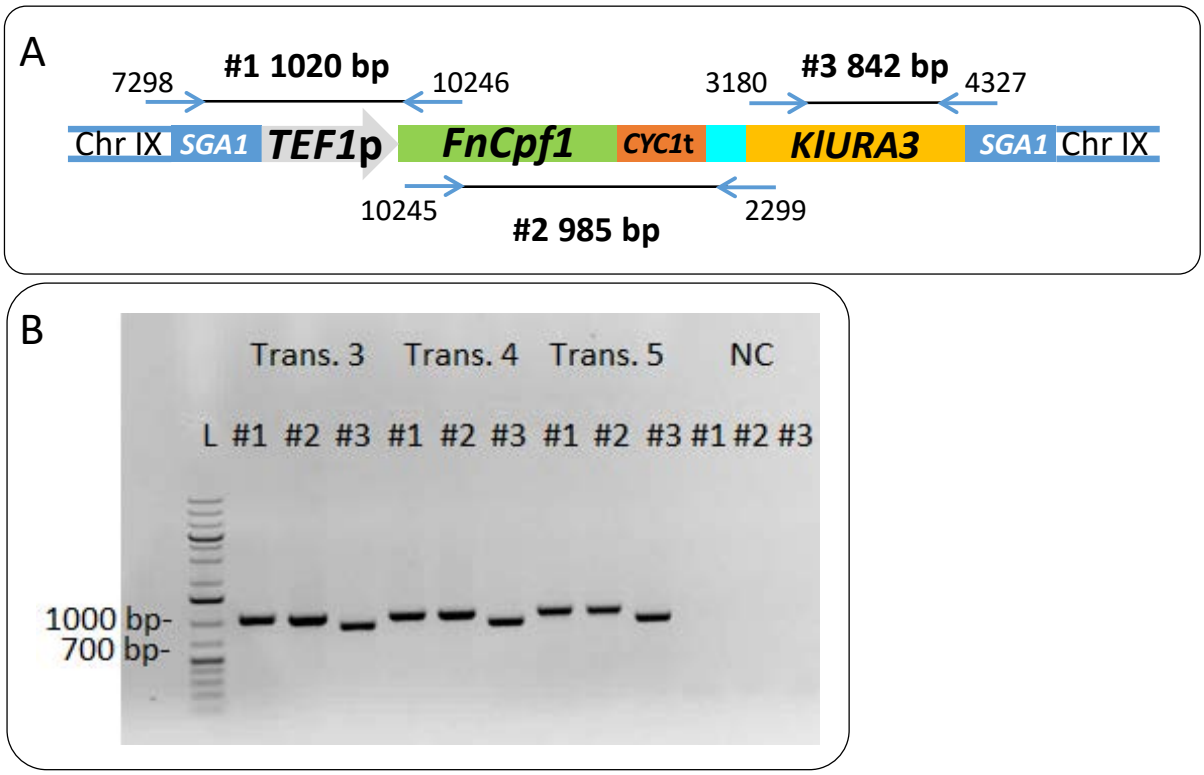
*Fn*Cpf1, a novel and efficient genome editing tool for *Saccharomyces cerevisiae*

Michal A. Świat, Sofia Dashko, Maxime den Ridder, Melanie Wijsman, John van der Oost, Jean Marc Daran, Pascale Daran-Lapujade

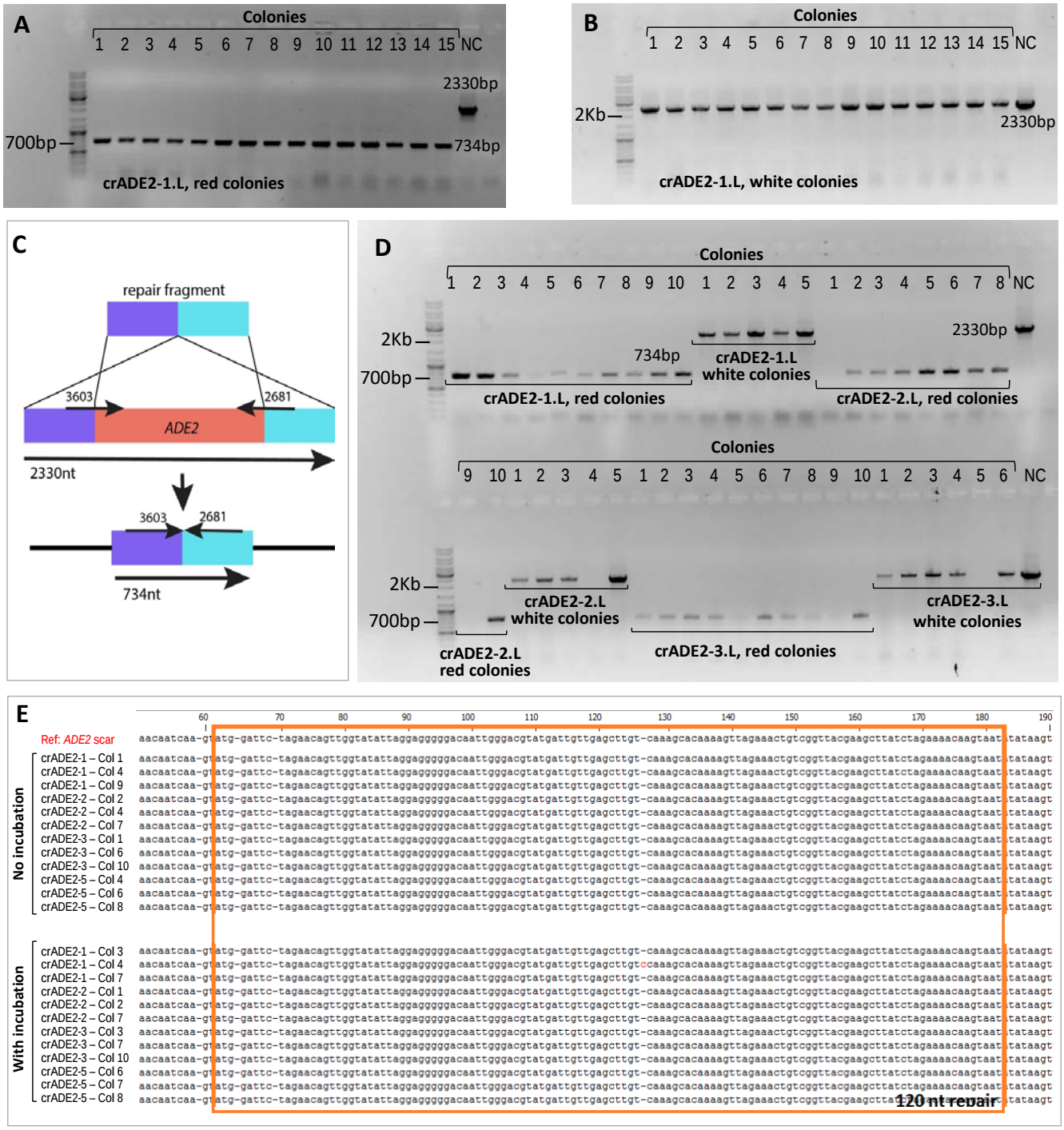
Supplementary material

Supplementary Figure S1

***Fncpf1* integration scheme and validation diagnostic PCR.** A – Schematic overview of the diagnostic PCR set up used to confirm correct integration of the *Fncpf1* and *KlURA3* expression cassettes into the *SGA1* locus. Primer numbers are shown. #1,2 and 3 represent corresponding PCR fragments of expected length. B – Agarose gel with PCR reactions shown in above section A. Transformants 3, 4 and 5 were positive for all three diagnostic reactions. NC – negative control (genomic DNA of CEN.PK113-7D)



Supplementary Figure S2

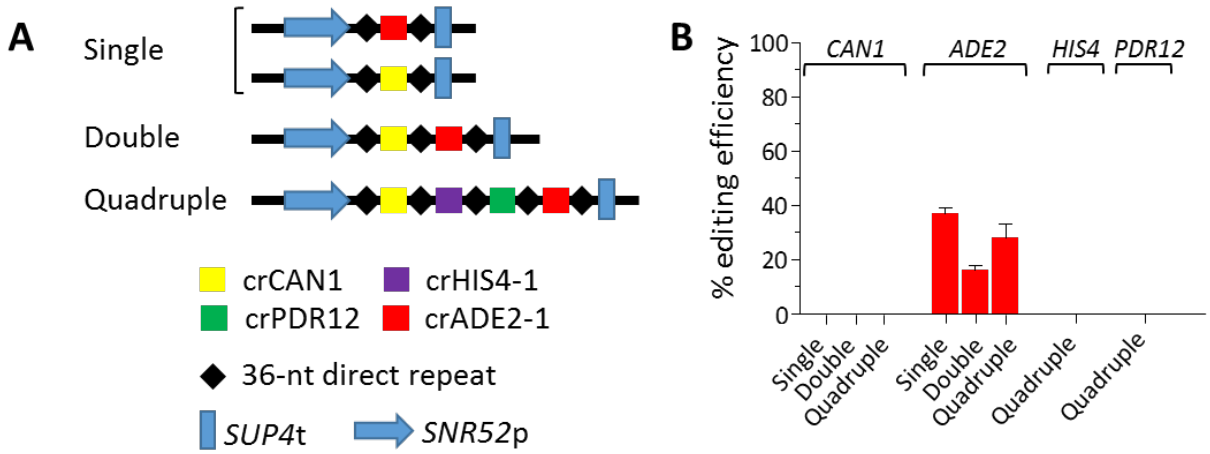


FnCpf1 mediated editing of *ADE2*: Diagnostic PCR and sequencing of IMX1139 (*TEF1*p::*Fncpf1*::*CYC1*t) transformed with crRNA arrays of different AT content targeting *ADE2*, with long direct repeats (36nt). A and B: Targeting with crADE2-1 (36% AT). NC: negative control, PCR with the control strain CEN.PK113-7D. A: Diagnostic PCR of red colonies, all colonies show a 734-bp band indicating deletion of *ADE2*; B: Diagnostic PCR of white colonies, the band at 2330 bp indicates that *ADE2* is intact, as shown by NC. C: schematic representation of the native and edited *ADE2* locus. Diagnostic PCR primers and fragments size are indicated. D: Diagnostic PCR after prolonged incubation between transformation and plating. Targeting with crADE2-1, crADE2-2 and crADE2-3 (36, 44 and 52% AT

respectively) are shown. Both red and white colonies were checked. NC: negative control, PCR with the control strain CEN.PK113-7D. E: sequence alignment of the *ADE2* locus after transformation with the CRISPR plasmids carrying crADE2-1, crADE2-2 and crADE2-3. Incubation was prolonged by 48 hours between transformation and plating. Only red colonies were checked. Sequencing confirms targeted editing of *ADE2* gene and repair by integration of the repair DNA fragment.

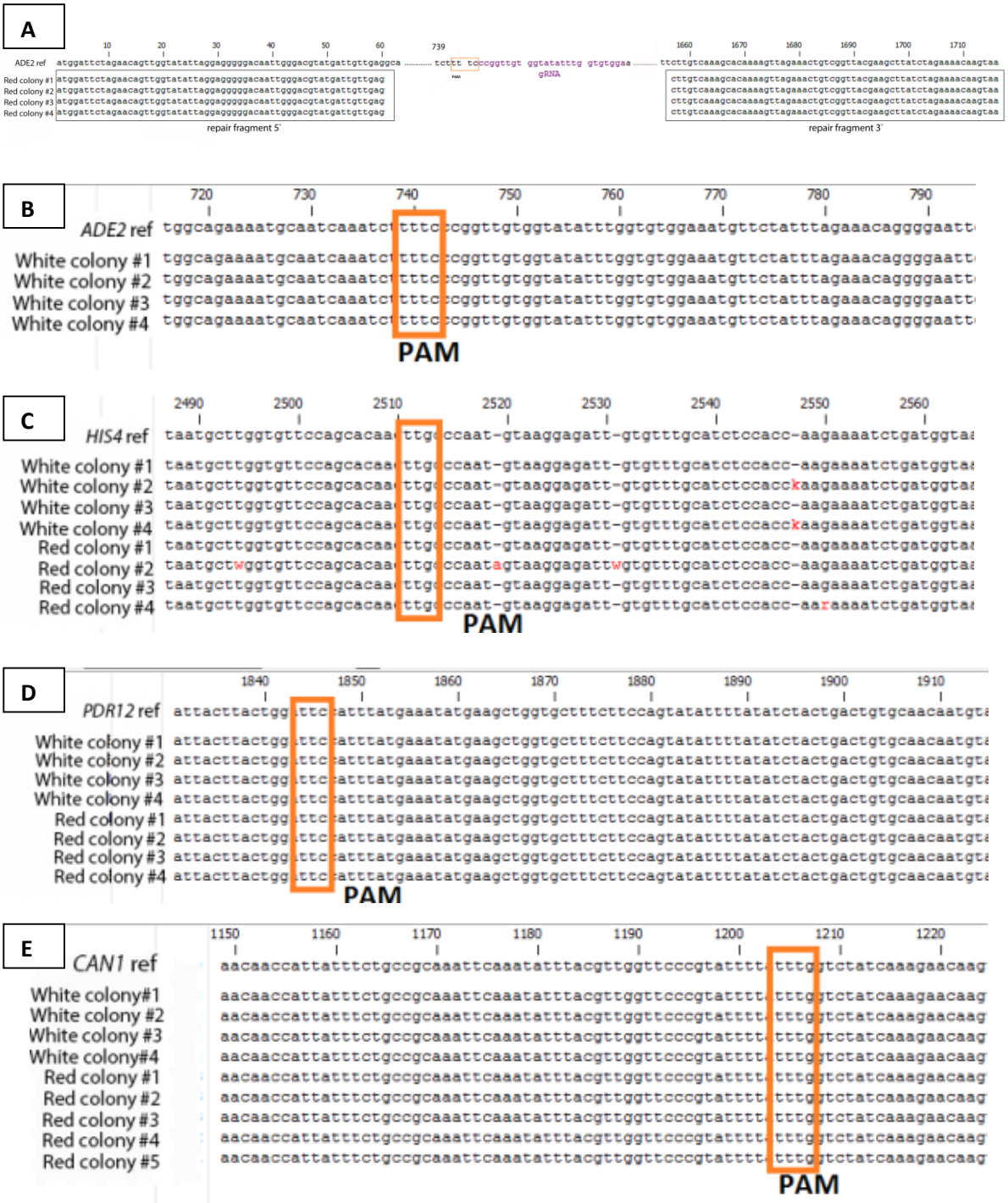
Supplementary Figure S3

***Fn*Cpf1-mediated multisite editing**. A: composition of the CRISPR arrays for single, double and quadruple genome editing target sites. 36-nt direct repeats were used and CRISPR plasmids were assembled *in vivo*. B: Efficiency of genome editing for single, double or quadruple deletions, following the design described in A. Genome editing efficiency is calculated as the fraction of positive colonies out of 10 colonies randomly picked and analysed by PCR. Values represent the mean and standard deviation of three independent transformations with five technical replicates each. Plating was performed just after transformation, without additional incubation.

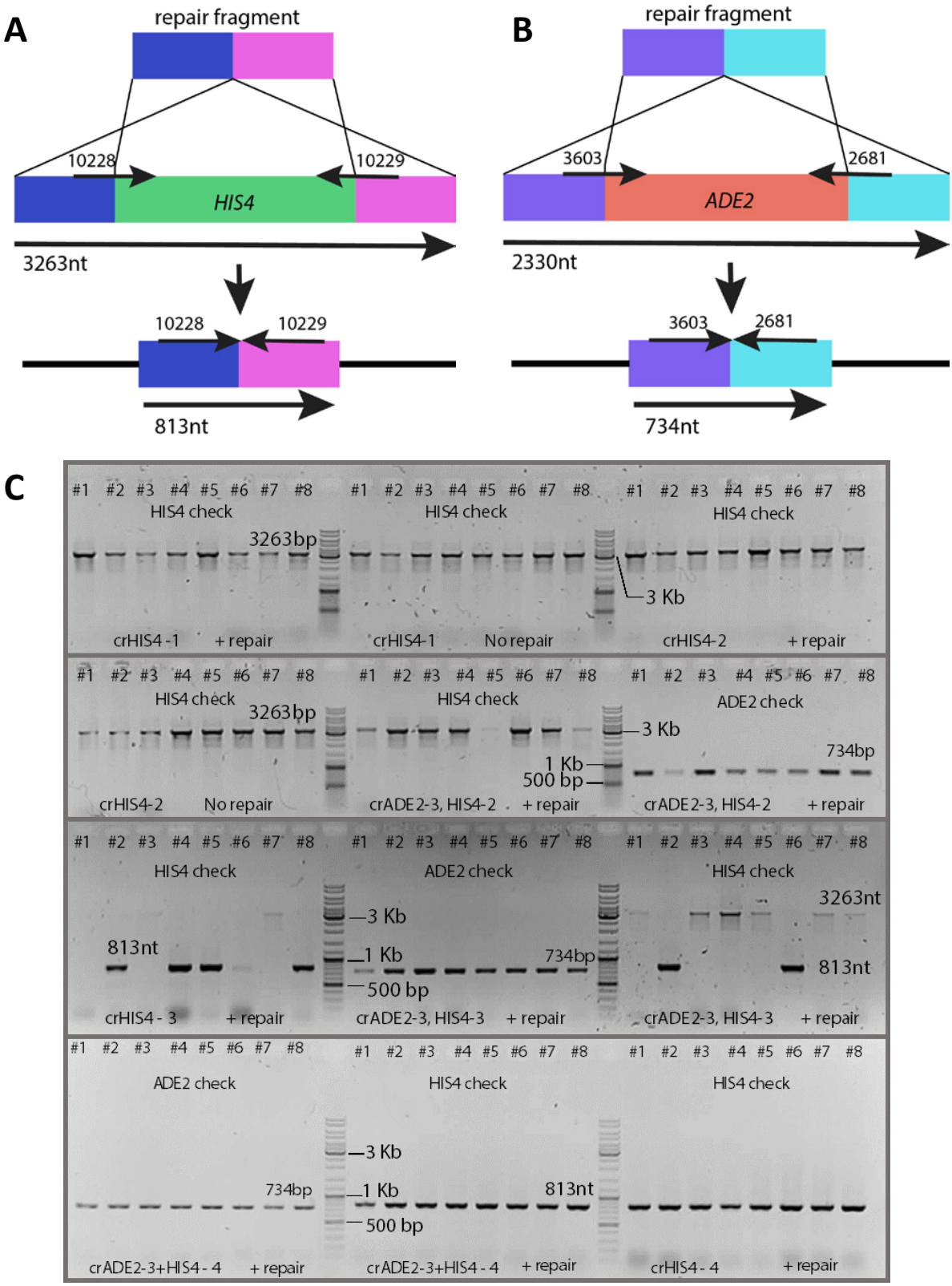


Supplementary Figure S4

Sanger sequencing of the targeted loci in clones transformed with the quadruple crRNA array targeting *CAN1*, *HIS4*, *PDR12* and *ADE2* loci. A: sequence alignment of *ADE2* gene of red colonies shows targeted *ADE2* editing with correct integration of repair fragment. B: sequence alignment of *ADE2* gene of white colonies shows intact *ADE2* gene with PAM still present, C: sequence alignment of *HIS4* gene of white and red colonies shows intact *HIS4* gene with PAM still present. D: sequence alignment of *PDR12* gene of white and red colonies shows intact *PDR12* gene with PAM still present. E: sequence alignment of *CAN1* gene of white and red colonies shows intact *CAN1* gene with PAM still present

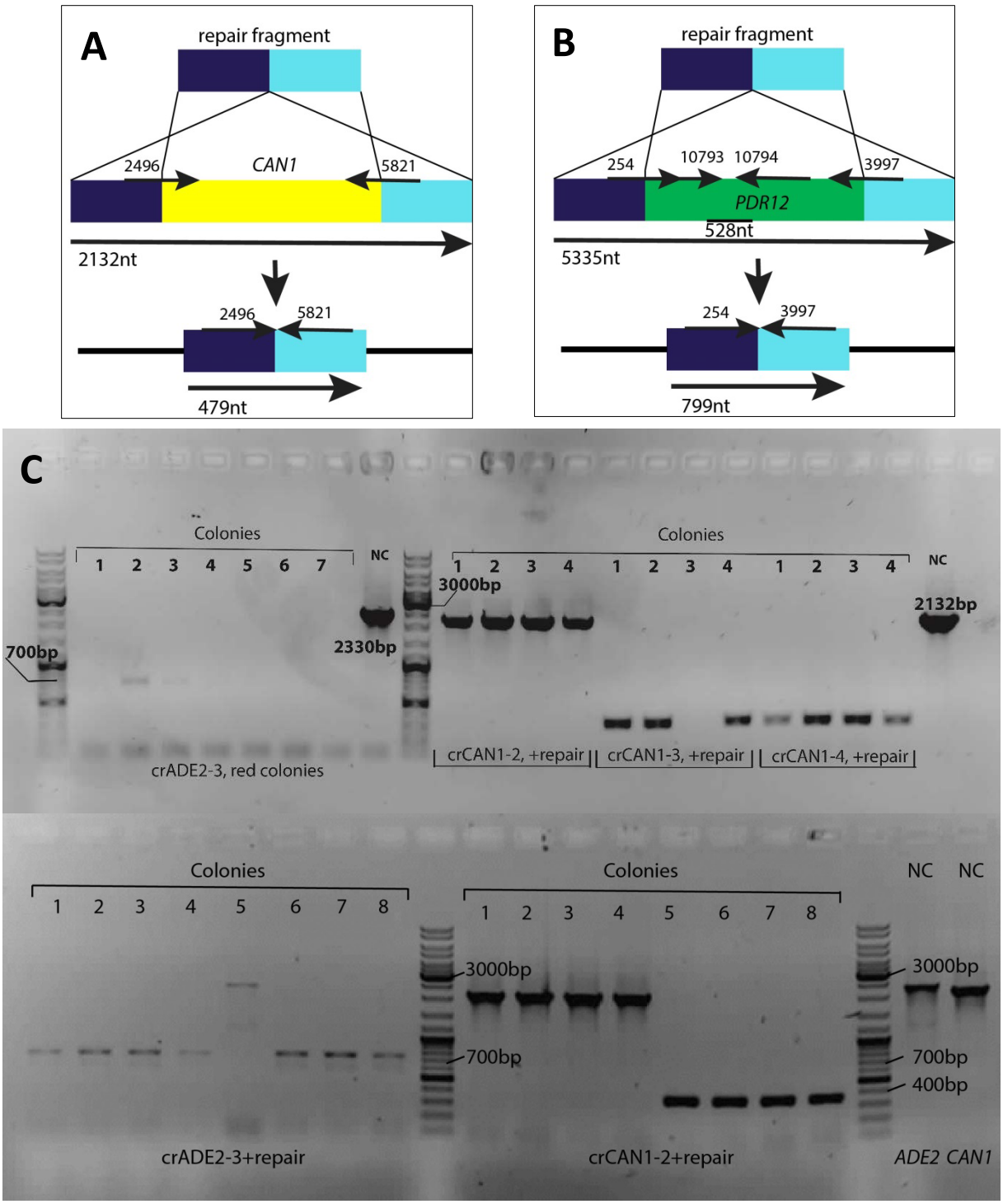


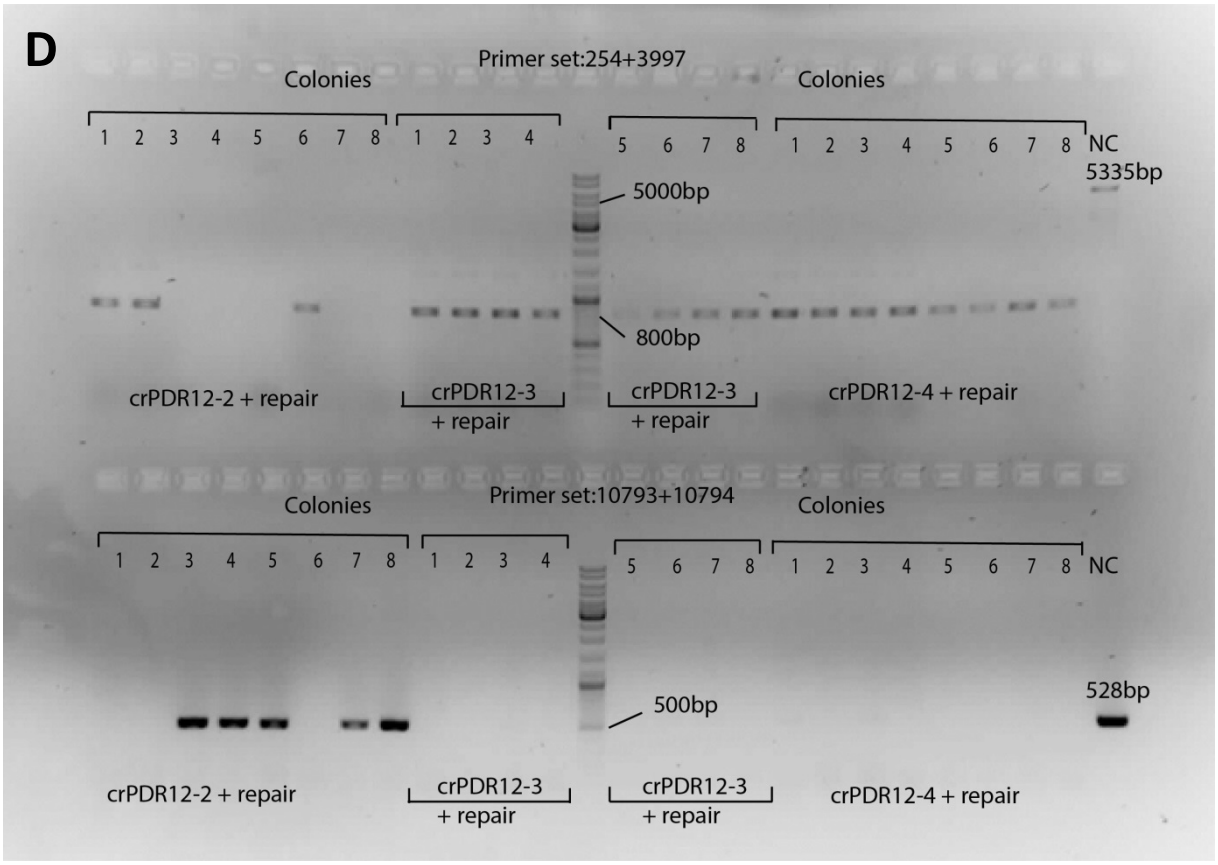
Supplementary Figure S5



Diagnostic PCR of IMX1139 transformed with crRNA arrays for *HIS4* single deletion (crHIS4-1, crHIS4-2, crHIS4-3, crHIS4-4) and double deletion (crADE2-3.crHIS4-2, crADE2-3.crHIS4-3, crADE2-3.crHIS4-4), using short DR (19nt). A,B: Schematic representation of the native and edited *HIS4* (A) and *ADE2* (B) locus. Diagnostic PCR primers and fragments size are indicated. C: diagnostic PCR of *ADE2* and *HIS4* editing after transformation with CRISPR arrays targeting one and two genes and repair DNA. Agarose gels with diagnostic PCR performed on eight colonies picked from each transformation. crRNA array, gene tested and obtained fragment size are indicated on the figure. When no editing was observed (crHIS4-1, crHIS4-2), colonies from plates in which repair DNA was omitted from the transformation mix were also tested (no repair).

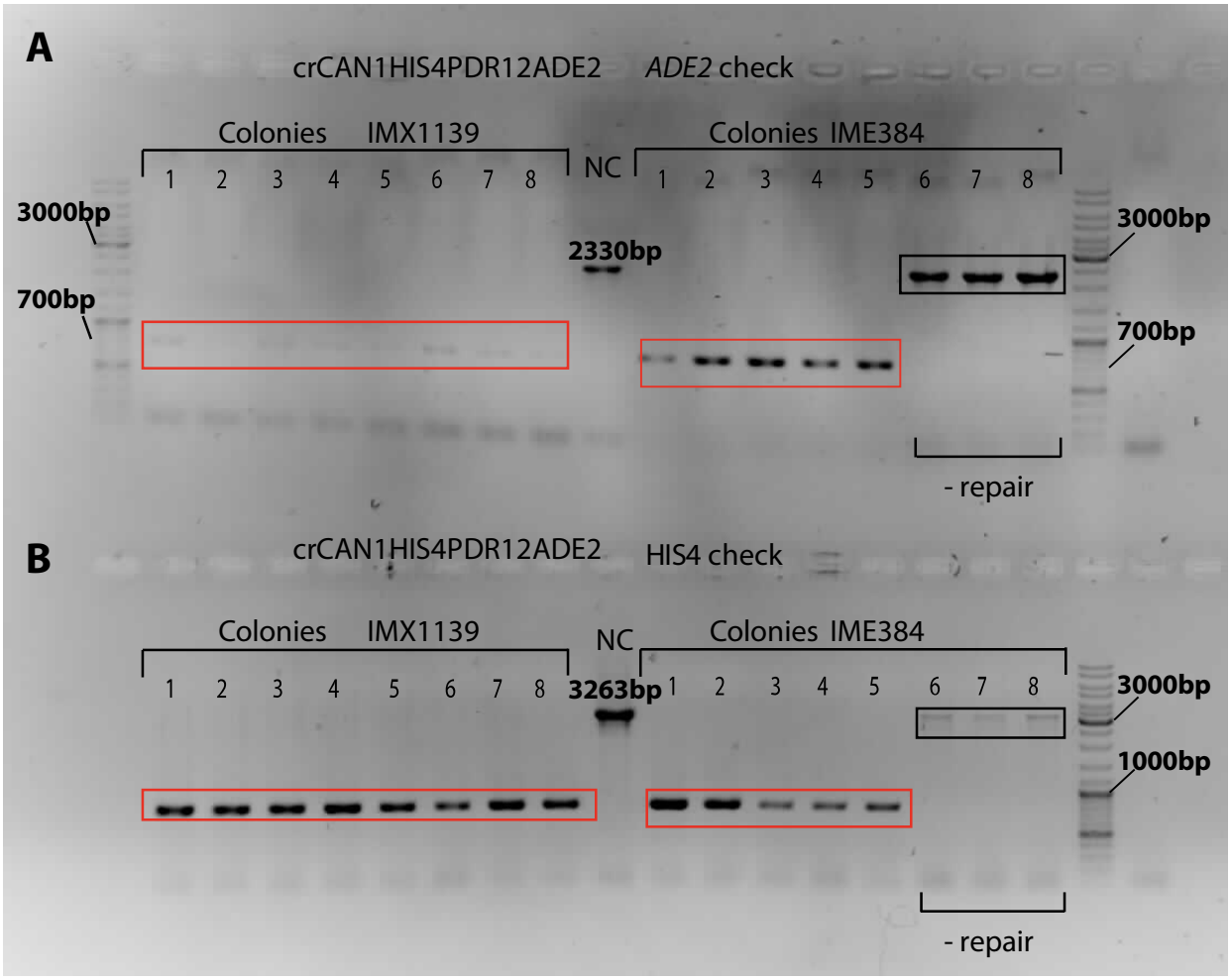
Supplementary figure S5

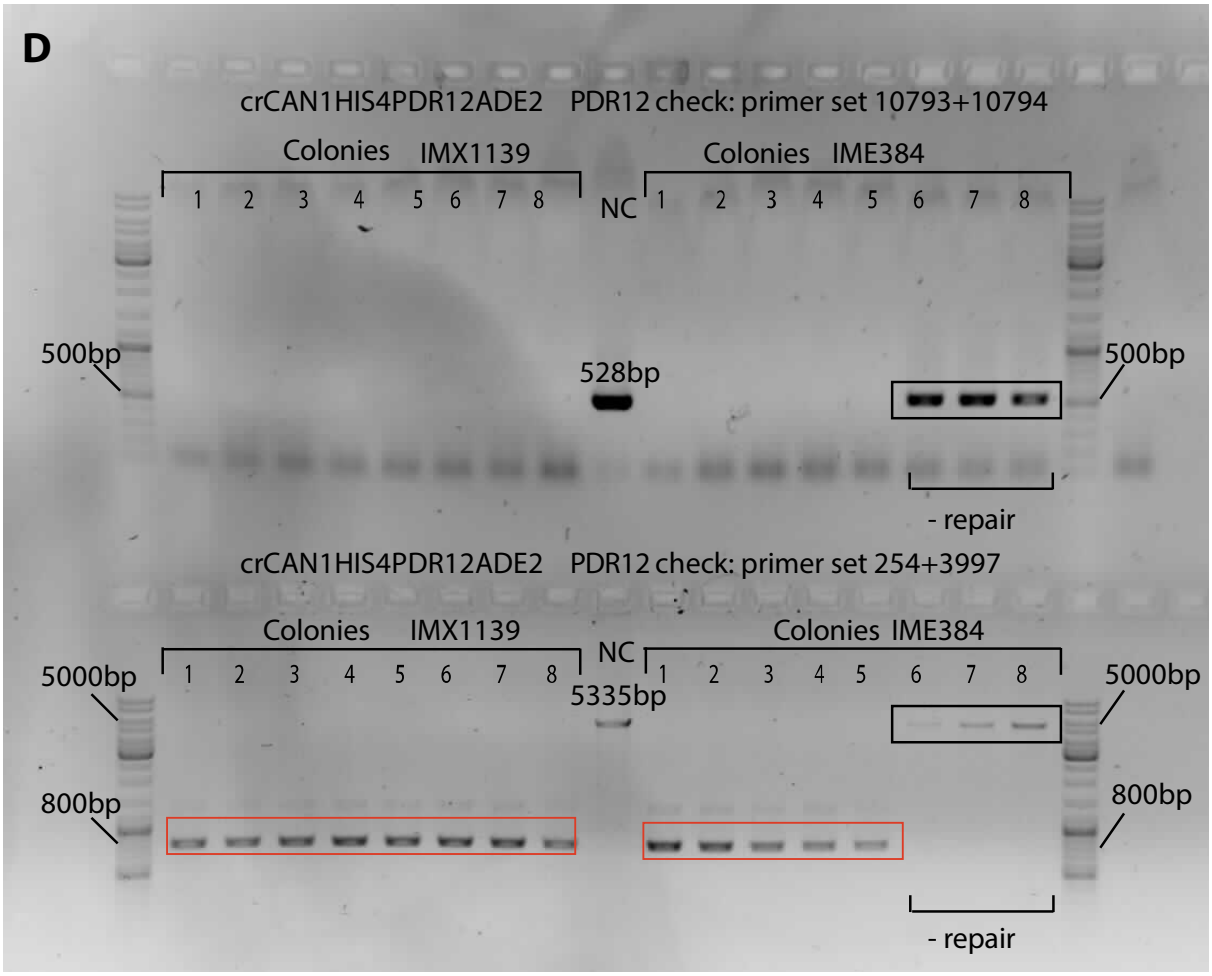




Diagnostic PCR of IMX1139 transformed with crRNA arrays for *CAN1* (crCAN1-2, crCAN1-3, crCAN1-4) and *PDR12* single deletions (crPDR12-2.crPDR12-3, crPDR12-4), using short DRs (19nt). A,B: Schematic representation of the native and edited *CAN1* (A) and *PDR12* (B) locus. Diagnostic PCR primers and fragments size are indicated. Two sets of primers are used for *PDR12* editing analysis. C,D: diagnostic PCR of *CAN1* and *PDR12* editing after transformation with CRISPR arrays and repair DNA. Agarose gels with diagnostic PCR performed on eight colonies picked from each transformation. crRNA array, gene tested and obtained fragment size are indicated on the figure.

Supplementary figure S6





Diagnostic PCR of IMX1139 (*Fncpf1* in genomic locus) and IME384 (*Fncpf1* on a multicopy plasmid) transformed with crRNA arrays for simultaneous editing of four targets: *CAN1, HIS4, PDR12* and *ADE2* (order corresponds to crRNA order in the array), using short DR (19nt). Diagnostic PCR of *ADE2* (A) and *HIS4* (B), *CAN1* (C) and *PDR12* (D) editing after transformation with CRISPR arrays and repair DNA. For *PDR12* (D), primer set 10793+10794 binds inside, and primer set 254+3997 outside of the coding sequence. Agarose gels with diagnostic PCR were performed on eight colonies picked from each transformation. In case of IME384 three colonies without addition of repair fragment were analysed as indicated on the figures A, B, C and D (lanes 6 to 8). crRNA array, targeted gene and obtained fragment size are indicated on the figure. Red boxes indicate expected fragment size in the case of DNA editing by *Fn*Cpf1, while black boxes indicate fragment size in the absence of DNA editing by *Fn*Cpf1. crRNA plasmid used: pUDE735.

Supplementary figure S7

***In vitro* assembly of crRNA expression plasmid using preassembled backbone and crRNA array -**This approach can be used to construct in a few steps any crRNA expressing plasmid of interest. To this end, the crRNA of any of the CRISPR plasmids available from Addgene (i.e. pUD628, pUDE710, pUDE714, pUD722, pUD724 and pUDE735, carrying crRNAs targeting *HIS4*, *ADE2*, *PDR2* and *CAN1* for single, double or quadruple deletion) can be replaced by a single or an array of crRNAs targeting the desired DNA loci, as described below (Figure S7a). For plasmid, primer sequences, reagents descriptions, please see Materials and methods section and Suppl. Table 1). Plasmid sequences are available from Addgene.

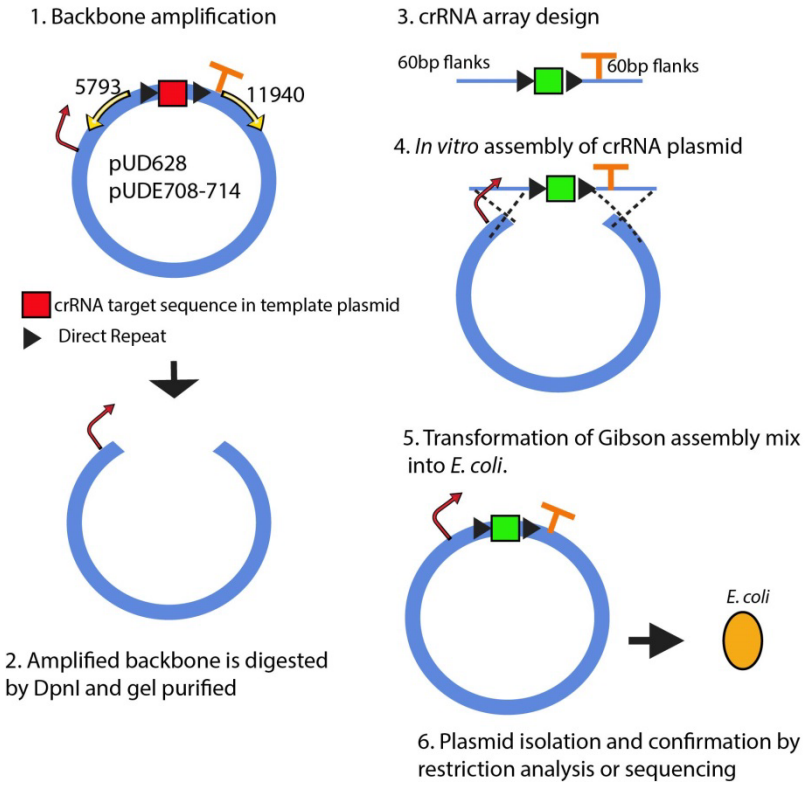


Figure S7a. Schematic overview of the crRNA expression plasmid construction workflow

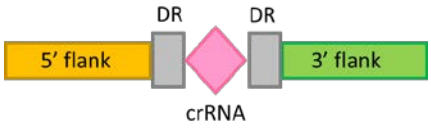
1. **Amplification of the backbone of crRNA plasmid**. For that, any of the CRISPR plasmids (pUD628, pUDE710, pUDE714, pUD722, pUD724 and pUDE735) can be used as a template. Primers 5793 and 11940 were used with the following PCR setup (Phusion polymerase):

$$98^0 - 30\ \text{s}$$
$$\begin{bmatrix} 98^0 - 10\ \text{s} \\ 65^0 - 30\ \text{s} \\ 68^0 - 1\ \text{min}\ 30\ \text{s} \end{bmatrix} \times 35$$
$$68^0 - 5\,\text{min}$$
$$4^0 - \text{hold}$$

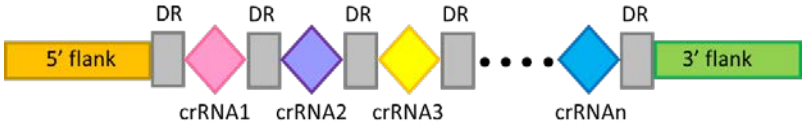
2. **Purification of the CRISPR plasmid backbone**. The amplicon was subsequently digested using DpnI and followed by a heat inactivation step (5 min at 80˚C). This DpnI digestion step allows to remove the circular template remaining after PCR amplification. This step helps to reduce the number of false positive colonies after transformation. The 5058 nt amplicon was separated using a 1% agarose gel, then excised from the gel and purified.

   Note: Gel purification is required as unspecific bands are present after the PCR amplification step.

3. **crRNA design**. The crRNA should match the following design criteria: (i) 5'-TTTV-3' PAM, located at the 5' end of the crRNA (ii) no thymine in the first position of the crRNA sequence, (iii) AT content between 30 and 70%, iv) 25 nt length. The crRNA should be flanked by direct repeats of 19 nt on each side, resulting in a DR-crRNA-DR construct. The sequence of the DR is 5'-AATTTCTACTGTTGTAGAT-3'. To enable *in vitro* assembly into the plasmid backbone (Figure 7a, steps 1,2) the DR-crRNA-DR construct should be flanked by the following sequences that are homologous to the plasmid backbone:
   5` flank: TGAAAGTTGGTGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATC
   3` flank: TTTTTTTGTTTTTTATGTCTTATCGTGACGCAGTCCCATGGGCCATTACAAACTCATGCA GACAGTCAGGGAATCCTAGC
   The crRNA array for a single target should be as follows:



   For multiplexing the crRNA array looks as follows:



4. **Ordering the crRNA array**. The crRNA array can be ordered as synthetic block (for example as gBlock from Integrated DNA Technologies, US).

   Note: when ordering from IDT, product amount up to 750 nt will be 500 ng. This is sufficient amount for in vitro assembly and the PCR amplification step can be avoided.

5. ***In vitro* assembly of the CRISPR plasmid**. The plasmid backbone and the custom-made array can be assembled using NEBuilder® HiFi DNA Assembly Master Mix (New England Biolabs). We used a total reaction volume of 5µl and a 1:3 backbone to array ratio and followed the manufacturer's instructions.
6. **Transformation of the CRISPR plasmid to *E. coli*.** 1µl of the assembly mix was transformed into *E.coli* competent cells (see Materials and methods section). Colonies were screened using PCR and restriction analysis. Colonies harbouring the correct plasmid were restreaked to single colony isolate, stocked and inoculated for plasmid isolation. 500 ng of CRISPR plasmids can be used for transformation to yeast.

The final plasmid is represented in Figure S7b.

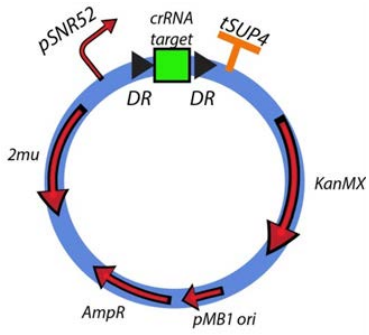


Figure S7b. Schematic representation of crRNA expression plasmid

Supplementary Table S1

List of primers used in the study

Primer	Primer name	Purpose	Sequence (5`-3`)
Construction of *Fn*Cpf1 expression plasmid			
10141	10141_10141_FnCpf1+pTEF1fl.Fw	Amplification of *Fncpf1*	TACAACTTTTTTTACTTCTTGCTCATTAGAAAGAAAG CATAGCAATCTAATCTAAGTTTTATGAGCATCTACCA GGAGTT
10144	10144_10144_Cpf1+pTEF1fl.Rv	Amplification of *Fncpf1*	TCCTTTTCGGTTAGAGCGGATGTGGGGGGAGGGCG TGAATGTAAGCGTGACATAACTAATTTAGGCATAGT CGGGGACAT
10145	10145_10145_pTEF1-CYC1tbackbFw	Amplification of p414-*TEF1p*-Cas9-*CYC1t* backbone	GGAAGGTGTGATCTCTTCTC
10146	10146_10146_pTEF1-CYC1tbackbRv	Amplification of p414-*TEF1p-SpCas9-CYC1t* backbone	CGAGCCCAATGGAGTACTTC
10147	10147_10147_Cpf1+Sga1flFw	Amplification of endonuclease cassette	TTTACAATATAGTGATAATCGTGGACTAGAGCAAGA TTTCAAATAAGTAACAGCAGCAAACATAGCTTCAAA ATGTTTCT
10189	10189_10189_CC-CYC1t	Amplification of endonuclease cassette	CATACGAATCTATACGTCGCCTGACGGTTAGAGTGC ATCTCACCATGTGCCGGAAGCTATCAAATTAAAGCC TTCGAGCG
10190	10190_10190_CC-Ura	Amplification of *URA3* cassette	ATAGCTTCCGGCACATGGTGAGATGCACTCTAACCG TCAGGCGACGTATAGATTCGTATGAACTGTCATCCT GCGTGAAG
10191	10191_10191_CD-Ura	Amplification of *URA3* cassette	AGTGAGACTGTGTGACATCTAGCTACCGAGTCGATC TGATGACTAGCGTCCATCTCGTTGAACTGTCATCCTG CGTGAAG
10192	10192_10192_Ura-SgaI flank	Amplification of *URA3* cassette	CTGCAAACGTGGTTGGGCTGGACGTTCCGACATAGT ATCTAATTAATTTATAATATCAGAAGTGTTGCACCGT GCCAATG
Construction of *Fn*Cpf1 expressing plasmid and empty backbone			

2055	2055_AmpRep-f+I	Amplification of *ampR* fragment of pMEL10	TATTCACGTAGACGGATAGGTATAGCCAGACATCAG CAGCATACTTCGGGAACCGTAGGCGAGAGGCGGTT TGCGTATTGG
4173	4173_FK249	Amplification of *ampR* fragment of pMEL10	GTTGAACATTCTTAGGCTGG
5976	5976_pSNR52 fw ol tag I-2	Amplification of *TEF1p –Fncpf1-CYC1t*	GCCTACGGTTCCCGAAGTATGCTGCTGATGTCTGGC TATACCTATCCGTCTACGTGAATACCCTCACTAAAGG GAACAAAAG
2629	2629_Amds rv + B	Amplification of *TEF1p –Fncpf1-CYC1t*	GTTGAACATTCTTAGGCTGGTCGAATCATTTAGACA CGGGCATCGTCCTCTCGAAAGGTGGGCCGCAAATT AAAGCCTTCGAG
12269	12269_TAG-B + TAG-I FW	Closure of pMEL10 empty backbone	GCCTACGGTTCCCGAAGTATGCTGCTGATGT CTGGCTATACCTATCCGTCTACGTGAATAGTT GAACATTCTTAGGCTGGTCGAATCATTTAGAC ACGGGCATCGTCCTCTCGAAAGGTG
12270	12270_TAGB+TAG-I RV	Closure of pMEL10 empty backbone	CACCTTTCGAGAGGACGATGCCCGTGTCTAA ATGATTCGACCAGCCTAAGAATGTTCAACTAT TCACGTAGACGGATAGGTATAGCCAGACATCA GCAGCATACTTCGGGAACCGTAGGC
Diagnostic primers to confirm integration of *Fncpf1*			
7298	7298_FW_sga1u_check	Confirmation of correct integration endonuclease	TTGTTCAATGGATGCGGTTC
10246	10246_10246_checkFnCpf1_sgaI_Rv	Confirmation of correct integration *Fncpf1*	CTGATGCACACGGAGCTCAG
10245	10245_10245_checkFnCpf1_sgaI_Fw	Confirmation of correct integration *Fncpf1*	TGGCTGATGTCAACGGAAAC
2299	2299_UA URA3	Confirmation of correct integration *URA3*	GGCCCAATCACAACCACATC
3180	3180_URA3 FW partial	Confirmation of correct integration *URA3*	TCATGCAAGTCCGGTTGCATC
4327	4327_URA3 Kl FW	Confirmation of correct integration *URA3*	AGTGTTGCACCGTGCCAATG
Diagnostic primers to confirm *Fn*Cpf1 expressing plasmid assembly and corresponding empty backbone			
2376	2376_I-reverse	Confirmation of correct *TEF1p –Fncpf1-CYC1t* assembly into the backbone	GCCTTTGAGTGAGCTGATACC
10408	10408_10408_FNCPF1_seq1_Rv	Confirmation of correct *TEF1p –Fncpf1-CYC1t* assembly into the backbone	GCTTCTCATCGTCCAGAATC
2750	2750_Fwd TAG D TEF1prom	Sanger Sequencing crRNA plasmids	GCTAAATGTACGGGCGACAG
2376	2376_I-reverse	Sanger sequencing of pUDE731 & pUD706	GCCTTTGAGTGAGCTGATACC

4661	4661_c URA fw	Sanger sequencing of pUDE731	CATATGATTGTCTCCGTAAGCTC
Construction of crRNA expression plasmids			
10224	10224_10224_CA-kanMX-I-Fw	Amplification of *KanMX* fragment	TATCGTGACGCAGTCCCATGGGCCATTACAAACTCA TGCAGACAGTCAGGGAATCCTAGCGACATGGAGGC CCAGAATAC
10225	10225_10225_CA-kanMX-I-Rv	Amplification of *KanMX* fragment	GCCTACGGTTCCCGAAGTATGCTGCTGATGTCTGGC TATACCTATCCGTCTACGTGAATACAGTATAGCGAC CAGCATTC
10313	10313_10313_A-2um FW	Amplification of *2µm* fragment	ACTATATGTGAAGGCATGGCTATGGCACGGCAGAC ATTCCGCCAGATCATCAATAGGCACCGCCCTCCACG CATTTAAGC
10314	10314_10314_2um-CB RV	Amplification of *2µm* fragment	CGGTCAGATGGGATACAATCTAGATAAGTTGCGCT GTAGCAGCAAGCTGAATAGCGATGCGTTCACGCCCT CCAACGAAG
2054	2054_Amprep-r+A	Amplification of a*mpR* fragment	GTGCCTATTGATGATCTGGCGGAATGTCTGCCGTGC CATAGCCATGCCTTCACATATAGTTGCGCGGAACCC CTATTTG
2055	2055_AmpRep-f+I	Amplification of *ampR* fragment	TATTCACGTAGACGGATAGGTATAGCCAGACATCAG CAGCATACTTCGGGAACCGTAGGCGAGAGGCGGTT TGCGTATTGG
10477	10477_10477_crRNAarray Fw	Amplification of crRNA array fragment	GCATCGCTATTCAGCTTGCTGC
10478	10478_10478_crRNAarray RV	Amplification of crRNA array fragment	GCTAGGATTCCCTGACTGTC
5793	pCAS9 rv	Amplification of pUD552 backbone	GATCATTTATCTTTCACTGCGGAG
11940	11940_tSUP4_fw	Amplification of pUD552 backbone	GTTTTTTATGTCTTATCGTGACGC
Diagnostic primers to confirm crRNA expression plasmids assembly			
3812	3812_KANMX4 rv	Diagnostic primer to confirm crRNA arrays integration	TAGATTGTCGCACCTGATTG
11966	11966_11966_ADE2 52% gRNA dg	Diagnostic primer for crADE2-3 confirmation	CCGGTTGTGGTATATTTGGTG
11967	11967_11967_HIS4-1 target dg	Diagnostic primer for crHIS4-2 confirmation	GATTCCAATCAATTCATGGTAAAAC
11968	11968_11968_HIS4-2 target dg	Diagnostic primer for crHIS4-3 confirmation	GATTCTAGCCCCACCAAACC

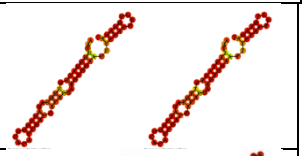
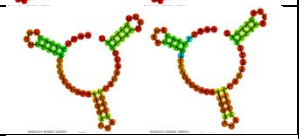
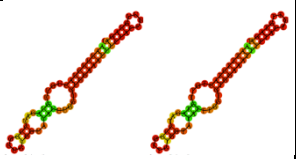
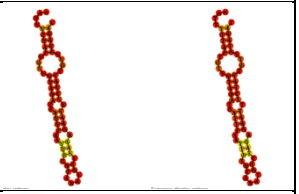
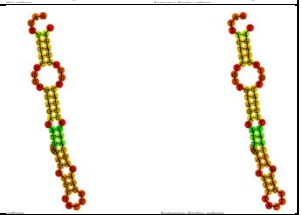
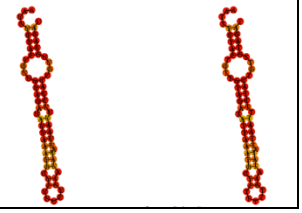
11969	11969_11969_HIS4-3 target dg	Diagnostic primer for crHIS4-4 confirmation	CATCTTGGCTAGCAATGAACAG
12192	12192_12192_dg CAN1-2 fw	Diagnostic primer for crCAN1-2 confirmation	GTTTATCCACACCTCTGACC
12193	12193_12193_dg CAN1-3 fw	Diagnostic primer for crCAN1-3 confirmation	GGTACTGAACTAGTTGGTATCAC
12194	12194_12194_dg CAN1-4 fw	Diagnostic primer for crCAN1-4 confirmation	CACATATCTTCAACGCTGTTATC
12195	12195_12195_dg PDR12-2 fw	Diagnostic primer for crPDR12-2 confirmation	GAGATCGAACCATGACGATG
12196	12196_12196_dg PDR12-3 fw	Diagnostic primer for crPRD12-3 confirmation	CACAAAGAATCAATATGGGTGTC
12197	12197_12197_dg PDR12-4 fw	Diagnostic primer for crPDR12-4 confirmation	GTAGATGCATATAAGCATGCTTGG
Repair fragments			
5614	5614_Oligo CAN 1 fw	Repair fragment for *CAN1* deletion	TATGAGGGTGAGAATGCGAAATGGCGTGGAAATGT GATCAAAGGTAATAAAACGTCATATTGCTATGCCTT TTTTTTTTTTGTTTTTACAGGAGTTAAGAAGTCTGA AGAACTCTGAAA
5615	5615_Oligo CAN 1 rv	Repair fragment for *CAN1* deletion	TTTCAGAGTTCTTCAGACTTCTTAACTCCTGTAAAAA CAAAAAAAAAAAAAGGCATAGCAATATGACGTTTTA TTACCTTTGATCACATTTCCACGCCATTTCGCATTCTC ACCCTCATA
10155	10155_10155_Ade2repairFw	Repair fragment for *ADE2* deletion	ATGGATTCTAGAACAGTTGGTATATTAGGAGGGGG ACAATTGGGACGTATGATTGTTGAGCTTGTCAAAGC ACAAAAGTTAGAAACTGTCGGTTACGAAGCTTATCT AGAAAACAAGTAA
10156	10156_10156_Ade2repairRv	Repair fragment for *ADE2* deletion	TTACTTGTTTTCTAGATAAGCTTCGTAACCGACAGTT TCTAACTTTTGTGCTTTGACAAGCTCAACAATCATAC GTCCCAATTGTCCCCCTCCTAATATACCAACTGTTCT AGAATCCAT
10226	10226_10226_His4 repair Fw	Repair fragment for *HIS4* deletion	CGATGTGTGTTGTACATACATAAAAATATCATAGCA CAACTGCGCTGTGTAATAGTAATACGAACACTAACG AAAATAATATGTATATATACATATATATATCAAACAA AATACAGTCTT
10227	10227_10227_His4 repair Rv	Repair fragment for *HIS4* deletion	AAGACTGTATTTTGTTTGATATATATATGTATATATA CATATTATTTTCGTTAGTGTTCGTATTACTATTACACA

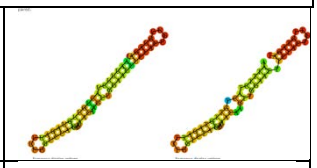
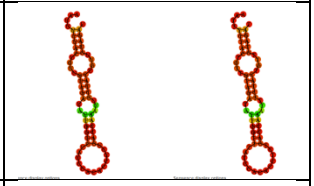
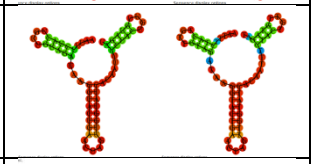
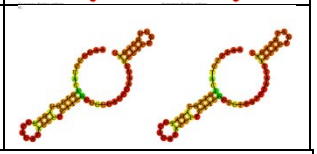
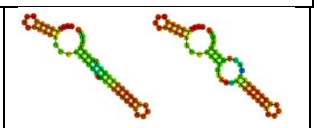
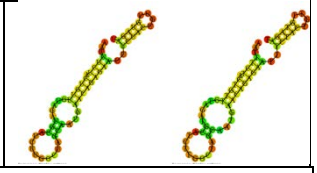
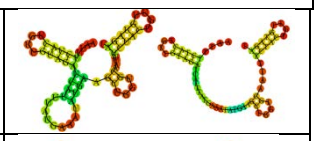
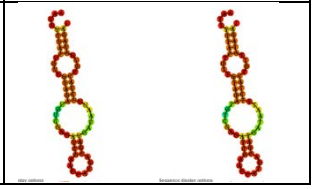
			GCGCAGTTGTGCTATGATATTTTTATGTATGTACAACACACATCG
10688	10688_10688_CAN1 repair FW	Repair fragment for *CAN1* deletion	ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCGGTCGTATGGGAAGATCATGAACCAAAGACTTTTTGGGACAAATTTTGGAATGTTGTAGCATAG
10689	10689_10689_CAN1 repair RV	Repair fragment for *CAN1* deletion	CTATGCTACAACATTCCAAAATTTGTCCCAAAAAGTCTTTGGTTCATGATCTTCCCATACGACCGGCTCATTGTACATATGCTTCTCCTCTATGTCGGCGTCTTCTTTTGAATTTGTCAT
11612	11612_11612_ADE2stopcod FW	Repair fragment for *ADE2* with premature stop codon	AGTTCCGGACTCCGTTCAACTTAAGGCGAAGTTGTTGGCAGAAAATGCAATCAAATCTTAACCCGGTTGTGGTATATTTGGTGTGGAAATGTTCTATTTAGAAACAGGGGAATTGCTTAT
11613	11613_11613_ADE2stopcod Rv	Repair fragment for *ADE2* with premature stop codon	ATAAGCAATTCCCCTGTTTCTAAATAGAACATTTCCACACCAAATATACCACAACCGGGTTAAGATTTGATTGCATTTTCTGCCAACAACTTCGCCTTAAGTTGAACGGAGTCCGGAACT
7042	7042_PDR12 Repair Oligo FW	Repair fragment for *PDR12* deletion	AAAATTGAAAATAAAAATTGTGTGTTAAACCACGAAATACAAATATATTTGCTTGCTTGTTTTTTTATTAATAAGAACAATAACAATAAATCTGTAAACCTTTTTTTTAAGTGAAAATTA
7043	7043_PDR12 Repair Oligo RV	Repair fragment for *PDR12* deletion	TAATTTTCACTTAAAAAAAAGGTTTACAGATTTATTGTTATTGTTCTTATTAATAAAAAAACAAGCAAGCAAATATATTTGTATTTCGTGGTTTAACACACAATTTTTATTTTCAATTTT
Diagnostic primers for target gene editing			
2496	2496_FW-conf-upstrm	Confirmation *CAN1* deletion	CGGGAGCAAGATTGTTGTG
2681	2681_ADE2dsHRV	Confirmation *ADE2* deletion	GGACACTTATATGTCGAGCAAGA
3603	3603_ade2-AMDS fw3	Confirmation *ADE2* deletion	TCTAAGTACATCCTACTATAACAATC
5821		Confirmation *CAN1* deletion	
10228	10228_10228_His4 del check Fw	Confirmation *HIS4* deletion	AACAGCCGTGGAATCGTTGC

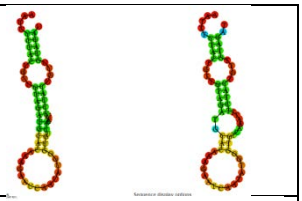
10229	10229_10229_His4 del check Rv	Confirmation *HIS4* deletion	TGGGTAGTGCCTTGTGATCC
10803	10803_10803_delADE2seqFW	Amplification of *ADE2* target site	CGGAAGCTTTGGAAGTACTG
10804	10804_10804_delADE2seqRv	Amplification of *ADE2* target site	GAGTCGCCAATGCTCTTTCG
10805	10805_10805_delCAN1seqFW	Amplification *CAN1* target site	AATCCGTTCCAAGAGCCATC
10806	10806_10806_delCAN1seqRv	Amplification *CAN1* target site	GCCGCATAATAAGCCAAGCC
10791	10791_10791_HIS4targetcheckFw	Amplification *HIS4* target site	CGTCCGCAAATTCCATGCTG
10792	10792_10792_HIS4targetcheckRv	Amplification *HIS4* target site	CACATCGGCATCTTCATCGG
10793	10793_10793_PDR12targetcheckFw	Amplification crPDR12-1 target site	ACCACGGCAGGTGCTTATTC
10794	10794_10794_PDR12targetcheckRv	AmplificationcrPDR12-1 target site	GGCGCTACACAACATTTCCC
254	254_PDR12 - CTRL RV	Confirmation *PDR12* deletion	GGAAGATAATAGGCGAGCTC
3997	3997_PDR12 KOfw	Confirmation *PDR12* deletion	CTTTCGGCTATGGCTCATAC

Supplementary Table S2.

Target sequences for Cpf1-mediated *ADE2*, *CAN1*, *HIS4* and *PDR12* editing. Different target sequences were designed to get a better understanding of the CRISPR-Cpf1 system in *S. cerevisiae*. Relevant characteristics are reported. The RNA secondary structure was predicted with the RNAfold web server, which provides two RNA secondary structures: minimum free energy (left) and centroid (right) prediction. The secondary structure is predicted with the crRNA consisting of the target sequence interspaced by 19 nt DR sequences after maturation by *Fn*Cpf1.

Plasmid names	crRNA name	Target sequence (5' to 3')	AT content (%)	Targeting position from ATG (nt)	PAM (5' to 3')	RNA secondary structure
		ADE2 (1716 nt)				
pUD520	crADE2-1	CGGGCACACCGATGACAGGAAGTGG	36	1438	T(TTA)	
pUD521	crADE2-2	CGGCGTACAAAGGACGATCCTTCAG	44	523	T(TTT)	
pUD438 pUD439 pUD440 pUD552 pUDE708 pUDE709 pUDE710	crADE2-3	CCGGTTGTGGTATATTTGGTGTGGA	52	743	T(TTC)	
pUD522	crADE2-4	CATTCAATTGTGCAAATGCCTAGAG	60	1498	T(TTA)	
pUD523	crADE2-5	ATTTGGGATGTTTTACTTGAAGATT	72	247	T(TTA)	
pUD524	crADE2-6	ATTAAATGCTCTTTTTGAATATATT	84	317	T(TTG)	

		CAN1 (1773 nt)				
pUD439 pUD440 pUD550	crCAN1-1	TTTGGTCTATCAAAGAACAAGTTGG	64	1204	T(TTA)	
pUDE720	crCAN1-2	ATTGGTTTATCCACACCTCTGACCA	64	322	T(TTC)	
pUDE721	crCAN-3	AAGGTACTGAACTAGTTGGTATCAC	60	893	T(TTC)	
pUDE722	crCAN1-4	CCACATATCTTCAACGCTGTTATCT	60	1123	T(TTG)	
		HIS4 (2400 nt)				
pUD440	crHIS4-1	CCCAATGTAAGGAGATTGTGTTTGC	56	1514	G(TTG)	
pUDE712 pUDE708	crHIS4-2	TCCAATCAATTCATGGTAAAACAAA	72	328	T(TTC)	
pUDE713 pUDE709	crHIS4-3	CTAAAGATTCTAGCCCCACCAAACC	52	730	T(TTA)	
pUDE714 pUDE710	crHIS4-4	GCATCTTGGCTAGCAATGAACAGAG	52	227	T(TTA)	
		PDR12 (4536 nt)				
pUD440	crPDR12-1	CATTTATGAAATATGAAGCTGGTGC	64	1847	A(TTC)	
pUDE723	crPDR12-2	GTCGAGATCGAACCATGACGATGAT	52	39	T(TTC)	

pUDE724	crPDR12-3	GCACAAAGAATCAATATGGGTGTCA	60	2674	T(TTA)	
pUDE725	crPDR12-4	GCATATAAGCATGCTTGGAGAAATT	62	2269	T(TTC)