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Construction of testers for reversion assay.

The ad-3 forward mutation assay system developed by de Serres and coworkers has been used in our laboratory for research on DNA repair end mutagenesis. This system gives much information on mutagenesis, but is expensive to use and is labor intensive. Therefore, we desired to develop a reversion assay system which is more convenient.

Dr. T. Ishikawa (University of Tokyo) had isolated and kept many ad-8 mutants in his laboratory. We screened revertability of seventy eight ad-8 mutants by the spot test method of Ong (1978, Mutation Res. 53:297-308) and selected two mutants on the basis of reversion specificity. One of them, ad-8(E193), was highly reverted by MNNG but not by ICR170 and the other, ad-8(E146) was highly reverted by ICR170 but not by MNNG. Each of these mutants was back-crossed to standard strain 74-OR31-14a(al-2 cot-1 pan-2 a) twice and two testers (T26 and T28) were constructed. Their genotypes are shown in Table 1. Responses of both testers to several mutagens are shown in Table 2. These data are from the plate test method of Ong. The results indicate that T26 responds to frameshift mutagens and T28 to basepair substitution mutagens. These testers may be useful along with Ong's strains N23 end N26, which had been constructed for the assay of environmental mutagens and are useful for quantitative and qualitative comparison of DNA repair ability and mutagen specificity.

Table 1. Tester strains

Tester	Strain #	FGSC #	Genotype (allele)			
T26	C3-T26-14a	5071	ad-8(E146)	al-2(Y112M38)	cot-1(C102(t))	pan-2(Y387-15.7)
T28	C3-T28-39a	5071	ad-8(E193)	al-2(Y112M38)	cot-1(C102(t))	pan-2(Y387-15.7)

Table 2. Induction of reverse mutations with plate test in T26 and T28

Mutagen	Concentration		No. of revertants per plate T26	T28	Mutagen	Concentration		No. of revertants per Plate T26	T28
MNNG	0	(ug/plate)	1	2	EMS	0	(ul/plate)	0	1
	1		0	60		10		0	51
	5		1	1070		20		2	290
	10		3	>1000					
					4NQO	0	(ug/plate)	0	2
ICR170	0	(ug/plate)	1	1		0.2		2	13
	5		84	3		0.5		4	32
	10		130	3					
	20		145	1	UV	0	(erg/mm2)	0	1
						1000		2	15
MMS	0	(ul/plate)	0	1		2000		8	23
	1		10	29		4000		11	20
	2		2	8					

Spontaneous reversion of T26 and T28 was 0.5 and 1.5 per plate, respectively, on average

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