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Abstract

Cloning structural and regulatory genes of Neurospora and their manipulation in vitro will allow detailed molecular study of gene structure and regulation.

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Cloning structural and regulatory genes of Neurospora and their manipulation ***in vitro*** will allow detailed molecular study of gene structure and regulation. An efficient transformation procedure is important in such cloning approaches and for the reintroduction of engineered sequences. Two methods for transformation of Neurospora are available, one of which employs spheroplasts prepared from germinated conidia (Case et al, PNAS 76: 5259, 1979), and a second which uses lithium acetate treatment of germinated conidia to make them competent for transformation (Dhawale et al, Current Genet. 19: 77, 1984).

An unanswered question of some interest is whether or not major differences occur between Neurospora strains in transformation frequency, and indeed whether some strains may not be transformable at all (or at a rate insufficient for experimental purposes). This consideration would be particularly important if a genomic library were being screened for a particular gene by complementation of the corresponding mutant. Transformation of Neurospora crassa using the cloned qa-2$^+$ gene has been routinely carried out with a standard strain (inl;qa-2;aro-9, alleles 4701, M246 and M6-11, respectively) constructed and made freely available to other laboratories by Dr. Mary Case. The purpose of the work reported here was to examine the transformation with qa-2 of a series of derived strains with varied genetic backgrounds to gain some insight into how the recipient may effect the transformation process.

The standard inl;qa-2;aro-9 transformation host strain was crossed with a series of wild-type stocks with different genetic backgrounds and with a few mutant strains of particular interest to our laboratory. Progeny with the inl;qa-2;aro-9 markers were isolated for each cross and, when appropriate, examined for the presence of other mutations. Transformation was accomplished with the lithium acetate protocol using selection of qa-2$^+$ which permits growth without added aromatic amino acids, Plasmid pSD3, a derivative of the qa-2$^+$-containing plasmid pVK57 that lacks the small BamHI fragment, was linearized with restriction endonuclease Bgl II, which cuts pSD3 at a single site within the cloned Neurospora DNA. This linear qa-2$^+$ plasmid DNA was used in all transformations. We first asked how much experimental variation in transformation frequency occurs when a single Neurospora strain is independently transformed a number of times. Table I summarizes the results of 11 separate transformations of the standard inl;qa-2;aro-9 host strain constructed by Mary Case. In these experiments, the viability of the lithium acetate-treated conidia averaged 29% and showed a range from as low as 11% up to 50%. Transformation rates were corrected for 100% survival so that meaningful comparisons could be made. The transformation frequency obtained with the standard strain varied over a U-fold range, with an average corrected value of 105 transformants per microgram of DNA (Table I). This variation was observed despite every attempt to eliminate obvious experimental differences, such as age of conidia or quality of the transforming plasmid DNA. We presume that the different degrees of viability and the different transformation rates observed in these experiments reflect natural variation inherent in this experimental procedure.

We then examined the transformation of the series of derived inl;qa-2;aro-9 strains obtained by crossing the standard host with various other stocks. The viability of lithium acetate-treated conidia of all of these strains falls within the same range observed with the standard host (Table II). Only two independent transformations were carried out with most of these strains because of the large amount of work required. Nevertheless, the results clearly indicate the presence of an obvious strain difference in transformation rate although all twelve stocks tested transformed at a readily detectable level (Table II). Overall, a 30-fold range in transformation frequency was observed among the 13 different strains examined, more than double the variation observed in repeated transformations with the same standard stock. It is noteworthy that none of strains tested transforms at a much higher frequency than the standard strain now routinely used. Strain 179A did appear to transform at a rate approximately 2-times greater than observed with the standard host, whereas strains 191A, 289 and 290 yield a transformation frequency comparable to standard strain. Other strains, e.g. numbers 288 and 305, showed an average transformation rate about 3-times lower than the standard stock, although these values are near the lower ones observed with the standard.

The inl;qa-2;aro-9 strains designated 283, 284, 288, 289 and 290 are all progeny of a single cross and yet show differences in transformation rate.It is noteworthy that those strains carrying additional mutations, such as nit-2, cys-3 and os-1 are all subject to qa-2^{+} transformation. When screening a genomic bank to directly clone a specific gene via complementation of a Neurospora mutant, we have previously constructed a mutant strain that also carried qa-2 and aro-9 so that it could be tested with the cloned qa-2^{+} gene to insure that it was capable of being transformed. The results presented in this paper demonstrate that 13 different derived strains with varied genetic backgrounds can all be transformed at readily detectable levels, although some strains are clearly less efficient as hosts. Thus, one should be able to utilize nearly any Neurospora strain with reasonable confidence that it should indeed be competent for transformation, although caution should be exercised when using a mutant that might affect recombination or have a possible influence on the transformation process. These results indicate that strain-dependent differences in the actual transformation rate occur in addition to the background of variation observed for multiple transformations of a single strain.The genetic basis for these differences is completely unknown. One particularly important outcome is our conclusion that the standard inl;qa-2;aro-9 mutant strain constructed by Dr. Mary Case and widely used by many laboratories is indeed an excellent host strain for transformation.

Table I

Results of independent transformation of the standard inl;qa-2;aro-9 strain

Experiment	Transformants/µg	Viability (%)	Corrected Transformants/µg*
1	56	25	224
2	10	31	32
3	15	11	136
4	82	30	273
5	20	33	61
6	39	42	93
7	16	30	53
8	23	30	77
9	10	31	32
10	17	11	155
11	11	50	22
		Mean = 29 Std. dev. = 11	Mean = 105 Std. dev = 79

*Only stable transformants were included; corrected for 100% viability.

Table II

Transformation of various derived strains

OSU Strain Number*	Outcrossed with strain (FCSC no.)	Mean viability %	(std. dev.)	qa-2$^+$ Transformant/µg DNA** %	(std. dev,)
249	(= standard strain)	29	(11)	105	(79)
179A	W.T. Lein 7A (847)	24	(0.5)	203	(41)
181A	W.T. Costa Rica (651)	21	(7)	41	(26)
187A	W.T. Puerto Rico (429	42	(3)	18	(5)
191A	W.T. Tatum	14	(0.5)	116	(8)
283	nit-2 (982)	39	(1)	49	(27)
284	nit-2 (982)	47	(5)	19	(2)
288	nit-2 (982)	33	(14)	15	(5)
289	nit-2 (982)	12	(1.5)	75	(15)
290	nit-2 (982)	21	(9)	94	(4)
305	os-1		(16)	12	(3)
197A	W.T. 74-OR23-1 (988)	33	--	15	--
268A	cys-3 (1090)	41	--	37	--

*All strains are inl;qa-2;aro-9, except that #305 (os-l;qa-2;aro-9), which was obtained from R.L. Metzenberg, lacks inl. Strains #283 also carry the nit-2 mutation and #268 carries cys-3.

**mean value, corrected to 100% viability, for 2 independent transformations is given, except that 197A and 268A values are for a single experiment.

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