

Ames MPF

Stability of Ames Tester Strains

“It’s not the destination, it’s the journey”
Ralph Waldo Emerson

Dimitrios Spiliotopoulos, PhD
2019



Testing for Stability of Ames Tester Strains

Shipping Simulation Experiments

We strive to guarantee the best shipping conditions for our kits.

Our Quality Control procedures are performed in order to guarantee that our products are functional after a 10-day storage at room temperature.

We also provide indications on how to preempt possible problems with **strains with higher spontaneous reversion rate**, such as

- *Salmonella* TA100
- *E. coli* WP2 pKM101
- *E. coli* WP2 *uvrA*[pKM101].

We focus on three issues with strains having higher spontaneous reversion rates:

- inappropriate shipping conditions (*e.g.*, a higher summer temperatures)
- extended storage of the vials at wrong temperatures (RT, 4°C, -20°C)
- more than one thawing-freezing cycle.



Shipping Simulation Experiments

Experimental Setup

It is possible that our strains are stored at warm temperatures during the shipment (*e.g.*, during shipments in the summer time).

We assessed if and how the performance of two potentially sensitive strains (*Salmonella* TA100 and *E. coli uvrA*[pKM101]) was impacted by such eventuality.

As per our usual Quality Control procedure, four vials were stored at room temperature for 10 days.

In this case, though, within the duration of this 10-day RT incubation, **a vial was put at 35°C for 1 day**, **a vial was put at 35°C for 2 days**, and **a vial was put at 35°C for 3 days**. In the meantime, **a control vial was just kept at room temperature**.

Shipping Simulation Experiments

Storage at 35°C

Upon storage at 35°C, condensation water was observed in the vials.

The extent of the condensation seemed to roughly depend on the time spent at 35°C.



Condensation water occasionally formed a distinct layer on top of the semisolid agar. Spinning the vial, it'll go to the bottom.

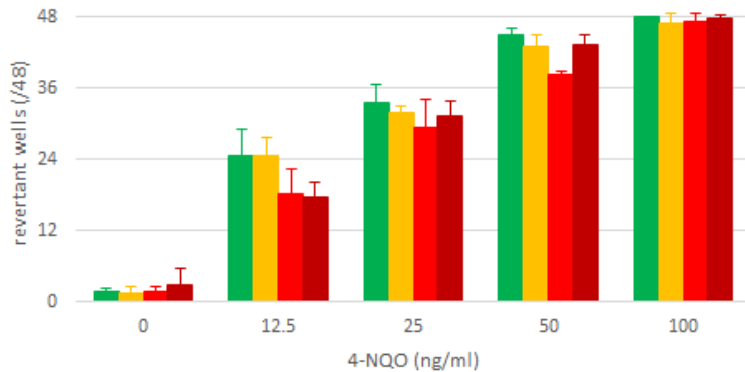


After four more days at RT, the layer was still there. Vials were frozen. The liquid layer was there upon thawing.

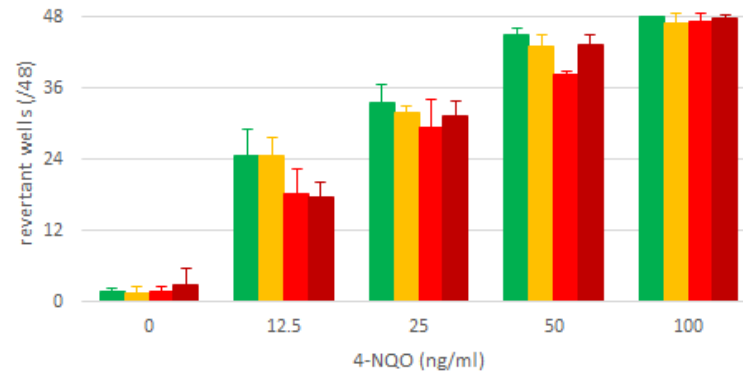
Shipping Simulation Experiments

Strain Performance

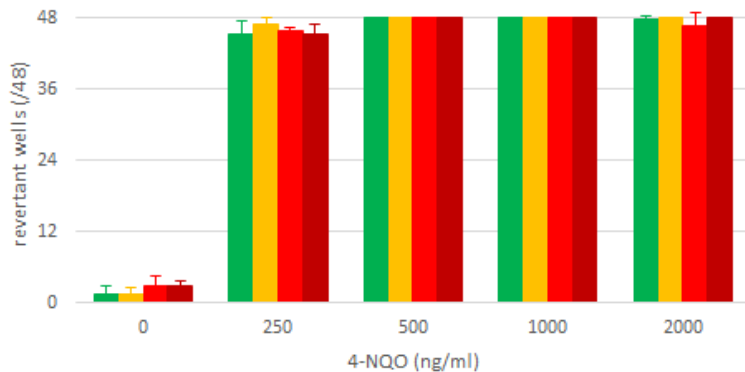
Shipping Simulation: TA100 (test #1)



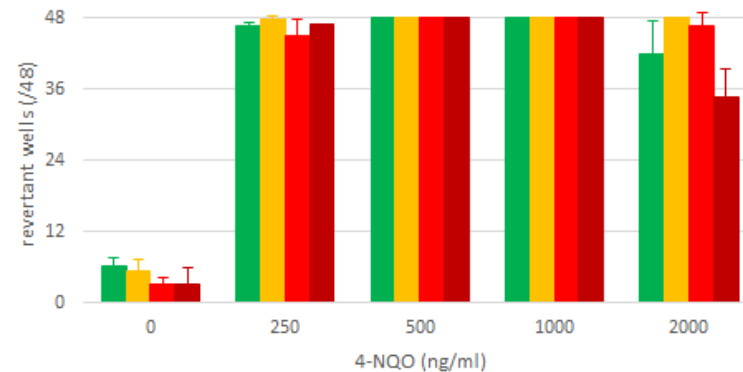
Shipping Simulation: TA100 (test #1)



Shipping Simulation: *uvrA*[pKM101] (test 1)



Shipping Simulation: *uvrA*[pKM101] (test 2)



control vial
 at 35°C for 1 day
 at 35°C for 2 days
 at 35°C for 3 days

Some like it warm



Shipping Simulation Experiments

Strain Performance

- Storage at high temperatures of the semi-solid vials seem to impact their performance to a limited extent only.
- The format is robust to different temperatures!



Extended Storage at Wrong Temperatures

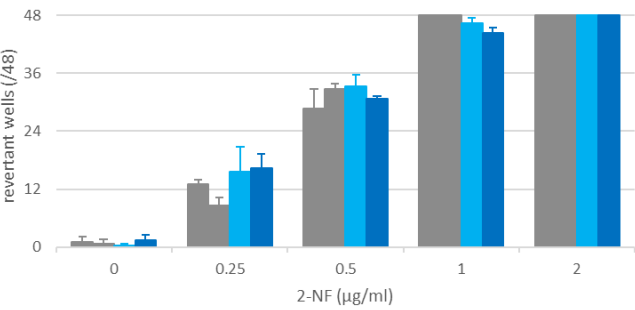
RT, 4°C, -20°C

■ 10 days at RT

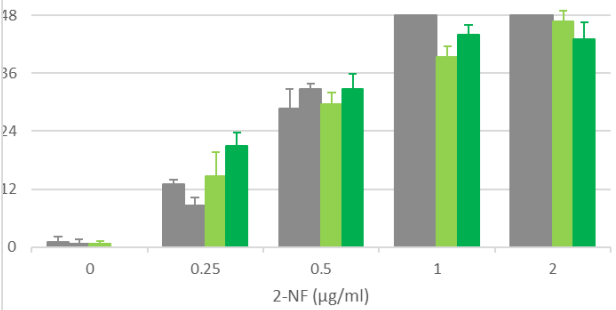
■ ■ ■ 14 days at ...

■ ■ ■ 30 days at...

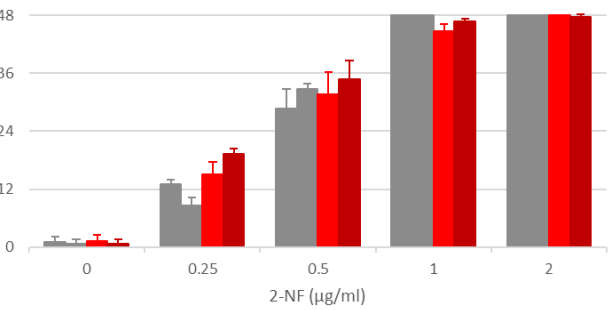
TA98 (semisolid) – storage at -20°C



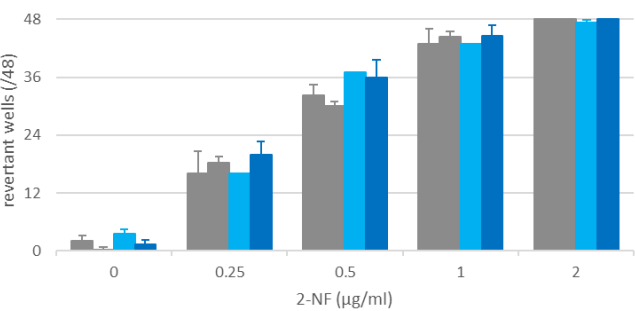
TA98 (semisolid) – storage at 4°C



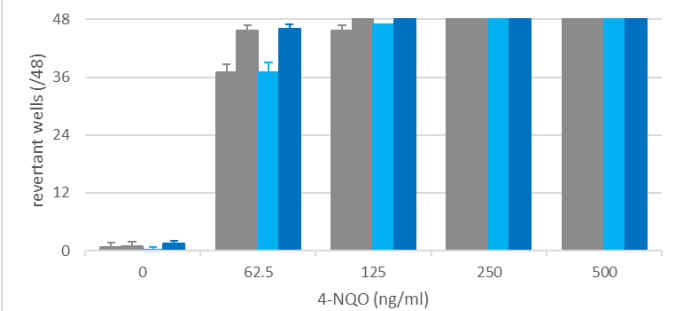
TA98 (semisolid) – storage at RT



TA98 (liquid) – storage at -20°C



TAMix (liquid) – storage at -20°C



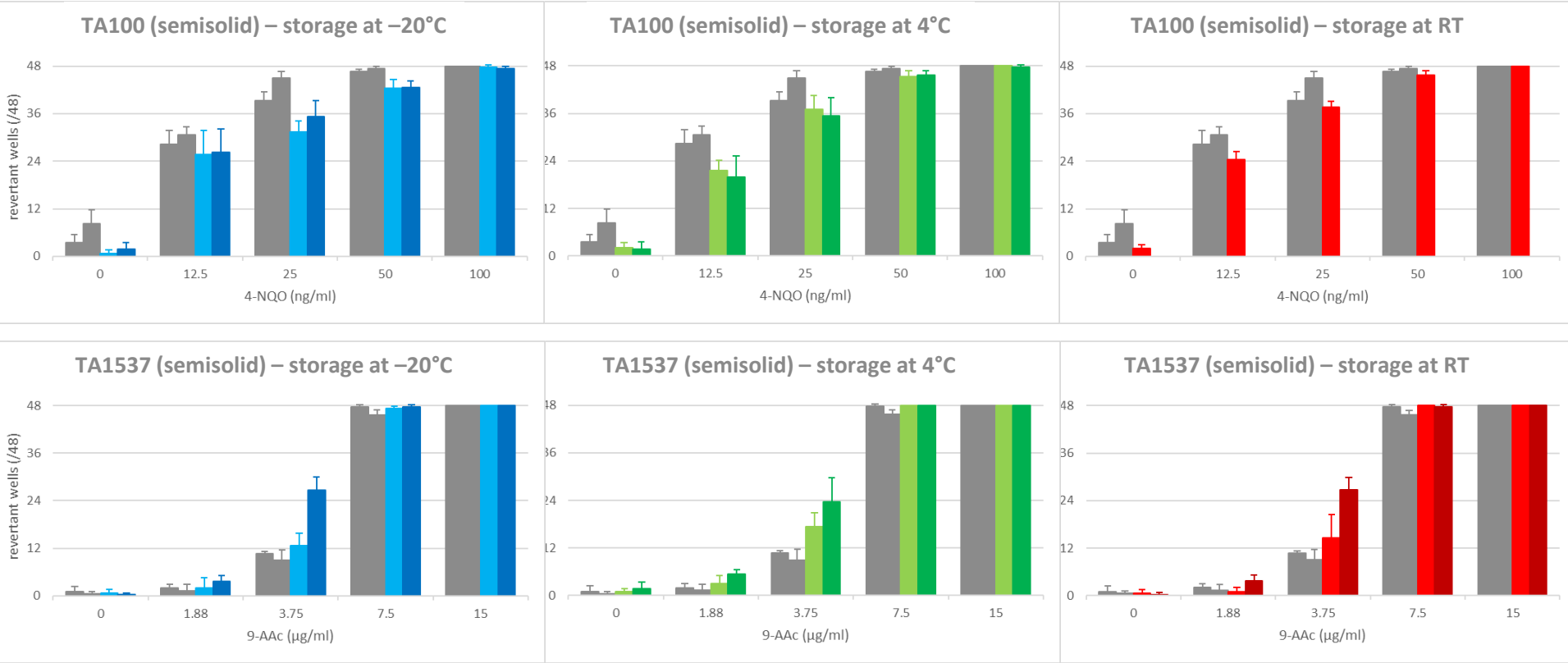
Extended Storage at Wrong Temperatures

RT, 4°C, -20°C

■ 10 days at RT

■ ■ ■ 14 days at ...

■ ■ ■ 30 days at...



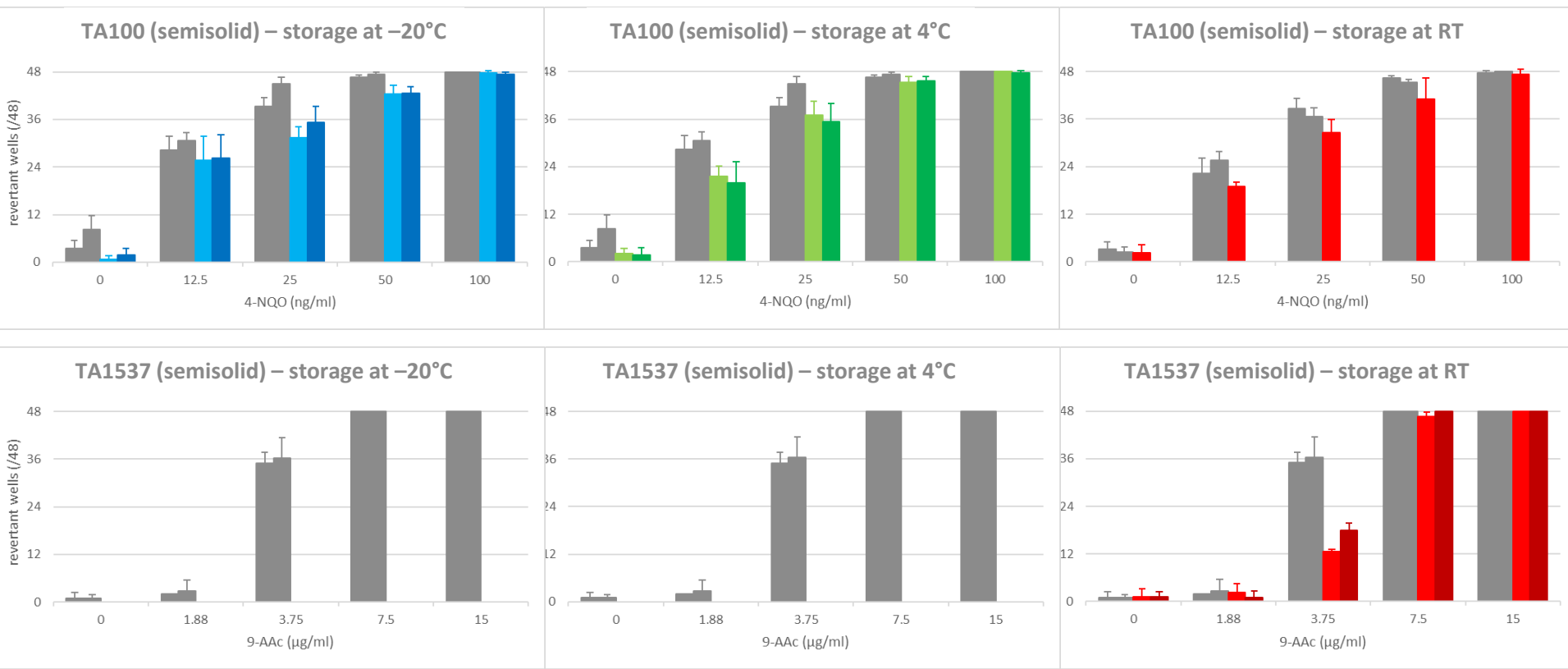
Extended Storage at Wrong Temperatures

RT, 4°C, -20°C

■ 10 days at RT

■ ■ ■ 14 days at ...

■ ■ ■ 30 days at...



Stability of the Ames Tester Strains

2019

Xenometrix AG

Storage

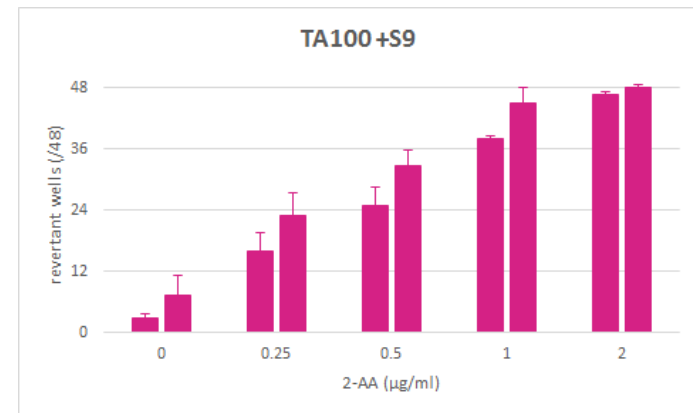
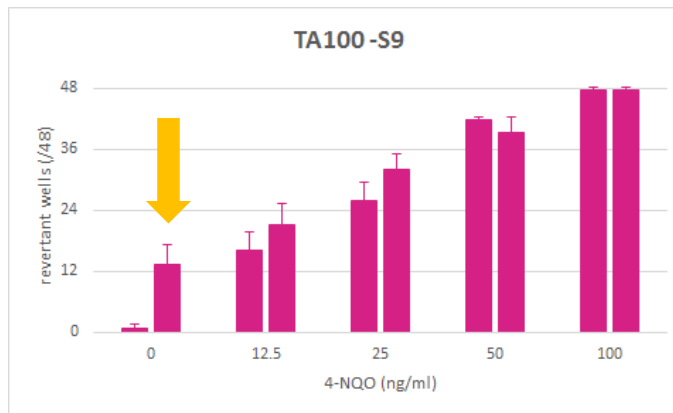


Multiple Freeze-Thaw Cycles

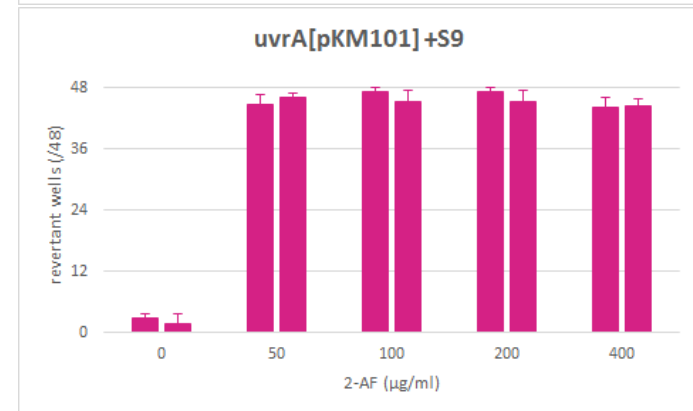
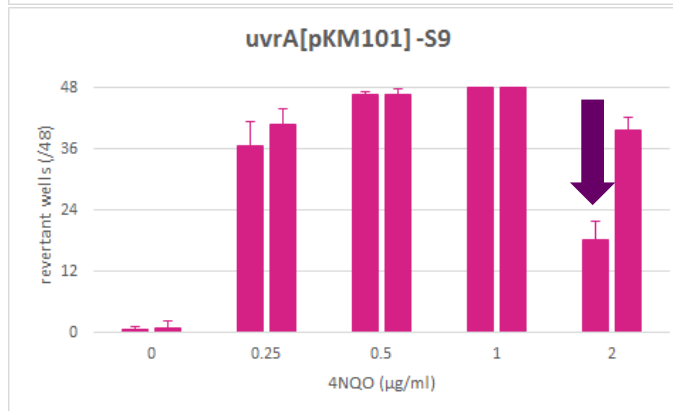
- What happens if the strain vials are frozen and thawed twice?
- We stored TA100 and *E. coli* WP2 uvrA[pKM101]
 - at RT for 10 days (usual QC)
 - back at -80°C for four days
 - back at RT for 3 days, and finally
 - froze them at -80°C .
- How can the Ames strain vials take such inappropriate storage?

Multiple Freeze-Thaw Cycles

TA100



E. coli WP2
uvrA[pKM101]



Conclusions

- Every batch of Ames tester strains manufactured at Xenometrix is **quality controlled after 10 days stored at room temperature** to simulate travel conditions.
- *Salmonella* and *E. coli* Ames tester strains are **very stable in the Xenometrix formats** also under non-optimal conditions.
- Storing the vials at the wrong temperatures for extended periods of time **might compromise their performance**.
- Semi-solid vials **may be thawed and frozen once**, but we would **not recommend it**: extensive tests were not run and tests were not standardized!

