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CRISPR/Cas9 genome editing in Candida albicans

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We use this protocol and it's working

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Abstract

This protocol aims to create a homozygous knockout strain in diploid *Candida albicans*, based primarily on the transient Cas9 expression method by Min et al. in *mSphere* (2016), with some modifications. Notably, it uses plasmid-digested Cas9 (pp2280) instead of PCR-generated Cas9, optimizing both time and cost. The knockout strain should be successfully obtained within one week of the transformation reaction.

Guidelines

This protocol combines the classic SAT1-Flipper system with Cas9-induced double-strand breaks to streamline the creation of homozygous knockouts in diploid *Candida albicans*.

Materials

pADH34_protocol_io.gbk 18KB
pp2280_protocol_io.gbk 72KB

Troubleshooting

Safety warnings

Due to the nature of the SAT1-Flipper system, the knockout strain will retain an FLP site scar. However, this scar can serve as a CRISPR/Cas9 target site for further modifications if needed.

Prepare for DNA required for transfomation

1 There are only three DNA components required for transformation:
1. P_{ENO1}-Cas9-t_{CYC1} construct (StuI digested pp2280)
2. P_{SNR52}-gRNA-scaffold-terminator
3. SAT1-Flipper donor DNA with homologous end

2 **Component 1.** P_{ENO1}-Cas9-t_{CYC1} construct (StuI digested pp2280)

This restriction enzyme digested fragment provides the transient Cas9 expression in *Candida albicans* without integrating into the genome, as it does not have homology sequence to the genome.

I modified the plasmid **pADH140** (Aaron Hernday) to pp2280 (see materials) so that it can be digested by StuI for transient Cas9 expression.

Gel extraction after StuI digestion is optional.

3 **Component 2.** P_{SNR52}-gRNA-scaffold-terminator
Two independent PCRs to amplify P_{SNR52}-gRNA, and gRNA-scaffold-terminator from **pADH143** (Aaron Hernday). Then followed by fusion PCR (alternatively, Gibson assembly or isothermal assembly also work) to fuse these two fragment together.

4 **Component 3.** SAT1-Flipper donor DNA with homologous end
PCR the classic SAT1-Flipper cassette with 80 bp flanking region to the targeted gene. Note: Generally speaking, the longer the flanking region, the higher the knockout efficiency. 60~100 bp flanking region should all work.

Transformation

1w 2d

5 Streak out strain from -80˚C to YPD plate, 30˚C sit for 2 days

2d

6 Pick single colony and inoculate to 3 mL YPD, roller drum 30˚C O/N

1d

7 In the morning, back dilute the O/N culture to 10 mL YPD per transformation reaction (~0.2 OD/mL to
start). 30˚C 200rpm shaking in flask

8 About 3~4 hours, the cell density reaches to ~0.8 OD/mL 4h

9 Spin down the cell by 3000xg 2min, wash with 10 mL water, and then spin down again 10m

10 Resuspend all cell with: (all need to be sterilized)

	A	B
	50 % PEG 3350	800 µL
	1 M Lithium Acetate	100 µL
	10 mg/mL heat denatured salmon sperm DNA	50 µL
	10X TE pH 8.0	100 µL
	Component 1	50 µL
	Component 2	50 µL
	Component 3	50 µL

11 44˚C heat shock for 15min (we use water bath here) 15m

12 Spin down cell at 3000xg 1min, wash twice with 1 mL YPD 10m

13 Resuspend all cell in 2 mL YPD, 30˚C roller drum for at least 4 hours (O/N is fine). 4h

14 Spin down all cell at 3000xg 1min, wash twice with water 10m

15 Resuspend all cell in 1 mL water and take 100 µL to YPD+NAT plate. 30˚C sit for >2days 2d

16 Pick several colonies to new YPD+NAT plate (Due to the high efficiency, 4 colonies should be enough)

17 Inoculate cells to 2 mL YP+2% maltose 30˚C O/N to Flip out SAT1 cassette 1d

18 Streak small amount of O/N culture to YPD. 30˚C sit for >2days 2d

19 Patch single colonies to YPD. Then test if it lack the NAT-resistance. (indicating the SAT1-Flipper has been removed) 1d

20 Store strains lacking NAT-resistance.

Appendix 1. PCR primer design information: Ade2 Knockout as an example

21 **Component 2.** P_{SNR52}-gRNA-scaffold-terminator (**pADH143** as template)

Component 2 PCR-A (P_{SNR52})

	A	B
	template	pADH143
	F1	catctaatcaactcccagat
	R1	caaattaaaaatagtttacgcaag

Component 2 PCR-B (gRNA-scaffold-terminator)

	A	B
	template	pADH143
	F2	cttgcgtaaactatttttaatttgCAACAATCATACGACCTAATgttttagagctagaaatagca
	R2	taaaaaaaCTCGAGAAAAAAAGCAC

F1, R1 and R2 are universal for different KO. Only F2 (contain gRNA) is gene specific

Component 2 PCR-C (fusion PCR of A and B)
This PCR product will be used for transformation)

	A	B
	template	PCR-A and PCR-B product, equal molar ratio (50ng each is good for 50 µL PCR reaction)
	F1	catctaatcaactcccagat
	R2	taaaaaaaCTCGAGAAAAAAAGCAC

The PCR-A and PCR-B product need to be DpnI digested and PCR clean up to remove both the template plasmid and primers.

Make sure to use the correct primer pair here: F1 and R2

Component 3. Primer for SAT1-Flipper donor DNA with homologous end (pADH34 as template)

	A	B
	template	pADH34 (or any plasmid with SAT1-Flipper should work)
	F	AAAAAACAACCAACCAACCCTTAACCCATTAACGAATTAAGATTTGTT CTATTTGACTACCAAGAATATAACCCATATTActagtgaattcgcgctcgag
	R	TTCTTTTTATATATTAATTATAATTTTATTGCACCATAACGTTTACTTGT TTAATATGCTATTGATATCTATATTTTTTTcgctctagaactagtggatc

Appendix 2. gRNA design

22 We design the gRNA using Benchling tools following the protocol in Nguyen *et. al., mSphere 2017* https://journals.asm.org/doi/suppl/10.1128/mspheredirect.00149-17/suppl_file/sph002172275s1.pdf

23 Alternatively, Candida albicans gRNA sequence for each gene can be found in the supplementary information in Vyas *et. al., Sci Adv. 2015*

Protocol references

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