb in prototype K-12 strain MG1655, allows rapid purification of the genes from any *E. coli* chromosomal interval as an isolated I-*Sce*I fragment.

The advent of in-vitro cloning of genomic DNA into multicopy plasmids laid the foundation for much of the rapid growth in biology seen over the last twenty years. Nevertheless, molecular cloning as a means of obtaining genomic segments for physical analyses, poses significant drawbacks: (*i*) DNA sequences containing genes that are lethal in multicopy may be refractive to cloning (1); (*ii*) overlapping genomic representations at the borders between clones may add significant redundancy into the process of comprehensive nucleotide sequencing (2); and (*iii*) artificial rearrangements in cloned DNA sequences may subvert accurate construction of genomic physical maps.

An attractive alternative to molecular cloning—the direct isolation, from whole-genome preparations, of desired DNA segments in sufficient quantities—was taken a step closer to practice by descriptions of mobile-intron-encoded endonucleases possessing ultrarare activities (3). In particular, the I-*Sce*I endonuclease from *Saccharomyces cerevisiae,* recognizing an 18-bp nonpalindromic site, presents the possibility of introducing unique cleavages into a genome as complex as that of humans (4). We present (*i*) a series of mini-Tn*10* insertions (containing the I-*Sce*I site) in *Escherichia coli* K-12 strain MG1655 and (*ii*) a method of using pairs of them in conjunction with I-*Sce*I digestion of genomic DNA, to purify targeted chromosomal segments from both laboratory and naturally occurring *E. coli.*

MATERIALS AND METHODS

Bacterial genetics techniques. Bacterial strains (Table I) were grown in LB with aeration or on solid LB or M9/glucose (5). Media were supplemented with thiamine (50 μg/ml; for K-12 strains), Km (25 μg/ml), Sp (100 μg/ml), and/or Cm (15 μg/ml) as required. Cultures were incubated at 37° C, or at 32° C for P1 infections of RS218 and RS218-chimera cultures (6). Cells were stored long term by suspending in LB/glycerol (85%/15%; v/v), and cooling to −80° C. Bacteriophage stocks were grown and stored as described by Sternberg and Maurer (7). MG1655 mutants containing Tn*10*dKanRCP2, Tn*10*dSpcRCP2, or Tn*10*dCamRCP2 insertions were generated by electrotransformation with plasmids pGI290, pGI300, or

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Abbreviations: bp, base pairs; CW, clockwise; Cm, chloramphenicol; CCW, counterclockwise; I-, intron-encoded (as in I-*Sce* I); Km, kanamycin; kb, kilobases or 1000 base pairs; LB, Luria-Bertani (medium); p, plasmid; PFGE, pulsed-field gel electrophoresis; RCP, rare-cutting polylinker; Tn, transposon; ::, novel junction (fusion or insertion).

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TABLE 1
Bacterial strains, plasmids, and phage used in this study

Name	Description	Source or Reference
Strains		
MG1655	*E. coli* K-12 prototype	(13)
RS218	*E. coli* newborn-meningitis prototype	(6)
χM2000-χM2128	MG1655::Tn*10*dRCP2 insertion mutants (see Table 2)	This study
χM2130	MG1655 *zija*-218::Tn*10*dSpcRCP2, *zijg*-224::Tn*10*dKanRCP2	This study
χM2131	RS218*zija*-218::Tn*10*dSpcRCP2, *zijg*-224::Tn*10*dKanRCP2	This study
CU267	*Bacillus subtilis* host strain for pGI-series plasmids	(9)
Plasmids		
pGI290	Gram$^+$ suicide plasmid carrying Tn*10*dKanRCP2	(22)
pGI300	Gram$^+$ suicide plasmid carrying Tn*10*dSpcRCP2	(22)
pGI310	Gram$^+$ suicide plasmid carrying Tn*10*dCamRCP2	(22)
Phage		
P1Δ*dam rev6*	large plaque revertant of P1 cm *c*1.100 r^- m^- *dam*ΔMB	(7)

pGI310 as described by Dower et al. (8): Briefly, plasmid DNA was column purified according to the manufacturer's specifications (Wizard Maxiprep column; Promega; Madison, WI) from *Bacillus subtilis* strain CU267 (9). Aliquots of 40 μl of competent MG1655 cells (10^{10} cfu/ml) mixed with 2 μl of plasmid DNA (10 μg/ml) were electroporated by use of a Gene Pulser (Bio-Rad; Hercules, CA) apparatus with settings of 2.5 kV, 25 μF, and 200 Ω. Routinely, 10^3–10^4 Km^R, Sp^R, or Cm^R colonies were isolated per 1 μg of *B. subtilis* plasmid DNA. Double insertion mutants of strain MG1655 and single- and double-insertion mutants of strain RS218 were generated by transducing recipient strains with P1Δ*dam rev6* lysates of MG1655 insertion mutants (10). Genomic structures of the resulting insertion mutants were screened for P1 transduction fidelity and for gene-order conservation between strains (10).

Genomic DNA biophysical techniques. Genomic DNAs were purified from 5 ml overnight cultures of *E. coli*::Tn*10*dRCP2 mutants in a manner suitable for yielding macrorestriction fragments (0.05–1.0 Mb) as described (10). Absence of nonspecific DNA degradation in stored agarose dots (TE pH 7.5 at 4° C) for up to 12 months was confirmed. After digestion of agarose-embedded DNAs with I-*Sce* I (Boehringer-Mannheim; Indianapolis, IN) for 1 h, *Not*I (New England Biolabs, Beverly, MA) for 4–5 h, or *Bln*I (Panvera; Madison, WI) overnight, according to the manufacturers' directions, and after reaction-buffer decanting, dots were melted (70° C) and gently pipetted with plastic 200 μl tips into sample wells in 1.3% agarose (PFGE-approved; FastLane; FMC, Portland, ME) gels for electrophoresis in 0.5X TBE buffer (0.045 M Tris borate/0.045 M boric acid/0.001 M EDTA) in a PFGE apparatus (Bio-Rad; DR-III) according to the manufacturer's instructions. Pulse-ramping parameters were determined as described elsewhere (11). After electrophoresis of samples with Megabase I and/or II DNA standards (Gibco/BRL, Bethesda, MD), gels were analyzed as described elsewhere (12).

RESULTS AND DISCUSSION

(a) Isolation of Rare-Restriction-Sites Insertions

A series of 129 insertions in prototype *E. coli* K-12 strain MG1655 (13) was assembled for purification of chromosomal segments without cloning. These were made with one of the three rare-restriction-sites-carrying transposable elements Tn*10*dKanRCP2, Tn*10*dSpcRCP2, or Tn*10*dCamRCP2 (delivered by plasmid vectors pGI290, pGI300, and pGI310, respectively, and referred to collectively as 'Tn*10*dRCP2' elements). Individual insertions were isolated from collections of random insertion mutants made with each of these varieties of insertion element, through a combination of the following methods: (*i*) screening with P1 lysates of pooled insertion mutants for P1 cotransductional linkage (14) to individual, previously established Tn*10*dRCP1 insertions (10); (*ii*) screening for insertions in known biosynthetic genes by a combination of

auxanography and rare-restriction mapping (12); and (*iii*) direct screening of insertions by rare-restriction mapping. The criteria for inclusion in the series were occurrence of (*i*) adjacent insertions in alternating antibiotic resistances and (*ii*) insertions at intervals allowing subdivision of the entire chromosome into 10, 20, or 70 equivalently sized contiguous/nonoverlapping segments.

(b) Locations of the Insertions on the NotI and BlnI Restriction Maps of the MG1655 Chromosome

Macrorestriction map coordinates of the Tn*10*dRCP2 insertions were determined to allow their use in purification of positionally-specified chromosomal segments (Table 2). This required both ordering of the insertions and orienting of their associated *Not*I and *Bln*I subfragments (CCW or CW about the insertions), a process that simultaneously resulted in reconstruction of the MG1655 *Not*I and *Bln*I maps (15). The physical distances between pairs of adjacent insertions averaged 35 kb, with the largest gap being ~110 kb.

Positions of the insertions on the *E. coli* genetic map (e.g., by P1-cotransductional or conjugational linkage to established genetic markers) were not directly determined in this study. However, their physical distances from other nearby markers (e.g., Tn*10*dRCP1 insertions that were previously subjected to both genetic and *Not*I/*Bln*I mapping; ref. 10) may be readily determined from the data in Table 2, and their genetic map locations estimated from this information (16). By the same approach, locus designations (e.g., *zab*, *zfb*, etc.) for the insertions [according to Chumley, Menzel, and Roth (17), with minor modification (10)] could be assigned (Table 2). The potential double mutants from the insertion set define thousands of different *E. coli* chromosomal intervals.

(c) Segmentation of the E. coli Chromosome at Pairs of Insertion Sites

The *Not*I, *Bln*I, and other rare restriction sites contained within the Tn*10*dRCP2 elements (including I-*Ceu*I, *Bsi*WI, *Srf*I, *Swa*I, *Xba*I, *Sna*BI, *Sph*I, *Sfi*I, *Pac*I, *Pme*I, *Bsp*I, and *Sse*8387I) may serve to facilitate mapping of their insertions. However, these sites which are shared with native *E. coli* sequences can also interfere with purification of insertion-delimited chromosomal segments from Tn*10*dRCP2 double mutants. This is because native sites in *E. coli* sequences may lead to either (*i*) fragmentation of insertion-delimited segments or, (*ii*) electrophoretic band contamination from fragments generated at other loci. However, the I-*Sce*I restriction site (5′ TAGGGATAA-CAGGGTAAT3′; ref. 3), which is also contained within the RCP2 sequence but not in native *E. coli* sequences, would be expected to allow purification of *E. coli* chromosomal segments (i.e., of segments delimited by pairs of Tn*10*dRCP2 insertions) by specifying cleavages at insertion sites only.

To test this approach, purification of an MG1655 segment flanked by different Tn*10*dRCP2 insertions was undertaken. Fig. 1 shows separation of the DNA between two insertions at ~90 and ~95 min from the rest of genomic DNA in strain χM2130, one of the progeny from crossing the MG1655 single insertion mutants χM2118 and χM2124. The DNA between these insertions was visualized upon I-*Sce*I digestion/PFGE as an isolated I-*Sce*I band of 240 kb (lane 3). Both the separation between these insertions as determined by MG1655 *Not*I mapping (~225 kb; Table 2) and the lack of additional bands was consistent with specific activity of the I-*Sce*I endonuclease at insertion sites only (18). The same procedure has also been applied recently at various other MG1655 loci, allowing gel purification of chromosomal segments in sufficient quantities for comprehensive, random subcloning and sequencing (18a). Thus pairs of Tn*10*dRCP2 insertions can be used to achieve, without cloning, physical purification of the genes located in any *E. coli* chromosomal interval. Conversely, a benefit of the purified products obtained from Tn*10*dRCP2 double mutants may be to allow manufacture of DNA filters, containing custom-made contiguous/nonoverlapping K-12 genomic segments (cf. refs. 15 and 19), for rapid and precise physical mapping of *E. coli* genes obtained by cloning.

TABLE 2
Locations of MG1655::Tn*10*dRCP2 Insertions

Insertion[a,b]	Native fragments interrupted[c]	*Not* I map coordinate[d]
	MG1655::Tn*10*dKanRCP2; Km^R	
zab-100	B_N (340, 18), H_B (80, 155)	7 kb
car-101	D_N (10, 269), H_B (100, 130)	35 kb
zac-103	D_N (70, 210), H_B (155, 80)	95 kb
zad-104	D_N (130, 150), H_B (225, 15)	155 kb
zah-106	D_N (235, 45), C_B (30, 30)	260 kb
zah-108	C_N (5, 355), J_B (20, 860)	309 kb
zaj-110	C_N (90, 270), J_B (100, 780)	394 kb
zbc-112	C_N (220, 140), J_B (235, 645)	524 kb
zbf-116	C_N (310, 50), J_B (320, 560)	614 kb
zbh-118	P_N (80, 25), J_B (440, 440)	742 kb
zbh-120	K_N (15, 140), J_B (480, 400)	780 kb
zbj-123	K_N (100, 55), J_B (560, 320)	865 kb
zcb-125	J_N (50, 165), J_B (670, 210)	968 kb
put-129	R_N (45, 50), M_B (5, 230)	1177 kb
zch-131	I_N (5, 85), M_B (60, 175)	1233 kb
trp-133	I_N (70, 20), M_B (120, 115)	1298 kb
zda-136	G_N (65, 210), M_B (200, 35)	1383 kb
zdb-137	G_N (100, 180), G_B (5, 195)	1418 kb
zdd-141	G_N (230, 45), G_B (140, 60)	1548 kb
zdg-144	O_N (45, 205), L_B (30, 1039)	1636 kb
zdj-147	O_N (175, 75), L_B (150, 960)	1766 kb
zea-150	O_N (230, 20), L_B (210, 860)	1821 kb
zed-152	N_N (80, 50), L_B (310, 760)	1922 kb
zeg-160	F_N (115, 135), L_B (530, 540)	2127 kb
zei-162	W_N (10, 5), L_B (640, 430)	2273 kb
*purF*164	M_N (115, 75), L_B (770, 300)	2392 kb
zfc-166	V_N (7, 7), L_B (850, 220)	2474 kb
zfg-169	E_N (125, 135), L_B (970, 90)	2606 kb
zfi-171	E_N (190, 70), L_B (1040, 30)	2671 kb
*pheA*173	E_N (225, 40), K_B (10, 1047)	2706 kb
*tyrA*174	E_N (225, 40), K_B (10, 1047)	2706 kb
zfj-175	E_N (250, 13), K_B (30, 1040)	2731 kb
zfi-176	A_N (35, 950), K_B (85, 972)	2779 kb
zga-179	A_N (100, 890), K_B (150, 907)	2844 kb
cys-180	A_N (115, 875), K_B (155, 902)	2859 kb
zgd-184	A_N (240, 750), K_B (295, 762)	2984 kb
zgf-187	A_N (320, 670), K_B (360, 700)	3064 kb
zgh-190	A_N (420, 570), K_B (465, 590)	3164 kb
*argG*193	A_N (520, 470), K_B (580, 480)	3264 kb
zhc-194	A_N (600, 390), K_B (644, 413)	3344 kb
zhc-195	A_N (620, 370), K_B (677, 380)	3364 kb
zhh-197	A_N (760, 230), K_B (807, 250)	3504 kb
zhi-200	A_N (850, 140), K_B (900, 160)	3594 kb
zhj-202	A_N (890, 100), K_B (927, 130)	3634 kb
zib-205	L_N (15, 190), K_B (1050, 5)	3751 kb
zid-207	L_N (90, 120), F_B (75, 75)	3826 kb
zif-209	L_N (160, 50), F_B (145, 5)	3896 kb
*ilvG*210	L_N (175, 35), D_B (5, 75)	3911 kb
*metE*213	S_N (29, 7), D_B (65, 20)	3972 kb
zii-214	T_N (30, 7), D_B (80, 5)	4009 kb
zij-216	H_N (25, 220), E_B (35, 95)	4042 kb
pur-220	H_N (140, 105), A_B (30, 5)	4157 kb

TABLE 2
Continued

Insertion[a,b]	Native fragments interrupted[c]	*Not* I map coordinate[d]
zje-222	B_N (14, 345), I_B (110, 250)	4276 kb
zjg-224	B_N (65, 295), I_B (160, 200)	4327 kb
zji-227	B_N (215, 145), I_B (300, 60)	4477 kb
	MG1655::Tn*10*dSpcRCP2; Sm^R	
car-102	D_N (10, 270), H_B (100, 130)	35 kb
zae-105	D_N (140, 140), H_B (230, 5)	165 kb
zah-107	D_N (245, 30), C_B (51, 10)	270 kb
zaj-109	C_N (35, 325), J_B (50, 830)	339 kb
zba-111	C_N (120, 240), J_B (135, 745)	424 kb
zbd-114	C_N (250, 110), J_B (260, 620)	554 kb
zbg-117	P_N (25, 80), J_B (395, 485)	687 kb
zbh-119	P_N (95, 5), J_B (460, 420)	757 kb
zbi-122	K_N (65, 90), J_B (530, 350)	830 kb
zca-124	J_N (35, 180), J_B (650, 230)	953 kb
zcc-126	J_N (85, 130), J_B (700, 180)	1003 kb
zcg-128	J_N (195, 20), J_B (800, 80)	1113 kb
zcg-130	R_N (80, 20), M_B (30, 205)	1212 kb
zci-132	I_N (45, 45), M_B (90, 145)	1273 kb
zda-135	G_N (5, 270), M_B (150, 85)	1323 kb
zdh-146	O_N (145, 105), L_B (130, 940)	1736 kb
zdj-149	O_N (200, 55), L_B (180, 890)	1791 kb
zea-151	N_N (15, 115), L_B (240, 830)	1857 kb
zed-154	U_N (10, 30), L_B (380, 690)	1981 kb
*his*A158	F_N (55, 195), L_B (470, 600)	2067 kb
zee-159	F_N (70, 180), L_B (485, 585)	2082 kb
zeh-161	F_N (185, 65), L_B (580, 490)	2197 kb
zfa-163	M_N (45, 145), L_B (700, 370)	2322 kb
zfc-167	V_N (7, 7), L_B (845, 225)	2474 kb
zfh-170	E_N (145, 115), L_B (1005, 65)	2626 kb
zfi-172	E_N (215, 50), L_B (1062, 7)	2696 kb
zga-178	A_N (70, 920), K_B (115, 942)	2814 kb
cys-181	A_N (115, 875), K_B (155, 902)	2859 kb
zgb-183	A_N (180, 810), K_B (220, 837)	2924 kb
zgd-185	A_N (250, 740), K_B (290, 770)	2994 kb
zgf-188	A_N (360, 630), K_B (405, 650)	3104 kb
zgj-191	A_N (515, 475), K_B (560, 495)	3259 kb
zhd-196	A_N (675, 315), K_B (718, 333)	3419 kb
zhh-198	A_N (777, 215), K_B (822, 235)	3521 kb
zhi-199	A_N (845, 145), K_B (888, 163)	3589 kb
zhj-201	A_N (870, 125), K_B (920, 143)	3614 kb
zib-203	A_N (960, 30), K_B (1020, 50)	3704 kb
zid-206	L_N (75, 130), F_B (60, 90)	3811 kb
zie-208	L_N (115, 100), F_B (100, 50)	3851 kb
*ilv*G211	L_N (175, 35), D_B (5, 75)	3911 kb
zig-212	S_N (3, 33), D_B (42, 42)	3946 kb
zja-218	H_N (85, 160), E_B (95, 35)	4102 kb
*arg*C219	H_N (100, 145), E_B (110, 20)	4117 kb
zje-221	B_N (5, 355), I_B (100, 260)	4267 kb
zjh-225	B_N (135, 225), I_B (230, 130)	4397 kb
zjh-226	B_N (160, 200), I_B (255, 105)	4422 kb
zaa-228	B_N (280, 80), H_B (26, 210)	4542 kb

TABLE 2
Continued

Insertion[a,b]	Native fragments interrupted[c]	*Not* I map coordinate[d]
	MG1655::Tn*10*dCamRCP2; Cm^R	
zbd-113	C_N (250, 105), J_B (265, 615)	554 kb
zbe-115	C_N (295, 65), J_B (310, 570)	599 kb
zbi-121	K_N (57, 96), J_B (520, 360)	822 kb
zcd-127	J_N (125, 90), J_B (740, 140)	1043 kb
zcj-134	I_N (87, 3), M_B (140, 95)	1315 kb
zdb-138	G_N (140, 130), G_B (45, 155)	1458 kb
zdd-139	G_N (205, 70), G_B (115, 85)	1523 kb
zdd-140	G_N (225, 50), G_B (135, 65)	1543 kb
zdd-142	G_N (235, 40), G_B (145, 55)	1553 kb
zdg-143	O_N (5, 245), G_B (190, 10)	1596 kb
zdh-145	O_N (75, 175), L_B (60, 1009)	1666 kb
zdj-148	O_N (200, 50), L_B (175, 935)	1791 kb
zed-153	N_N (105, 25), L_B (340, 730)	1947 kb
zed-155	U_N (35, 5), L_B (405, 665)	2006 kb
zee-156	F_N (15, 235), L_B (430, 640)	2027 kb
zee-157	F_N (40, 210), L_B (455, 615)	2052 kb
zfc-165	M_N (145, 45), L_B (800, 270)	2422 kb
zfe-168	E_N (75, 185), L_B (920, 140)	2556 kb
zfj-177	A_N (45, 940), K_B (95, 962)	2789 kb
zga-182	A_N (130, 860), K_B (170, 987)	2874 kb
zgf-186	A_N (285, 705), K_B (325, 735)	3029 kb
zgh-189	A_N (400, 590), K_B (445, 610)	3144 kb
zgi-191	A_N (465, 525), K_B (510, 550)	3209 kb
zib-204	L_N (7, 200), K_B (1060, 10)	3743 kb
zii-215	H_N (20, 225), E_B (30, 90)	4037 kb
zii-217	H_N (35, 210), E_B (45, 85)	4052 kb
zig-223	B_N (65, 295), I_B (160, 200)	4327 kb

[a] Locus designations either were according to Chumley, Menzel, and Roth (17), with minor modifications (10), or were determined by auxanography (12). In instances of auxotrophies that may be conferred by mutations at any one of multiple loci, specific designations were determined where possible by alignments between the insertions' physical-map coordinates and the *E. coli* genetic map.

[b] Strain numbers for the χM-series strains containing the insertions (see Table 1) were obtained by adding 1900 to their insertions' allele numbers (e.g., dictating that the strain containing the *zab-100*::*Tn10*dKanRCP2 insertion allele be named strain χM2000).

[c] The *Not*I and *Bln*I fragments from the MG1655 chromosome were designated according to Perkins, et al. (15), with the addition of the subscripts N or B denoting fragments from the *Not*I or *Bln*I maps, respectively. The pairs of *Not*I and *Bln*I subfragments generated by each insertion were as indicated (CCW, CW). Both the sequential ordering of insertions and the CCW/CW orientations of subfragments were inferred from the two sets of data for each insertion (one set from each rare-restriction pattern). This was possible because only one ordering and one combination of orientations allowed agreement of the physical distances between neighboring insertions on both the *Not*I and *Bln*I maps (10).

[d] The *Not*I map coordinates of insertions (clockwise from 0 kb) were determined by adding, to the *Not*I coordinate of the first insertion in the series (*zab*-100::Tn*10*dKanRCP2), the lengths of the *Not*I subfragments between it and each successive insertion. The *Not*I coordinate of the first insertion in the series (7 kb) was designated by its position relative to the MG1655 native *Not*I site nearest the *car* locus (12). Note that these physical-coordinate determinations may disagree with their *Bln*I-map counterparts from the same chromosome. This is partly explained, given the size-range-specific variation in accuracy of PFGE measurements (11), by identical chromosomal regions being contained within *Not*I and *Bln*I fragments of different sizes.

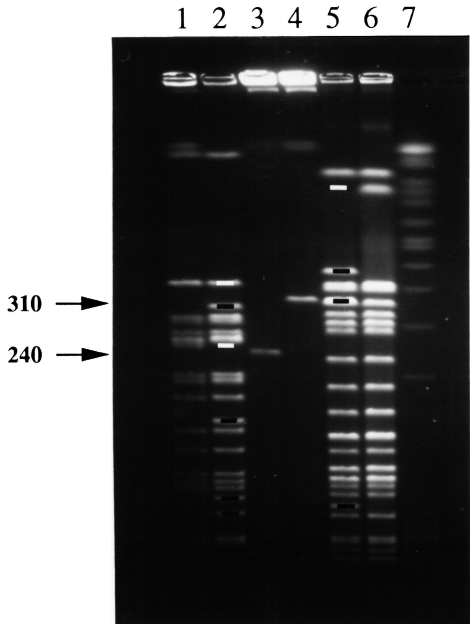

FIG. 1. PFGE separation of genomic DNA segments between Tn*10*dRCP2 insertions. Lanes 1, 2, 5, and 6: wild-type MG1655, χM2130, χM2131, and wild-type RS218, respectively, digested with *Not*I. Lanes 3 and 4: χM2130, χM2131, respectively, digested with I-*Sce*I. Lane 7: Megabase I standards. The MG1655 native *Not*I fragments H (245 kb) and B (360 kb; causing a doublet band with fragment C) were cleaved into pairs of subfragments of 80 and 170 kb, and 70 and 290 kb, respectively, in the double insertion mutant χM2130 (as predicted from the *Not*I digestion data from the single insertion mutants containing the same two insertions; Table 2). The *Not*I pattern of genomic DNA from strain χM2131 (containing the identical two insertions in the RS218 background) showed double cleavage of the RS218 native fragment B (confirming that this fragment contains the RS218 chromosomal interval corresponding to ~90–95 min; ref. 10) resulting in three subfragments of 70, 310, and 400 kb. Digestion of χM2130 and χM2131 genomic DNAs with I-*Sce*I, in contrast to *Not*I, produced isolated bands. (However, a small number of additional faint bands caused by cleavage at I-*Sce*I secondary sites are not uncommon; ref. 18.) This allowed direct side-by-side PFGE comparison of allelic restriction fragments (240 kb_{MG1655} and 310 kb_{RS218}) with corresponding endpoints. Note that following I-*Sce*I digestion the majority of genomic material remained as high molecular-weight DNA in the loading well.

The MG1655::Tn*10*dRCP2 insertion alleles should be especially useful for biophysical comparisons of allelic genomic segments from different *E. coli* isolates. This is because I-*Sce*I digestion at insertion sites only is expected to obviate the potential for interference that is posed by native-restriction-site degeneracy between different strains. Thus a comparison of the above MG1655 segment containing 90–95 min, with the corresponding chromosomal segment from a pathogenic *E. coli* strain was undertaken. Separation of the segment between the identical two χM2130 insertions transferred into the pathogenic *E. coli* RS218 background (strain χM2131), is shown in lane 4 of Fig. 1. I-*Sce*I digestion/PFGE resulted in an isolated 310-kb segment, indicating a difference of 70 kb between strains MG1655 and RS218 in the size of the interval. This difference provided confirmation of comparative *Not*I/*Bln*I mapping from these strains indicating an ~66-kb segment of RS218-specific DNA at ~91–93 min (10). However, by contrast with the multiband measurements required to calculate comparative *Not* I/*Bln* I maps, I-*Sce*I digestion at pairs of corresponding Tn*10*dRCP2 insertion sites allowed direct side-by-side PFGE comparisons of isolated bands. The expected precision of this approach over comparative *Not*I/*Bln*I mapping (as the later may suffer from both the requirement for added measurements per se and the size-range-specific variation in accuracy that is a prominent characteristic of PFGE measurements; ref. 11) should allow improved detection of strain-specific *E. coli* chromosomal segments encoding virulence and/or other strain-specific traits (20).

We conclude that I-*Sce*I digestion/PFGE of DNAs from Tn*10*dRCP2 double insertion mutants allows rapid isolation of *E. coli* genomic segments without cloning. This method (which can be applied in other species supporting Tn*10* transposition, or in other species that can undergo genetic exchanges leading to incorporation of *E. coli* insertions) should be particularly useful for purifying native contiguous/nonoverlapping genomic DNA segments (for Southern analysis or sequencing), and for comparing allelic chromosomal segments from different strains (21). The procedure is complementary to the polymerase-chain-reaction amplification method, being (*i*) applicable to much larger genomic segments and (*ii*) easier to use in instances where flanking insertions are more readily obtained than flanking sequences.

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