Is there space for an industry focused research in academia?
II. An industry point of view

Antonio Rodríguez

Department of Toxicology, ESTEVE

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Interpretation of Results: False Positives-*in vitro*

- General Postulate in the genotoxicity field:
  - Academia groups doing basic research generate know-how
    - Mechanism on DNA Replication, Repair and Mutagenesis
    - Industry and CROs apply this know-how to the genotoxicity assessment

Gentoxicity Assays

Assay (Regulatory)	Academic group	Year	Publication
Ames Test	U. of California	Early 70's	Ames 1971
***In vitro* mammalian mutation assay**	MIT U. of North Carolina Research Triangle Park,	Late 70's	Clive 1979 MLA Skopek 1978 HA
***In vivo* and *in vitro* micronucleus assay**	U. of California U. of Zurich	Early 70's	Schmid 1975 Heddle 1973
Assay (Screening)	**Academic group**	**Year**	**Publication**
GreenScreen	U. of Manchester	Late 90's	Walmsley 1997

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- New techniques to assess gentoxicity potential
- Refinement of the existing assays

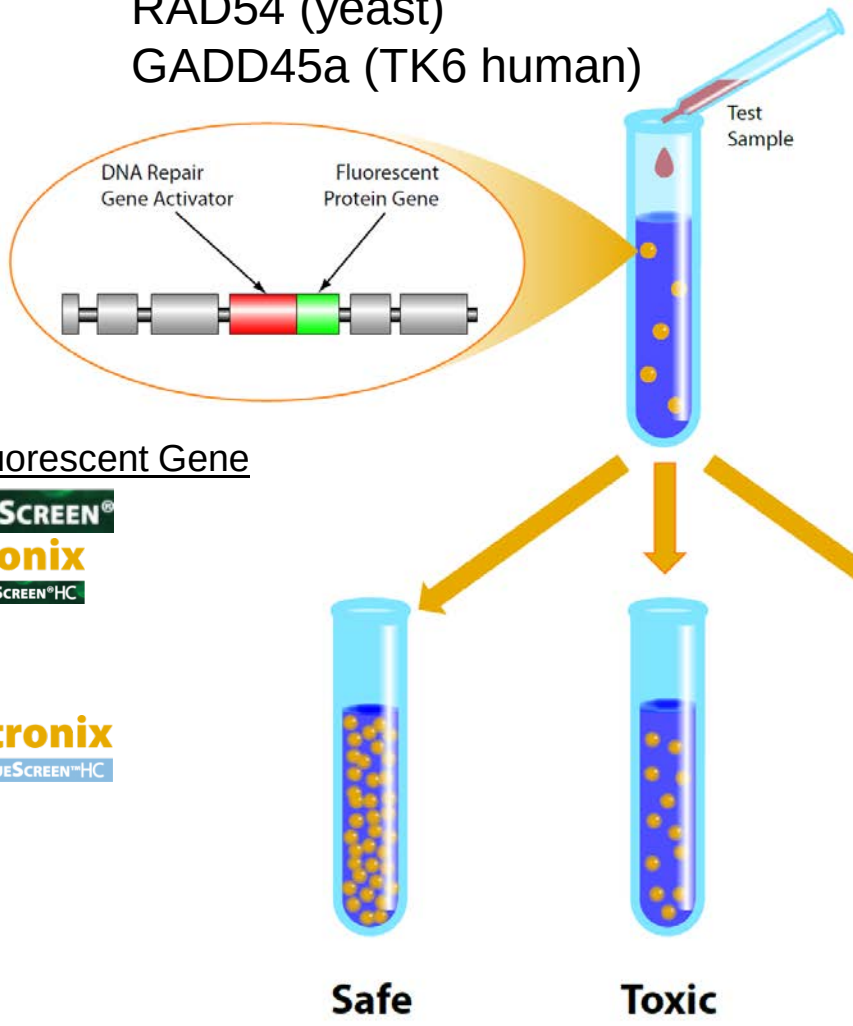
GreenScreen

gentronix

MANCHESTER 1824
The University of Manchester

Richard Walmsley

RAD54 (yeast)
GADD45a (TK6 human)



Reporter Fluorescent Gene

GFP GreenScreen® gentronix GreenScreen®HC

Gluc gentronix BlueScreen™HC

New techniques to assess gentoxicity potential

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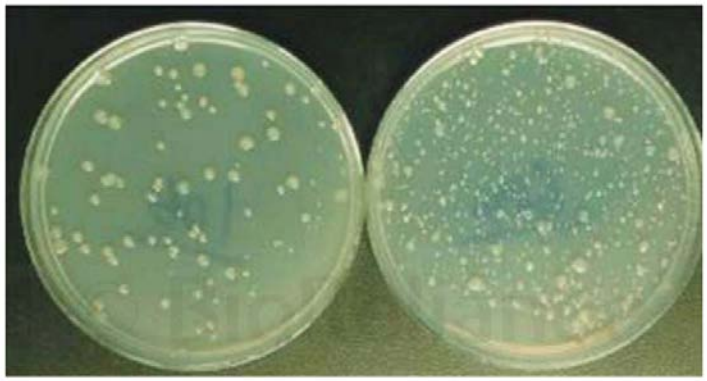
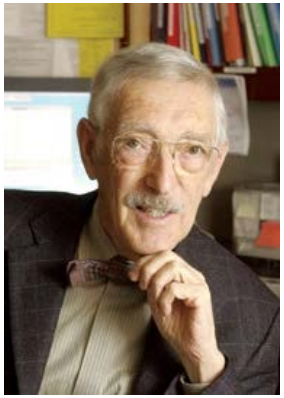
Companies using Greenscreen

CROs using Greenscreen

Ames Test



Solvent control plate Positive mutagen plate



Bruce Ames

Refinement of the existing assays

ELSEVIER

Mutation Research 483 (2001) 1–11

Fundamental and Molecular Mechanisms of Mutagenesis

www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Accelerated publication

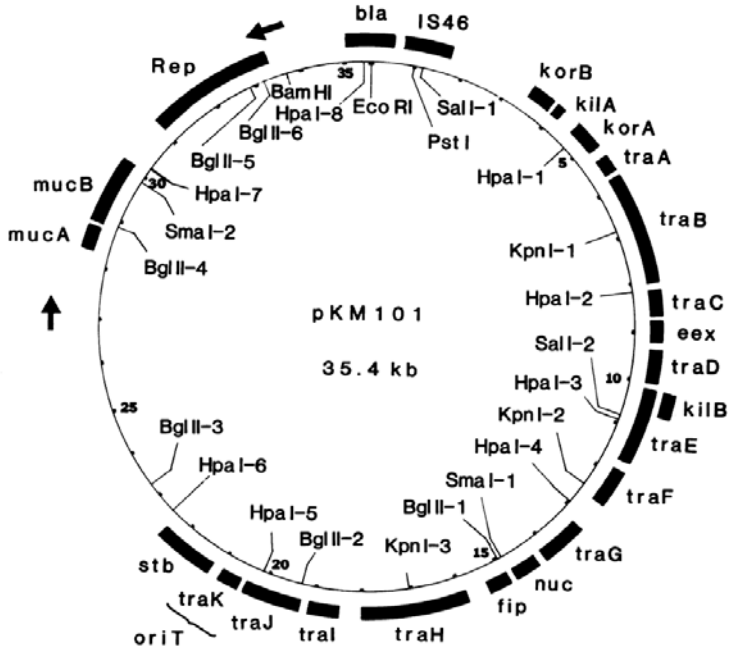
The *ΔuvrB* mutations in the Ames strains of *Salmonella* span 15 to 119 genes

Steffen Porwollik[a], Rita Mei-Yi Wong[a], Simon H. Sims[b], Roel M. Schaaper[c], David M. DeMarini[d], Michael McClelland[a,*]

[a] *Sidney Kimmel Cancer Center, 10835 Altman Row, San Diego, CA 92121, USA*
[b] *Sigma-Genosys, 1442 Lake Front Circle, The Woodlands, TX 77380, USA*
[c] *Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA*
[d] *Environmental Carcinogenesis Division (MD-68), US Environmental Protection Agency, Research Triangle Park, NC 27711, USA*

Received 5 June 2001; received in revised form 17 July 2001; accepted 25 July 2001

Ames Tester Strain	Number of deleted genes	Kb deleted	% deleted genome
S. typhimurium TA98	119	125	2.6
S. typhimurium TA100	47	50	1.0
S. typhimurium TA1535	47	50	1.0
S. typhimurium TA1537	87	96	1.9

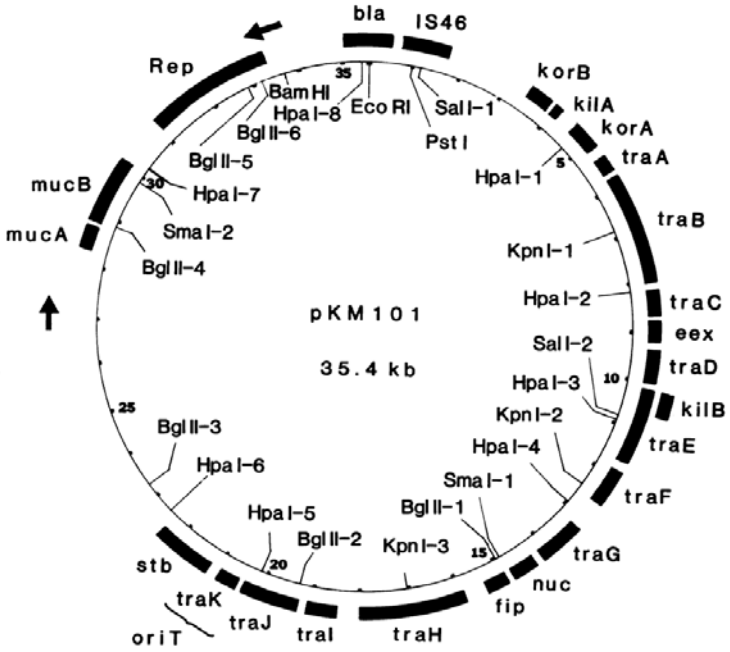


pKM101 Plasmid

Refinement of the existing assays

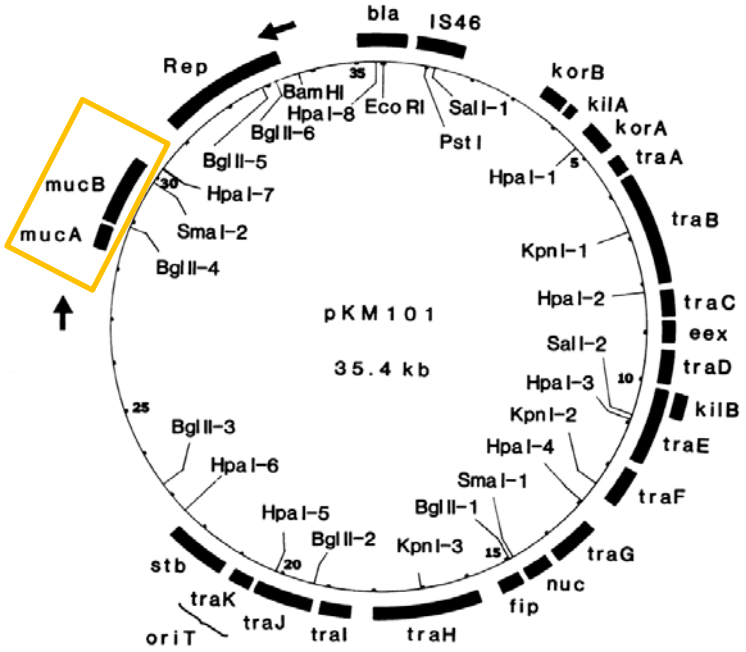
Bacterial strain	Target allele	Additional genotype	pKM101	Mutation detected
S. typhimurium				
TA97/TA97a [4]	*hisD6610*	*rfa*, Δ*uvrB*	+	Frameshift
TA98[1]	*hisD3052*	*rfa*, Δ*uvrB*	+	Frameshift
TA100[2]	*hisG46*	*rfa*, Δ*uvrB*	+	Base substitution
TA102[5]	*hisG428*	*rfa*, pAQ1 (Tc^R)	+	Base substitution, oxidative and cross-linking
TA1535[3]	*hisG46*	*rfa*, Δ*uvrB*	–	Base substitution
TA1537[4]	*hisC3076*	*rfa*, Δ*uvrB*	–	Frameshift
E. coli				
WP2 *uvrA*[5]	$trpE^{oc}$	*uvrA*	–	Base substitution and cross-linking
WP2 *uvrA* pKM101[5]	$trpE^{oc}$	*uvrA*	+	Base substitution and cross-linking

Refinement of the existing assays



- As pKM101 clearly enhanced UV induced mutagenesis in *S. typhimurium,* there was a widespread believing that *samAB* and *umuDC*$_{St}$ were poorly active since:
  - *umuDC*$_{St}$ showed a low level of UV-induced mutagenesis in *E. coli*
  - *samAB* was not contributing to UV-induced *hisG* reversion in *S. typhimurium*
  - *samAB* were not complementing *umuDC*$_{Ec}$ function when it was expressed in a low copy number plasmid

Refinement of the existing assays



Year 1975	Year 2000
Funtion of *mucAB* unknown	Function of *mucAB* known
umuDC as analogues accessory prot of DNA Pol III	Y-family DNA pol (PolRI)
mucAB genes enhance mutagenesis	How this new function is influencing Ames Test results?

Summary of ideas

- How Academic groups could help industry on the gentox field:
  - Training new professionals to be incorporated in the industry sector
  - New tools for gentox assessment (always updated know-how)
  - Participation in the validation process of new techniques
  - Refinement of the existing techniques
    - Ames test strains with genome deletions/pKM101
    - Cell lines used in the mammalian gentox test
      - CHO cell line chromosome number instability
      - Metabolic activity proficient cells (now S9)
  - Setting platforms to help industry on gentox assessment
    - Having the know-how and developing new techniques, why not offering services to the industry?
    - GLP compliant?

Thank you