

# ***Comparison of different Genotoxicity tests for selected Migratox FCM samples***

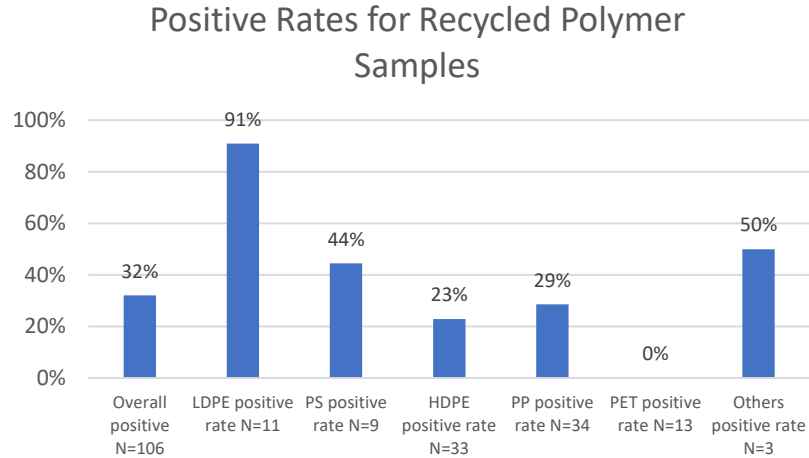
SECTION PACKAGING AND RESOURCE MANAGEMENT



# Overview

- Confirmation tests and false negative/positives
- Test method overview
- Results of selected FCM samples
- Conclusion and Outlook

# Background and Research Question



>High positivity rate detected in recycled PE/PP/PS

>90% of positive results in TA98+S9

>**Are our Ames positive samples true positives? Or are they Ames-specific artifacts?**

# Sensitivity and Specificity

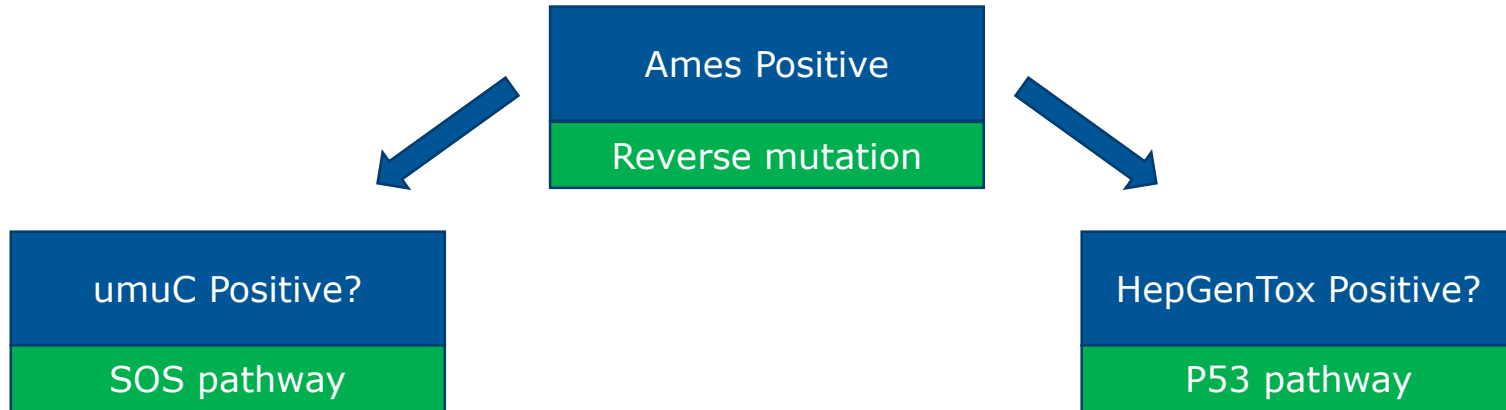
- > Endpoint: rodent carcinogenicity
- > Sensitivity: the ability to detect a carcinogenic substance as positive
- > Specificity: the ability to detect non-carcinogens as negative
- > Ames test results: ~60-80% correct
- > Data is for pure substances only!

Published performance parameters for a selection of *in vitro* genotoxicity assays

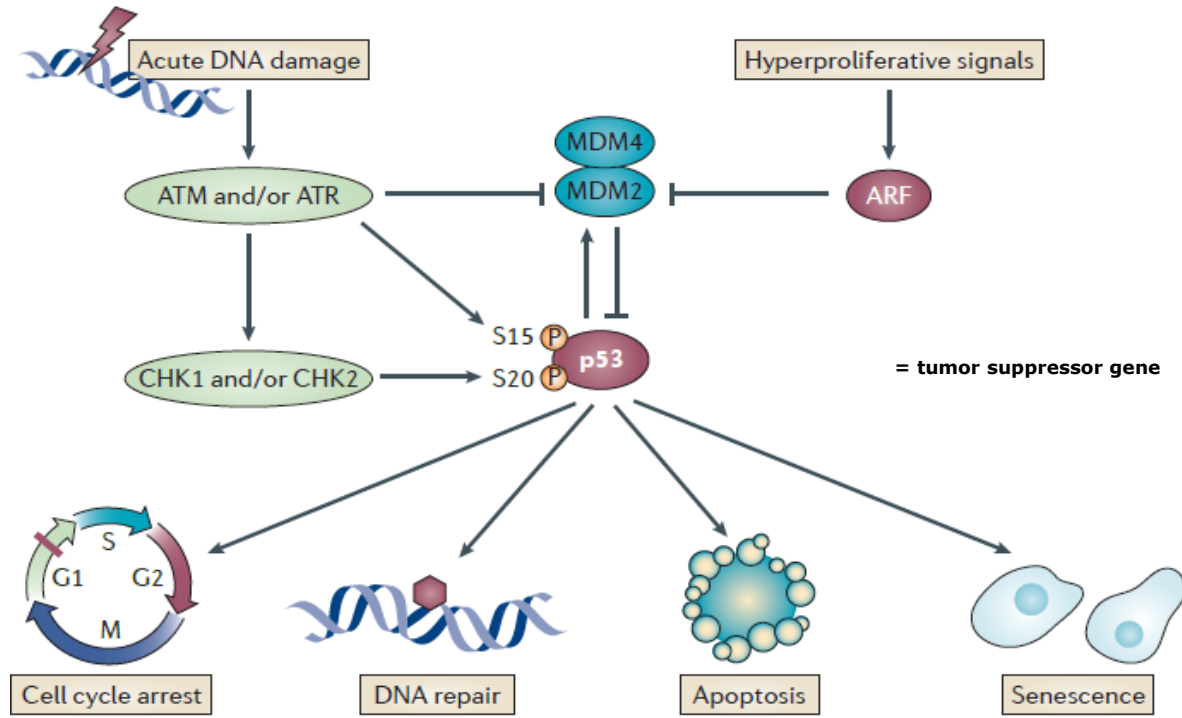
| Test name                  | Sensitivity (%) | Specificity (%) | References                                   |
|----------------------------|-----------------|-----------------|--|
| 1.Regulatory               |                 |                 |  |
| Bacterial reversion (Ames) | 60              | 77              | <a href="#">Kirkland et al., 2005</a>        |
| Chromosome aberrations     | 70              | 55              | <a href="#">Kirkland et al., 2005</a>        |
| Mammalian mutation         | 81              | 48              | <a href="#">Kirkland et al., 2005</a>        |
| 2.Screening                |                 |                 |  |
| Bacterial                  |                 |                 |  |
| SOS Umu C                  | 62              | 72              | <a href="#">Reifferscheid and Heil, 1996</a> |
| Ames MPF                   | 58              | 63 <sup>a</sup> | <a href="#">Kamber et al., 2009</a>          |
| Yeast                      |                 |                 |  |
| RAD54-GFP                  | 39              | 82              | <a href="#">Knight et al., 2007</a>          |
| DEL                        | 86              | 80 <sup>a</sup> | <a href="#">Brennan and Schiestl, 2004</a>   |
| Mammalian                  |                 |                 |  |
| MNT                        | 81              | 54              | <a href="#">Kirkland et al., 2005</a>        |
| GADD45a-GFP <sup>b</sup>   | 87              | 95              | <a href="#">Hastwell et al., 2009</a>        |

# Confirmation Tests

- > Best option: substance identification via HPLC-MS
- > Confirmation with Ames Tests on individual standard substances
- > Second best option: confirmation via *in vitro* bioassays
- > Different assay principles/cellular targets

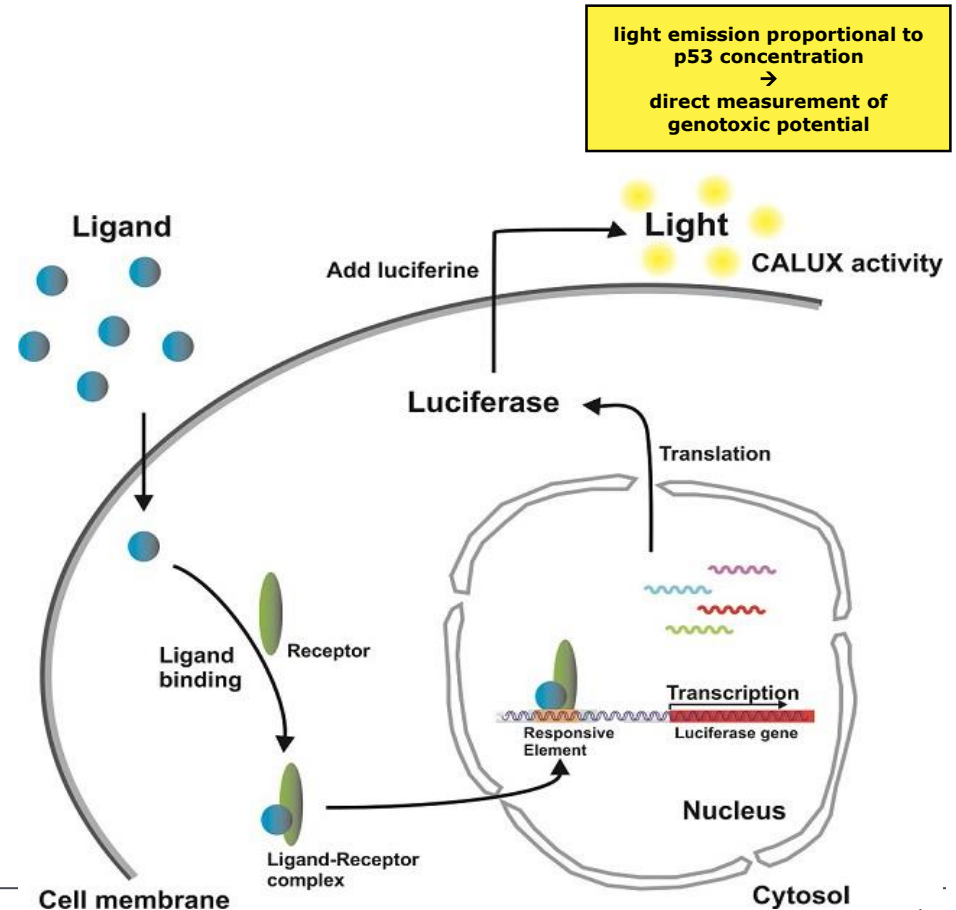


# HepGenTox: P53-Pathway



# HepGenTox Assay

- > used cell line: human hepatocellular carcinoma cells (HepG2)
- > no external activation system (S9) required!



# umuC-Test - Principle

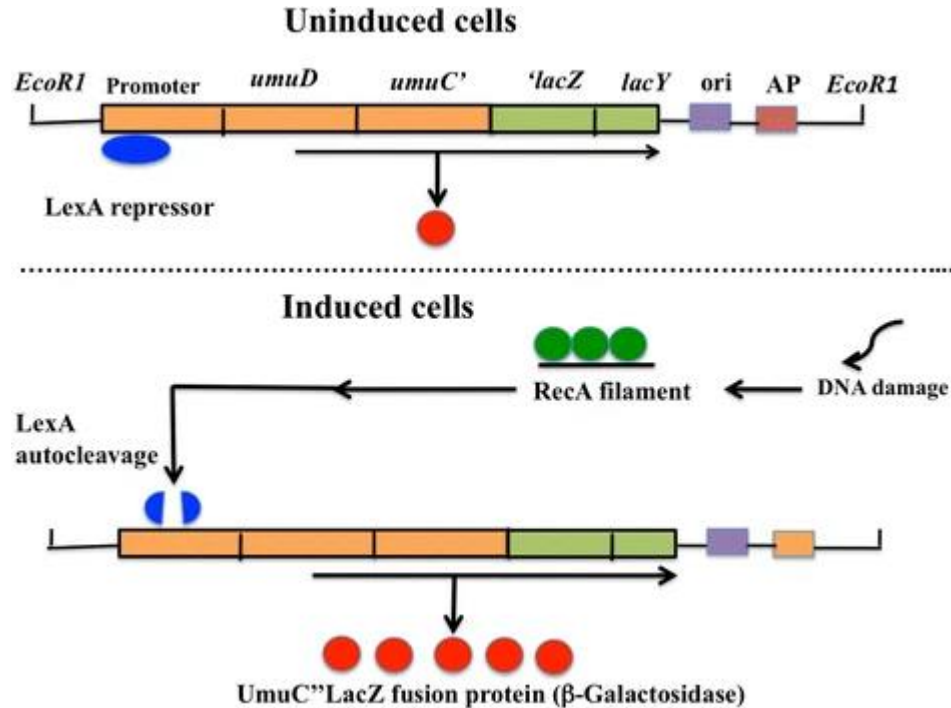


Figure: Schema showing the principle of umu test using *umuC''lacZ* fusion gene.

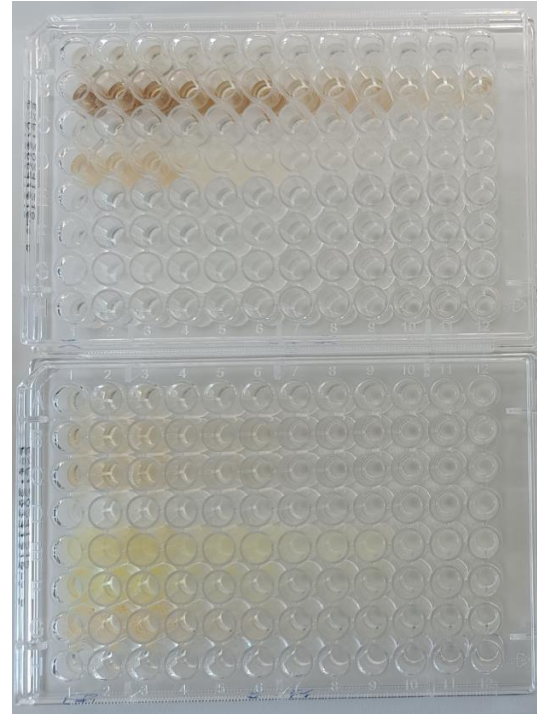


# umuC-Test

- > Test performed in 96-well plates or on thin layer plates
- > Fast and cheap
- > Several advantages/disadvantages when compared to Ames

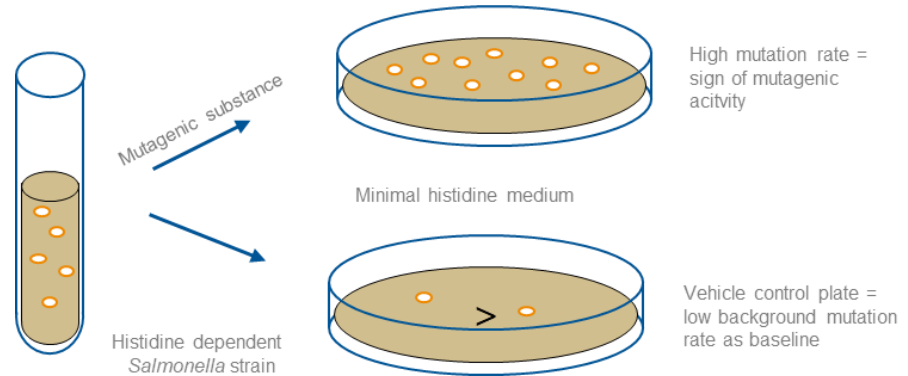
Comparison of *umu* test results and chemicals tested for rodent carcinogenicity [22]

| Carcinogenicity | umu test |           |   | Total |
|-----------------|----------|-----------|---|-------|
|                 | +        | —         | ± |       |
| +               | 119      | 72        | 2 | 193   |
| —               | 11       | 39        | 0 | 50    |
| ±               | 0        | 5         | 0 | 5     |
| Total           | 130      | 116       | 2 | 248   |
| Sensitivity     | 65 %     | (119/193) |   |       |
| Specificity     | 78 %     | (39/50)   |   |       |
| Accuracy        | 65 %     | (158/243) |   |       |



# Ames Test Principle

- **In vitro bioassay** to detect direct DNA reactive (mutagenic) substances
- **Genetically modified bacteria**  
E.g. *Salmonella Typhimurium* that cannot produce histidine.
- **Mutagenic substances** can lead to mutations (reversion of histidine auxotrophy).
- **Bacterial growth** indicates the presence of mutagenic substances.



# Sample Overview

- **16 Ames-positive samples**

- 2 bioplastic samples
- 14 polymer samples

- **7 Ames-negative samples**

- 1 bioplastic sample
- 1 control sample (solvent only)
- 5 polymer samples

- **2 Ames-inhibiting samples**

- 1 bioplastic sample
- 1 polymer sample

- **Total: 25 samples**

- Two independent test runs

- Identical migrate applied

- Ames: TA98/100 +/-S9

- umuC: +/-S9

# Test Results

- Overall concordance is high
- Ames results are not „set in stone“
- Chemical analysis is limited for confirmation of positives

| Code | Type       | Ames MPF   | HepGenTox | umuc       |
|------|------------|------------|-----------|------------|
| B1   | bioplastic | -          | -         | +          |
| B2   | bioplastic | +          | +         | +          |
| B3   | bioplastic | +          | +         | +          |
| B4   | bioplastic | inhibition | +         | +          |
| P1   | HDPE       | -          | -         | -          |
| P2   | LDPE       | +          | +         | +          |
| P3   | Control    | -          | -         | -          |
| P4   | PET        | -          | -         | +          |
| P5   | PP         | +          | -         | +          |
| P6   | HDPE       | -          | -         | eq         |
| P7   | HDPE       | inhibition | +         | inhibition |
| P8   | PS         | -          | -         | -          |
| P9   | HDPE       | -          | -         | -          |
| P10  | LDPE       | +          | -         | eq         |
| P11  | PS         | +          | +         | +          |
| P12  | LDPE       | +          | +         | +          |
| P13  | HDPE       | +          | +         | +          |
| P14  | LDPE       | +          | +         | +          |
| P15  | PP         | +          | -         | +          |
| P16  | PS         | +          | -         | +          |
| P17  | LDPE       | +          | +         | +          |
| P18  | PE/PP      | +          | +         | +          |
| P19  | LDPE       | +          | +         | +          |
| P20  | PP         | +          | +         | +          |
| P21  | PS         | +          | +         | +          |

|                           | Ames MPF | Hep | umuC |
|---------------------------|----------|-----|------|
| Concordance with Ames     | 25       | 19  | 20   |
| Concordance with Ames - % | 100%     | 76% | 80%  |

# Concordance: Ames-positive Samples

| Code | Type       | Ames MPF | HepGenTox | umuc |
|------|------------|----------|-----------|------|
| B2   | bioplastic | +        | +         | +    |
| B3   | bioplastic | +        | +         | +    |
| P2   | LDPE       | +        | +         | +    |
| P5   | PP         | +        | -         | +    |
| P10  | LDPE       | +        | -         | eq   |
| P11  | PS         | +        | +         | +    |
| P12  | LDPE       | +        | +         | +    |
| P13  | HDPE       | +        | +         | +    |
| P14  | LDPE       | +        | +         | +    |
| P15  | PP         | +        | -         | +    |
| P16  | PS         | +        | -         | +    |
| P17  | LDPE       | +        | +         | +    |
| P18  | PE/PP      | +        | +         | +    |
| P19  | LDPE       | +        | +         | +    |
| P20  | PP         | +        | +         | +    |
| P21  | PS         | +        | +         | +    |

- 75% of positive results were confirmed by the HepGenTox assay
- 94% of positive results were confirmed by the umuC assay
- Only one sample did not test positive in any other assay

|                       | Ames MPF | HepGenTox | umuc |
|-----------------------|----------|-----------|------|
| Positive results      | 16       | 12        | 15   |
| Concordance with Ames | 100%     | 75%       | 94%  |

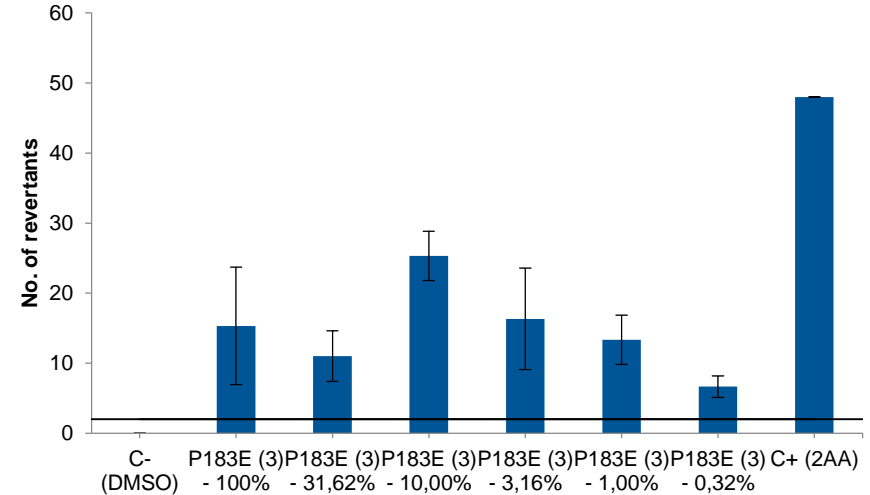
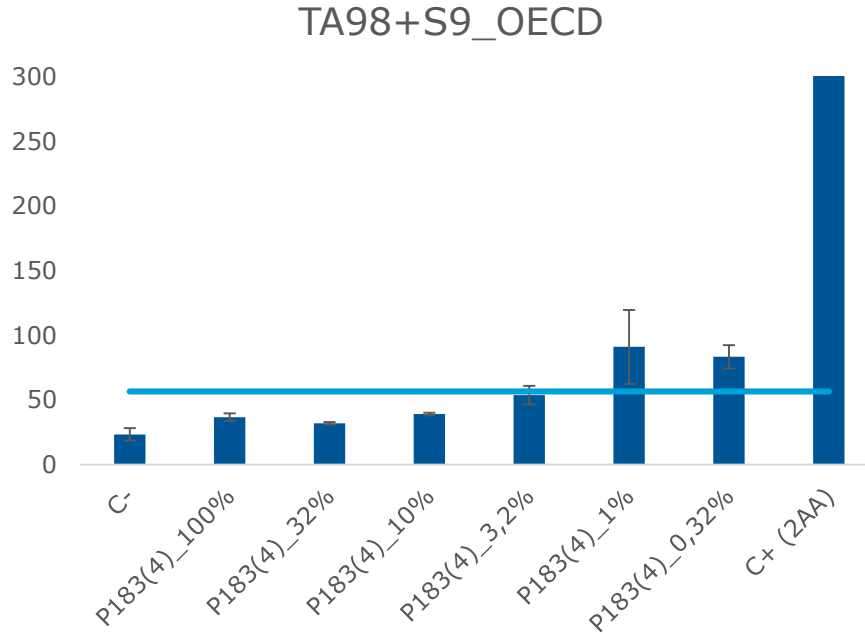
# Concordance: Ames-negative Samples

| Code | Type       | Ames MPF | HepGenTox | umuc |
|------|------------|----------|-----------|------|
| B1   | bioplastic | -        | -         | +    |
| P1   | HDPE       | -        | -         | -    |
| P3   | Control    | -        | -         | -    |
| P4   | PET        | -        | -         | +    |
| P6   | HDPE       | -        | -         | eq   |
| P8   | PS         | -        | -         | -    |
| P9   | HDPE       | -        | -         | -    |

|                       | Ames MPF | HepGenTox | umuc |
|-----------------------|----------|-----------|------|
| Negative results      | 7        | 7         | 4    |
| Concordance with Ames | 100%     | 100%      | 57%  |

- 100% Ames-negative samples also negative in HepGenTox
- 2 additional samples scored positive in umuC, 1 additional equivocal

# Full OECD Ames vs. Ames MPF



# Discussion

- >Overall Concordance with the Ames Test is high (>76%)
- >Positive results could be confirmed with two additional test systems
- >umuC – issues with sample turbidity, higher positivity rate
- >HepGenTox – slightly lower positivity rate, less issues with toxicity than expected
- >Identification (HPLC-MS) still needed





**Thank you for your attention!**

**Open Questions?**

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