A novel mutation assay with a non-selective protocol using a next-generation DNA sequencer

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GENOTOXICITY TESTS

In vitro tests
(using cell culture or bacteria)

1. Rec assay
2. *umu* test
3. **Reverse mutation assay (Ames test)**
5. Forward mutation assay
6. Unscheduled DNA synthesis test
5. Sister chromatic exchange test
6. Chromosomal aberration test
7. Micronucleus assay
8. Comet assay

etc.

In vivo tests
(using animals)

1. Micronucleus assay
2. Sister chromatic exchange test
3. Chromosomal aberration test
4. Unscheduled DNA synthesis test
5. *Pig-a* assay
6. Transgenic animal gene mutation assay
7. Drosophila Spot test
8. Comet assay

etc.

GENOTOXICITY TEST Conventional method

Ames test

- Data can be available within a week
- No need of special facilities
- Reproducible and reliable data
- Accumulation of reliable data in accordance with GLP
- Data are the number of colonies

GENOTOXICITY TEST

From a different perspective

Identification of whether the chemical is hazardous is the principal aim of Ames test.

We can directly detect the mutation induced by the chemical in the genome with NGS

A NEXT GENERATION SEQUENCER MUST BE USEFUL !

GENOTOXICITY TEST

Novel method

Whole genome sequencing

- Data can be available within a week ✓
- The special facility is required ↓
- Reproducible and stable data ✓
- No data at present ↓

◆Data is **ACTUAL** base changes !

COMPARISON

Ames test	Whole genome sequencing
5 strains and 4 or more doses	One strain is enough and not many doses are needed
Frameshift or base substitution	Any type of mutation
Minimal plates for two-night incubation	LB plates for one night incubation
His^+ selection	Non-selective method
5000 µg/plate for a top dose, and one hundred plates	Lower doses, ~10 plates
Precipitation or killing effect of samples may disturb the assay	Colony formation is minimum requirement

PROCEDURE

Conventional Ames test

Test chemical: AF-2 (0.01μg/plate)
DMSO as a solvent control
Strain: TA100 , without S9mix

Randomly pick up the revertants
Few clones for each condition
Prepare the genomic DNA

Whole genome sequencing of the clones using an NGS, Miseq

The expected base substitutions at the target site of His^+ reversion

Treatment	Clone ID	Frequency (%)	Coverage	Gene	Site	Mutation	Amino Acid Change
DMSO	1	100	14	*hisG*	206	C>A	Pro69His
	2	100	17	*hisG*	205	C>A	Pro69Ile
	3	100	14	*hisG*	206	C>A	Pro69His
	4	100	14	*hisG*	205	C>A	Pro69Ile
	5	100	17	*hisG*	205	C>T	Pro69Phe
AF-2	6	100	29	*hisG*	206	C>A	Pro69His
	7	100	15	*hisG*	206	C>A	Pro69His
	8	100	37	*hisG*	206	C>T	Pro69Leu
	9	100	10	*hisG*	205	C>T	Pro69Phe
	10	100	26	*hisG*	205	C>G	Pro69Val

Matsuda et al., Genes & Environment, 35, 53-56, 2013

RESULTS - 1

Base Substitutions Induced on the Whole Genome of the Ames tester Strain TA100 Treated with AF-2

Treatment	Clone ID	Frequency (%)	Coverage	Gene	Site	Mutation	Seq. context	Amino acid change
DMSO	2	100	31	*hypE*	831	T>A	GAT**[T]**GCC	
AF-2	6	100	29	*yraP*	25	G>A	GCA**[G]**TCC	V 19 I
	8	96.7	30	*cueO*	964	C>T	CCG**[C]**TGC	
	8	100	29	*ybiR*	832	C>A	GCA**[C]**TGT	L278M
	8	100	38	*xseA*	996	C>G	GGC**[C]**AGG	
	10	100	27	*hisG*	231	G>C	GCT**[G]**GAA	

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RESULTS - 2

ENU treated strain YG7108*

Induced Reverse Mutations in Ames Test and Base Substitutions Induced in the Whole Genome

ENU	Ames test	Whole genome sequence (per genome)			
µg/plate	revertants/plate	total	G:C to A:T	A:T to G:C	others
0	17	0	0	0	0
		0	0	0	0
		0	0	0	0
		0	0	0	0
50	3,250	12	12	0	0
		19	16	2	1(GCtoCG)
		14	14	0	0
		8	8	0	0
250	8,953	64	63	1	0
		62	57	4	1(GCtoTA)
		48	44	4	
		71	65	5	1(Gcto CG)

ADVANCED PROTOCOL

No selection!

I. Treatment with the test chemical is conducted in liquid medium.

II. The treated culture is spread onto an LB plate after appropriate dilution.

III. Colonies are obtained without phenotypic selection.

IV. Genomic analysis with a next-generation DNA sequencer is performed for a few randomly chosen colonies.

RESULTS - 3

Mutation spectrum of the genome in TA1535 treated with ENU with no selection

Pre-incubation time (min)	x 10^6 cells	Mutation/ genome	Ave.	GC to AT	GC to TA	GC to CG	AT to GC	AT to CG	AT to TA	Insertion	Deletion
0	295	0	0	0	0	0	0	0	0	0	0
		0		0	0	0	0	0	0	0	0
		0		0	0	0	0	0	0	0	0
20	47	19	22	16	0	0	3	0	0	0	0
		18		17	0	0	1	0	0	0	0
		29		27	0	0	1	0	0	0	0
40	46	4	9	4	0	0	0	0	0	0	0
		22		20	0	0	1	0	0	0	1
		1		1	0	0	0	0	0	0	0
60	43	16	16	16	0	0	0	0	0	0	0
		9		6	0	0	2	0	1	0	0
		23		21	0	0	2	0	0	0	0

CONCLUSION

Whole genome sequencing would realize

THAT

ANY MUTATIONS ! can be detected

in **ONE STRAIN !**

with **MORE PRECISE DATA !**

at **LESS COST !**

In the Future

- Considering the remarkable recent advances in the performance of DNA sequencers, it would soon be possible to easily determine whole genomes of rodents or cultured human cells exposed to chemicals.
- However, taking into consideration of the principal aim of Ames test, that is, determining of hazard identification on DNA induced by chemicals, bacteria still have an advantage due to its small size of genome and identical composition of DNA to the other organisms.