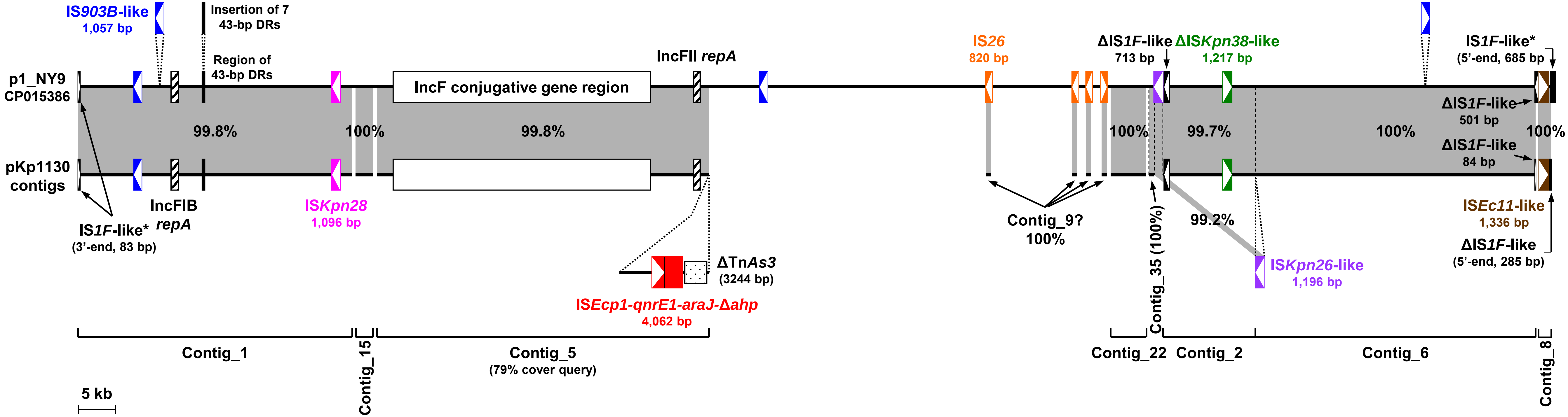

FIG. S1. Comparison of the contigs of pKp1130 with the plasmid p1 of *K. pneumoniae* NY9 (199,497 bp; GenBank accession number CP015386). In the BLAST analysis of the complete set of 14 pKp1130 contigs, the p1_NY9 sequence was the GenBank entry that showed high similarity scores with the largest number (9) of contigs (shown in the figure). DNA sequences are represented by thick horizontal lines. The regions of p1_NY9 sharing high identity with the pKp1130 contigs are indicated by grey-shaded areas and the corresponding % of identity is shown. Relevant genetic features shown are: genes encoding plasmid replication proteins (diagonally hatched bars); the following color-coded insertion sequences (white triangles indicate the sense of *tnpA* transcription): blue, IS*903B*-like; fuchsia, IS*Kpn28*; red, IS*Ecp1*; orange, IS*26*; violet, IS*Kpn26*-like; black, IS*1F*-like (the asterisks indicate consecutive fragments); green, IS*Kpn38*-like and brown, IS*Ec11*-like, and regions containing 43-bp-long direct repeats (DRs, thick vertical lines); genes encoding functions for conjugative plasmid transference (white boxes); a Tn*3* family truncated transposon Tn*As3* (ΔTn*As3*, dotted box), and the *qnrE1*-containing region mobilized by IS*Ecp1* (red box). DNA insertions are indicated by dotted lines. The Contig_5 was the unique that showed a cover query <100%. Both the position and orientation, as well as the number of copies of Contig_9 in pKp1130 could not be unequivocally determined because it comprises an IS*26* fragment and four copies of this insertion sequence were found in p1_NY9. Considering the remaining 5 contigs of pKp1130 that did not show a relevant similarity to p1_NY9, three of them displayed the best BLAST hits with plasmids of *Serratia marcescens*, *Yersinia pestis* or *Shigella dysenteriae*, respectively, while the other two comprised an IS*2* or a *dhfrVIII* gene[1], respectively.

1. Sundström L, Jansson C, Bremer K, Heikkilä E, Olsson-Liljequist B, Sköld O. 1995. A new *dhfrVIII* trimethoprim-resistance gene, flanked by IS*26*, whose product is remote from other dihydrofolate reductases in parsimony analysis. Gene **154:**7–14.

A

	qnrA	*qnrB*	*qnrC1*	*qnrD*	*qnrS*	*qnrVC*	*qnrE1*
qnrA		**48.8**	**59.5**	**48.4**	**59.0**	**60.2**	**50.3**
qnrB	**51.2**		**49.4**	**63.1**	**50.2**	**51.0**	**75.0**
qnrC1	**40.5**	**50.6**		**49.6**	**61.2**	**69.3**	**47.9**
qnrD	**51.6**	**36.9**	**50.4**		**47.9**	**51.6**	**63.0**
qnrS	**41.0**	**49.8**	**38.8**	**52.1**		**64.5**	**49.8**
qnrVC	**39.8**	**49.0**	**30.7**	**48.4**	**35.5**		**51.1**
qnrE1	**49.7**	**25.0**	**52.1**	**37.0**	**50.2**	**48.9**	

B

```
                10        20        30        40        50        60        70
qnrBcon ATGRSWCTDGYRYTVRTNDGCGWWAAAATTRRCAGRAABHGNTTCACYRGKGHRAAARTYGAWARYRGYA
qnrE1   ......T..A.T..T.A............A..TC.T........T.....A........A..G....CA.

                80        90       100       110       120       130       140
qnrBcon CDTTTTTYWWHTGYGATTTTTCRGGNRCSGAYHTNWGYRGYACTGARTTTATYGGCTGYCARTTHTATGA
qnrE1   TC...CG.........C.....C.............C....T.G..........................

               150       160       170       180       190       200       210
qnrBcon TCGHGARAGYCARAAAGGVKGYAAWTTYAGYCGYRCDAWVYTRARRGATRCYATTTTYAAAAGYWGYGAT
qnrE1   C..G.........C.G........C....A...G...C....T...........................

               220       230       240       250       260       270       280
qnrBcon YTMTCMATGGYNGRTTTYMGVAAYDYVARYGCNYTKGRHATHGARATTHSYCAYTGYMGNGCDCARGGBD
qnrE1   .....G...............C.............A...........C...G.A................

               290       300       310       320       330       340       350
qnrBcon CRGATTTYCGCGGYGCVAGYTTYATGAAYATGATYAYYAYNCGNACYTGGTTYTGYAGYGCNTAYATMAC
qnrE1   .C...........G..........................................T.............

               360       370       380       390       400       410       420
qnrBcon BARYWCNAAYYTRWSYTAYGCCAAYTTTTCDAARGYHGTVYTKGARAARTGYGARYTRTGGGARAAYCGY
qnrE1   A..A.G..............................G..T..............................

               430       440       450       460       470       480       490
qnrBcon TGGATRGGDRCYCAGRTRVYKGGBRCRACGTTBAGTGGWTCVGAYYTYKYBGGHGGYGARTTTWCRDCBT
qnrE1   ....AC..C..G..............C..C.....C..C........G.....G........C.......

               500       510       520       530       540       550       560
qnrBcon TCGACTGGCGRRCVGSRAAYKTHACRCAYTGYGAYYTSACHAAYTCRGARYTRGGBRRYYTMGAYRYYCG
qnrE1   .T..............C........C...................................G.....G..

               570       580       590       600       610       620       630
qnrBcon BVKNGTNGATTTRCARGGHGTYAARYTRGAYARCTAYCAGGCNKYDYTGMTSMYGGARCGDCTTGGYATC
qnrE1   ....A....................................T...C.A.T....................

               640
qnrBcon GCKRTNATKGGHTRR
qnrE1   ......T.A......
```

FIG. S2. Comparison of *qnr* genes. **(A)** Paired comparisons of *qnr* families and *qnrE1*. Averages of the % of identity (upper triangle), or the % of nucleotide difference (lower triangle), are shown. The maximal % of identity and the minimal % of difference are indicated in red. **(B)** Sequence alignment of *qnrE1* and a consensus (*qnrB*con) of all the *qnrB* alleles previously known (accession numbers are indicated in Table S2). Dots indicate nucleotide identities. The consensus was done considering all the different nucleotides found in a given position for all the *qnrB* alleles and the variants found are indicated by the corresponding IUPAC codes for nucleotide mixtures (2, 3 and 4 nucleotides, blue, orange and fuchsia, respectively). The nucleotide changes of *qnrE1* located in positions fully conserved among all the *qnrB* alleles previously described are highlighted with a green background.

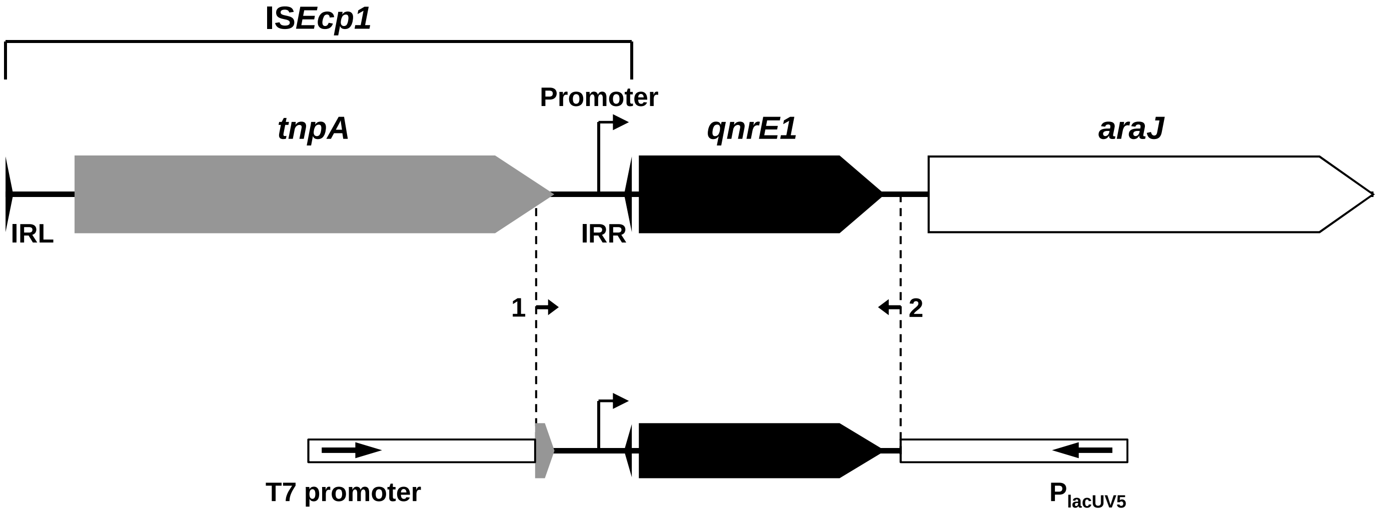

FIG. S3. Genetic context of *qnrE1*. A region of 3,619 bp of the Contig_5 of pKp1130 (see Fig. S1) is depicted. The horizontal square bracket indicates IS*Ecp1* (100% identical to GenBank accession number AJ242809). Genes are represented by arrow-shaped boxes. The black triangles indicate the inverted repeats of IS*Ecp1* (IRL and IRR), and the promoter located next to the IRR, which likely accounted for the expression of *qnrE1*, is marked. The numbered arrows indicate the binding sites of the primers ISEcp1-Fout and pQNR1130-comp-R (1 and 2, respectively, see Table S1), which were used to completely amplify *qnrE1*. The 965-bp-long amplicon obtained with these primers is depicted between vertical dashed lines, and the multiple cloning site of the vector pJET1.2/blunt, where this fragment was cloned to give pJET1.2-1130, is represented by horizontal empty boxes (not drawn to scale). The vector promoters, T7 and P_{lacUV5}, and their transcriptional senses are indicated by arrows.

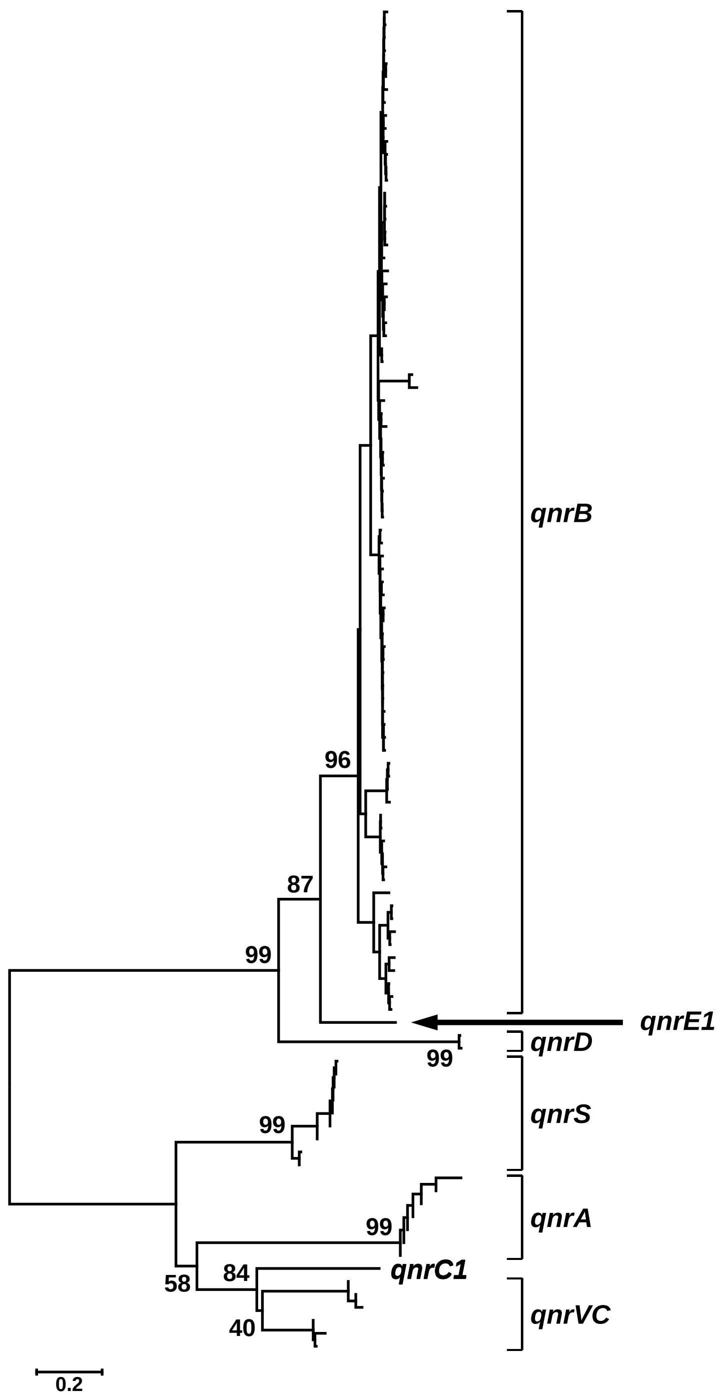

FIG. S4. NJ tree generated from an alignment of *qnrE1* and all the *qnr* alleles currently known (accession numbers are indicated in Table S2). For simplicity, the taxa names of *qnr* alleles were excluded (see Table S3) and only the bootstrap percentages (over 1,000 replicates) for relevant nodes are shown. The clustering of the alleles for each *qnr* family is indicated with square brackets at the right. The branch lengths were drawn to the scale shown, which indicates number of substitutions/site..

TABLE S1. PCR primers used in this study. The primer pair used in each PCR assay (1) is indicated by a different background.

Target	Primer[a]	Sequence (5'−3')	Temp[b]
Screening of *qnrVC* genes:[c]			
qnrVC1, qnrVC3 and *qnrVC6*	qnrVC136-F	GTGAACTTCTCACATCAGGACT	55ºC
	qnrVC136-R	GCCACGAGCATATTTTTACACC	
qnrVC4, qnrVC5 and qnrVC7	qnrVC457-F	CACATCAAGACATGAGTGGTCA	55ºC
	qnrVC457-R	GCCACGAACAGATTTTTACACC	
Complete amplification/Sanger sequencing of *qnrE1* :			
tnpA of IS*Ecp1C*	ISEcp1-Fout	GGAAAACTATCCGTACAAGG	52ºC
Region between *qnrE1* and *araJ*	pQNR1130-comp-R	GGCTGACAGGTTAATCCATT	
Amplification/Sanger sequencing of the vicinities of the *ahp* interruption point:[d]			
araJ	araJ-F	GACCTCGCTGTTGATGTATG	55ºC
Resolvase-encoding gene located upstream of ∆*ahp*	R2	AAGTGTTTGCGCTCGATGTC	

[a] F, forward; R, reverse.

[b] Annealing temperature used in the corresponding PCR assay.

[c] Given the broad genetic difference among the *qnrVC* alleles, two PCR assays were implemented for the screening of all the members of this family.

[d] An amplicon of 663 bp was generated.

References

1. Melano R, Corso A, Petroni A, Centrón D, Orman B, Pereyra A, Moreno N, Galas M. 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum β-lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. J Antimicrob Chemother **52:**36–42..

TABLE S2. GenBank accession numbers of the known *qnr* sequences.

***qnr* family**	***qnr* sequence**	**Accession number**
qnrA	qnrA1	AY070235
	qnrA2	AY675584
	qnrA3	DQ058661
	qnrA4	DQ058662
	qnrA5	DQ058663
	qnrA6	DQ151889
	qnrA7	GQ463707
qnrB	qnrB1	DQ351241
	qnrB2	DQ351242
	qnrB3	DQ303920
	qnrB4	DQ303921
	qnrB5	DQ303919
	qnrB6	EF520349
	qnrB7	EU043311
	qnrB8	EU043312
	qnrB9	EF526508
	qnrB10	DQ631414
	qnrB11	EF653270
	qnrB12	AM774474
	qnrB13	EU273755
	qnrB14	EU273757
	qnrB15	EU302865
	qnrB16	EU136183
	qnrB17	AM919398
	qnrB18	AM919399
	qnrB19	EU432277
	qnrB20	AB379831
	qnrB21	FJ611948
	qnrB22	FJ981621
	qnrB23	FJ981622
	qnrB24	HM192542
	qnrB25	HQ172108
	qnrB26	HQ386846
	qnrB27	HM439641
	qnrB28	HM439643
	qnrB29	HM439649
	qnrB30	HM439650
	qnrB31	HQ418999
	qnrB32	JN173054
	qnrB33	JN173055
	qnrB34	JN173056
	qnrB35	JN173057
	qnrB36	JN173058
	qnrB37	JN173059
	qnrB38	JN173060
	qnrB39	NG_050501 [a]
	qnrB40	JN166689
	qnrB41	JN166690
	qnrB42	JN680743
	qnrB43	JQ349151
	qnrB44	JQ349153
	qnrB45	JQ349152

	qnrB46	JQ349154
	qnrB47	JQ349155
	qnrB48	JQ762640
	qnrB49	JQ582718
	qnrB50	JX440357
	qnrB51	JX440358
	qnrB52	EF488762
	qnrB53	HQ704413
	qnrB54	HE820727
	qnrB55	KF730650
	qnrB56	JX259317
	qnrB57	JX259318
	qnrB58	JX259319
	qnrB59	JX259320
	qnrB60	AB734055
	qnrB61	AB734053
	qnrB62	JX987101
	qnrB64	KC580653
	qnrB65	KC580654
	qnrB66	KC580655
	qnrB67	KC580656
	qnrB68	KC580657
	qnrB69	KC580658
	qnrB70	KC580659
	qnrB71	KC580660
	qnrB72	KC741443
	qnrB73	KF443075
	qnrB74	KJ415247
	qnrB75	KF207591
	qnrB76	KM985469
	qnrB77	KM985470
	qnrB78 [b]	KM985471
	qnrB80	KM985473
qnrC	qnrC1	EU917444
qnrD	qnrD1	FJ228229
	qnrD2	KF055448
qnrS	qnrS1	AB187515
	qnrS2	DQ485530
	qnrS3	EU077611
	qnrS4	FJ418153
	qnrS5	HQ631377
	qnrS6	HQ631376
	qnrS7	KF730651
	qnrS8	KF730652
	qnrS9	KF732714
qnrVC	qnrVC1	EU436855
	qnrVC3	HM015626
	qnrVC4	GQ891757
	qnrVC5	JN408080
	qnrVC6	KC202804
	qnrVC7	KM555152

[a] The record NZ_ABWL02000005 (http://www.lahey.org/qnrstudies/) was removed by RefSeq staff.

[b] The *qnrB79* allele (KM985472) is 100% identical to *qnrB78* and was not considered.

Table S3. The taxa names of the trees depicted in Fig. 2 (Maximum Likelihood) and Fig. S3 (Neighbor-Joining) are indicated from top (number 1) to bottom (number 104) of the corresponding tree. The *qnr* families are indicated with different background colors and *qnrE1* is shown in red.

Number	Maximum Likelihood tree	Neighbor-Joining tree
1	*qnrB43*	*qnrB43*
2	*qnrB57*	*qnrB57*
3	*qnrB76*	*qnrB76*
4	*qnrB13*	*qnrB13*
5	*qnrB24*	*qnrB24*
6	*qnrB29*	*qnrB29*
7	*qnrB48*	*qnrB48*
8	*qnrB23*	*qnrB23*
9	*qnrB45*	*qnrB45*
10	*qnrB54*	*qnrB54*
11	*qnrB49*	*qnrB49*
12	*qnrB20*	*qnrB20*
13	*qnrB2*	*qnrB2*
14	*qnrB52*	*qnrB52*
15	*qnrB9*	*qnrB9*
16	*qnrB32*	*qnrB32*
17	*qnrB78*	*qnrB78*
18	*qnrB7*	*qnrB7*
19	*qnrB44*	*qnrB44*
20	*qnrB64*	*qnrB64*
21	*qnrB41*	*qnrB41*
22	*qnrB15*	*qnrB15*
23	*qnrB14*	*qnrB14*
24	*qnrB58*	*qnrB58*
25	*qnrB18*	*qnrB18*
26	*qnrB30*	*qnrB30*
27	*qnrB16*	*qnrB16*
28	*qnrB80*	*qnrB80*
29	*qnrB42*	*qnrB31*
30	*qnrB1*	*qnrB53*
31	*qnrB74*	*qnrB42*
32	*qnrB6*	*qnrB1*
33	*qnrB3*	*qnrB74*
34	*qnrB75*	*qnrB6*
35	*qnrB26*	*qnrB3*
36	*qnrB77*	*qnrB75*
37	*qnrB17*	*qnrB26*
38	*qnrB66*	*qnrB77*
39	*qnrB31*	*qnrB17*
40	*qnrB53*	*qnrB66*
41	*qnrB5*	*qnrB5*
42	*qnrB19*	*qnrB19*
43	*qnrB10*	*qnrB10*
44	*qnrB56*	*qnrB56*
45	*qnrB59*	*qnrB59*
46	*qnrB62*	*qnrB62*
47	*qnrB46*	*qnrB46*
48	*qnrB47*	*qnrB47*
49	*qnrB50*	*qnrB50*

50	*qnrB40*	*qnrB40*
51	*qnrB51*	*qnrB51*
52	*qnrB36*	*qnrB61*
53	*qnrB71*	*qnrB71*
54	*qnrB61*	*qnrB36*
55	*qnrB67*	*qnrB67*
56	*qnrB70*	*qnrB68*
57	*qnrB68*	*qnrB70*
58	*qnrB72*	*qnrB72*
59	*qnrB28*	*qnrB28*
60	*qnrB33*	*qnrB33*
61	*qnrB27*	*qnrB27*
62	*qnrB73*	*qnrB73*
63	*qnrB8*	*qnrB8*
64	*qnrB21*	*qnrB21*
65	*qnrB25*	*qnrB25*
66	*qnrB35*	*qnrB38*
67	*qnrB38*	*qnrB35*
68	*qnrB60*	*qnrB60*
69	*qnrB39*	*qnrB39*
70	*qnrB4*	*qnrB4*
71	*qnrB55*	*qnrB55*
72	*qnrB22*	*qnrB22*
73	*qnrB65*	*qnrB65*
74	*qnrB37*	*qnrB37*
75	*qnrB69*	*qnrB69*
76	*qnrB12*	*qnrB12*
77	*qnrB11*	*qnrB11*
78	*qnrB34*	*qnrB34*
79	***qnrE1***	***qnrE1***
80	*qnrD1*	*qnrD1*
81	*qnrD2*	*qnrD2*
82	*qnrA4*	*qnrS3*
83	*qnrA5*	*qnrS8*
84	*qnrA3*	*qnrS1*
85	*qnrA6*	*qnrS9*
86	*qnrA7*	*qnrS4*
87	*qnrA1*	*qnrS7*
88	*qnrA2*	*qnrS5*
89	*qnrS8*	*qnrS2*
90	*qnrS1*	*qnrS6*
91	*qnrS3*	*qnrA1*
92	*qnrS9*	*qnrA2*
93	*qnrS4*	*qnrA6*
94	*qnrS7*	*qnrA7*
95	*qnrS5*	*qnrA5*
96	*qnrS2*	*qnrA4*
97	*qnrS6*	*qnrA3*
98	*qnrC1*	*qnrC1*
99	*qnrVC1*	*qnrVC6*
100	*qnrVC3*	*qnrVC1*
101	*qnrVC6*	*qnrVC3*
102	*qnrVC7*	*qnrVC5*
103	*qnrVC4*	*qnrVC7*
104	*qnrVC5*	*qnrVC4*