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Viral sensitizer technology improves yield of modified vaccinia ankara from available cell substrates

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Viral Sensitizer Technology Improves Yield of Modified Vaccinia Ankara from Available Cell Substrates

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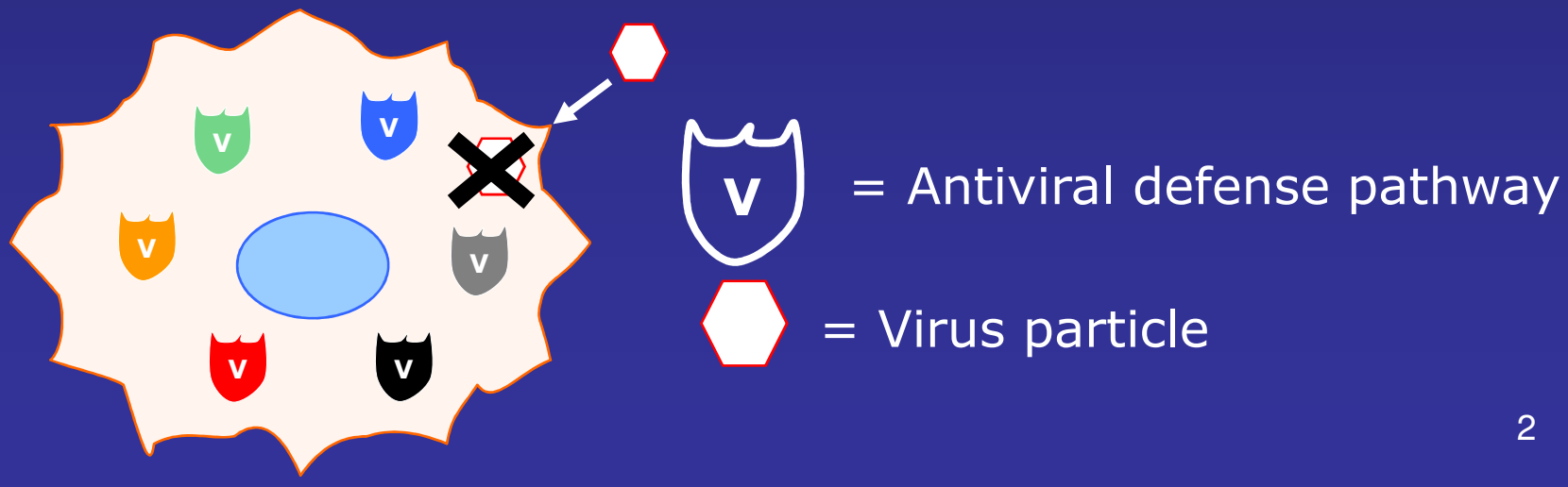
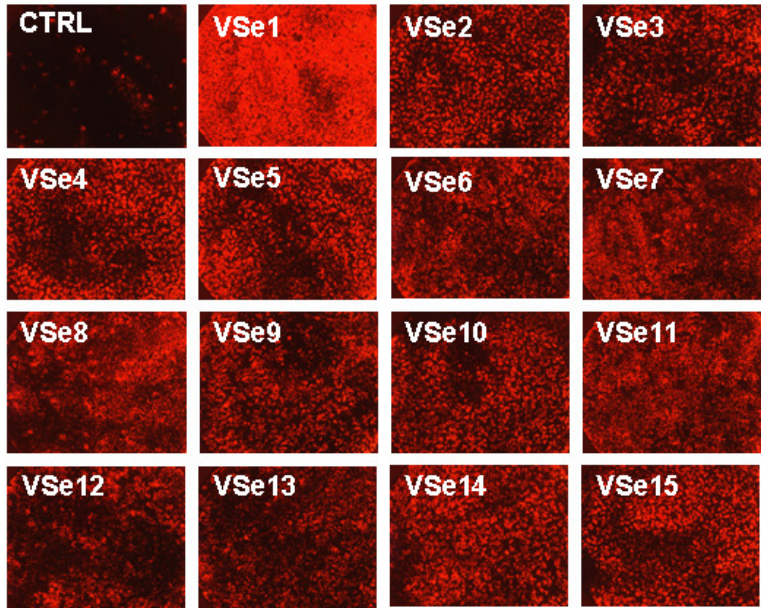
The Problem

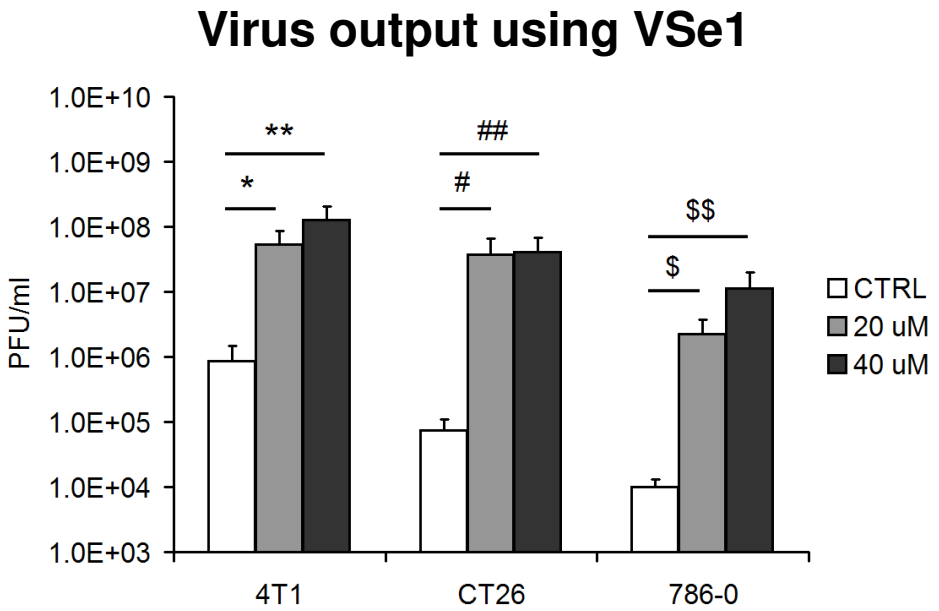
- There is a rising global demand for virus-based vaccines and therapeutics
- Viruses need to be propagated in cells
- Innate cellular antiviral responses are a primary hurdle for efficient viral replication



The solution: Viral Sensitizer Technology (VST)

Viral sensitizer technology (VST) encompasses a collection of small molecules identified by high-throughput screening that enhance viral growth in some cases up to ***over 1000-fold***



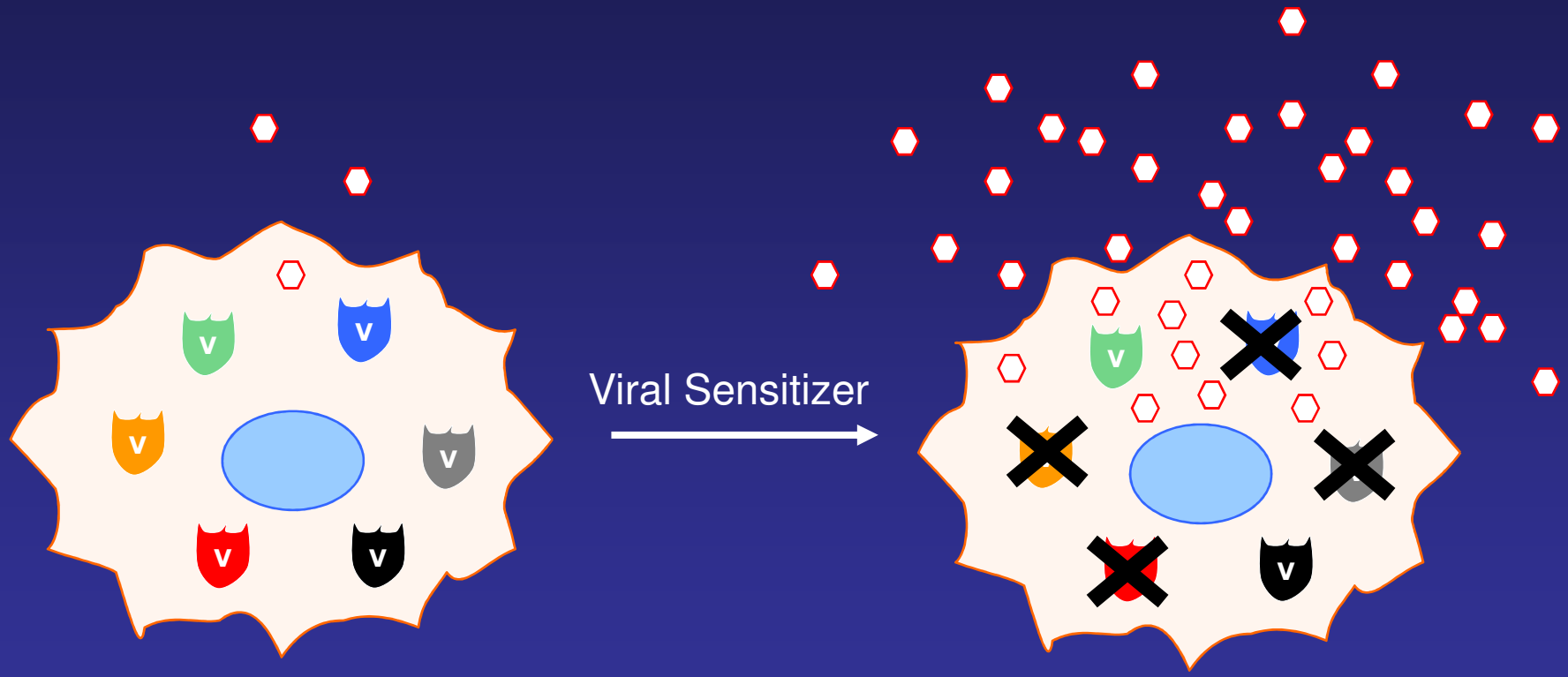


Red = Viral Growth

$$ = over 1000-fold increase

Diallo JS et al. Mol Ther, 2010

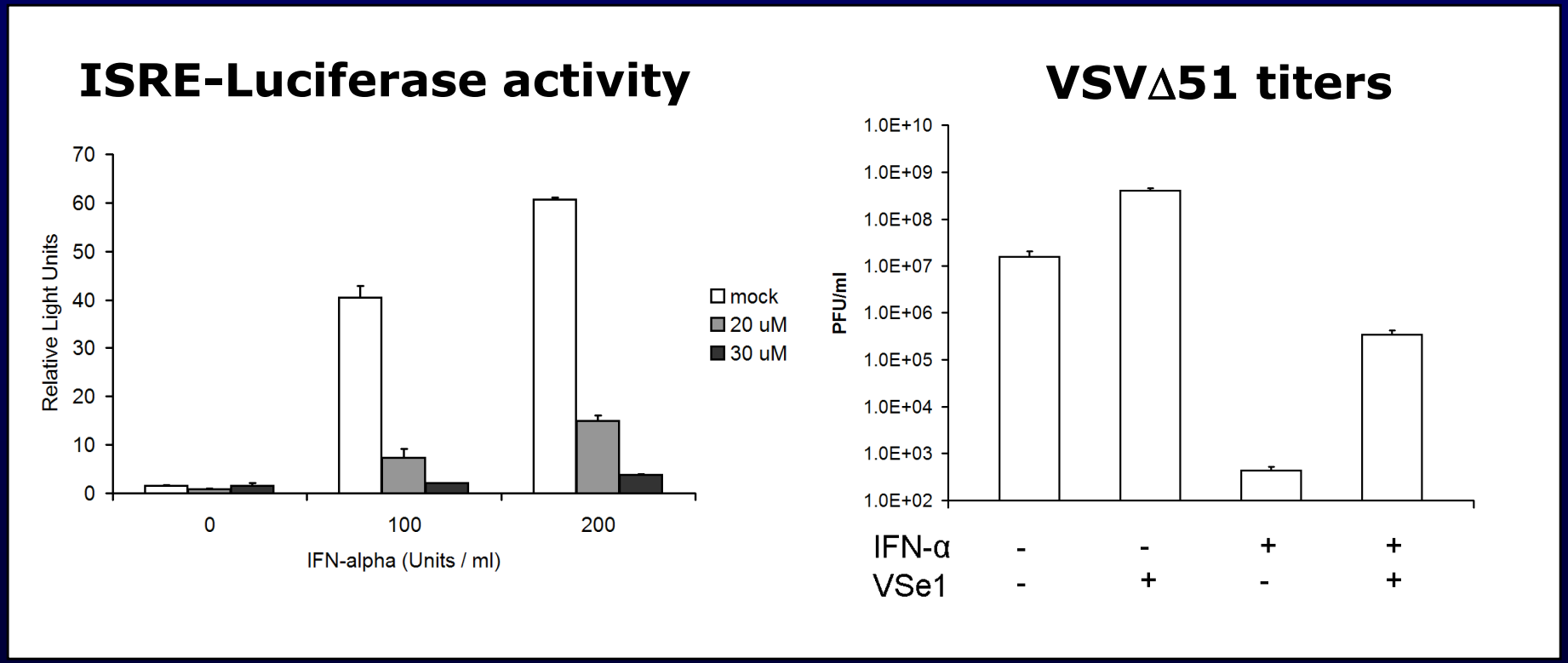
Viral sensitizer compounds, through a variety of mechanisms, affect the innate cellular antiviral response in order to promote more efficient growth of attenuated viruses



= Antiviral defense pathway

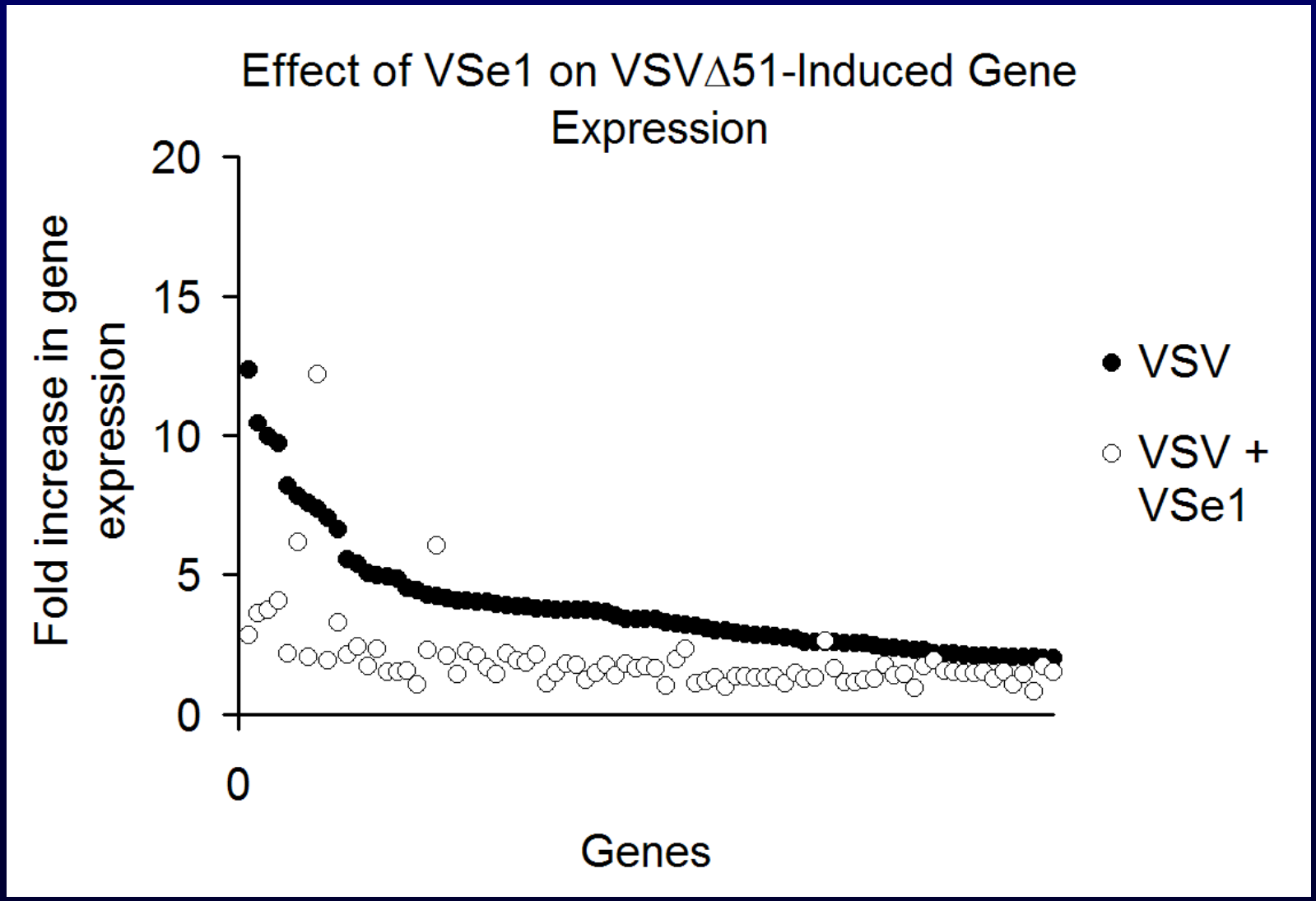
= Virus particle

Viral sensitizers repress cellular antiviral response



Diallo JS et al. Mol Ther, 2010

VST represses cellular antiviral response



Diallo JS et al. Mol Ther, 2010

CELL-BASED VACCINE MANUFACTURING

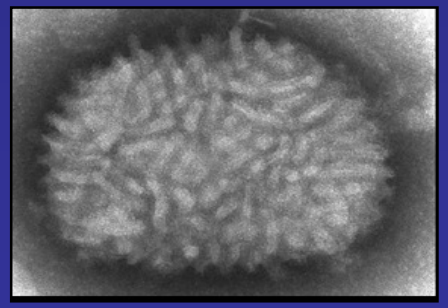
50+ year old method

New method

- Most vaccines are virus-based and many are poorly growing attenuated virus strains
- Production of several vaccines (eg. Influenza, MVA) is dominated by egg-based methods which have a number of issues
- Producers are starting to move towards continuous cell-line based productions and chemically defined media
- Few cell lines are approved for vaccine manufacturing
- **The innate antiviral response can limit the production of virus from cells, providing an opportunity for VST**

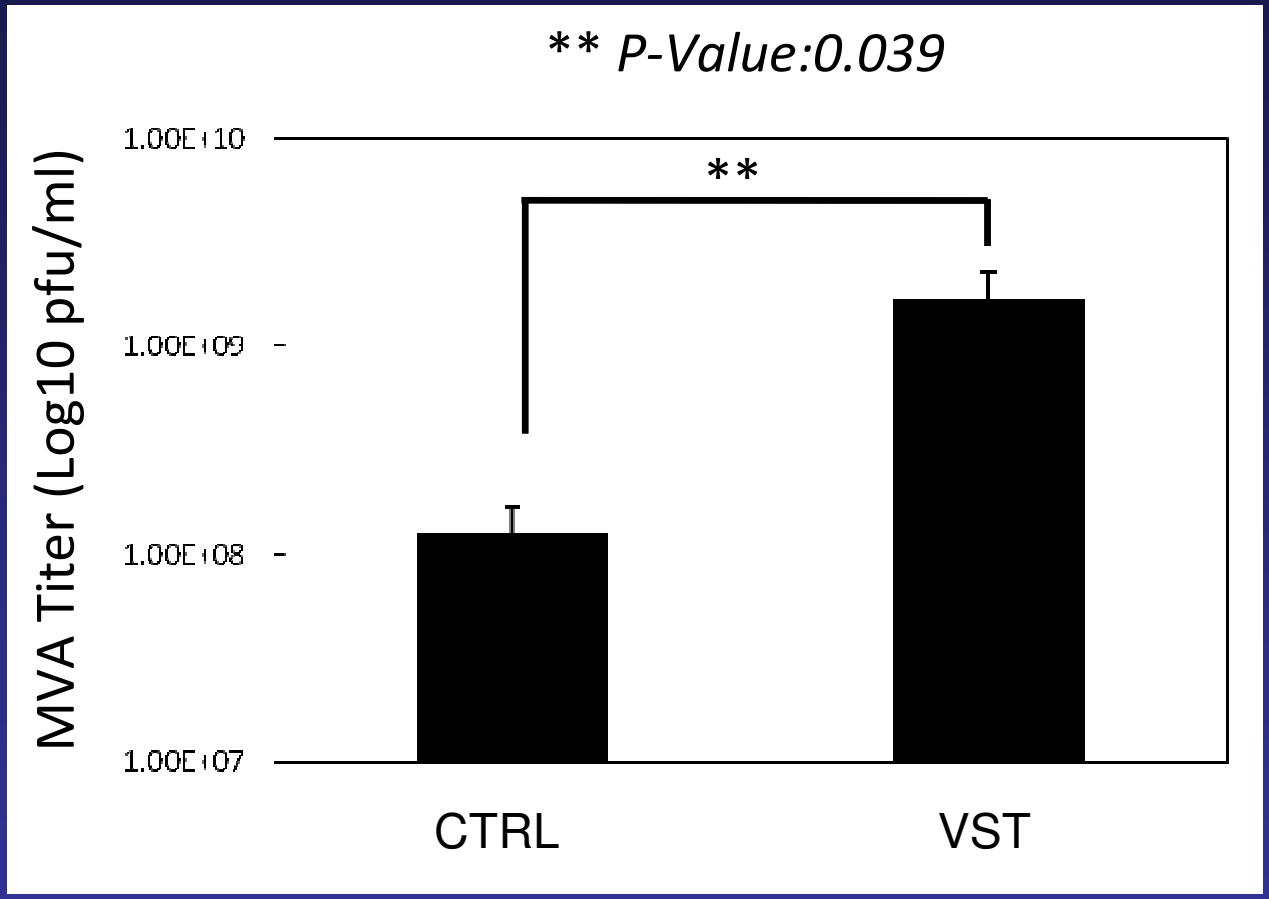
VST APPLICATION: MODIFIED VACCINIA ANKARA (MVA)

- **Modified Vaccinia Ankara (MVA)** is a multi-application vaccine platform approved for smallpox vaccination and in Phase II/III evaluation for several indications including Malaria, HIV, Influenza, and Cancer
- MVA is currently produced in eggs or egg-derived CEF cells.
- BHK21 cell line is the **only continuous mammalian cell line** that supports MVA production



MVA

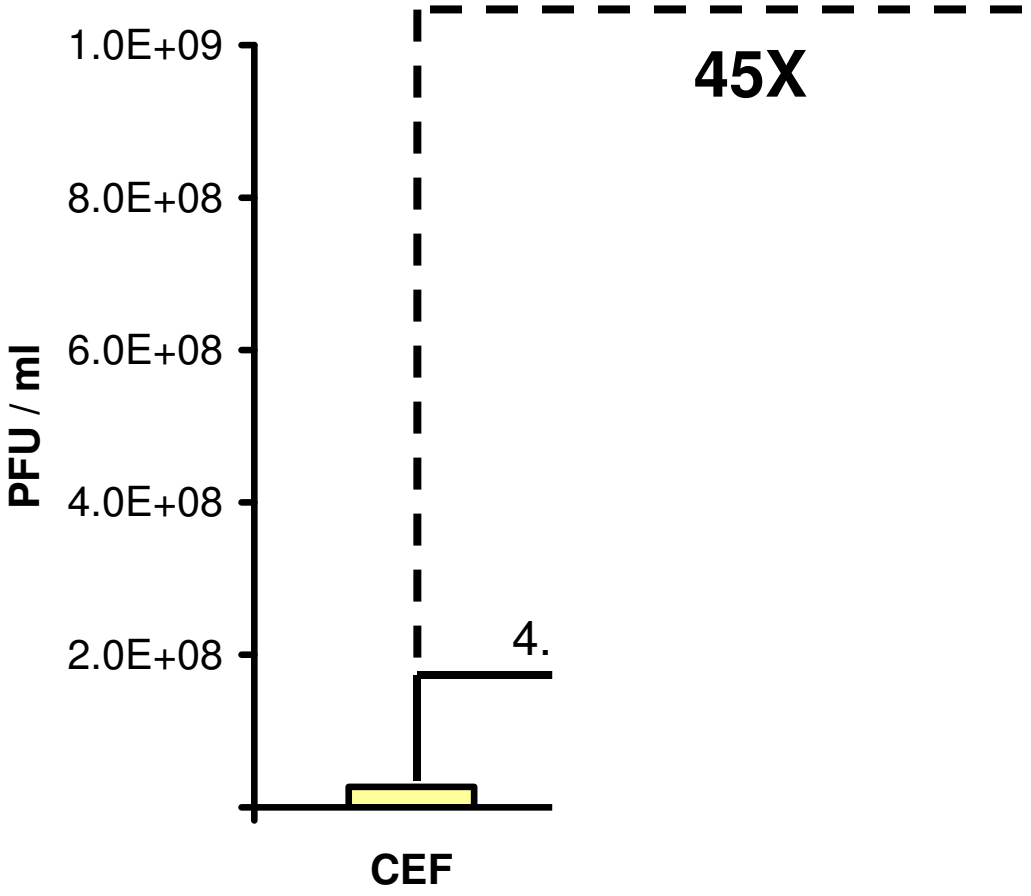
VST INCREASES YIELD OF MVA IN BHK21



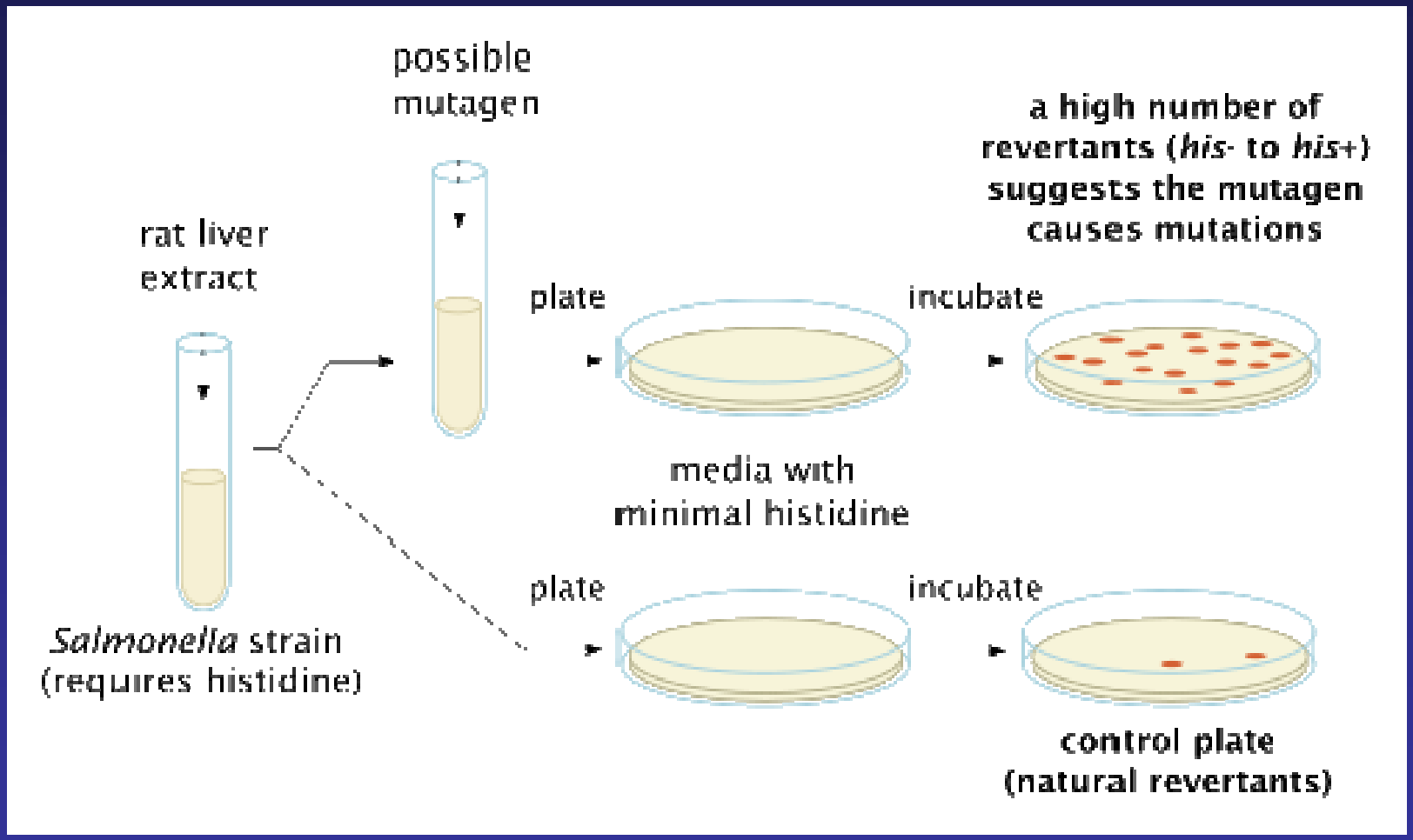
>10-fold increase in viral titers

** Average of four separate experiments in sextuplicate

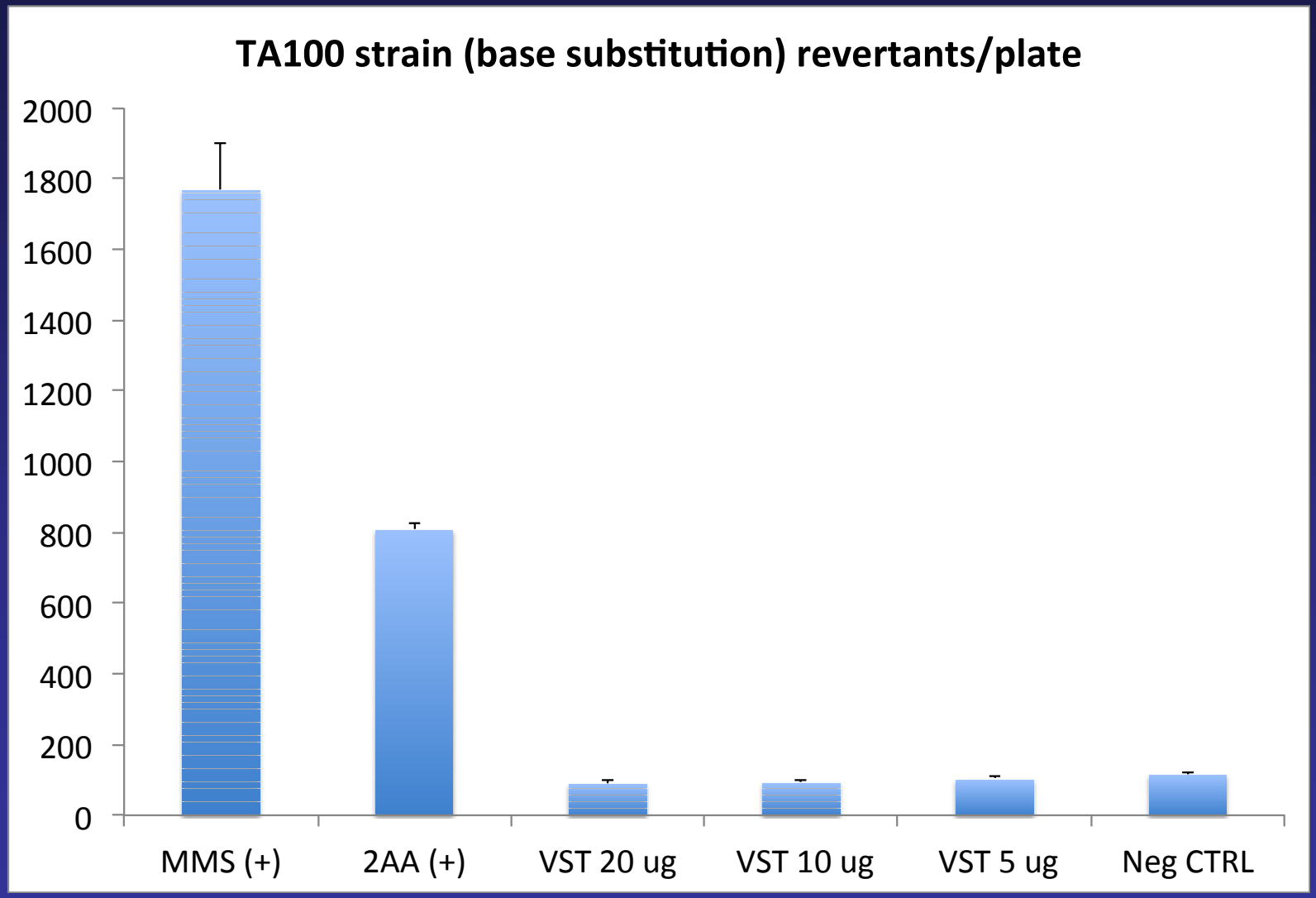
VST SUBSTANTIALLY IMPROVES MVA YIELDS COMPARED TO STANDARD METHODS AT ROLLER BOTTLE SCALE



The AMES mutagenicity test



VST IS NOT DNA REACTIVE IN SALMONELLA



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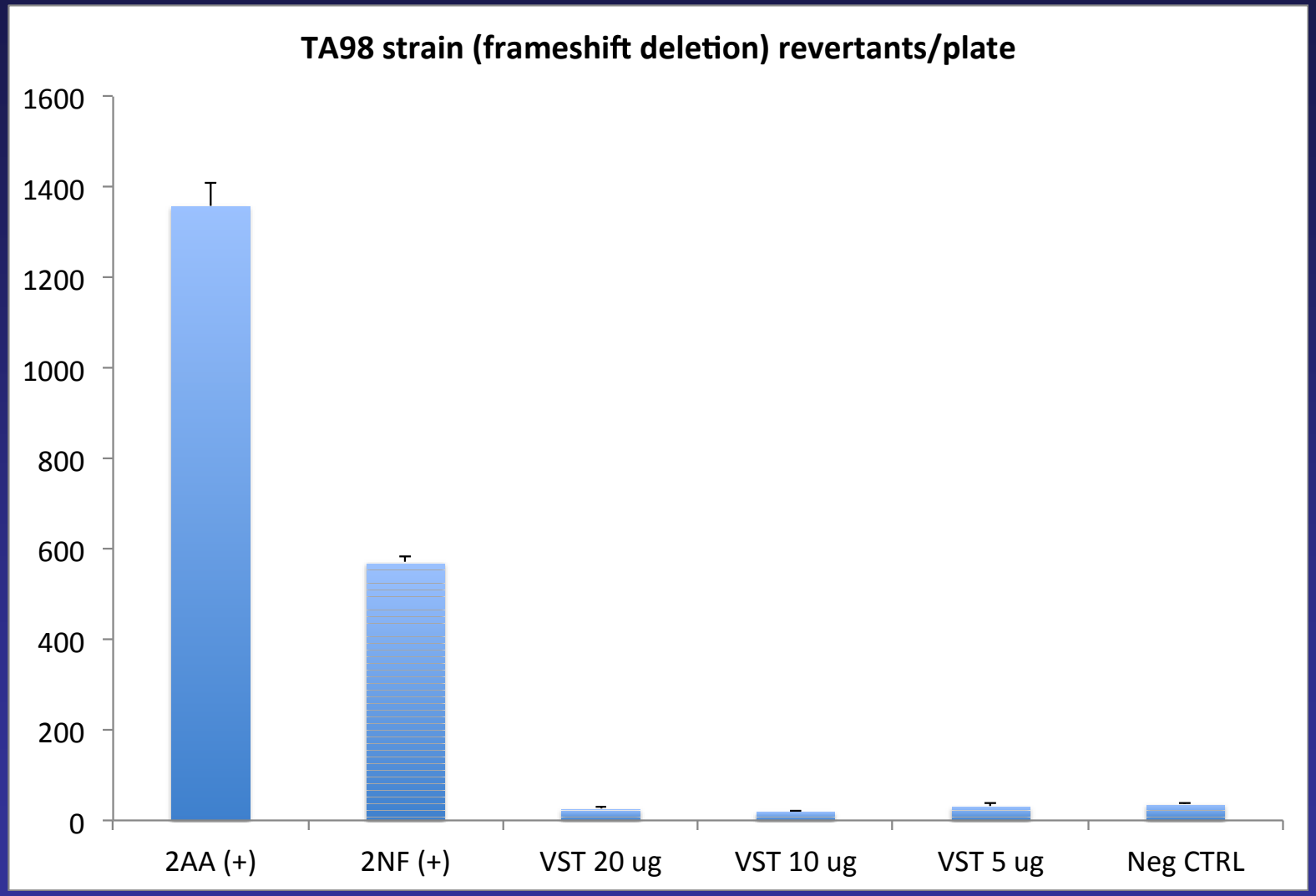
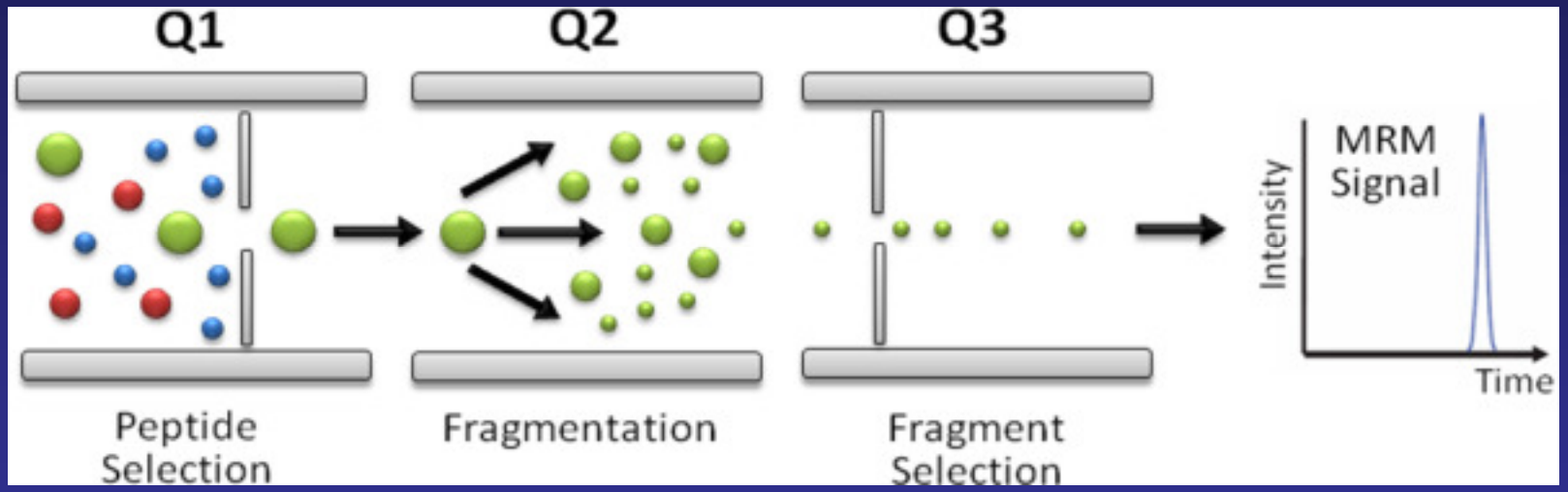


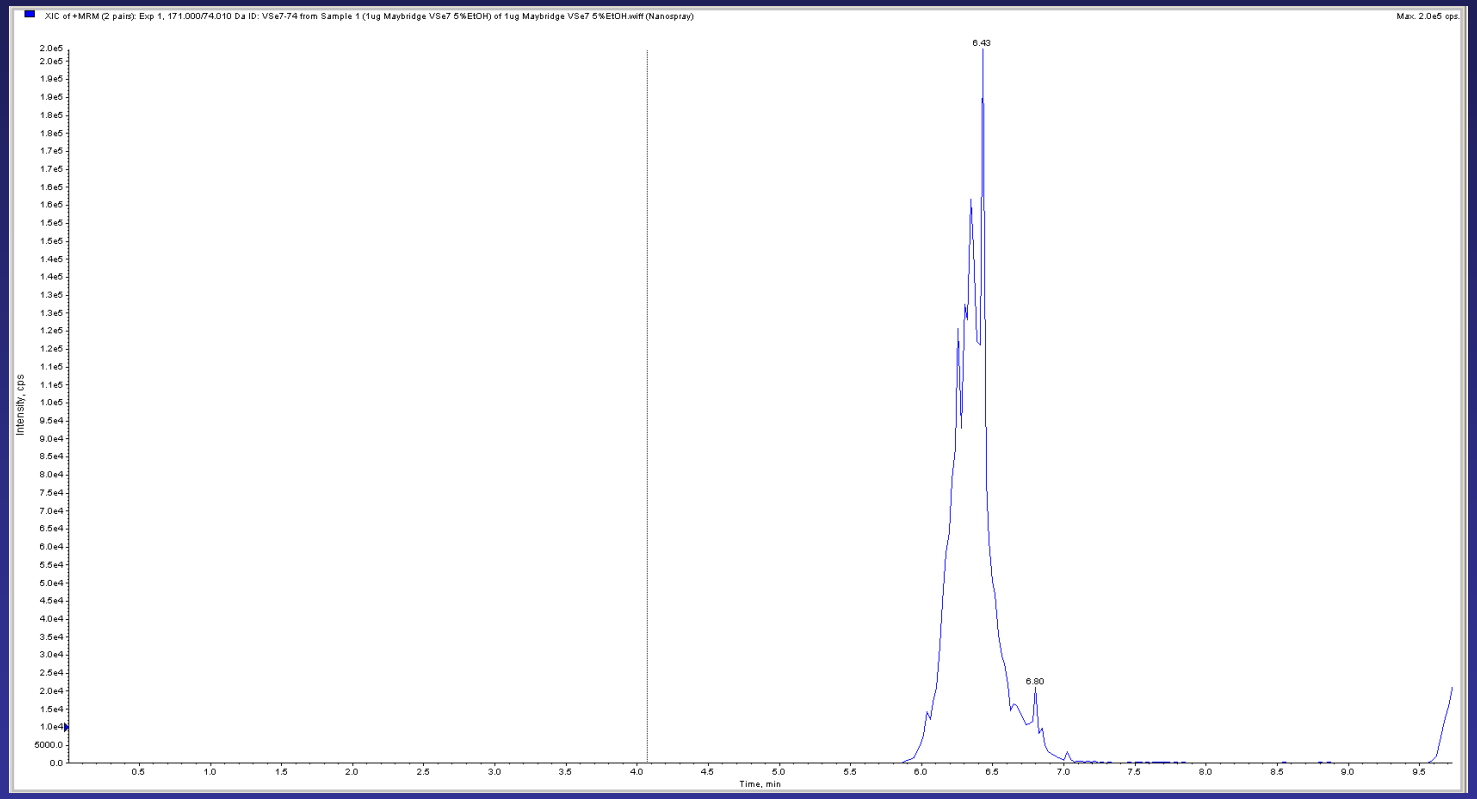
VST does not increase frameshift deletion rate

MRM method for detection of VST in purified virus cultures



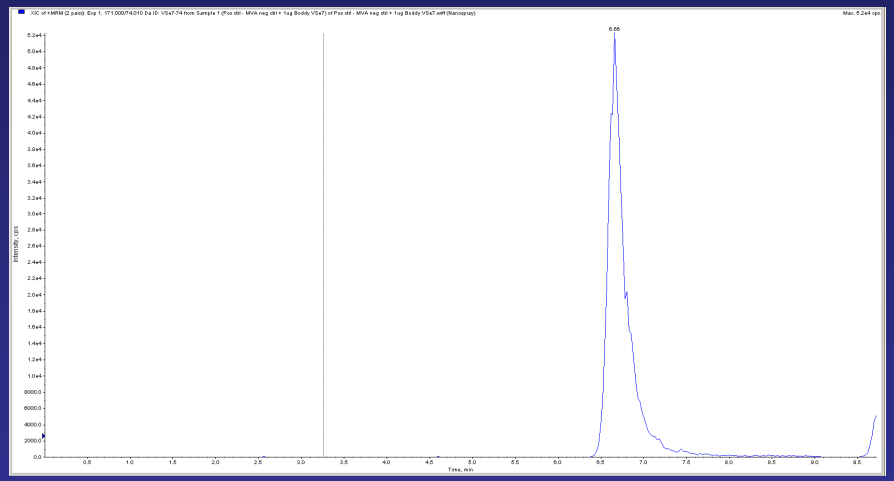
MRM is a sensitive mass spectrometry-based method that allows for specific detection of peptides or molecules even in complex mixtures

MRM method for detection of VST in purified virus cultures

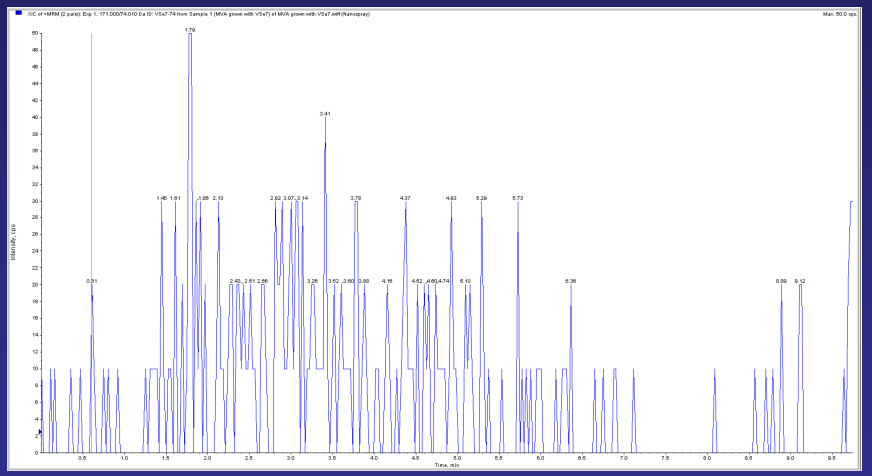


We can easily detect MRM transition of purified VST (1ug)

MRM method for detection of VST in purified virus cultures



MVA preparation spiked with VST => Compound is easily detected



VST-assisted MVA preparation flowthrough => No compound-associated MRM transition signal detected (background)

Conclusions

- VST can increase yields of MVA by 4.5X in CEF and > 10-fold in BHK21 cells
- VST activity is maintained at the roller bottle scale
- VST is not DNA reactive based on AMES tests
- MRM methodologies can be used to detect VST, which is absent from purified VST-assisted MVA cultures

Acknowledgements

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