

Table S2: Primers used for plasmid construction and qPCR

Primer name	Sequence (5' - 3') ¹	Target ²	Purpose ³
tdsP984	gtcaaacactgatagtttCTTGGTGATGCTAAAGGG	pFC332	GA: pPTS608-Cas9-hyg; amplify Cas9 & hyg backbone
tdsP985	attgtggtgtaaacattaatGTGTGCGCCCTTATTCGAC	pFC332	GA: pPTS608-Cas9-hyg; amplify Cas9 & hyg backbone
tdsP986, FragA F (common)	ccgctgagggtttaatCGTAAAGCTCCCTAATTG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: CRISPR targeting constructs
tdsP989, FragB R (common)	cggctgaggctttaatGAGCCAAGAGCGGATTCC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: CRISPR targeting constructs
tdsP991, FragA R (Pra1)	GACGAGCTTACTCGTTTCGTCTCACGGACTCATCAGCTGGGCGGTGATGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-Pra1 sgRNA
tdsP992, FragB F (Pra1)	GAAACGAGTAAGCTCGTCTGCTGGTCCCAATCTGCATGGTTTLAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-Pra1 sgRNA
gckP001, PRA1 F	TCACCATGAGGCTCCCCATA	<i>B. d. PRA1</i> (BDFG 05357)	Screening & sequencing targeted strains
gckP002, PRA1 R	GCCATACCAGAGCACATGGT	<i>B. d. PRA1</i> (BDFG 05357)	Screening & sequencing targeted strains
gckP011, PRA1 F	GACTATCCCATCCACAAC	<i>B. d. PRA1</i> (BDFG 05357)	qPCR
gckP012, PRA1 R	TACAGAGCGGAATCTTTG	<i>B. d. PRA1</i> (BDFG 05357)	qPCR
gckP015, Cas9 F	GAGCATGCCGAAGTCAATA	Cas9 (pPTS608-Cas9-hyg)	qPCR
gckP016, Cas9 R	CCCAATCCTTCTTTGAGCG	Cas9 (pPTS608-Cas9-hyg)	qPCR
gckP021, GAPDH F	ACCCCCGCTCCTCCATCTTC	<i>B. g. GAPDH</i> (BDBG 07959)	qPCR
gckP022, GAPDH R	GAGTAGCCCCACTCGTTGTCATACC	<i>B. g. GAPDH</i> (BDBG 07959)	qPCR
gckP025, RPL34 F	AAAGTCCGCATCATCAAGAC	<i>B. g. RPL34</i> (BDBG 01400)	qPCR
gckP026, RPL34 R	GAACGCCTGGGAGTTTG	<i>B. g. RPL34</i> (BDBG 01400)	qPCR
gckP027, FragA R (Zrt1 #1)	GACGAGCTTACTCGTTTCGTCTCACGGACTCATCAGATAGCACGGTGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-neo-Zrt1.1 sgRNA
gckP028, FragB F (Zrt1 #1)	GAAACGAGTAAGCTCGTCTATAGCAAGGCAATACGTAGGGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-neo-Zrt1.1 sgRNA
gckP029, FragA R (Zrt1 #2)	GACGAGCTTACTCGTTTCGTCTCACGGACTCATCAGCATGGTGGTGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-neo-Zrt1.2 sgRNA
gckP030, FragB F (Zrt1 #2)	GAAACGAGTAAGCTCGTCCATGGTATGGCAGTCGGAGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-neo-Zrt1.2 sgRNA
gckP034, F	gcggaacataactgggcccgggaagatctGCGTAAGCTCCCTAATTGG	pPTS608-Cas9-hyg-Zrt1 sgRNA #1	GA: pPTS608-Cas9-hyg-2X-Pra1-Zrt1 #1 sgRNAs
gckP035, R	tcagcggaaacagctatgacctgagatctGAGCCAAGAGCGGATTCC	pPTS608-Cas9-hyg-Zrt1 sgRNA #1	GA: pPTS608-Cas9-hyg-2X-Pra1-Zrt1 #1 sgRNAs
gckP042, F	ACAATCAGCCGTGTTTGTGA	<i>B. d. BLENG2</i> (BDFG 08749)	Screening & sequencing targeted strains
gckP043, R	ATTCCTTCTGCGCATCCTTT	<i>B. d. BLENG2</i> (BDFG 08749)	Screening & sequencing targeted strains
gckP044, FragA R (mCherry v1, v2)	GACGAGCTTACTCGTTTCGTCTCACGGACTCATCAGCGCTTCGGTGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-mCherry sgRNA
gckP045, FragB F (mCherry v1)	GAAACGAGTAAGCTCGTCCGCTTCAAGGTGCGATGGGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-mCherry sgRNA
gckP048, PgpA-Pra1_sgRNA F	tagctgtttccgctgagggtttaattaaGCGTAAGCTCCCTAATTGGC	pPTS608-Cas9-hyg-Pra1 sgRNA	GA: pPTS608-Cas9-hyg-2XSP-Pra1-Zrt1 #1 sgRNAs
gckP049, PgpA-Pra1_sgRNA R	gcactctcaatgtacaagGTCCATTCCGATGCCG	pPTS608-Cas9-hyg-Pra1 sgRNA	GA: pPTS608-Cas9-hyg-2XSP-Pra1-Zrt1 #1 sgRNAs
gckP050, Zrt1.1_sgRNA F	tggcgaatgggacctgtacattgagagTGCTATCTGATGAGTCCGTGAG	pPTS608-Cas9-hyg-Zrt1 sgRNA #1	GA: pPTS608-Cas9-hyg-2XSP-Pra1-Zrt1 #1 sgRNAs
gckP053, TtrpC R	attctgctgtctcgctgaggctttaattaaGAGCCAAGAGCGGATTCC	pPTS608-Cas9-hyg-Zrt1 sgRNA #1	GA: pPTS608-Cas9-hyg-2XSP-Pra1-Zrt1 #1 sgRNAs
gckP062, ZRT1 F	TATACCTCTATCCAAGTCCAACTA	<i>B. d. ZRT1</i> (BDFG 09159)	Screening & sequencing targeted strains
gckP064, ZRT1 R	CTCCTATGCAAAATCTAGATGGAA	<i>B. d. ZRT1</i> (BDFG 09159)	Screening & sequencing targeted strains
gckP065, mCherry F	GGAATAAGTGGTGCAACAAGC	Bad1 TRΔ20 Eα mCherry T85-14.5 gDNA	Screening & sequencing targeted strains
gckP068, mCherry R	CAGCTTCACCTTGATGAACTC	Bad1 TRΔ20 Eα mCherry T85-14.5 gDNA	Screening & sequencing targeted strains

Primer name	Sequence (5' - 3') ¹	Target ²	Purpose ³
gckP069, FragB F (mCherry v2)	GAAACGAGTAAGCTCGTCGCGCTTCAAGGTGCACATGGGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-mCherry sgRNA #2
gckP070, FragA R (mCherry v3)	GACGAGCTTACTCGTTTTCGTCCTCACGGACTCATCAGGCGCATCGGTGATGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-mCherry sgRNA #3
gckP071, FragB F (mCherry v3)	GAAACGAGTAAGCTCGTCGCGCATGAACTCCTTGATGAGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-mCherry sgRNA #3
P1 FragA R (BI-Eng2 #1)	GACGAGCTTACTCGTTTTCGTCCTCACGGACTCATCAGAGTACTCGGTGATGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-BIEng2 sgRNA #1
P2 FragB F (BI-Eng2 #1)	GAAACGAGTAAGCTCGTCAGTACTCAGCTTGGCTAGAGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-BIEng2 sgRNA #1
P3 FragA R (BI-Eng2 #2)	GACGAGCTTACTCGTTTTCGTCCTCACGGACTCATCAGGAAGTCCGGTGTCTGCTCAAG	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-BIEng2 sgRNA #2
P4 FragB F (BI-Eng2 #2)	GAAACGAGTAAGCTCGTCGAAGTCGTCAAGAAGTTCGGTTTTAGAGCTAGAAATAGC	pFC334 or pPTS608-Cas9 sgRNA vectors	GA: pPTS608-Cas9-hyg-BIEng2 sgRNA #2

¹Color coding for Gibson Assembly Frag A & B primers: Hammerhead (HH) ribozyme, HH IR or IR' in protospacer, protospacer, sgRNA backbone, gpd promoter, trpC terminator

²B.g., *Blastomyces gilchristii*; B.d., *Blastomyces dermatitidis*

³GA: Gibson Assembly