FH CAMPUS WIEN
UNIVERSITY OF APPLIED SCIENCES

DIE HOCHSCHULE,
DIE MEHR KANN.

SenseAmes: Optimization of a miniaturized Ames test for better detection limits"

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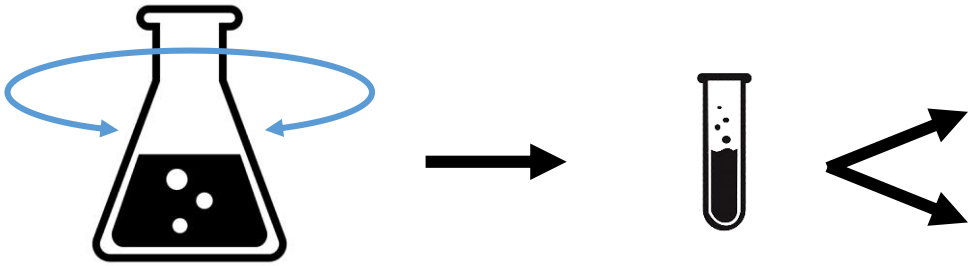
Ames Test

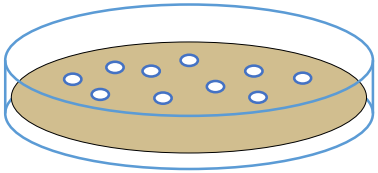
Salmonella bacteria with mutation in his pathway, strains TA100 and TA98

O/N culture
with external his

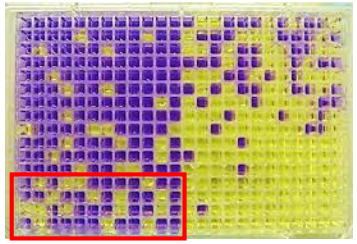
exposure
-> reverse
mutations

medium without
his: only mutated
bacteria will grow





pre-
incubation



liquid
culture /
MPF™

SenseAmes:
goals for a new Ames version

- Micro version of Ames Test
- Optimized for mixtures of genotoxins
- Low detection limits
- High throughput

SenseAmes

Micro version of Ames Test with high throughput

	SenseAmes:	MPF™	pre-incubation
• exposure volume	20 µL	250 µL	500 µL
• DMSO tolerance	10%	4-8%	4-8%
• typical sample volume	2 µL	10-20 µL	20-40 µL
• high throughput version	mult. plate	mult. plate	Petri dish
• samples / person / day	288	128	50

Advantages of low volume

- enriched samples have low volume

  typically starting material 300 mL – 1000x enriched 300 µL – 10.000x 30 µL
- dramatically improved statistics possible

Optimization for mixtures

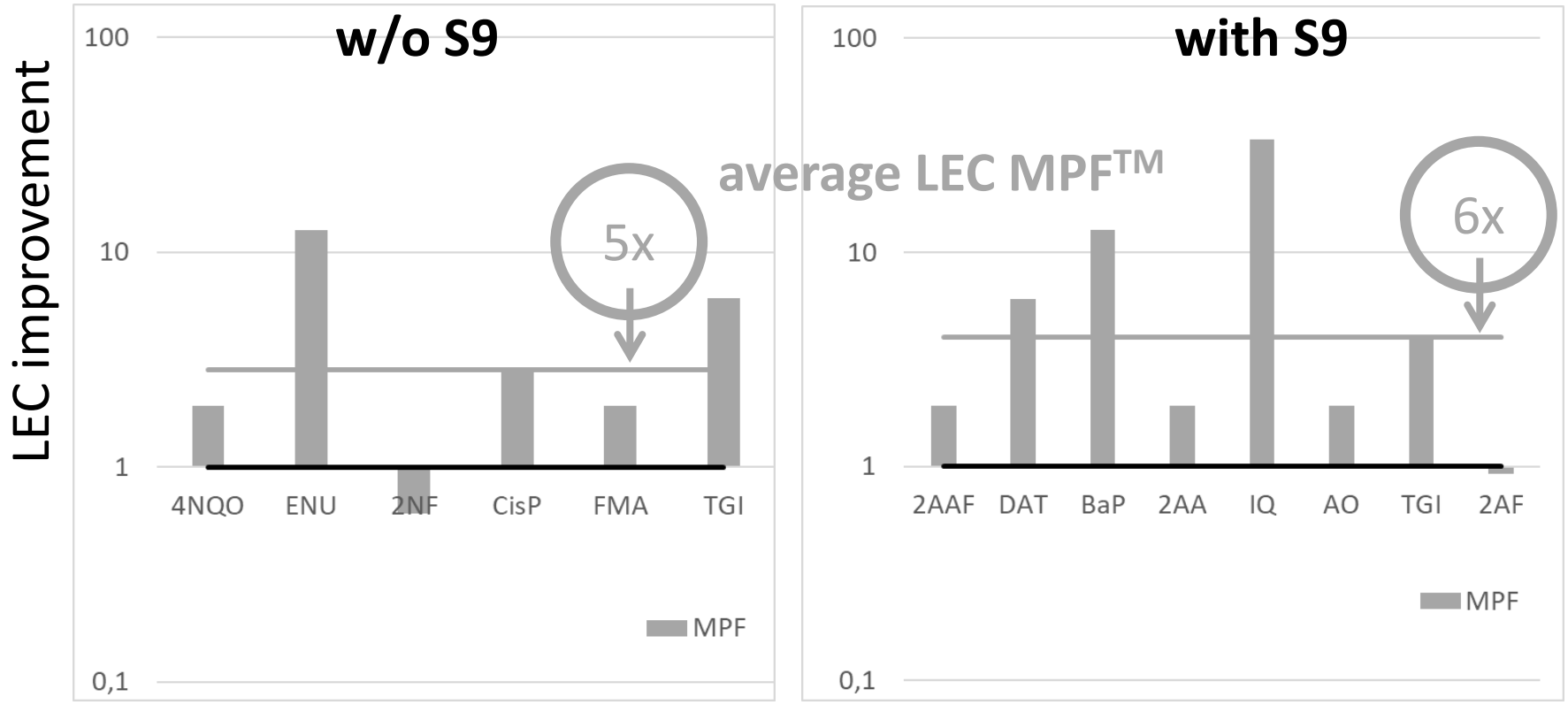
Genotoxins can be present at low concentrations - detection limits (LEC) of critical importance

- optimization of culture conditions/buffers/incubation conditions
- main goal: improvement of LEC values
- different results for different genotoxins – compromise necessary

High tolerance for toxic effects of samples

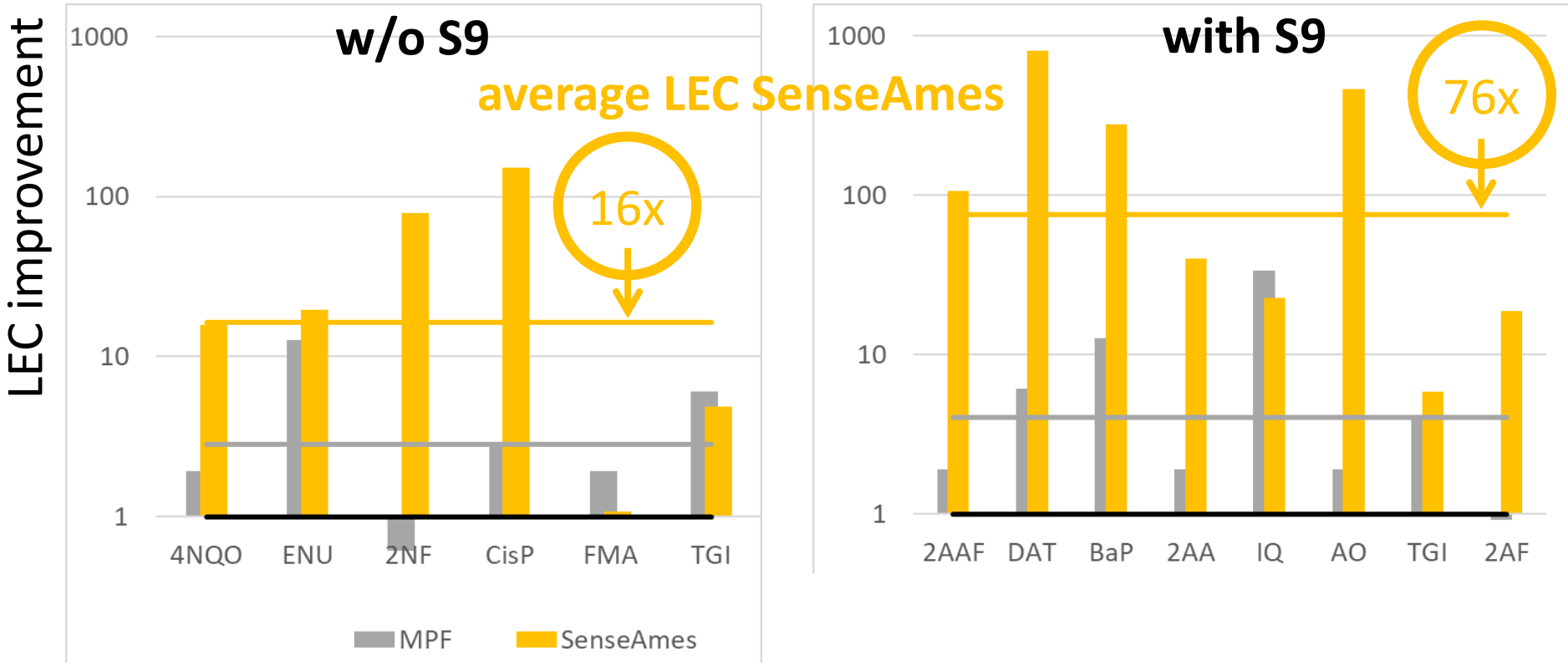
LEC improvement TA98 MPF™

calculated for final concentration of genotoxin in exposure buffer (independent from volume reduction)

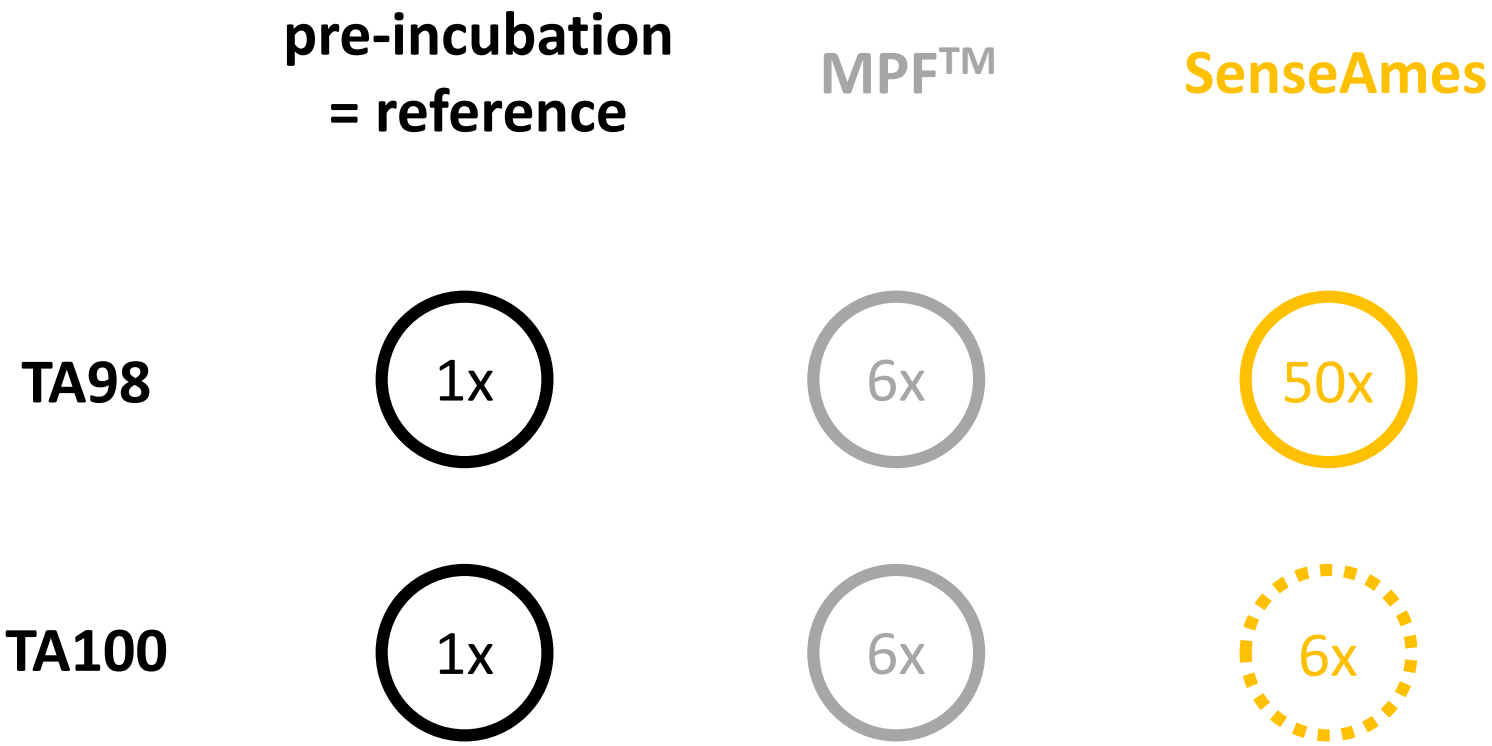


LEC reference pre-incubation = 1

LEC improvement TA98 SenseAmes



LEC improvements



Optimization for mixtures

Genotoxins can be present at low concentrations - detection limits (LEC) of critical importance

- optimization of culture conditions/buffers/incubation conditions
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High tolerance for toxic effects of samples

- toxic/inhibitory effects of samples
- spike experiments: reduced number of colonies for same amount of genotoxin
- optimisation: dependence on genotoxin / sample
- complex optimisations (buffer/conditions): different effects depending on extract and genotoxin

Reducing toxic effects

Samples w/o S9

- improved buffering (more PO4 buffer)

- higher cell number improves survival
  might titrate away toxic components

- optimisation of buffer components

Reducing toxic effects

Samples with S9

- improved buffering (more PO4 buffer)

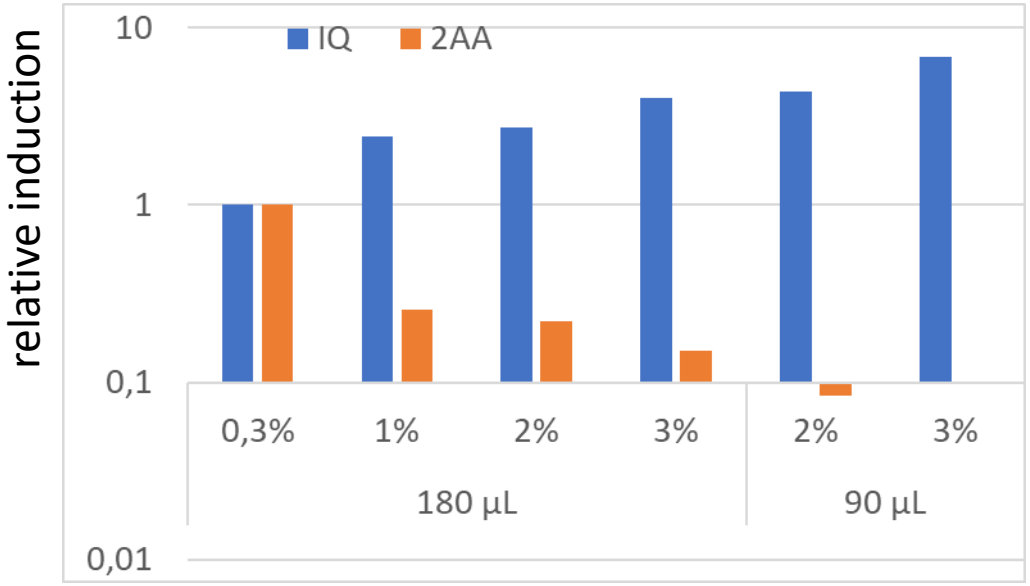
- higher cell number improves survival
  might titrate away toxic components

- amount S9

Amount S9

vol bact	180 µL				90 µL	
IQ	0,3%	1%	2%	3%	2%	3%
rel induction	1,0	2,4	2,8	4,0	4,4	6,9

vol bact	180 µL				90 µL	
2AA	0,3%	1%	2%	3%	2%	3%
rel induction	1,0	0,3	0,2	0,2	0,1	



Amount of S9 critical for pure substances and extracts

Summary

SenseAmes

- high throughput version of miniaturized Ames test
- low sample volume
- adaptations for mixtures
  - dramatic improvement of detection limits for TA98
  - optimizations for TA100 under way
  - high tolerance against toxic samples

Thank you for your attention!

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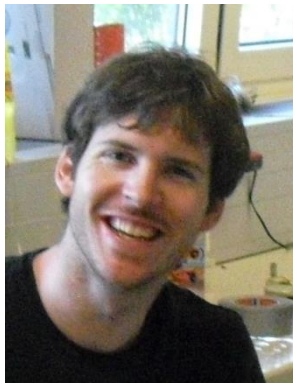

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