

In situ* real-time evaluation of radiation-responsive promoters in the extremely radioresistant microbe *Deinococcus radiodurans

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Supplementary material

Supplementary table 1. Plasmids and recombinant strains used in this study

Bacterial strain/ plasmid	Description	Source
Bacterial strains		
<i>D. radiodurans</i> (DRA)	ATCC BAA-816, Wild-type strain	Daly M J
<i>D. radiodurans</i> - $\Delta pprI$	<i>D. radiodurans</i> strain in which <i>pprI</i> gene (DR0167) is replaced with spectinomycin resistance cassette	This study
Recombinant DRA strains	<i>D. radiodurans</i> harboring one of the recombinant plasmids listed below	
<i>E. coli</i> (EC)		
DH5 α (EC)	F ⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ 80dlacZ Δ M15 Δ (<i>lacZYA-argF</i>)U169, hsdR17 ($r_K^- m_K^+$), λ^-	Lab collection
<i>E. coli</i> BL21	F ⁻ ompT gal demhsdS _B ($r_B^- m_B^-$) λ (DE3) pLysS(cm^R)	Lab collection
Recombinant EC strains	<i>E. coli</i> harbouring one or more of the recombinant plasmids listed below	This study
Plasmids		
pRAD1	6280 bp, EC-DRA shuttle vector, Amp ^R , Cm ^R	Meima and Lidstrom 2000
pUC4K	3914 bp, Kan ^R	Pharmacia Biotech
pAM1956	EC-Anabaena shuttle vector with a promoter less <i>gfp-mut2</i> , Kan ^R	Yoon and Golden 1998
pBluescriptII SK(+)	3000 bp, Amp ^R	Stratagene
pBS- <i>gfp</i>	pBS with cloned <i>gfp</i> gene, Amp ^R	This study
pBS-T- <i>gfp</i>	pBS with transcriptional terminator and <i>term-116</i> and <i>gfp</i> gene, Amp ^R	This study
pBS-Spec	pBS, Spec ^R cassette (1104 bp) inserted at SmaI site, Amp ^R , Spec ^R	This study
pBS- $\Delta pprI$	pBS-Spec carrying 500bp <i>pprI</i> -upstream and <i>pprI</i> -downstream DNA sequence, Amp ^R , Spec ^R	This study
pRAD-Kan	pRAD1 with Cm ^R and Amp ^R replaced with Kan ^R , Kan ^R	This study
pKTG	pRAD-Kan with cloned transcriptional terminator <i>term-116</i> and <i>gfp</i> gene, Kan ^R	This study
pKGX	pKTG with MCS, Kan ^R	This study
pKG	4745 bp, 386 bp between pUC Ori and Term116 and 305 bp between kanamycin resistance cassette and <i>repU</i> removed from pKGX, Kan ^R	This study

Supplementary table 1 (continued)

Bacterial strain/ plasmid	Description	Source
pKG-P _{DR0053}	367 bp putative promoter sequence of DR0099 (Chr. I 49915-49549) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0070}	400bp promoter sequence (Chr. I 65604-66004) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0099}	367 bp putative promoter sequence of DR0099 (Chr. I, 100091-100458) cloned in pKG (<i>EcoRV</i>), Kan ^R	This study
pKG-P _{DR0219}	300 bp promoter sequence (Chr. I 219026-219326) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0423}	300 bp promoter sequence (Chr. I 424005-423705) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0596}	506 bp promoter sequence of DR0596 (Chr. I 609955-609448) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0606}	261 bp putative promoter sequence of DR0606 (Chr. I 617609-617870) cloned in pKG (<i>EcoRV</i>), Kan ^R	This study
pKG-P _{DR0694}	348 bp putative promoter sequence of DR0694 (Chr. I 709674-710021) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR0906}	375 bp promoter sequence (Chr. I 911842-912217) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR1143}	390 bp promoter sequence (Chr. I 1153428-1153038) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR1720}	515 bp promoter sequence (Chr. I 1743794-1744309) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR1913}	260 bp promoter sequence (Chr. I,1932139-1932399) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR2220}	300 bp putative promoter sequence of DR2220 (Chr. I 2217380-2217680) cloned in pKG (<i>EcoRI/EcoRV</i>), Kan ^R	This study
pKG-P _{DR2275}	177bp promoter sequence (Chr. I,2272809-2272986) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pKG-P _{DR2238}	315 bp promoter sequence (Chr. I 2335583-2335896) cloned in pKG (<i>EcoRI/SpeI</i>), Kan ^R	This study
pET21a (+)	Protein overexpression vector for <i>E. coli</i>	Lab collection
pET-ddrO	The <i>D. radiodurans</i> gene ddrO cloned in pET21a(+) vector	This study
P13840	p11830 PSpac-term 116, Spc ^R	Nguyen <i>et al.</i> 2009

Supplementary table 2. List of primers used in this study

Sr. No.	Name	Sequence	Restriction sites used for cloning
1	Term116-F	5'-AAAAAATCCCCCGGTGGCAATCCGGGGGTTTTT-3'	-
2	Term116-R	5'-TTTTTtagggggccaccgTTAGGCCCCCCAAAAA-3'	-
3	GFP-F	5'-GGAATTCATATGAGTAAAGGAGAAGAAGTTCCTACTG-3'	<i>EcoRI</i>
4	GFP-R	5'-CGGGATCCTTATTTGTATAGTTCATCCATGCCAT-3'	<i>BamHI</i>
5	Kan-F	5'-TATTCGTTACAACCAATTAACCAATTCTG-3'	-
6	Ori-R	5'-GGAAGTAGACCCGTAAAAAGGCCG-3'	-
7	pRAD-F	5'-TCGCGAGGCCTCGAGGTCAAAA-3'	-
8	pRAD-R	5'-GGTCAGGCCTGCTTCTGTGCTTCCCT-3'	-
9	MCS-F	5'-ACTAGTAGCGCCGCTCTAGAGTCGACGATATC-3'	-
10	MCS-R	5'-GATATCGTCGACTCTAGAAGCGCCGCACTAGT-3'	-
11	P0053F	5'-GGACTAGTAGTTCGGCAGGTTGG-3'	<i>SpeI</i>
12	P0053R	5'-GGAATTCCTTCTTTCCTCCTGA-3'	<i>EcoRI</i>

Supplementary table 2 (continued)

Sr. No.	Name	Sequence	Restriction sites used for cloning
13	P0070F	5'-GG <u>ACTAGT</u> TCGCAGCGTAAAGGCA-3'	<i>SpeI</i>
14	P0070R3	5'-GGAATTCCTGCCTCCTCCTTACGTA-3'	<i>EcoRI</i>
15	P0099-F	5'-CCGAGAAGGATTACAATCTAGAACG -3'	-
16	P0099-R	5'-CATGCCTCGGGCCATATGAAATTCT -3'	-
17	P0219-F	5'-GG <u>ACTAGT</u> ACATGGCCCAACTGAGG-3'	<i>SpeI</i>
18	P0219-R	5'-GGAATTCGCTTAGGGATTATATCT-3'	<i>EcoRI</i>
19	P0423-F	5'-GG <u>ACTAGT</u> CGTGCCCGGTGGCGG-3'	<i>SpeI</i>
20	P0423-R	5'-GGAATTCGCATTTAGTTTAGAA-3'	<i>EcoRI</i>
21	P0596-F	5'-GG <u>ACTAGT</u> AACTCGTGACGGTGGGA-3'	<i>SpeI</i>
22	P0596-R2	5'-GGAATTCGCCAGCTTGCTTGTG-3'	<i>EcoRI</i>
23	P0606-F3	5'-GTGGCCGCCACTAGTGTTTCAGGGAT-3'	<i>SpeI</i>
24	P0606-R	5'-GGAATTCGTGGGGTCTCCTGT-3'	<i>EcoRI</i>
25	P0694-F	5'-GG <u>ACTAGT</u> AGCATCTGACAACAGAC-3'	<i>SpeI</i>
26	P0694-R	5'-GGAATTCCTCCCTCCTCCTTTC-3'	<i>EcoRI</i>
27	P0906F	5'-GG <u>ACTAGT</u> TGACCTTTCCCGCA-3'	<i>SpeI</i>
28	P0906R	5'-GGAATTCCTGCTCATTGGGTTC -3'	<i>EcoRI</i>
29	P1143F	5'-GG <u>ACTAGT</u> AGTGATGCTTATCCGC-3'	<i>SpeI</i>
30	P1143R	5'-GGAATTCGGTTGGGCTGAAGTGC-3'	<i>EcoRI</i>
31	P1720-F	5'-GG <u>ACTAGT</u> AAGTGGGCAGCGGC-3'	<i>SpeI</i>
32	P1720-R	5'-GGAATTCGGGTAAATCCTCCTTG-3'	<i>EcoRI</i>
33	P1913-F	5'-GG <u>ACTAGT</u> CTCAATGGGGGTACA-3'	<i>SpeI</i>
34	P1913-R	5'-GGAATTCGGCACTCCAATCGGG -3'	<i>EcoRI</i>
35	P2220-F	5'-ACCCGATATCGCGAGGTGCAGCAGG-3'	<i>EcoRV</i>
36	P2220-R	5'-AAAAGAATTCTGCGAGTCTCCCGA-3'	<i>EcoRI</i>
37	P2275-F	5'-GG <u>ACTAGT</u> TTTCGACATGCGGAC-3'	<i>SpeI</i>
38	P2275-R	5'-GGAATTCCTCCCGTGCCACCGGC-3'	<i>EcoRI</i>
39	P2238-F	5'-GG <u>ACTAGT</u> CCTCGTGACGAAAC	<i>SpeI</i>
40	P2238-R	5'-GGAATTCCTGATGATTTCTGCTA-3'	<i>EcoRI</i>
41	DdrO-F	5'-GCCATATGACATTGAAACTGCACGA-3'	<i>NdeI</i>
42	DdrO-R	5'-CGAAGCTTTCACCTCCTGGGCTGCGGCG-3'	<i>HindIII</i>
43	pprI-up-F	5'-CCCAAGCTTCCTCAAGCTCTACGCCCTTAC-3'	<i>HindIII</i>
44	pprI-up-R	5'-AACTGCAGATGGCAGTGATTTTCGCTGTTTGC-3'	<i>PstI</i>
45	pprI-dn-F	5'-CGGGATCCACTGGACGGGCCGTATCCACGAGC-3'	<i>BamHI</i>
46	pprI-dn-R	5'-GCTCTAGACTGGTGCTGCATGGTGCGCGGCTC-3'	<i>XbaI</i>
47	pprI-F	5'-CGCATATGCCAGTGCCAACGTCAGCCCCCTT-3'	-
48	pprI-R	5'-CGAAGCTTCTGTGCAGCGTCTGCGGCTCGTC-3'	-
49	pKG-1	5'-CTGTCATGTTTCAGATAAGT-3'	-
50	pKG-2	5'-TTCCAAATGAGTTTCCGCC-3'	-
51	pKG-3	5'-TCGTAGAAGGCGGACTTCT-3'	-
52	pKG-4	5'-TTGTCACGCAAAGGCCCG-3'	-
53	pKG-5	5'-AGGCGGTGCTACAGAGTTCT-3'	-
54	pKG-6	5'-CTTGCTCGAGGCCGCGA-3'	-
55	pKG-7	5'-GGCTGGCCTGTTGAACAAG-3'	-
56	pKG-8	5'-GACGGTATCGATAAGCTTGA-3'	-
57	pKG-9	5'-ACAACATTAAGATGGAAGCG-3'	-

The restriction site sequence contained in the primer, and used for cloning subsequently, is underlined.