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Abstract

For targeted gene disruption in wild-type *Neurospora crassa*, 1000-bp of homologous sequences on either side of the cassette used for disruption is sufficient to give more than 10 % homologous recombination. We report here that varying the length of homology on each side seems to have different effects on the homologous recombination frequency.

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For targeted gene disruption in wild-type *Neurospora crassa*, 1000-bp of homologous sequences on either side of the cassette used for disruption is sufficient to give more than 10 % homologous recombination. We report here that varying the length of homology on each side seems to have different effects on the homologous recombination frequency.

Gene disruption based on homologous recombination is a critical strategy for genome analysis. We analyzed the effects of homology length and transformation methods on gene disruption by a simple method. We chose *ad-3A* as a target gene, because loss of gene function could be identified by pigment accumulation.

A 6-kb genomic fragment which covered the whole *ad-3A* gene and a 5-kb adjacent region was cloned on pBluescript® SK$^+$. We inserted the 1.4-kb *hyg*^{r} gene of pCB1003 (supplied by FGSC) at the *Eag*I site of *ad-3A*. DNA fragments of varying lengths were amplified by PCR from this plasmid. Primers were designed to produce constructs that had 100 to 2000-bp *ad-3A* genomic sequence on each side of the *hyg*^{r} gene. The albino strain (FGSC 4934: *a al-2 cot-1 pan-2*) was used as the transformation recipient. Transformation of the DNA fragments was done in two ways, electroporation and classical spheroplast fusion. Electroporation was done based on published methods (Ninomiya *et al.* 2004). Five micrograms of DNA was mixed with 100 microliter of the suspension in 2-mm electroporation cuvets. Pulse conditions were as follows. Charging voltage: 1.5 kV, Maximum voltage range/timing mode: 2.5 kV/resistance, Capacitance timing: 50 uF, Resistance timing: R6 (186 ohm). After a one-shot pulse, the suspension was immediately mixed with 1 ml of ice-cooled minimal medium supplemented with adenine and incubated for three hours at 30 C before plating. Three ml of top agar medium kept at 50 C was mixed with the suspension and poured on minimal medium containing hygromycin, Ca-pantothenate and adenine sulfate. Spheroplast formation and transformation were done as described (Tomita *et al.* 1993) with a few modifications. The LYSING ENZYMES preparation from *Trichoderma harzianum* (SIGMA, L-1412) was used at 10 mg/ml for digestion and was incubated with conidia for 30 to 60 min. Five micrograms of DNA was used for transformation of 10^7-10^8 conidia. In both methods, colonies were collected after three-day incubation on the selection medium and transferred to the same medium in culture tubes. Conidial and hyphal color of transformants was examined after two weeks, and DNA was isolated if needed.

The results in Table 1 show that length of homology affects homologous insertion as expected. Ratios of colored transformants (disruptants) were low in these experiments because we selected by eye, and only reliably scorable colonies were counted. Most transformants were white, which suggests that most events were ectopic insertions. However, coloration by *ad-3A* disruption is dependent on the ratios of nuclei containing disrupted and nondisrupted genes . If transformants are heterokaryotic for transformation, the appearance of coloration by ad-3A disruption would be less apparent. Thus, we surely underestimated insertion frequencies. This inaccuracy in scoring homologous disruptants, which would underestimate their number, did not affect the trends observed, however.

We did PCR for all transformants of the 3000-3000 group in experiment 1. Two sets of primers were constructed for checking transformation events. One contained a primer set to detect an original *ad-3A* region. The two primers of this set corresponded about 3kbp upstream and 4kbp downstream, respectively, of the *ad-3A* translation start site. Neither of these sequences was contained in the DNA fragments used in transformation. The other set contained a primer deduced from a central region of *hyg*^{r} gene and one of the primers of the first set. We did PCR until one of the sets or both sets amplified appropriate length fragments. In addition to the 26 transformants initially identified by eye, half of the rest (42/84) were proved to have target disruption.
These results indicate that (1) *At least 1000 bp of homology to both sides of a target gene is needed to get a reasonable number of disruptants*. Longer homology seems better, but even 1000bp homology on both sides results in more than half of the transformants containing a disrupted gene. (2) *Homology length on one side affects insertion frequency differently from that on the other side.* Homology in the 5' direction in the target gene seems more important than that in the 3' direction. Another possibility we cannot exclude is that the direction of the marker gene with respect to the homologous flanking sequences affects homologous recombination. The *hyg*^{r} gene was a reverse direction to the *ad-3A* gene in this study.
In comparison of transformation methods, the both methods gave similar efficiencies of targeted disruption. However, more transformants were obtained in electroporation with a less tedious procedure. We recommend electroporation to get disruptants.

Table 1. Effect of homology length on gene disruption

Homology length (bp)		Exp. 1		Exp. 2		Exp. 3		Average % of
left side	right side	Drug resistant	% of colored	Drug resistant	% of colored	Drug resistant	% of colored	colored
Electroporation								
3000	3000	110	23.6	140	12.9			18.3
2000	2000	126	12.7	100	9			10.9
1000	1000	126	11.9	120	13.3			12.6
500	500	127	6.3	100	6			6.2
200	200	34	0	50	2			1
100	100	125	0.8	100	1			0.9
2000	500	23	8.7	115	12.5	100	14	10.6
500	2000	16	6.3	100	6	100	10	6.2
2000	200	100	13	136	12.5			12.8
200	2000	100	2	135	3.7			2.9
Enzymatic method								
2000	2000	28	3.6	35	11.4			7.5

References

Ninomiya, Y., K. Suzuki, C. Ishii and H. Inoue, 2004. Highly efficient gene replacements in Neurospora strains deficient for nonhomologous end-joining. Proc. Natl. Acad. Sci. USA 101**:** 12248-12253.

Tomita, H., T. Soshi and H. Inoue, 1993. The *Neurospora uvs-2* gene encodes a protein which has homology to yeast Rad18 with unique zinc finger motifs. Mol. Gen. Genet. 238**:** 225-233.