Supplementary Data of the following Manuscript:

Title- Characterization of DNA processing protein A (DprA) of the radiation-resistant bacterium *Deinococcus radiodurans*

Authors- Dhirendra Kumar Sharma[1,2], Hari S. Misra[2], Ishu Soni[2], Yogendra S. Rajpurohit[1, 2#],

Affiliation-

[1]Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai- 400085, India

[2]Life Science, Homi Bhabha National Institute (DAE- Deemed University), Mumbai- 400094, India

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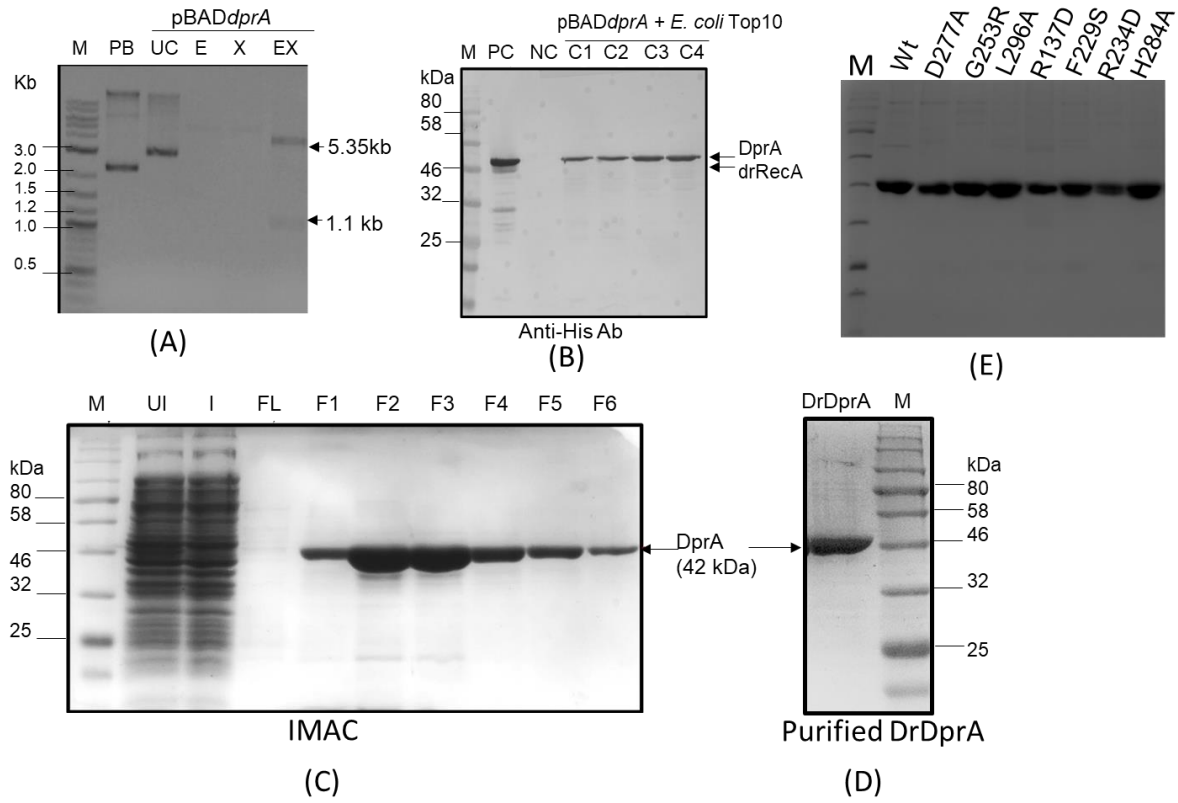

Figure S1; Cloning of dr*dprA* in pBAD vector and DrDprA protein purification. The *dprA* gene (*dr_0120*) was PCR amplified using *D. radiodurans* genomic DNA and gene specific primers and cloned in *pBAD* vector. (A) 1.1 kb PCR DNA was cloned in pBAD expression vector at XhoI (X) and EcoR1 (E) restriction sites. Restriction digestion of selected clone showed release of 1.1kb DNA with double digestion (EX) while linear band of 6.4kb with either of single restriction digestion (E/X) observed. M-DNA ladder, PB-pBAD plasmid DNA uncut, UC-pBAD*dprA* recombinant plasmid uncut.

(B) DrDprA protein overexpression confirmation by anti-His antibody in *E. coli* Top10 cells harboring pBAD*dprA* plasmid. M-Protein ladder, PC-positive control (DrRecA protein), NC-negative control (*E. coli* Top10 cells harboring empty pBAD plasmid), C1 to C4- selected clones *E. coli* Top10 cells harboring pBAD*dprA* plasmid.

(C) Purification of His-tag DrDprA by immobilized-metal affinity chromatography (IMAC). M-protein ladder, UI-uninduced sample, I-arabinose induced sample, FL- column flow through, Eluted fractions (F1 - F6).

(D) SDS-PAGE showing purified DrDprA protein.

(E) SDS-PAGE showing purified wild type DrDprA and its various mutant proteins.

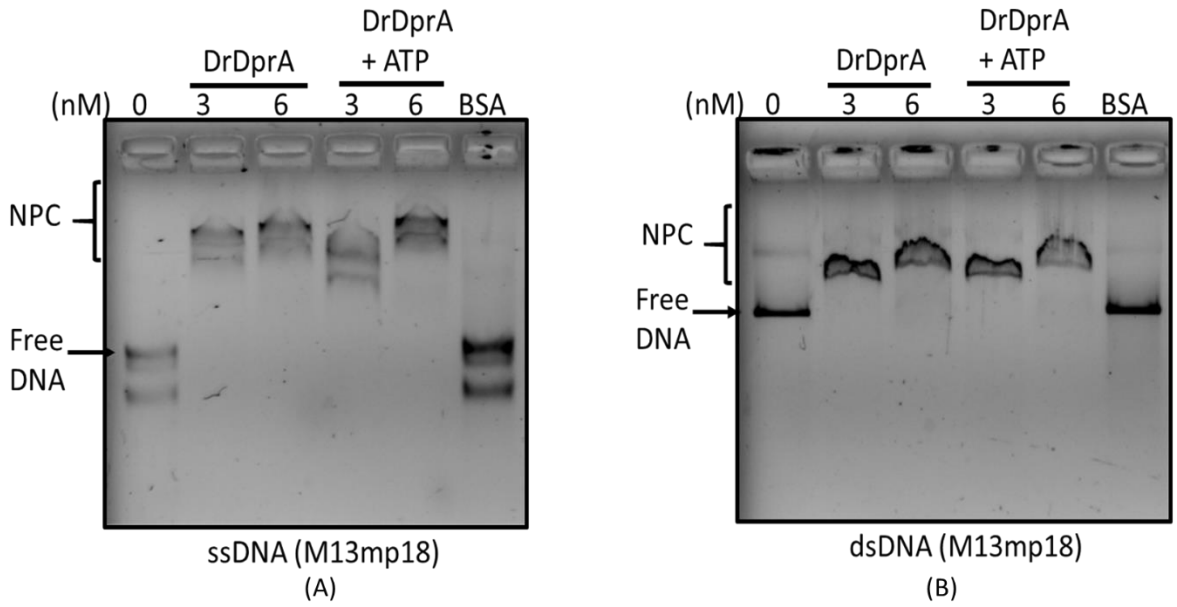

Figure S2; DrDprA binding with M13mp18 ssDNA (A) and dsDNA (B) with and without ATP (1mM) monitored on agarose gel (0.8%). Data shown are representatives of the reproducible experiments repeated two times.

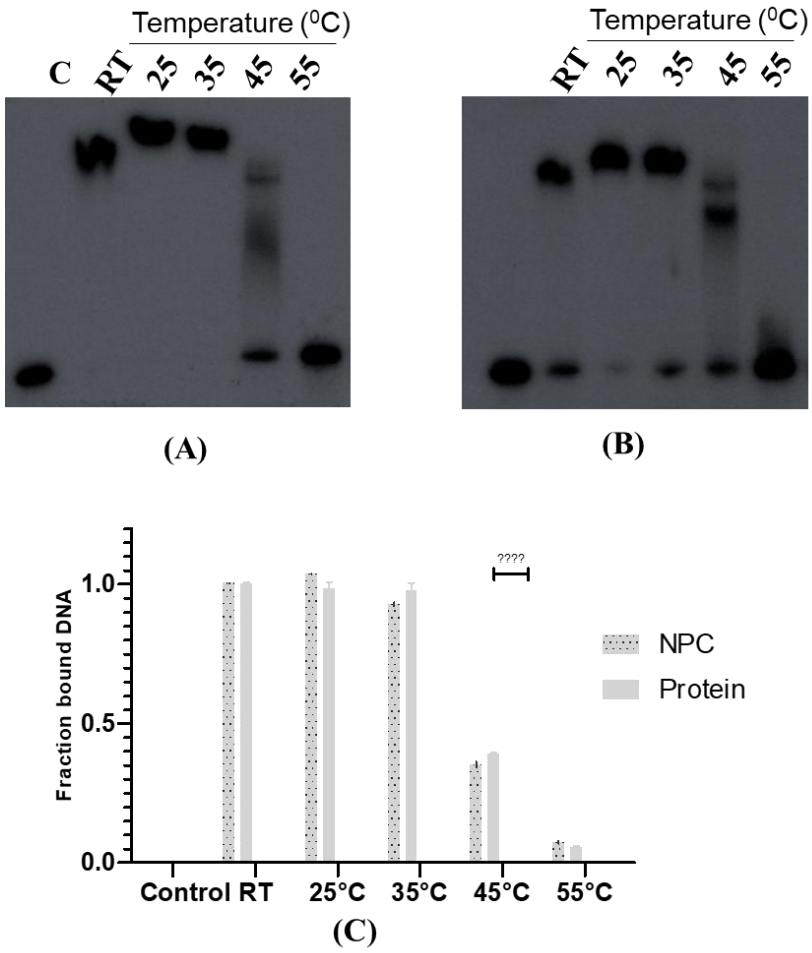

Figure S3; Temperature stability of DrDprA protein or its nucleoprotein complex (NPC) with ssDNA. (A) The DrDprA protein incubated at different temperatures for 30 min., followed by ssDNA binding checked by EMSA on 8% native PAGE. (B) DrDprA bound to ssDNA nucleoprotein complex (NPC) incubated at different temperatures for 30 min followed by NPC resolved on 8% native PAGE. (C) Bound DNA band intensities were quantified densitometrically and percent bound fractions were calculated. Results were plotted and analyzed using GraphPad Prism software.

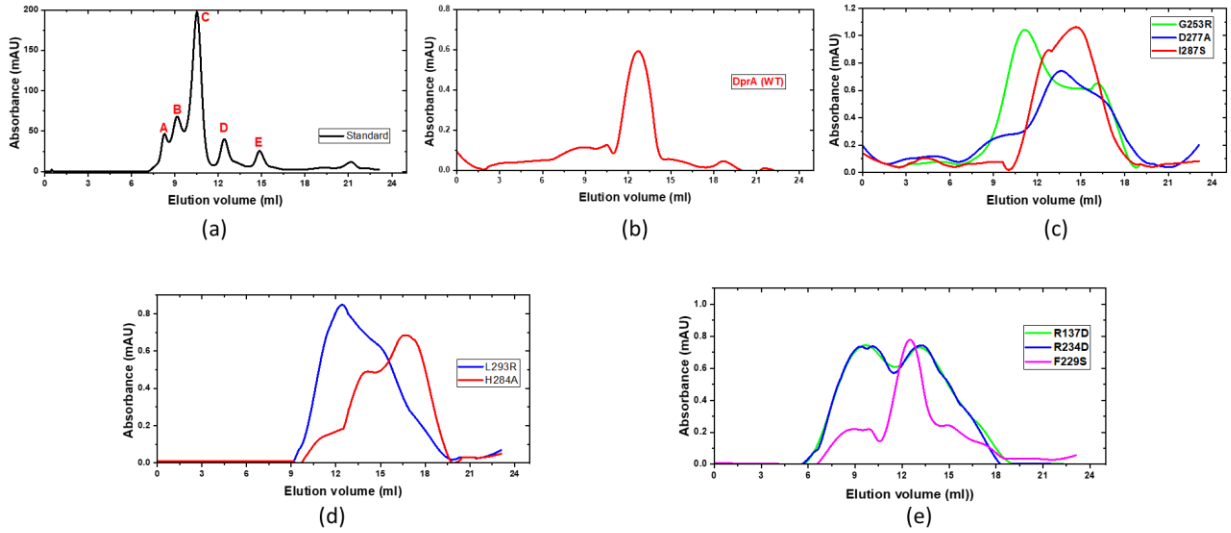

Figure S4; Size exclusion chromatography for wild type DrDprA and its mutants. Size exclusion chromatography done using Supdex-200 Increase 10/300 GL, column and the Absorbance (mAu) was plotted as a function of elution volume (ml) using GraphPad Prism. (a) Size exclusion standards; (A) Thyroglobulin; 669kDa, (B) Ferritin; 450kDa, (C) Catalase; 232kDa, (D) Aldolase; 160kDa, and (E) Ovalbumin, 45kDa., (b) wild type DrDprA, (c) predicted RecA-DrDprA interaction mutants (G253R, D277A, and I287S) profile, (d) DrDprA oligomerization mutants (L293R and H284A) profile, and (e) DrDprA DNA binding mutants (R137D, R234D, and F229S) profile.

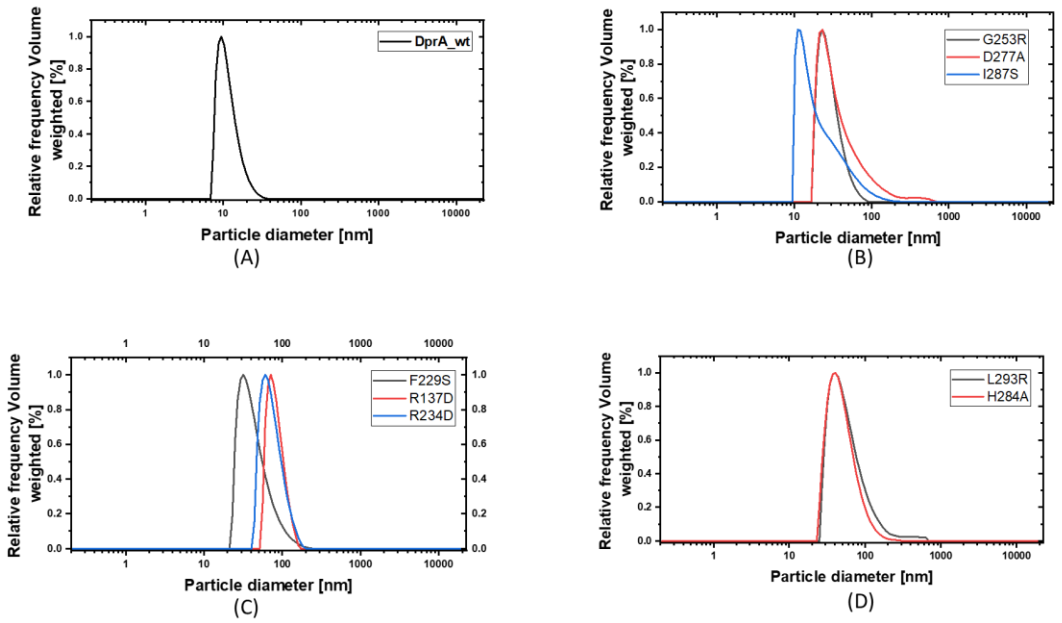

Figure S5; Dynamic light scattering (DLS) analysis of wild type DrDprA and its mutants. DLS data acquisition done for 10 second at 37 ºC by taking 5 µM purified proteins in a buffer (20mM Tris pH 8, 100mM NaCl and 0.1mM EDTA). (A) Wild type DrDprA; (B) RecA-DrDprA interaction mutant proteins (G253R, D277A, and I287S); (C) DNA binding mutant proteins (R137D, R234D, and F229S); (D) Oligomerization mutant proteins (L293R and H284A).

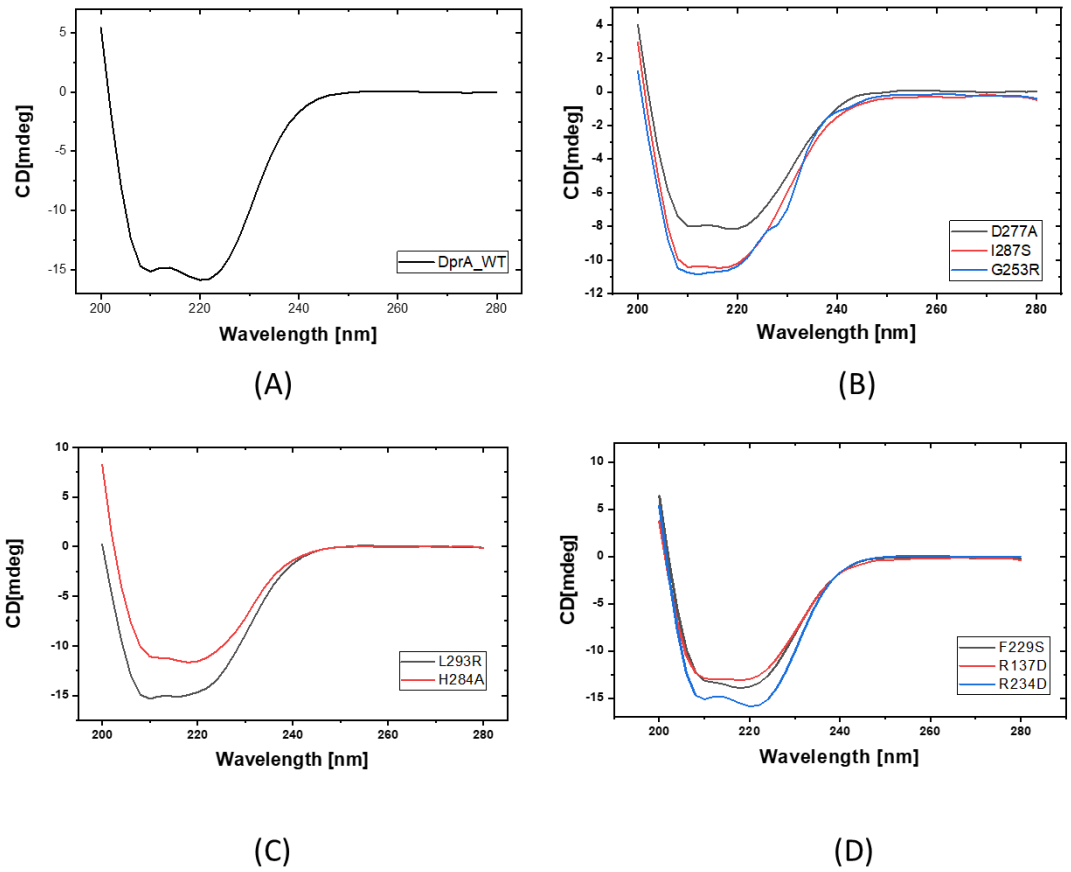

Figure S6; CD Spectra of wild type DrDprA and its various mutants. Proteins were diluted in PBS buffer and spectra was recorded using CD spectrophotometer (JASCO, J815, Japan). (A) Wild type DrDprA; (B) RecA-DrDprA interaction mutant proteins (G253R, D277A, and I287S); (C) Oligomerization mutant proteins (L293R and H284A); and (D) DNA binding mutant proteins (R137D, R234D, and F229S).

Table S1 Bacterial strains, plasmids, Primers and used in this study:

Bacterial strains	**Genotype**	**Source**
D. radiodurans R1	Wild type strain ATCC13939	Lab stock
E. coli Novablue	*end*A1 *hsd*R17*(r* $_{K12}^{-}$ *m* $_{K12}^{+}$*) sup*E44 *thi-1 rec*A1 *gyr*A96 *rel*A1 *lac*F''*[pro*A^{+}B^{+} *lac*I^{q} *Z*ΔM15*::*T*n*10] (Tet^{R})	Invitrogen
E. coli BTH 101	F^{-}, *cya*-99, *ara*D139, *gal*E15, *gal*K16, *rps*L1 (Str ^{r}), *hsd*R2, *mcr*A1, *mcr*B1	Lab stock
E. coli Top10	F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 Δ lacX74 recA1 araD139 Δ(araleu)7697 galU galK rpsL (StrR) endA1 nupG	Lab stock
E. coli BL21(DE3)	*fhu*A2 *(lon)omp*T *gal*(λDE3)*(dcm)* Δ*hs*dS	Lab stock
Δ*dprA*	*dprAΩspec*	In this study

Plasmids:

Names	**Characteristics and Source**	**MW of protein**
pUT18	pUC19 derivative, MCS at N-terminal of T18 fragments of adenylate cyclase, ~3 kb, AmpR [29]	18 kDa
pKNT25	pSU40 derivative, MCS at N-terminal of T25 fragment of adenylate cyclase, ~3.4 kb, KanR [29]	25 kDa
pUTDr*recA*	pUT18 carrying dr*recA* at *Bam*HI and *Kpn*I [31]	56 kDa
pKNDr*recA*	pKNT25 carrying dr*recA* at *Bam*HI and *Kpn*I [31]	63 kDa
pUT*dprA*	pUT18 carrying *drpprA at BamHI and KpnI* [this study]	56 kDa
pVHS559	A shuttle vector between *D. radiodurans* and *E. coli* (Spec^{R}) [lab stock]	-
pRADgro	pRAD1 carrying 261bp *Bgl*II-*Xba*I fragment of promoter (Pgro) from *D. radiodurans* [lab stock]	-
pBADdr*dprA*	pBAD carrying dr*dprA* at *XhoI* and *EcoRI* [this study]	41 kDa
pRadHisRecA	pRADgro carrying *recA*His from pETdr*recA* at *Apa*I & *Xba*I [31]	41 kDa
pVHSM*dprA*	pVHS559 carrying *dprA*-T18 from pUT*dprA* at *Nde*I & *Xho*I [this study]	

Primer details

SI.No.	**Name of primer**	**Nucleotide sequence of primer (5' to 3')**	**Purpose**
1	DprAG253R_F	GTGGTGGAAGGCCGGCGCAAGTCCG	G253R/SDM
2	DprAG253R_R	CGGACTTGCGCCGGCCTTCCACCAC	
3	DprAD277A_F	GGGCGGGCCGGCGCCCCCCGCGCGAGTG	D277A/SDM
4	DprAD277A_R	CACTCGCGCGGGGGGCGCCGGCCCGCCC	
5	DprAR137D_F	CATCGTGGGCACGGAGGCAGCGAGTCC	R137D/SDM
6	DprAR137D_R	GGACTCGCTGCCTCCGTGCCCACGATG	
7	DprAF229S_F	CGCAGCACCACTCCCCGAGCCGCAAC	F229S/SDM
8	DprAF229S_R	GTTGCGGCTCGGGGAGTGGTGCTGCG	
9	DprAR234D_F	CACTTCCCGAGCGACAACCGCGTCATC	R234D/SDM
10	DprAR234D_R	GATGACGCGGTTGTCGCTCGGGAAGTG	
11	DprAL293A_F	CGGGGCTGTCCGCACCGAGTCGG	L296R/SDM
12	DprAL293A_R	CCGACTCGGTGCGGACAGCCCCG	
13	DprAH284A_F	CGAGTGGCCCCGCCGCCCTGATTCG	H284A/SDM
14	DprAH284A_R	CGAATCAGGGCGGCGGGGCCACTCG	
15	DprApBAD XhoI_F	CCGCTCGAGGTGACCCTTCCCTCCCCT	*dprA* pBAD cloning
16	DprApBAD EcoRI_R	CGGAATTCTCAGCGACTCCAACGCCC	
17	Oligo167F	CTGCTTTATCAAGATAATTTTTCGACTC ATCAGAAATATCCGTTTCCTATATTTAT TCCTATTATGTTTTATTCATTTACTTATT CTTTATGTTCATTTTTTATATCCTTTACT TTATTTTCTCTGTTTATTCATTTACTTAT	EMSA

		TTTGTATTATCCTTATCTTATTTA	
18	Oligo167R	GACGAAATAGTTCTATTAAAAAGCTGA GTAGTCTTTATAGGCAAAGGATATAAA TAAGGATAATACAAAATAAGTAAATGA ATAAGAAATACAAGTAAAAAATATAGG AAATGAAATAAAAGAGACAAATAAG TAAATGAATAAAACATAATAGGAATA GAATAAAT	
19	Oligo40F	TAATACAAAATAAGTAAATGAATAAACAGAGAAAATAAAG	EMSA
20	Oligo40R	CTTTATTTTCTCTGTTTATTCATTTACTTATTTTGTATT	EMSA
21	BTDprA_F	GCGGATCCGATGACCCTTCCCTCCCCTG	*dprA* BTH cloning
22	BTDprA_R	GGGGTACCCGTCAGCGACTCCAACGCCC	