

Appendix E Optional Strain Handling to Reduce the Risk of Occasional Elevated Baseline Values

Salmonella TA100 and *E.coli* wp2 pKM101 have a higher spontaneous reversion rate than the other strains available in the Ames MPF™ format. This leads to a higher frequency of overnight cultures with elevated baseline values making it difficult to evaluate mutagenic agents, especially weak ones. While Xenometrix makes every effort to sell bacterial strains with optimal sensitivities and low spontaneous reversion frequencies, emergence of unsuitable overnight cultures can still occur. Depending on the lot, storage, handling and growth conditions such unfavorable TA100 and wp2 pKM101 overnight cultures typically occur with frequencies in the range of 5 - 10%. While this can be just inconvenient with readily available samples, it can be very frustrating and annoying with valuable samples or under conditions where results must become available in a very short time.

Xenometrix has therefore developed a method to minimize the risk of performing an experiment with a unsuitable TA100 or pKM101 overnight culture. This risk reduction comes at the price of an additional day of experimentation, and consumption of somewhat more plasticware and media.

Note: The spontaneous revertants of TA100 may already be scored after 24 h, producing representative values for starting the actual experiment immediately. However, the spontaneous revertants of wp2 pKM101 can not be reliably scored after 24 hrs. We therefore suggest the following procedure schedule when testing *E.coli* wp2 pKM101 or both strains concurrently:

Procedure:

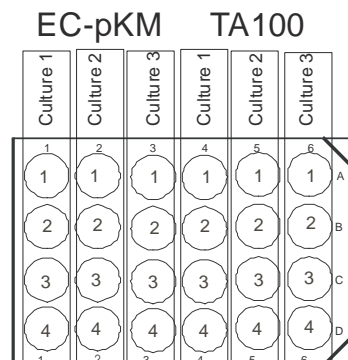
Overnight culture (Thursday evening):

Start 3 instead of 1 overnight cultures using stock from 1 vial.

Pre-experiment (Friday morning):

Pipet 1 ml of each overnight culture into fresh sterile 50 ml tubes. Add 1 ml of fresh growth medium to each tube and mix the contents by gently swirling. Store the diluted cultures in a refrigerator. Ensure sufficient aeration by using loose caps or tubes with filter caps (highly recommended to avoid airborne contaminations).

Perform a small Ames MPF assay with solvent only for the determination of the spontaneous reversion rate with each of the 3 undiluted overnight cultures. We recommend 4 replicates for each culture (a re-incubation of TA100 is not necessary):



Experimental day (Monday morning):

Score the 384-well plates obtained with the 3 cultures from Friday for spontaneous revertants. Retrieve the culture with the best (lowest) spontaneous reversion rate from the refrigerator. Swirl the content of the tube. Add 2 ml of Growth Medium to the refrigerated culture and incubate at 37°C and 250 rpm for approximately 2 hrs until the OD₆₀₀ reaches about 75% of the density of the original overnight culture.

Proceed to the exposure step as described on page 11 to perform the actual Ames MPF assay.

Note:

The same procedure can be used for the other Ames MPF™ strains. Due to their low spontaneous reversion rate the risk of obtaining an unfavorable overnight culture with the standard procedure is very small and the benefit of this new procedure is accordingly smaller.

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