University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

US Army Research

U.S. Department of Defense

2013

Role of Nitrogen Limitation in Transformation of RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) by *Gordonia* sp. Strain KTR9

Karl J. Indest

U.S. Army Engineer Research and Development Center, Karl.J.Indest@usace.army.mil

Dawn E. Hancock

U.S. Army Engineer Research and Development Center

Carina M. Jung

U.S. Army Engineer Research and Development Center

Jed O. Eberly

U.S. Army Engineer Research and Development Center

William W. Mohn

University of British Columbia

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/usarmyresearch

Indest, Karl J.; Hancock, Dawn E.; Jung, Carina M.; Eberly, Jed O.; Mohn, William W.; Eltis, Lindsay D.; and Crocker, Fiona H., "Role of Nitrogen Limitation in Transformation of RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) by *Gordonia* sp. Strain KTR9" (2013). *US Army Research*. 245. https://digitalcommons.unl.edu/usarmyresearch/245

This Article is brought to you for free and open access by the U.S. Department of Defense at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in US Army Research by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors Karl J. Indest, Dawn E. Hancock, Carina M. Jung, Jed O. Eberly, William W. Mohn, Lindsay D. Eltis, and Fiona H. Crocker				



Role of Nitrogen Limitation in Transformation of RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) by *Gordonia* sp. Strain KTR9

Karl J. Indest,^a Dawn E. Hancock,^a Carina M. Jung,^a Jed O. Eberly,^a William W. Mohn,^b Lindsay D. Eltis,^b Fiona H. Crocker^a

U.S. Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, Mississippi, USA^a; Department of Microbiology & Immunology, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada^b

The transcriptome of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)-degrading strain *Gordonia* sp. strain KTR9 and its *glnR* mutant were studied as a function of nitrogen availability to further investigate the observed ammonium-mediated inhibition of RDX degradation. The results indicate that nitrogen availability is a major determinant of RDX degradation and *xplA* gene expression in KTR9.

ilitary training activities and the production of explosives such as RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) have resulted in the contamination of soils with these compounds at military facilities around the world. As a result, there are numerous examples of RDX migrating into groundwater at military manufacturing and testing/training sites (1–4). *In situ* bioremediation offers an attractive, cost-effective cleanup option for contaminated sites with limited access. Microbially mediated RDX biodegradation has been reported under a number of conditions (5–14), with only a few genes implicated in RDX degradation (15–17). Of these, the cytochrome P450 system encoded by *xplAB* is the best characterized (18, 19).

Previously, we isolated and characterized environmental *Actinomycetes* able to use RDX as a sole nitrogen source (14). RDX degradation by a *Gordonia* sp. strain KTR9 isolate was inhibited in the presence of competing inorganic nitrogen sources, and reduction in RDX degradation correlated to expression of *xplA* (20). Recently, a role for the global nitrogen regulator, GlnR, in RDX degradation has been suggested for KTR9 since *glnR* mutants of KTR9 are unable to grow on RDX as a sole nitrogen source (21). To further investigate nitrogen limitation in the catabolism of RDX, gene expression analyses of KTR9 and a *glnR* KTR9 deletion mutant were conducted under excess and limiting nitrogen growth conditions.

KTR9 was grown as described previously (20), and triplicate cultures in the late exponential phase of growth were used to inoculate media containing the following nitrogen-containing compounds: (i) 4 mM (NH₄)₂SO₄, (ii) 0.9 mM (NH₄)₂SO₄, (iii) 40 mg liter⁻¹ (180 μM) RDX, and (iv) 40 mg liter⁻¹ RDX plus 4 mM (NH₄)₂SO₄. RDX concentrations were determined by high-performance liquid chromatography (HPLC) analysis (14), and ammonium concentrations were verified using the AQUANAL-plus test kit for ammonium (Sigma, St. Louis, MO). Total RNA extraction, cDNA synthesis/labeling, and microarray experimentation were performed as described previously (22). Microarray hybridizations were carried out using custom Gordonia sp. strain KTR9 arrays (Roche, Madison, WI) developed from the annotated genome (23). The microarray data set is available from NCBI (www.ncbi.nlm.nih.gov/geo) under accession number GSE42342.

Transcript levels were compared across the different growth conditions using mid-exponential stage (36-h) samples of RDX-grown cultures and late-exponential stage (48-h) samples of am-

monium-grown cultures of KTR9 (Fig. 1). At these times, 10 mg liter $^-$ (45 μ M) and 25 mg liter $^{-1}$ (112 μ M) RDX remained in the medium for cultures grown in the presence of RDX and RDX-(NH₄)₂SO₄, respectively. In comparison, all of the ammonium had been consumed in the low-nitrogen growth condition [0.9] mM (NH₄)₂SO₄], but a significant amount of ammonium remained in the nitrogen-rich growth condition [4 mM (NH₄)₂SO₄]. Using the 4 mM (NH₄)₂SO₄ growth condition as a baseline for the transcriptome comparison, a 76% overlap was observed between the transcriptome of cells grown on 0.9 mM $(NH_4)_2SO_4$ and the transcriptome of cells grown on RDX (Fig. 2). A total of 100 genes involved in nitrogen transport, nitrogen assimilation, amino acid and nucleoside catabolism, transcription, and RDX degradation were common to both conditions (Table 1). A cluster of at least three hypothetical genes (KTR9_4925 to KTR9_4927) of unknown function, contiguous with the xplAB gene locus, were also upregulated. In contrast, the expression of these genes was significantly reduced in cells grown on RDX-4 $mM (NH_4)_2SO_4 (Table 1)$.

Additional transcriptome studies using KTR9 and a global nitrogen regulation (glnR) mutant of KTR9 were conducted with cultures grown under nitrogen-rich [4 mM (NH₄)₂SO₄] and nitrogen-limiting conditions [0.9 mM (NH₄)₂SO₄]. Cells for transcriptome analysis were harvested at 48 h, when nitrogen became depleted in the low-nitrogen growth condition (Fig. 3). Effects of the glnR mutation on the expression of genes revealed significant reductions in transcript levels under nitrogen-limiting conditions in the mutant compared to those of the wild-type strain (Table 2). The glnR mutant was significantly impaired in its ability to upregulate key genes involved in nitrogen transport and assimilation, consistent with the regulatory role of GlnR in response to nitrogen starvation (24–27). In addition, the magnitude of upregulation of xplAB and additional genes surrounding xplAB was

Received 17 December 2012 Accepted 20 December 2012

Published ahead of print 28 December 2012

Address correspondence to Karl J. Indest, Karl J. Indest@usace.army.mil.

Supplemental material for this article may be found at http://dx.doi.org/10.1128

/AFM 03905-12

Copyright © 2013, American Society for Microbiology. All Rights Reserved. doi:10.1128/AEM.03905-12

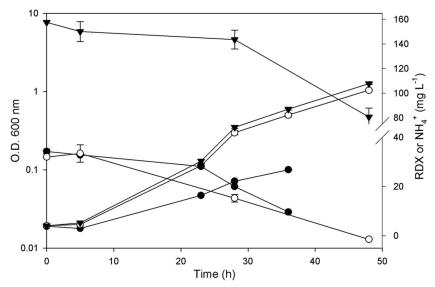


FIG 1 Growth and nitrogen utilization by *Gordonia* sp. KTR9 on 4 mM (NH₄)₂SO₄ (∇), 0.9 mM (NH₄)₂SO₄ (\bigcirc), and 180 μ M RDX (\blacksquare) as sole sources of nitrogen. Error bars represent the standard deviations from three replicates.

also reduced under nitrogen limitation. Conversely, the lack of GlnR *cis*-acting elements upstream of *xplAB* may suggest that GlnR does not directly regulate *xplAB* expression. Real-time PCR analyses as described previously (20) confirmed the relative changes in transcript levels for genes listed in Table 2.

Atrazine catabolic genes are induced under nitrogen-limiting conditions by a combination of global nitrogen regulators and a specific regulator of the catabolic genes (28–30). We identified a regulator located upstream of the *xplAB* gene cluster, designated *xplR*, which is transcriptionally upregulated in the presence of RDX and subject to nitrogen repression (20). We hypothesized that XplR may repress *xplAB* in the presence of preferred nitrogen sources such as (NH₄)₂SO₄. To test this hypothesis, a kanamycin resistance marker (Km^r) was inserted into the *xplR* coding region (KTR9_4921) (31). Wild-type and *xplR* mutant cells were resuspended in cold 0.25 mM phosphate buffer containing 25 mg liter⁻¹ RDX, and RDX levels were monitored over 24 h. No significant differences in degradation rates were observed between the

29 100 235 4 8 29

FIG 2 Analysis of gene induction patterns 2-fold or greater across the 0.9 mM (NH₄)₂SO₄ (dark gray), 180 μ M RDX (light gray), and 4 mM (NH₄)₂SO₄-180 μ M RDX (medium gray) transcriptomes using the 4 mM (NH₄)₂SO₄ growth condition as a baseline condition.

two strains, indicating that XplR does not play a role in RDX degradation.

The coordination of *xplAB* with the cell's response to nitrogen limitation may represent a partial adaptation to scavenging xenobiotic sources of nitrogen under nitrogen-limiting conditions. In lieu of evolving specific regulators that respond directly to the presence of exogenous xenobiotics, the bacterium has instead evolved regulators that respond to the more common environmental state of nitrogen limitation. Perhaps the current regulation of *xplAB* in strain KTR9 represents a transition state toward specific regulation of RDX degradation, as the evolution of new catabolic activities can precede their respective regulators (32). The observation that nitrogen limitation induces RDX degradation in some bacteria has important practical implications for *in situ* RDX bioremediation projects that rely on natural attenuation, bioaug-

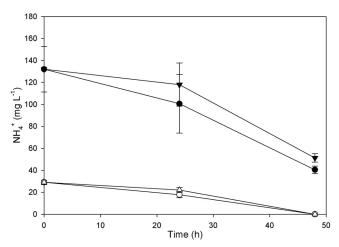


FIG 3 Nitrogen utilization by *Gordonia* sp. KTR9 (circles) and a *glnR* KTR9 mutant (triangles) grown under excess [4 mM (NH₄)₂SO₄; black] and nitrogen-limiting [0.9 mM (NH₄)₂SO₄; white] conditions. Error bars represent the standard deviations from three replicates.

TABLE 1 Genes induced in the presence of either 40 mg liter $^{-1}$ (180 μ M) RDX or 0.9 mM (NH₄)₂SO₄ a

			Fold change			
			0.9 mM		RDX + 4 mM	
		Cellular	ammonium	DDV	ammonium	D 1
Locus	Function	role	sulfate	RDX	sulfate	P value
KTR9_1218	Proline dehydrogenase	A	24	12	1.6	4.0E - 07
KTR9_1219	Putative delta-1-pyrroline-5-carboxylate dehydrogenase	A	25	12	1.4	1.4E - 06
KTR9_1224	Glutamine synthetase, type III	N	4.3	9.0	1.3	1.4E - 04
KTR9_1306	NAD(P)H-nitrite reductase	N	7.7	13	1.4	4.6E - 05
KTR9_1307	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	N	6.2	12	1.2	3.4E-05
KTR9_1309	Putative nitrate reductase/sulfite reductase	N	5.0	9.1	1.2	1.3E - 04
KTR9_2009	Ammonia permease	T	5.2	7.0	0.9	1.5E - 04
KTR9_2010	Nitrogen regulatory protein PII	R	3.0	4.3	0.9	7.0E - 04
KTR9_2091	Alanine dehydrogenase	A	15	5.1	1.4	1.4E - 03
KTR9_2634	Urea amidohydrolase (urease) gamma subunit	U	2.2	4.2	1.0	8.3E - 05
KTR9_2769	Branched-chain amino acid ABC-type transport system, permease components	T	3.2	2.9	2.1	4.6E-02
KTR9_2770	ABC-type branched-chain amino acid transport system, permease component	T	3.4	4.2	1.7	1.9E-04
KTR9_2772	ABC-type branched-chain amino acid transport systems, ATPase component	T	2.8	4.4	1.6	1.5E-03
KTR9_2773	ABC-type branched-chain amino acid transport systems, periplasmic component	T	6.5	8.1	2.3	2.4E-03
KTR9_2930	Ethanolamine ammonia-lyase, large subunit	A	2.1	3.1	1.3	2.7E-05
KTR9_2938	Glutamate synthase, NADH/NADPH, small subunit	N	2.6	2.4	0.5	1.4E-03
KTR9_3418	Branched-chain amino acid ABC-type transport system, permease	T	2.1	9.2	1.4	2.9E-03
KTR9_3419	components ABC-type branched-chain amino acid transport systems,	T	3.0	15	1.4	6.2E-05
WTD0 2425	periplasmic component	T	6.0	10	1.2	0.05 05
KTR9_3425	Formate/nitrite family of transporters	T	6.9	12	1.3	8.8E-05
KTR9_3426	Cyanate lyase	A	2.8	4.6	1.1	5.0E-04
KTR9_3533	Uroporphyrinogen III synthase	3.7	7.9	14	1.1	3.1E-05
KTR9_3534	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	N	21	30	1.1	2.8E-07
KTR9_3535	NAD(P)H-nitrite reductase	N	15	24	1.0	4.1E - 06
KTR9_3536	Nitrate/nitrite transporter	T	11	16	1.2	1.0E - 06
KTR9_3594	Xanthine/uracil permeases	T	2.3	2.2	1.3	4.0E - 03
KTR9_3723	Cytosine permease/uracil permease/thiamine permease/allantoin permease	T	4.5	2.9	1.4	2.9E-03
KTR9_3824	Allophanate hydrolase subunit 2	U	8.1	6.0	1.1	3.0E - 04
KTR9_3825	Urea carboxylase-associated protein 1	U	25	14	1.1	1.2E - 07
KTR9_3826	Urea carboxylase-associated protein 2	U	16	11	1.1	5.4E - 06
KTR9_3827	Permease, urea carboxylase system	T	12	8.7	1.2	1.0E-05
KTR9_4060	ABC-type nitrate/sulfonate/bicarbonate transport system, permease component	T	3.2	5.4	1.4	3.2E-02
KTR9_4061	ABC-type nitrate/sulfonate/bicarbonate transport systems, periplasmic components	T	3.4	5.7	1.4	4.4E-04
KTR9_4063	Putative creatinine amidohydrolase	A	2.2	4.4	1.1	1.7E-04
KTR9_4065	Cytosine deaminase and related metal-dependent hydrolases	A	2.1	2.9	1.2	1.1E-03
KTR9_4672	Ferredoxin-dependent glutamate synthase	N	7.7	2.5	1.2	5.1E-05
KTR9_4673	Glutamate synthase domain 3	N	12	4.6	1.3	3.0E-06
KTR9_4674	Glutamate synthase domain 1	N	9.2	3.3	1.1	4.9E-06
KTR9_4676	Ammonia permease	T	2.4	2.4	1.3	6.2E-03
KTR9_4922	Cytochrome P450	X	10	11	0.8	2.0E-05
KTR9_4923	GlnA-XplB fusion protein,glutamine synthetase, GlnA, flavodoxin reductase, XplB	X	10	10	0.5	6.1E-06
KTR9_4924	Flavodoxin-cytochrome P450 XplA	X	10	8.5	0.6	2.5E-05
KTR9_4925	Hypothetical protein	X	20	17	0.7	1.7E-08
KTR9_4925 KTR9_4926	Hypothetical protein	X	25	18	1.0	1.0E-05
KTR9_4920 KTR9_4927	Hypothetical protein	X	6.4	6.9	1.1	6.8E-06
	ransport (T), nitrogen assimilation (N), urea degradation (U), amino acid, nuclei					

^a Cellular roles: transport (T), nitrogen assimilation (N), urea degradation (U), amino acid, nucleoside, and other ammonia generating catabolic process (A), regulation (R), and RDX degradation (X). Fold change values were based on baseline comparisons, with transcriptome data from cells grown on 4 mM ammonium sulfate.

TABLE 2 Effects of a glnR mutation on the expression of genes induced by nitrogen limitation in wild-type KTR9^a

		Microarray fold change		Real-time PCR fold change	
Locus	Function	KTR9 wild type	KTR9 ΔglnR strain	KTR9 wild type	KTR9 $\Delta glnR$ strain
KTR9_0797 ^b	Permease, MFS superfamily	1.0	1.1	1.4	1.0
KTR9_1218	Proline dehydrogenase	31	120	4.6	171
KTR9_1219	Putative delta-1-pyrroline-5-carboxylate dehydrogenase	18	219	4.5	346
KTR9_1306	NAD(P)H-nitrite reductase	9.2	1.3	12	1.4
KTR9_1307	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	4.8	1.2	12	1.3
KTR9_1309	Putative nitrate reductase/sulfite reductase	8.2	1.7	7.9	1.2
KTR9_1685 b	DNA polymerase III, alpha subunit	1.1	1.2	2.4	1.1
KTR9_2010	Nitrogen regulatory protein PII	3.8	1.9	1.1	1.5
KTR9_3071	Glutamine synthetase, type I	2.3	0.2	0.2	0.1
KTR9_3533	Uroporphyrinogen III synthase	14	0.8	6.7	0.7
KTR9_3534	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	38	0.9	23	0.7
KTR9_3825	Urea carboxylase-associated protein 1	18	1.4	4.3	0.8
KTR9_3826	Urea carboxylase-associated protein 2	10	1.1	4.2	0.9
KTR9_3827	Permease, urea carboxylase system	12	1.4	1	1.4
KTR9_3954 ^b	Isochorismate synthase	0.9	0.9	1.8	0.5
KTR9_4922	Cytochrome P450	1	3.5	9.0	2.1
KTR9_4923	GlnA-XplB fusion protein	14	2.3	19	1.1
KTR9_4924	Flavodoxin-cytochrome P450 XplA	19	1.5	14	0.9
KTR9_4925	Hypothetical protein	36	1.0	7.8	0.6
KTR9_4926	Hypothetical protein	16	1.4	8.7	1.0

^a Fold change values were based on baseline comparisons with microarray and/or real-time PCR data from corresponding cells grown on 4 mM ammonium sulfate. A correlation of 0.76 was observed for gene expression values generated by microarray and real-time PCR.

mentation, or biostimulation. These approaches may be more suitable for sites with low inorganic background nitrogen levels, since aerobic RDX denitration may function more efficiently under these geochemical conditions.

ACKNOWLEDGMENTS

This research was funded in part by the U.S. Army Corps of Engineers Environmental Quality Research Program, the Strategic Environmental Research and Development Program (Project ER-1609), and Genome BC.

Views, opinions, and/or findings contained herein are those of the authors and should not be construed as an official Department of the Army position or decision unless so designated by other official documentation.

REFERENCES

- Beller HR, Tiemeier K. 2002. Use of liquid chromatography/tandem mass spectrometry to detect distinctive indicators of in situ RDX transformation in contaminated groundwater. Environ. Sci. Technol. 36:2060– 2066.
- Bordeleau G, Martel R, Schafer D, Ampleman G, Thiboutot S. 2007. Groundwater flow and contaminant transport modelling at an air weapons range. Environ. Geol. 55:385

 –396.
- Clausen J, Robb J, Curry D, Korte N. 2004. A case study of contaminants on military ranges: Camp Edwards, Massachusetts, U.S.A. Environ. Pollut. 129:13–21.
- 4. Wani AH, Neal O BR, Davis JL, Hansen LD. 2002. Treatability study for biologically active zone enhancement (BAZE) for in situ RDX degradation in groundwater. ERDC/EL TR-02-35. U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS.
- 5. Adrian NR, Arnett CM. 2004. Anaerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Acetobacterium malicum* strain HAAP-1 isolated from a methanogenic mixed culture. Curr. Microbiol. 48:332–340.
- Adrian NR, Arnett CM, Hickey RF. 2003. Stimulating the anaerobic biodegradation of explosives by the addition of hydrogen or electron donors that produce hydrogen. Water Res. 37:3499–3507.

- 7. **Beller HR.** 2002. Anaerobic biotransformation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by aquifer bacteria using hydrogen as the sole electron donor. Water Res. 36:2533–2540.
- Boopathy R, Gurgas M, Ullian J, Manning JF. 1998. Metabolism of explosive compounds by sulfate-reducing bacteria. Curr. Microbiol. 37: 127–131.
- Bradley PM, Dinicola RS. 2005. RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) biodegradation in aquifer sediments under manganese-reducing conditions. Biorem. J. 9:1–8.
- Coleman NV, Nelson DR, Duxbury T. 1998. Aerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) as a nitrogen source by a *Rhodococcus* sp. strain DN22. Soil. Biol. Biochem. 30:1159–1167.
- 11. Freedman DL, Sutherland KW. 1998. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) under nitrate-reducing conditions. Water Sci. Technol. 38:33–40.
- Hawari J, Halasz A, Sheremata T, Beaudet S, Groom C, Paquet L, Rhofir C, Ampleman G, Thiboutot S. 2000. Characterization of metabolites during biodegradation of hexahydro-1, 3,5-trinitro-1,3,5-triazine (RDX) with municipal anaerobic sludge. Appl. Environ. Microbiol. 66: 2652–2657.
- Sherburne LA, Shrout JD, Alvarez PJ. 2005. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) degradation by *Acetobacterium paludosum*. Biodegradation 16:539–547.
- Thompson KT, Crocker FH, Fredrickson HL. 2005. Mineralization of the cyclic nitramine explosive hexahydro-1,3,5-trinitro-1,3,5-triazine by Gordonia and Williamsia spp. Appl. Environ. Microbiol. 71:8265–8272.
- Bryant C, DeLuca M. 1991. Purification and characterization of an oxygen-insensitive NAD(P)H nitroreductase from *Enterobacter cloacae*. J. Biol. Chem. 266:4119–4125.
- Fuller ME, McClay K, Hawari J, Paquet L, Malone TE, Fox BG, Steffan RJ. 2009. Transformation of RDX and other energetic compounds by xenobiotic reductases XenA and XenB. Appl. Microbiol. Biotechnol. 84: 535–544.
- Seth-Smith HM, Rosser SJ, Basran A, Travis ER, Dabbs ER, Nicklin S, Bruce NC. 2002. Cloning, sequencing, and characterization of the hexahydro-1,3,5-trinitro-1,3,5-triazine degradation gene cluster from *Rhodo-coccus rhodochrous*. Appl. Environ. Microbiol. 68:4764–4771.
- Jackson RG, Rylott EL, Fournier D, Hawari J, Bruce NC. 2007. Exploring the biochemical properties and remediation applications of the un-

^b Housekeeping genes shown not to be regulated in response to RDX or high- or low-nitrogen growth conditions.

- usual explosive-degrading P450 system XplA/B. Proc. Natl. Acad. Sci. U. S. A. 104:16822–16827.
- Sabbadin F, Jackson R, Haider K, Tampi G, Turkenburg JP, Hart S, Bruce NC, Grogan G. 2009. The 1.5-A structure of XplA-heme, an unusual cytochrome P450 heme domain that catalyzes reductive biotransformation of royal demolition explosive. J. Biol. Chem. 284:28467–28475.
- Indest KJ, Jung CM, Chen HP, Hancock D, Florizone C, Eltis LD, Crocker FH. 2010. Functional characterization of pGKT2, a 182-kilobase plasmid containing the *xplAB* genes, which are involved in the degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine by *Gordonia* sp. strain KTR9. Appl. Environ. Microbiol. 76:6329–6337.
- Zhu S, Reuther J, Eltis LD, Mohn WW, Crocker FH. 2010. Gene expression during RDX degradation by *Gordonia* sp. KTR9. Partners in environmental technology technical symposium and workshop: meeting DoD's environmental challenges. SERDP and ESTCP, Arlington, VA. http://symposium2010.serdp-estcp.org/content/download/8863/107188/version/3/file/SymposiumProgramGuide_2010-Rev.pdf.
- 22. Hancock DL, Indest KJ, Gust KA, Kennedy A. 2012. The effects of C_{60} on the *Salmonella typhimurium* TA100 transcriptome expression: insights into C60 mediated growth inhibition and mutagenicity. Environ. Toxicol. Chem. 31:1438–1444.
- Chen HP, Zhu SH, Casabon I, Hallam SJ, Crocker FH, Mohn WW, Indest KJ, Eltis LD. 2012. Genomic and transcriptomic studies of an RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)-degrading actinobacterium. Appl. Environ. Microbiol. 78:7798–7800.
- 24. Amon J, Brau T, Grimrath A, Hanssler E, Hasselt K, Holler M, Jessberger N, Ott L, Szokol J, Titgemeyer F, Burkovski A. 2008. Nitrogen control in *Mycobacterium smegmatis*: nitrogen-dependent expression of ammonium transport and assimilation proteins depends on the OmpR-type regulator GlnR. J. Bacteriol. 190:7108–7116.
- 25. Beckers G, Strosser J, Hildebrandt U, Kalinowski J, Farwick M, Kramer

- R, Burkovski A. 2005. Regulation of AmtR-controlled gene expression in *Corynebacterium glutamicum*: mechanism and characterization of the AmtR regulon. Mol. Microbiol. 58:580–595.
- Pullan ST, Chandra G, Bibb MJ, Merrick M. 2011. Genome-wide analysis of the role of GlnR in *Streptomyces venezuelae* provides new insights into global nitrogen regulation in actinomycetes. BMC Genomics 12:175–189.
- Tiffert Y, Supra P, Wurm R, Wohlleben W, Wagner R, Reuther J. 2008.
 The Streptomyces coelicolor GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. Mol. Microbiol. 67:861–880.
- Garcia-Gonzalez V, Govantes F, Porrua O, Santero E. 2005. Regulation of the *Pseudomonas* sp. strain ADP cyanuric acid degradation operon. J. Bacteriol. 187:155–167.
- Garcia-Gonzalez V, Govantes F, Shaw LJ, Burns RG, Santero E. 2003. Nitrogen control of atrazine utilization in *Pseudomonas* sp. strain ADP. Appl. Environ. Microbiol. 69:6987–6993.
- Garcia-Gonzalez V, Jimenez-Fernandez A, Hervas AB, Canosa I, Santero E, Govantes F. 2009. Distinct roles for NtrC and GlnK in nitrogen regulation of the *Pseudomonas* sp. strain ADP cyanuric acid utilization operon. FEMS Microbiol. Lett. 300:222–229.
- 31. van der Geize R, Hessels GI, van Gerwen R, van der Meijden P, Dijkhuizen L. 2001. Unmarked gene deletion mutagenesis of kstD, encoding 3-ketosteroid D¹-dehydrogenase, in Rhodococcus erythropolis SQ1 using sacB as a counter-selectable marker. FEMS Microbiol. Lett. 205:197–202.
- 32. de Las Heras A, Chavarría M, de Lorenzo V. 2011. Association of *dnt* genes of *Burkholderia* sp. DNT with the substrate-blind regulator DntR draws the evolutionary itinerary of 2,4-dinitrotoluene biodegradation. Mol. Micrbiol. 82:287–299.

Table S1. Bacterial strains, constructs and primers used in study.

of xplR (KTR9_4921) Gordonia sp. KTR9/AglnR KTR9 strain with glnR (KTR9_3806) deletion Escherichia coli Top 10 Invitrogen #C4040 Plasmid constructs pKTR9-4921:kan 1614 bp fragment containing insertion of 929 bp kan gene (gb AAB63351.1) into 685 bp KTR9_4921 (xplR) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_07977 CCTGCCACTCCGGGATT This study KTR9_1218f AACCGGCGGACCACCTT KTR9_1218f CGCAGCGACGACGGATCAG This study KTR9_1219f ACACCGATCGTGCGATGAG This study KTR9_1219r CCTTCGACTCCCGGATC This study KTR9_1306f CATCTCGAAGGACCAGAG KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1307f GGCCCCATGGAGGACCACTT This study KTR9_1309r CGAACCGCGATCTGGAG KTR9_1309r CGATCCGTGTATCGAGTTCG This study KTR9_1309r CGATCCGTGTATCGAGTTCC This study KTR9_1309r CGATCGTGTATCGAGGATCAG This study KTR9_1309r CGATCGTGTATCGAGGATCAG This study KTR9_1685f CCGAGCTGTCGCCATTTCC This study KTR9_1685f CCAAGGTCTGCGGTGAGAA This study KTR9_2010r CTCGCAATTTCCGATTCC This study KTR9_2010r CTCGCAATTTCCGATTCCC This study KTR9_3533f CCAAGGTCTGCCCTTCTTC This study KTR9_3533r CCAGGCGAACTACACTACAA This study KTR9_3533r CCAGGCGAACTCCGTTCTTC This study KTR9_3533r CCAGGCGAACTCCGTTCTCT This study KTR9_3533r CCAGGCGAACTCCGTTCTCT This study KTR9_3533r CCAGGCCGATCTCCTTCTG This study KTR9_3533r CCAGGCCGATCTCCTCTTCT This study KTR9_3533r CCAGGCCGATCCGTTCTCT This study KTR9_3533r CCAGGCCGATCCGTTCTCT This study KTR9_3533r CCAGGCCAGTCGGTAT This study KTR9_3533r CCAGGCCAGTCCGTACTCC This study KTR9_3533r CGCGCCCTGGGTGAT This study KTR9_3534r CGCGGCAGTCGGAACACACAC This study KTR9_3825r TGCGGGGAACACGCACTCCGTAACC This study KTR9_3825r TGCGGGGCAACCACCCGGAACC This study KTR9_3827f TGGGTGAACAGTCCAAGACC This study KTR9_3954f CCCGCAGCCGCTCCCAACCC This study KTR9_3954f TGGCTGAACCACCCC This study KTR9_3954f	Bacterial strains, constructs, and primers	Catalog number, sequence, or description	Reference
Gordonia sp. KTR9/xplR:kan KTR9 strain with kan insertional inactivation of xplR (KTR9_4921) Gordonia sp. KTR9/AglnR KTR9 strain with glnR (KTR9_3806) (48) Escherichia coli Top 10 Invitrogen #C4040 Plasmid constructs pKTR9-4921:kan 1614 bp fragment containing insertion of 929 bp kan gene (gb AAB63351.1) into 685 bp KTR9_4921 (xpl) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_07971 ACCGCGATGATGGTCATGA This study KTR9_1218f AACCGGCGGACCACTCT This study KTR9_1218f CGCAGCGAACGGATCAG This study KTR9_1219f ACACCGGTGGGATGAG This study KTR9_1219f ACACCGATCACCAGGACAGAA This study KTR9_1306f CATCCGAGTACCAGGACAGAA This study KTR9_1306f CATCCGAGGACGACTCCT This study KTR9_1300f CGGAGCAACGCACCTCT This study KTR9_1307f GGCCCCATGGAGACGAACTGA This study KTR9_1307f CGGAACACCGCATCTCT This study KTR9_1309f CGATCCGTGTATCGAGTTTCC This study KTR9_1309f CGATCCGTGTATCGAGTTTCC This study KTR9_1309f CGATCCGTGTATCGAGTTTCC This study KTR9_1685f TCGCCAATTTCGGTTTTCC This study KTR9_1685f CCAAGGTCATCAGAACAGAA This study KTR9_1685f CCAAGGTCTGAGTAGAACTCGAGTTTCC This study KTR9_1685f CCAAGGTCTGAGAACTACAA This study KTR9_2010f TGAAGCTGATCACTGCAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAGTAGAA This study KTR9_2010f CCAGGCGGAGTCACACTCCTTCTG This study KTR9_3533f TCGACGGCCATTCTCT This study KTR9_3533f TCGACGGCAACTCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCAGTCGGTAACT This study KTR9_3534f ACGACACGCAGCGGTATCG This study KTR9_3534f ACGACACGCAGCGGTATCG This study KTR9_3825f TGCCGGTGGGCAACC This study KTR9_3825f TGCCGGTGGGCAACC This study KTR9_3826f TGACACGCCGCGCAACC This study KTR9_3827f TGGGTGAACAGTCCAGACT This study KTR9_3827f TGGGTGAACAGTCCCGTAACCT This study KTR9_3827f TGGGTGAACCACTCCGTAACCT This study KTR9_3827f TGGGTGAACCACTCCGTAACCT This study KTR9_3827f TGGGTGAACCACTCCGTAACCT This study KTR9_3827f TGGGTGAACACTCCGGAACCT This study KTR9_3824f TCGACGCCGCGCAACCT This	<u>Strains</u>		
of xplR (KTR9_4921) Gordonia sp. KTR9/AglnR KTR9 strain with glnR (KTR9_3806) deletion Escherichia coli Top 10 Invitrogen #C4040 Plasmid constructs pKTR9-4921:kan 1614 bp fragment containing insertion of 929 bp kan gene (gb AAB63351.1) into 685 bp KTR9_4921 (xplR) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_07977 CCTGCCACTCCGGATT This study KTR9_1218f AACCGGCGACCACCTT KTR9_1218f ACCGCGGACCACCTT This study KTR9_1219r CCTTCGACTGCGATTAG KTR9_1219r CCTTCGACTGCGATGAG This study KTR9_1306f CATCTCGAAGGACAGGAAC KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1307f GGCCCCATGGCAGACTCTC KTR9_1307f CGGAACACCGCGATCTGT KTR9_1309r CGATCCGTGTATCGAGGATCTG KTR9_1309r CGATCCGTGTATCGAGTTCC KTR9_1309r CGATCCGTGTATCGAGTTCC KTR9_1309r CATTGAATTCCGAGGATCAG This study KTR9_1685f CCAAGGTCTGCGCATTTCC This study KTR9_1685f CCAAGGTCTGCGGTGAGTAGA This study KTR9_1685f CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTTCC This study KTR9_2010r CTCGGCAATTTCGGATTCC This study KTR9_3533f CCAAGGCGAACTACACAA This study KTR9_3533f CCAGGCGAACTCGCGTTCTT This study KTR9_3533f CCAGGCGAACTCGGTTTCC This study KTR9_3533f TCGACGGCCAGTCTCTT This study KTR9_3533f TCGACGGCCAGTCTCTT This study KTR9_3533f TCGACGGCCAGTCGTTATCCGATTGTC This study KTR9_3533f TCGACGGCCAGTCGTTCTT This study KTR9_3533f TCGACGGCCAGTCGTTCTT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3534f CGCGCCCTGGGGAAACACTCCT This study KTR9_3534f CGCGCCGTGGGGAACACACTC This study KTR9_3534f CGCGCAGTCGGAACACACAC This study KTR9_3825f TGCCGGAACACGCACTCCGTAACT This study KTR9_3827f TGGGTGAACAGTCCAGACGAT This study KTR9_3954f GCCGCAGTCTCCAACCTC This study KTR9_3954f TGGATGACCGCCGTCCAA This study	Gordonia sp. KTR9		(43)
Real-time PCR Primers (5' to 3') KTR9_4921:kan	Gordonia sp. KTR9/xplR:kan		This study
Plasmid constructs pKTR9-4921:kam 1614 bp fragment containing insertion of 929 bp kan gene (gb AAB63351.1) into 685 bp KTR9_4921 (xplR) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_0797f ACCGCGATGATGGTCATGA KTR9_0797f CCTGCCACTCCGCGATT This study KTR9_1218f AACCGGCGACCACCTT This study KTR9_1219f ACACCGATCGTGCGATGATG KTR9_1219r CCTTCCACATCGCGGATGATG KTR9_1306f CATCTCGAAGGCCAACTTC This study KTR9_1307f GGCCCCATGTGCGATGTAG KTR9_1307f GGCCCCATGTGCGAT KTR9_1307f GGCCCCATGTCCGCATTT This study KTR9_1309f CGATCCGTGTATCGAG KTR9_1309f CGATCCGTGTATCGAG KTR9_1309f CGATCCGTGTATCGAGTTTGC This study KTR9_1685f TCGCCAATTTCGAGTTTCC This study KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_3071f CCAGGCGGAGATCAACTACAA This study KTR9_3071f CCAGGCGGAGATCACTCTT This study KTR9_3533f TCGACGCACTTCCCTTCT This study KTR9_3533f TCGACGCACTTCCCTTCT This study KTR9_3533f TCGACGCACTTCCCTTCT This study KTR9_3533f TCGACGCCACTTCCCTTCT This study KTR9_3533f TCGACGCCAGTCTCTCT This study KTR9_3533f TCGACGCCAGTCTCCT This study KTR9_3533f TCGACGCCAGTCTCTCT This study KTR9_3533f TCGACGCCAGTCTCCCTTCT This study KTR9_3533f TCGACGCCAGTCTCCCTTCT This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f ACGACACCCAGTCTCCTTCT This study KTR9_3534f ACGACACCCAGTCTCCGTAACTC This study KTR9_3534f ACGACACCCAGTCTCCGTAACTC This study KTR9_3534f ACGACACCCAGTCCGTAACTC This study KTR9_3534f ACGACACCCAGTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCCGTAACTC This study KTR9_3825f TGCCGGTGGTCAGAACA This study KTR9_3825f TGCCGGTGGTCAGAACA This study KTR9_3825f TGCCGGTGGTCAGAACA This study KTR9_3825f TGCCGGTGGTCAGAACA This study KTR9_3825f TGCCGGTGGTCAA This study KTR9_3825f TGCGGTGGTCAACACTCC This study KTR9_3825f TGCACGTCCCGAACCC This study KTR9_3825f TGCACGCCCCATCCCCAACCCT This study TTR9_3825f	Gordonia sp. KTR9/∆glnR	g .	(48)
pKTR9-4921:kan 1614 bp fragment containing insertion of 929 bp kan gene (gb AAB63351.1) into 685 bp KTR9-4921 (xplR) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_0797f ACCGCGATGATGGTCATGA KTR9_0797r CCTGCACTCCGCGATT This study KTR9_1218f AACCGGCGGACCACCTT KTR9_1218f AACCGGCGGACGACGGATCAG KTR9_1219f ACACCGATGCGGATGTAG KTR9_1219r ACACCGATGCGAACGGATCAG This study KTR9_1306f CATCTCGAGTACCAGGGACAGA KTR9_1306f CATCTCGAGTACCAGGGACAGA KTR9_1300f CGGTGTCCCCATGTCGA KTR9_1300r CGGAACACCGCGATCTG This study KTR9_1300r CGATCCGTGTATCGAGTTTGC This study KTR9_1300r CGATCCGTGTATCCGAGTTTGC This study KTR9_1300r CGATCCGTGTATCGAGTTTGC This study KTR9_1685f TCGCCAATTTCCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGAAGA This study KTR9_1685r CCAAGGTCTGCGGTGAGAAGA This study KTR9_2010r CTCGGTGTGGCCCTTCTG This study KTR9_3071r TGAGCTGATCACTGCAATTGTC This study KTR9_3071r TGAGCGGAACACCGCTTCTG This study KTR9_3071r TGAGCGCAGTTCCGTTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study MTR9_3534f ACGACACGCACTCCGTAACTC This study MTR9_3534f ACGACACGCACTCCGTAACTC This study MTR9_3534f ACGACACGCACTCCGTAACTC This study MTR9_3534f ACGACACGCACTCCGTAACTC This study MTR9_3825f TGCCGGTGGTCAGAACACTC This study MTR9_3826f TGCACGCCGATCCCGAAACC This study MTR9_3826f TGCACGCCCATCCCGCAACC This study MTR9_3827f TGGGTGAACACTCCCAACCC This study MTR9_3827f TGGGTGAACACTCCCAACCC This study MTR9_3824f TGGATGACCCCCGCACCCCCAACCC This study MTR9_3824f TGGATGACCCCCGCACCCCCAACCCC This study MTR9_3824f TGGATGACCCCCACTCCCCCAACCCC This study MTR9_3824f TGGATGACCCCCCATCCCCCAACCCC This study MTR9_3824f TGGATGACCCCCCGTCAACCCC This study MTR9_3824f TGGATGACCCCCCCTCAACCCCC This s	Escherichia coli Top 10	Invitrogen #C4040	
bp kan gene (gb AAB6335 I.1) into 685 bp KTR9_4921 (xp/R) near midpoint of gene cloned into BamH1 site of pK18mobsacB Real-time PCR Primers (5' to 3') KTR9_0797f: ACCGCGATGATGGTCATGA This study KTR9_0797r CCTGCCACTCCGCGATT This study KTR9_1218f AACCGGCGGACCACCTT This study KTR9_1218r CGCAGCGAACGGATCAG This study KTR9_1219f ACACCGATGTGCGGATGAG This study KTR9_1219r CCTTCGAGTACCAGGGACAGAA This study KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1300f CGGTGTCCCCATGTCGAA This study KTR9_1307f GGCCCCATGAGGGATCTG This study KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309f CGATCCGTGTACGAGTTTGC This study KTR9_1309f CGATCCGTGTACGAGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCCGTGTAGA This study KTR9_1685f CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGACTGCAATTGTC This study KTR9_2010f TGAAGCTGACGCACTTCTG This study KTR9_3071f CCAGGCGGATCACAA This study KTR9_3071f CCAGGCGGATCACAA This study KTR9_3071f TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCACTCGTAACTC This study KTR9_3533f TCGACGGCCAGTCGGTAAT This study KTR9_3533f TCGACGGCCAGTCGGTAAT This study KTR9_3533f GCCGCCCTGGGTGAT This study KTR9_3533f TCGACGGCCAGTCGGTAACTC This study KTR9_3533f GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f ACGACACGACTCCGTAACTC This study KTR9_3534f ACGACACGACACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACGCACTCCGTAACTC This study KTR9_3534f ACGACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACGCACTCCGGAACC This study KTR9_3825f TGCCGGTGGTCAGGAAC This study KTR9_3825f TGCCGGTGGTCAGGAAC This study KTR9_3826f CGGACTCCGCGAACC This study KTR9_3827f TGGGTGAGCCGCGCGTCAACT This study KTR9_3954f GCCGCAGTCTCCGACCT This study KTR9_3954f GCCGCAGTCTCCGACCT This study	Plasmid constructs		
KTR9_0797f	pKTR9-4921: <i>kan</i>	bp <i>kan</i> gene (gb AAB63351.1) into 685 bp KTR9_4921 (<i>xplR</i>) near midpoint of gene	This study
KTR9_0797f	Real-time PCR Primers (5' to 3')		
KTR9_0797r		ACCGCGATGATGGTCATGA	This study
KTR9_1218f AACCGGCGGACCACCTT This study KTR9_1218r CGCAGCGAACGGATCAG This study KTR9_1219r ACACCGATCGTGCGATGTAG This study KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1306r CGGTGTCCCCATGTCGAA This study KTR9_1307r CGGAACACCGGATCTG This study KTR9_1309r CATTCGAATCCAGGATCTGC This study KTR9_1309r CATTGAATTCCGAGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATCAG This study KTR9_1685f TCGCCAATTTCGT This study KTR9_2010f TGAAGCTGGTAGAA This study KTR9_2010r CTCGGTGTACCAGATTGC This study KTR9_3071f CCAGGCGATCTGC This study KTR9_3071r TGACCGATTCGCATTGC This study KTR9_3071r TGACCGACTTCTG This study KTR9_3533f TCGACGGCATCTGT This study KTR9_3533f TCGACGGCAGTCAA This study KTR9_3533r GCCGCGCTGGTAT This study KTR9_3534f ACGACACCACTCGTAACTC This study KTR9_3534f ACGACACCACTCACTC This study KTR9_3534f ACGACACCACTCACTC This study KTR9_3825f TGCCGGTGGTCAGTACC This study KTR9_3825f TGCCGGTGGTCAGGAACCT This study KTR9_3825r TGCACGTCCAGACACCT This study KTR9_3825r TGCACGTCCAGACACC This study KTR9_3826r TCGACTCCCGGAACC This study KTR9_3827r TGGATGACCGCTCCAACCT This study KTR9_3827r TGGATGACCCCTTCCCCCGAACCT This study KTR9_3827r TGGATGACCCCTTCCCCCGAACCT This study KTR9_3827r TGGATGACCCCTTCCCCCTCCCCCCCCCCCCCCCCCCCC			•
KTR9_1218r			•
KTR9_1219f ACACCGATCGTGCGATGTAG This study KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1306r CGGTGTCCCCATGTCGAA This study KTR9_1307f GGCCCCATGGAGGACTTGG This study KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309f CGATCCGTGTATCGAGTTTGC This study KTR9_1309r CATTGAATTCGAGTTTGC This study KTR9_1685f TCGCCAATTTCGT This study KTR9_2010f TGAAGCTGCGGTAGTAGA This study KTR9_2010r CTCGGTGTACCAATTGTC This study KTR9_3071r CGGAACACTGCTTTCC This study KTR9_3071r TGACGGATCACTGT This study KTR9_3533f TCGCCGATTTCG This study KTR9_3533f TCGACGCTGTTCTG This study KTR9_3533f TCGACGCCTTCTG This study KTR9_3533f TCGACGCCATTGCAATTGTC This study KTR9_3533f TCGACGCCATTCTG This study KTR9_3533f TCGACGCCATTCTG This study KTR9_3533f TCGACGCCAGTCGTAT This study KTR9_3534f ACGACACCACTCCGTAACTC This study KTR9_3534f TCGACGCACTCCGTAACTC This study KTR9_3825f TGCCGGTGAGAACACTACTA This study KTR9_3825f TGCCGGTGAGAACACTACCA This study KTR9_3825f TGCCGGTGAACACTACCA This study KTR9_3825f TGCCGGTCAGAACACTACCA This study KTR9_3825f TGCCGGTCAGAACACACCACTCCGTAACCT This study KTR9_3826f CGGACTCCCGAAACC This study KTR9_3826f CGGACTCCCGAAACC This study KTR9_3827f TGGATGACCCGCTCAA This study KTR9_3827f TGGATGACCCGCTCAA This study KTR9_3827r TGGATGACCCGCTCAACCT This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_1219r CCTTCGAGTACCAGGGACAGAA This study KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1306r CGGTGTCCCCATGTCGAA This study KTR9_1307f GGCCCCATGGAGGATCTG This study KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309f CGATCCGTGTATCGAGGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_2010r CTCGGTGTGCCCTTCTG This study KTR9_3071f CCAGGCGAGATCACTACAA This study KTR9_3071r TGACCGACTTCTG This study KTR9_3533f TCGACGCCATTCTG This study KTR9_3533r GCCGCCTTCTG This study KTR9_3533r GCCGCCTGGGTAT This study KTR9_35334 ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGACT This study KTR9_3825f TGCCGTGGTCAGAAGA This study KTR9_3825f TGCCGTGTGTCAGAAGA This study KTR9_3825f TGCCGTCCGAACC This study KTR9_3825r GACACGTCCCGAATCC This study KTR9_3826f CGGACTCCCCGAACC This study KTR9_3826f CGGACTCCCCGAACC This study KTR9_3826f CGGACTCCCCGAACC This study KTR9_3826f CGGACTCCCCGAACC This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3827r TGGATGACCCCCGTAACT This study KTR9_3827r TGGATGACCCCCTCCAACCT This study KTR9_3827r TGGATGACCCCCTCCAACCT This study KTR9_3827r TGGATGACCCCCCGTAACCT This study KTR9_3827r TGGATGACCCCCCTCCCTTCAACCT This study KTR9_3827r TGGATGACCCCCCCTCCCTTCAACCT This study KTR9_3827r TGGATGACCCCCCCTCCCTTCAACCT This study KTR9_3827r TGGATGACCCCCCTTCCCCCCCCCCCCCCCCCCCCCCCC			•
KTR9_1306f CATCTCGAAGGCCAACTTCCT This study KTR9_1306r CGGTGTCCCCATGTCGAA This study KTR9_1307f GGCCCCATGGAGGATCTG This study KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309f CGATCCGTGTATCGAGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_2010r CTCGGTGTGCCCTTCTG This study KTR9_3071f CCAGGCGAGATCACTACAA This study KTR9_3071r TGACCGACTTGCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGTAT This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_35334f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825f TGCCGGTGCCGGAAGCA This study KTR9_3826f CGGACTCCCGAACCG This study KTR9_3826f TGCACGTCCGGAACC This study KTR9_3826f TGGACTCCCGGAACC This study KTR9_3827f TGGATGACCGCGTCAA This study KTR9_3827f TGGATGACCGCGTCAA This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_1306r KTR9_1307r GGCCCCATGGAGGATCTG This study KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309r CATTGAATTCCGAGGATGCA KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGAA KTR9_2010f KTR9_2010r CTCGGTGTGCCCTTCTG This study KTR9_3071f CCAGGCGGAGATCAA This study KTR9_3071r TGACCGACTTGCGGTCAA This study KTR9_3533f TCGACGGCCAGTCTGT This study KTR9_3533r CCAGGCCGGTGAT This study KTR9_3533r CCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGAA This study KTR9_3534r CGCTGGCAAGAACT This study KTR9_3825f TGCCGGTTGCCAACTC This study KTR9_3825r GACACGCCAGTCAGAAA This study KTR9_3826r TCGACGCCGAATCA This study KTR9_3826r TCGACTCCCGAATC This study KTR9_3826r TCGACTCCCGGAAC This study KTR9_3827r TGGATGACCCGCTCAA This study KTR9_3827r TGGATGACCCCGCTCAA This study KTR9_3827r TGGATGACCCCGCTCAA This study			-
KTR9_1307f			•
KTR9_1307r CGGAACACCGCGATCTGT This study KTR9_1309f CGATCGTGTATCGAGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_2010r CTCGGTGTGCCCTTCTG This study KTR9_3071f CCAGGCGGAGATCAACTACAA This study KTR9_3071r TGACCGACTTGCGGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCCTGGGTAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGACTC This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825f GACACGTCCAGAAGA This study KTR9_3826f CGGACTCCGCGAATCC This study KTR9_3826f CGGACTCCCGAACCC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827r TGGATGACCAGCACTCCAGACGAT This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3827r TGGATGACCCCGTAACCT This study KTR9_3827r TGGATGACCCCCGTAACCT This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_1309f CGATCCGTGTATCGAGTTTGC This study KTR9_1309r CATTGAATTCCGAGGATGCA This study KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_2010r CTCGGTGTGCCCTTCTG This study KTR9_3071f CCAGGCGAGATCAACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534r ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGTCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCCGGAATCC This study KTR9_3826r TCGACTCCCGAATCC This study KTR9_3826r TCGACTCCCGAATCC This study KTR9_3826r TCGACTCCCGAATCC This study KTR9_3827r TGGATGACCAGCACTCCAGACGAT This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3827r TGGATGACCCCGTCAACCT This study KTR9_3827r TGGATGACCCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_1309r KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA KTR9_2010f KTR9_2010r KTR9_3071f CCAGGCGGAGATCACTACAA KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCTCTG This study KTR9_3533r GCCGCGCTGGGTAT KTR9_3534f ACGACACGCACTCCGTAACTC KTR9_3534r CGCTGGGCAAGACTC KTR9_3825f TGCCGGTGGTCAGAACT TGCCGGTGTGCCATTCT This study KTR9_3825r CGCACGCCCGAATCC This study CTR9_3826r TCGACCGCCGAAAC This study CTR9_3827r TGGATGACACACACACA This study			•
KTR9_1685f TCGCCAATTTCGGTTTCC This study KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010f TGAAGCTGATCACTGCAATTGTC This study KTR9_3071f CCAGGCGGAGACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCACTC This study KTR9_3534r CGCTGGGCAAGCAGACTC This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCCGCGAATGC This study KTR9_3826r TCGACTCTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827r TGGATGACCAGGTCAA This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3827r TGGATGACCCCGAACCT This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_1685r CCAAGGTCTGCGGTGAGTAGA This study KTR9_2010r TGAAGCTGTGCGTTGCGAATTGTC This study KTR9_3071f CCAGGCGGAGATCACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGTCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGTCT This study KTR9_3825f TGCCGGTGGTCAGAAGA This study KTR9_3825r GACACGTCCAGAAGA This study KTR9_3825r GACACGTCCAGACGTATTCG This study KTR9_3826f CGGACTCGCGAATCC This study KTR9_3826r TCGACTCTCGCCGAAAC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827r TGGATGACCAGCGTCAA This study KTR9_3827r TGGATGACCCGCGAACCT This study KTR9_3954f GCCGCAGTCTCCGAACCT			•
KTR9_2010f KTR9_2010r CTCGGTGTGGCCCTTCTG This study KTR9_3071f CCAGGCGAGATCACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATT This study KTR9_3826f CGGACTCCGCGAATCC This study KTR9_3826f TCGACTCTCGCCGAATCC This study TGGACTCTCGCCGGAAAC This study TTR9_3826r TCGACTCTCGCCGGAAAC This study TTR9_3827r TGGATGACCGCTCAA This study TTR9_3827r TGGATGACCGCGTCAA This study TTR9_3827r TGGATGACCGCGTCAA This study TTR9_3827r TGGATGACCCGCGTCAA This study TTRS_3827r TGGATGACCCGCGTCAA This study TTRS_3827r TGGATGACCCGCGTCAA This study			-
KTR9_2010r CTCGGTGTGGCCCTTCTG This study KTR9_3071f CCAGGCGAGATCAACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCCGCAATCC This study KTR9_3826f CGGACTCCCGAATCC This study KTR9_3826r TCGACTCTCGCCGAATCC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827f TGGATGACCAGGAT This study KTR9_3827r TGGATGACCCGCGTCAA This study KTR9_3827r TGGATGACCCCGCGTCAA This study KTR9_3827r TGGATGACCCCCGCTCCCGCAACCT This study			•
KTR9_3071f CCAGGCGAGATCAACTACAA This study KTR9_3071r TGACCGACTTGCCGTTCTG This study KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCGAATGC This study KTR9_3826r TCGACTCTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827r TGGATGACAGTGCCAGGAT This study KTR9_3827r TGGATGACCGCGTCAA This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_3071r KTR9_3071r TGACCGACTTGCCGTTCTG KTR9_3533f TCGACGGCCAGTCGGTAT This study KTR9_3533r GCCGCGCTGGGTGAT KTR9_3534f ACGACACGCACTCCGTAACTC KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAG KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCGAATGC This study KTR9_3826f TCGACTCTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGACCCGCGTCAA This study This study This study TTROMATCAGCCGCGTCAA This study TTROMATCAGCCGCGTCAA This study TTROMATCAGCCGCGTCAA This study TTROMATCAGCCCGCTCCAACCT This study TTROMATCAGCCCGCTCCAACCT This study TTROMATCAGCCCCGTCCAACCT This study TTROMATCAGCCCCCGTCCCCCCCCCCCCCCCCCCCCCCCCCCC			•
KTR9_3533f KTR9_3533r GCCGCGCTGGGTAT This study KTR9_3534r KCRY_3534r CGCTGGGCAAGCACTCCGTAACTC KTR9_3825f KTR9_3825r GACACGTCCAGACAGAAGA KTR9_3825r GACACGTCCAGACAGAAGA KTR9_3826r CGGACTCGCAATGC This study KTR9_3826r TCGACTCTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGAATGC This study KTR9_3827r TGGGTGAACAGTGCCAGACG This study			-
KTR9_3533r GCCGCGCTGGGTGAT This study KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGACCGCGTCAA This study KTR9_3827r TGGATGACCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3534f ACGACACGCACTCCGTAACTC This study KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGACCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_3534r CGCTGGGCAAGCAGATCT This study KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
KTR9_3825f TGCCGGTGGTCAGGAAGA This study KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3825r GACACGTCCAGACGGTATTCG This study KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study	-		-
KTR9_3826f CGGACTCGCCGAATGC This study KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3826r TCGACTCTCGCCGGAAAC This study KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3827f TGGGTGAACAGTGCCAGGAT This study KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3827r TGGATGAGCCGCGTCAA This study KTR9_3954f GCCGCAGTCTCCGAACCT This study			-
KTR9_3954f GCCGCAGTCTCCGAACCT This study			•
· · · · · · · · · · · · · · · · · · ·			•
WILLIAM STORY TO THE STORY OF T	KTR9_3954f KTR9_3954r	TTCAGGACCGTACGGAGTTTG	This study This study

KTR9_4922f	GGTATCGACGCGAAATGCA	This study
KTR9_4922r	TCACCTGGTCGGAAGTGATTG	This study
KTR9_4923f	CAACGTCGCCATGGACATC	This study
KTR9_4923r	GGTGTCCGGGTGCAGATC	This study
KTR9_4924f	CGACGAGGAGGACATGAGATG	This study
KTR9_4924r	GCAGTCGCCTATACCAGGGATA	This study
KTR9_4925f	AGGCATCTTCGTGCTGAACA	This study
KTR9_4925r	AGGTCAGCTGGCGAATCG	This study
KTR9_4926f	ACGACGAATGCATGTGAACAG	This study
KTR9_4926r	GGTGCGGGTATTCGACTATCC	This study
Miscellaneous Primers (5' to 3')		
gntR_knockout_F	ATGCAGATCGGAAGCATCTCC	This study
gntR_knockout_R	AACACTGCCAGCGCATCAAC	This study
gntR_568r	CGGTCACCGTGCTCATCAC	This study