

1 **Using CRISPR-Cas9-mediated genome editing to generate *C. difficile* mutants defective**
2 **in selenoproteins synthesis**

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13 Running Title: CRISPR-Cas9 mutagenesis of *C. difficile*

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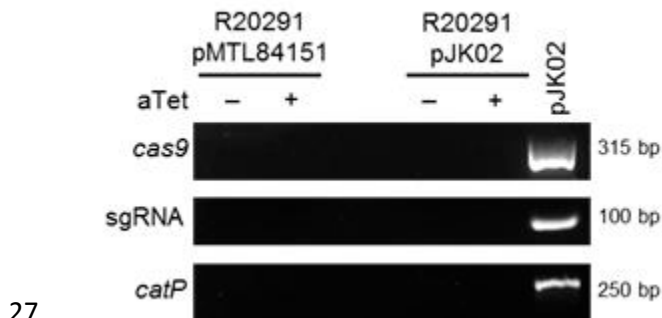
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20 **Supplemental Information**

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22 **Figure S1: No DNA contamination in RNA samples of *C. difficile* R20291 vectors.**

23 RT-PCR without reverse transcriptase showing the comparison of *C. difficile* R20291 pMTL8151
 24 and *C. difficile* R20291 pJK02 induced without aTet and those induced in the presence of aTet
 25 to turn on the expression of *cas9*. Also tested was the sgRNA and *catP*, as positive controls.
 26 The full, uncropped gels corresponding to this data can be found below.



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30 Table S1: Strains and plasmids used in this study.

Strain	Description/Phenotype	Source or Reference
<i>E. coli</i> DH5 α	F ⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ 80d/lacZ Δ M15 Δ (lacZYA-argF)U169, hsdR17(r _K ⁻ m _K ⁺), λ -	51
<i>E. coli</i> HB101 pRK24	<i>lavYI galK2 xyl-6 mtl-I repsL20</i> carrying pRK24	B. Dupuy
<i>B. subtilis</i> BS49	Tn916 donor strain, Tet ^R	52
<i>C. difficile</i> JIR8094	<i>erm</i> -sensitive derivative of 630	14
<i>C. difficile</i> LB-CD7	<i>selD</i> TargeTron mutant	This Study

<i>C. difficile</i> LB-CD12	<i>grdA</i> TargetTron mutant	19
<i>C. difficile</i> LB-CD4	<i>prdB</i> TargetTron mutant	19
<i>C. difficile</i> LB-CD8	<i>prdR</i> TargetTron mutant	19
<i>C. difficile</i> R20291	Wild type, ribotype 027	53
<i>C. difficile</i> KNM5	<i>pyrE</i> targeted CRISPR- <i>cas9</i> mutant, uracil auxotroph	This study
<i>C. difficile</i> KNM6	<i>selD</i> targeted CRISPR- <i>cas9</i> mutant	This study
Plasmids		
pCE240	Derivative of pJIR750ai (Sigma-Aldrich)	45
pBL38	<i>selD</i> TargetTron in pCE240	This study
pBL54	pMC123-containing group II intron from pBL38	This study
pJK02	<i>traJ</i> in pKM71	This study
pJS116	<i>B. subtilis</i> - <i>C. difficile</i> shuttle vector (pCD6 ColE1 <i>Tn916 oriT</i> Cm ^R)	6
pJS170	<i>selD</i> homology region in pJK02	This study
pJS187	<i>selD</i> -targeted sgRNA in pJS170	This study
pJS194	<i>tn916 oriT</i> in pJS187	This study
pKM22	<i>spoVAC</i> - targeted sgRNA in pJS116	This study
pKM46	codon-optimized wildtype <i>cas9</i> in pKM22	This study
pKM48	codon-optimized <i>cas9</i> _{D10A} in pKM22	This study
pKM54	<i>gdh</i> promoter with sgRNA in pKM46	This study
pKM55	<i>gdh</i> promoter with sgRNA in pKM48	This study
pKM64	<i>pyrE</i> homology region in pKM54	This study
pKM65	<i>pyrE</i> homology region in pKM55	This study
pKM71	<i>pyrE</i> -targeted sgRNA in pKM64	This study
pKM72	<i>pyrE</i> -targeted sgRNA in pKM65	This study

pKM93	<i>traJ</i> in pKM72	This study
pKM142	<i>selD</i> with 500 bp upstream in pJS116	This study
pMC123	<i>C. difficile</i> shuttle vector	54
pMK-RQ-Bs-cas9	codon-optimized <i>cas9</i> for <i>C. difficile</i> in pMK-RQ-Bs	Invitrogen, This study
pMTL84151	<i>E. coli-C. difficile</i> shuttle vector (pCD6 ColE1 <i>traJ</i> Cm ^R)	55
pRPF215	tetracycline-inducible P _{tet} promoter	R. Fagan ¹⁶

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32 Table S2: Oligonucleotides used in this study.

Oligonucleotide	Sequence
gRNA_1_for	AAATACGGTGTTTTTGTACCCTAAGTTTCATAAAAATAAGAAGC CTGCAAATGCAGGCTTCTATTTTTATGGTTTAAACCCGCATTATT AAA
gRNA_2_rev	CTTTTCTTATATTTTTATTTTTTAATTTTATGTATACAAAAAGTA ACAAAATTGTAATTTTTTTAATAATGCGGGTTTAAACCATAAAAA T
gRNA_3_for	AATATTAATAAAAATAAAAATATAAGAAAAAGTTTATATCTTTTGGTTA ATTATTACAATAAGTCTCATTTATTGAAATAATATCAAATATATATT A
gRNA_4_rev	ATTTCTAGCTCTAAAACAATAAAGAAAAATCTATTTTTATTATTTT TTCCTTATTTACCAATTATAATATATATTTGATATTATTTCAATAAA T
gRNA_5_for	GATTTTTCTTTATTGTTTTAGAGCTAGAAATAGCAAGTTAAAATA AGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG CTTT
gRNA_6_rev	TAAAAATAGTTGCAGAGCTTTGTACACTAGTCAGACATCATGCTG ATCTAGATTTCTCCATAGAAAAAAGCACCGACTCGGTGCCACTT TTTCA
gRNA_7_rev	ATTTTGGTCATGAGATTATCAAAAAGGAGTTTAATAAAAAATAAAA ATAAGCTCTGCAACCATCTAAAAATAGTTGCAGAGCTTTGTACAC TAGT
5'gRNA	AAATACGGTGTTTTTGTACCCTAAGTTTCATAAAAATAAGAAGC CTGCAAATGCAGGC
3'gRNA	TTGGTCATGAGATTATCAAAAAGGAGTTTAATAAAAAATAAAAA AGCTCTGCAACCA
5'MTL_tetRprom	CCATGGAGATCTCGAGGCCTGCAGACATGCGCGATCGCAGACC CACTTTCACATTTAAG
3'tetR_Cas9	ATCTAAGCCTATTGAGTATTTCTTATCCATTTAATTAAAAAACCT CCTAGTATTATTGAGC
5'tetR_CO_cas9	CTGAGCTCAATAACTAGGAGGTTTTTTTAATTAATGGATAAAA AATATAGTATAGGATTAGATATAGGAAC

3'MTL_CO_cas9	TCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTTTAATCAC CACCTAATTGAGATAAATCTAT
5'tetR_CO_cas9_D10A	CTGAGCTCAATAATACTAGGAGGTTTTTTAATTAATGGATAAAA AATATAGTATAGGATTAGCTATAGGAACAAATAG
5'gdh	TGCAGGCTTCTTATTTTTATGGTTTAAACGGTTTTAGCTGGGATAT CG
3'gdh_gRNA	AAAACAATAAAGAAAAAATCTATTTGGTACCTTTACAGTTTAATTA TAGCACACTTTATT
5'gRNA_gdh	AGTGTGCTATAATTAAGTGTAAAGGTACCAAATAGATTTTTCTT TATTGTTTTAG
3'gRNA 2	CATCTAAAATAGTTGCAGAGCTTACGCGTCTAGTCAGACATCAT GCTGA
5'pyrE_UP	TTATCAGGAAACAGCTATGACCGCGGCCGCGACGTGATTTTTAA TGGGTA
3'pyrE_UP	TAAGTAACACTATAAATAATTAAGTTTTTAATTATTTTTCCTCCATG TTAATG
5'pyrE_DOWN	ATAACATTAACATGGAGGAAAAATAATTA AAAACTTAATTATTTAT AGTGTACTTAAAA
3'pyrE_DOWN	GTGGGTCTGCGATCGCGCATGTCTGCAGGCCTCGAGAAGCATT GATGTTCTTCT
pyrE_gRNA_gBlock	GGTACCGAAAAGTGATGCATTGTTGGGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTTCTATGGAGAAATCTAGATCAGCATG ATGTCTGACTAGACGCGT
5'traJ	GCGAGGAAGCGGAAGAGCGCCCAATACGCAGGGCCCCCTGCTT CGGGGTCA
3'traJ	AATTTATCTACAATTTTTTATCCTGCAGGGGGCCCGATCGGTCT TGCCTTG
5'MTL_selID_UP	TTATCAGGAAACAGCTATGACCGCGGCCGCATTAATAAAAGTGA TAAATTTCTTTTCAT
3'MTL_selID_UP	TCAAAACAATCACTCTTCTCTATAATTTTTGAGATAAAACTGAT GCCA
5'selID_MTL_DN	CCAGAGGTTCTGGCATCAGTTTTATCTCAAATATTATAGAGAAA GAGTGATTGTTTT
3'selID_MTL_DN	CTGCGATCGCGCATGTCTGCAGGCCTCGAGTCTAGTAAGTTGAT TTTTCTTCATT
CRISPR_selID_183	GGTACCTGCTATGGGAGGCAAACCTTGTTTTAGAGCTAGAAATA GCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTTTTTTTCTATGGAGAAATCTAGATCAGCATG ATGTCTGACTAGACGCGT
5'Tn916ori	AAGCGGAAGAGCGCCCAATACGCAGGGCCCTAACATCTTCTATT TTTCCCA
3'Tn916ori	TATCTACAATTTTTTATCCTGCAGGGGGCCCTAAAGGGAATGT AGATAAATTATTAG
5'selID_comp	CAATTTTTTATCAGGAAACAGCTATGACCGCGGCCGCACCTAAA ATAGGTGAAGCAAC
3'selID_comp	GCCAGTGCCAAGCTTGCATGTCTGCAGGCCTCGAGTTATAAAAC TGTAATATTTTTCC
5' tcdB	TTACATTTGTTTGGATTGGAGGTC

3' tcdB	AGCAGCTAAATTCCACCTTTCTACC
5' catP 3	ATGGTATTTGAAAAAATTGATAAAAATAG
3' catP 2	TTAACTATTTATCAATTCCTGCAATTCG
5'pyrE 2	GTCCAGTGTTCTGGGGAG
3'pyrE 2	AAAATTTACATTTTTTAAGTAACACTATAAATAATTAAGTTTTTA
5'selD	GAGCTTCCTAAAAATGAAGTAAATATCAATAAACAG
3'selD	TTTTGCTCAAAACAATCACTCTTTCTCTATAATATT
5'COcas9_RT	GTGTAGGATGGGCAGTAATAAC
3'COcas9_RT	CCAAATATTGGATGTCTTTTCGTG
5'gRNA_RT	GTTTTAGAGCTAGAAATAGCAAG
3'gRNA_RT	CTGATCTAGATTTCTCCATAG
5'catP_RT	CTTTGCAAGTGTACCTTGTACC
3'catP_RT	GTCAGACTTACACTCAGTCC
oLB70	AAAAGCTTTTGCAACCCACGTCGATCGTGAAGAGCCTAAATATGT GCGCCAGATAGGGTG
oLB71	CAGATTGTACAAATGTGGTGATAACAGATAAGTCAAATATGGTAA CTTACCTTTCTTTGT
oLB72	CGCAAGTTTCTAATTTTCGGTTGGCTCTCGATAGAGGAAAGTGTCT
EBS-universal	CGAAATTA GAACTTTCGTTTCAGTAAAC
oLB76	ATGACAACAGCAGGTGGTTGAGCCGC
oLB77	ATTTGTGCCATTTCTATTGCACC

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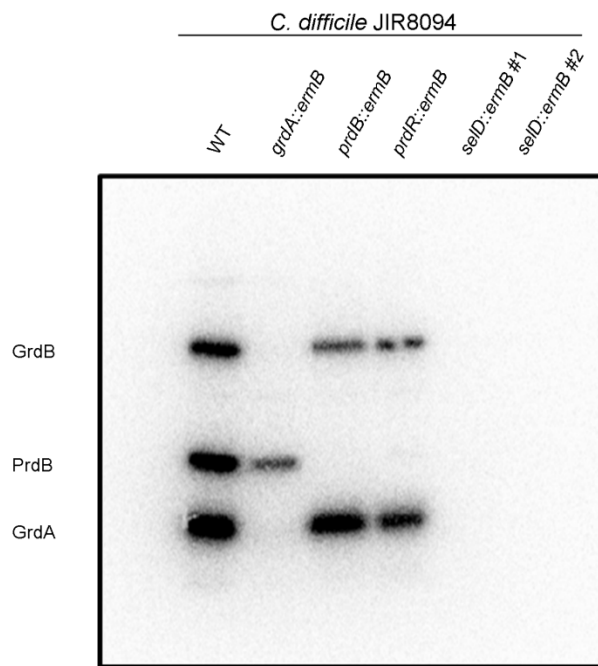
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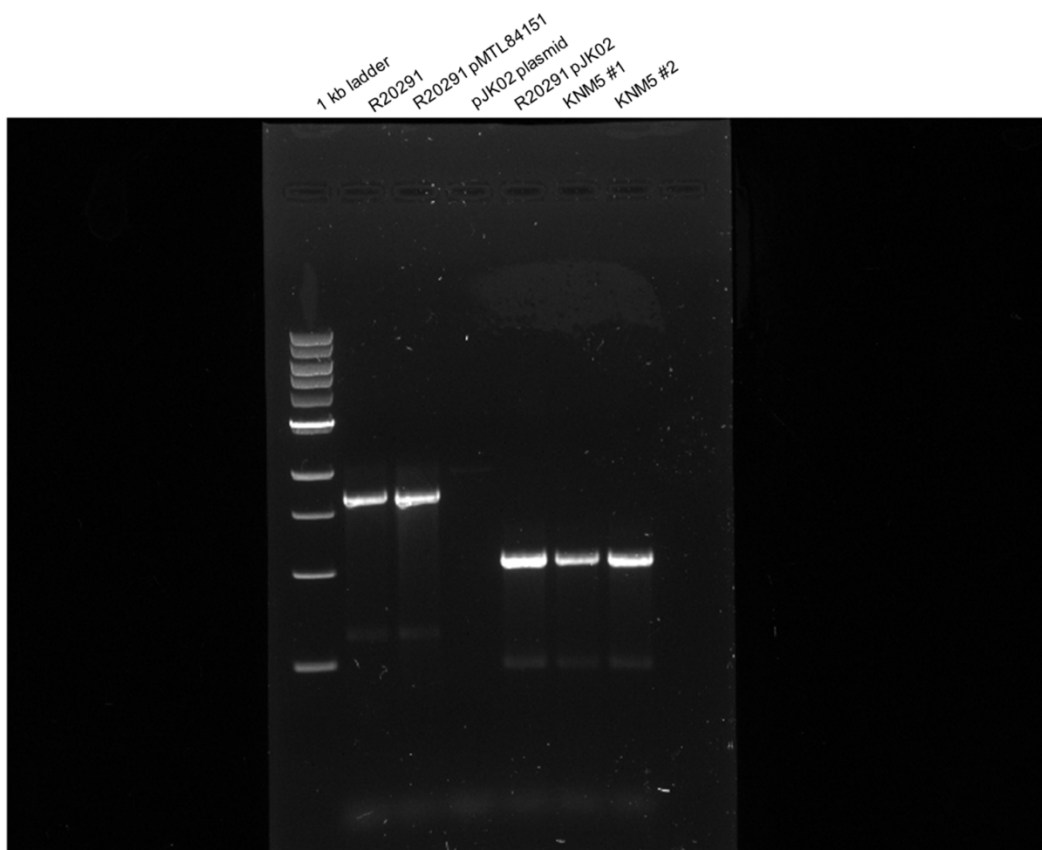
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36 **Uncropped Gel images (labeled)**

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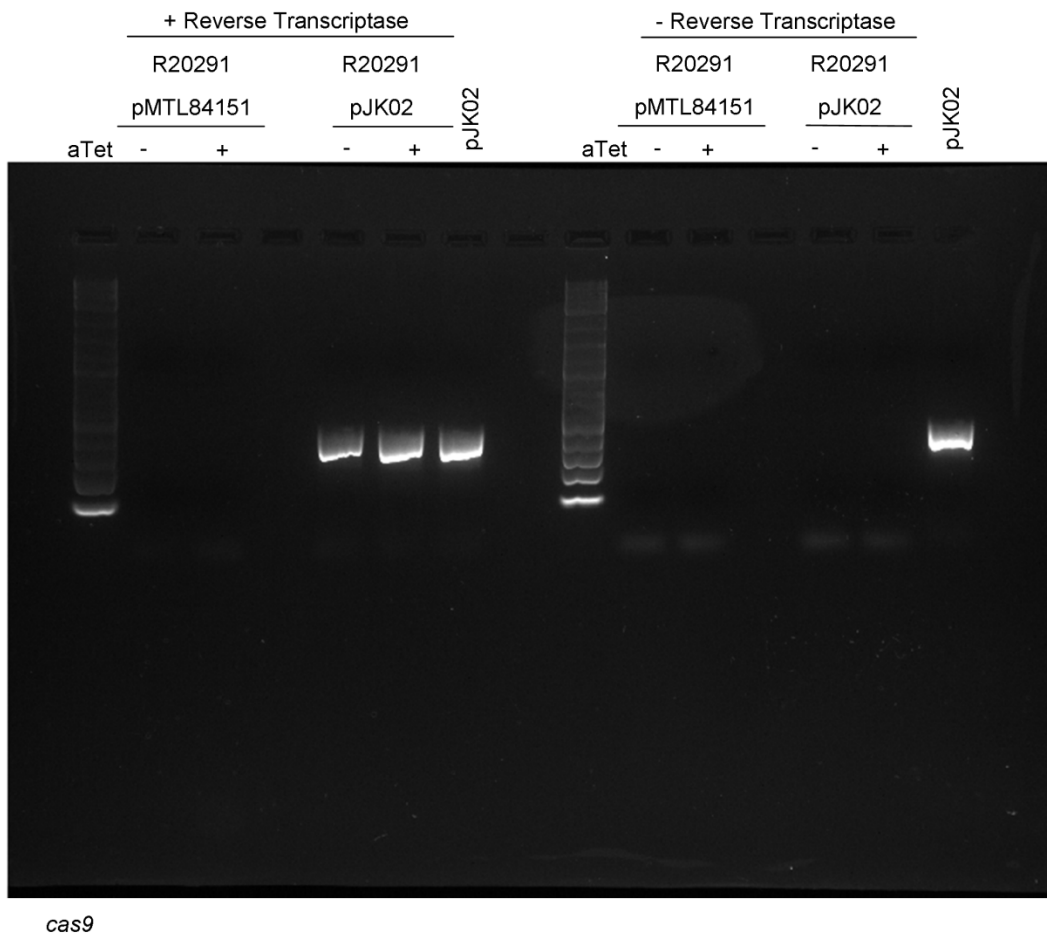




pyrE PCR

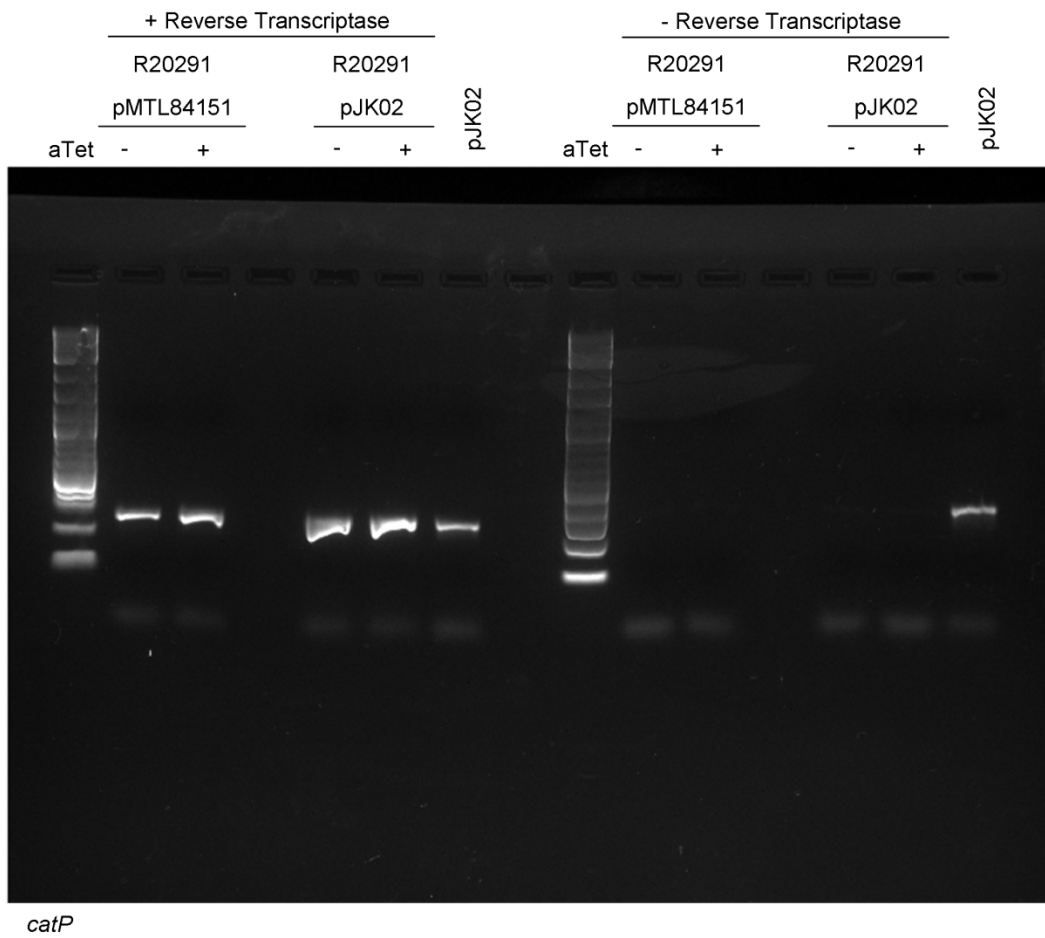
40 PCR of *pyrE* (Figure 3B). Labels added for clarity but the gel images is unaltered / uncropped

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42 **cas9 RT-PCR (Figure 3E).** Labels added for clarity, but the image is unaltered / uncropped.

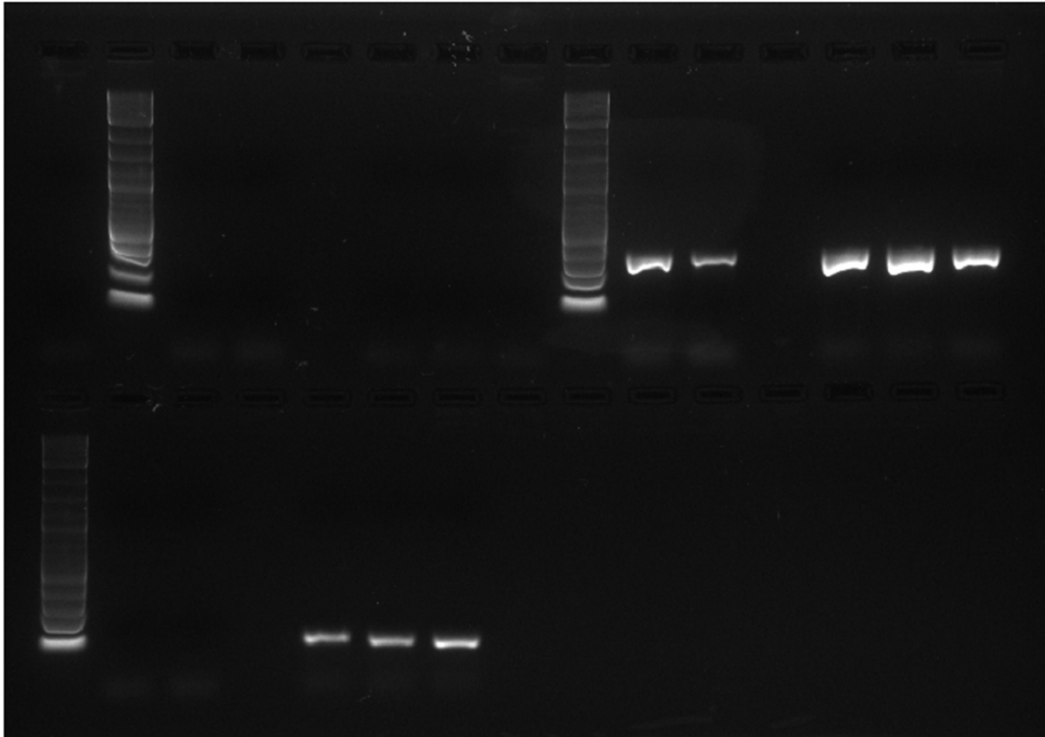
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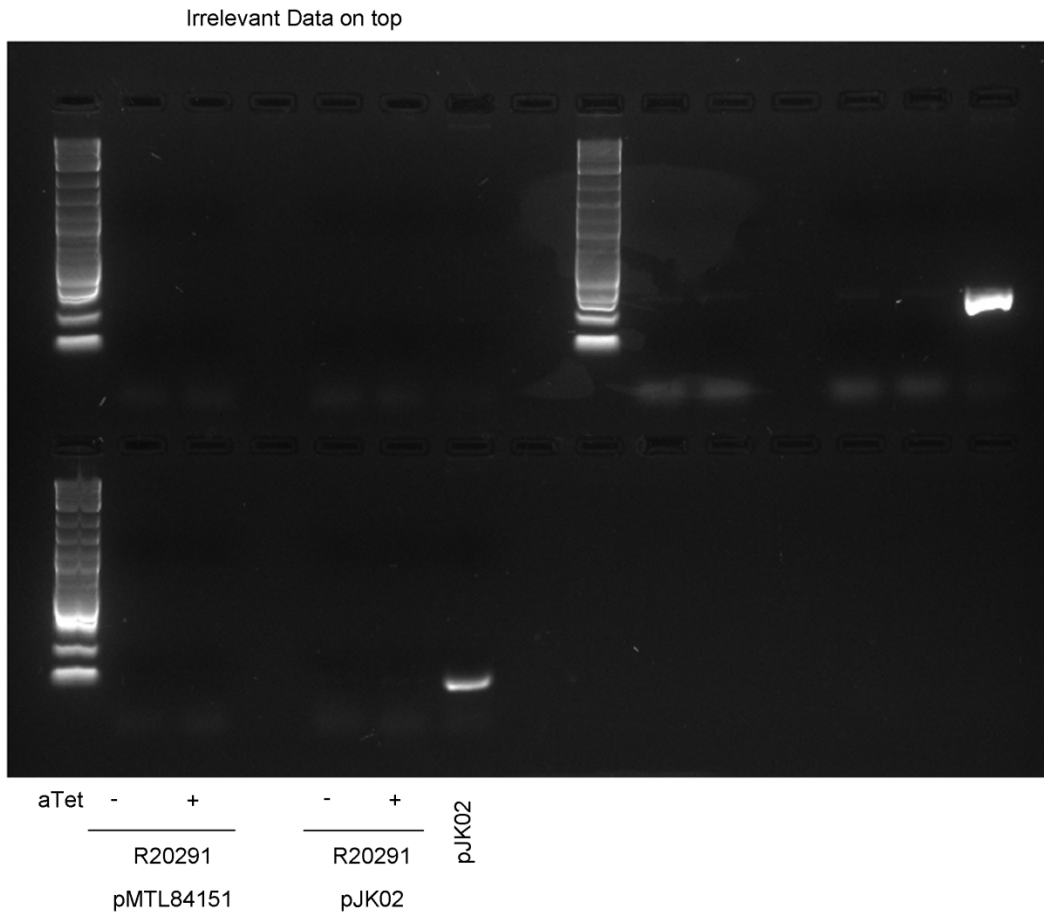
45 **cat9** RT-PCR (Figure 3E). Labels added for clarity, but the image is unaltered / uncropped.

Irrelevant data on top



aTet	-	+	-	+	pJK02
	R20291		R20291		
	pMTL84151		pJK02		

46 **sgRNA RT-PCR (Figure 3E).** Plus-reverse transcriptase conditions for the sgRNA RT-PCR on
47 the bottom. Irrelevant data on top. Labels added for clarity, but the image is unaltered /
48 uncropped.



50 **sgRNA RT-PCR (Figure 3E).** No-reverse transcriptase conditions for the sgRNA RT-PCR on
 51 the bottom. Irrelevant data on top. Labels added for clarity, but the image is unaltered /
 52 uncropped

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