PMA-qPCR for *E. coli* viability detection

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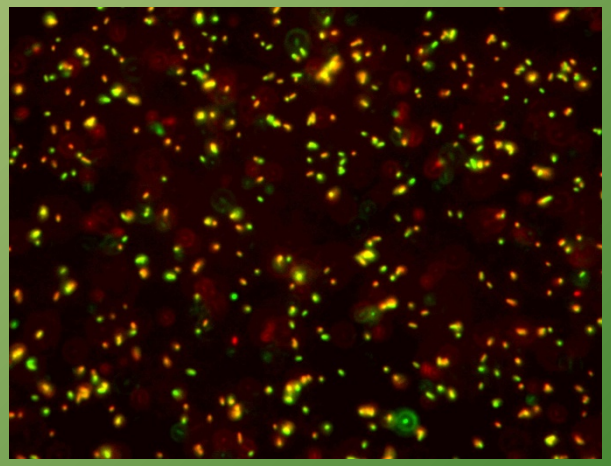
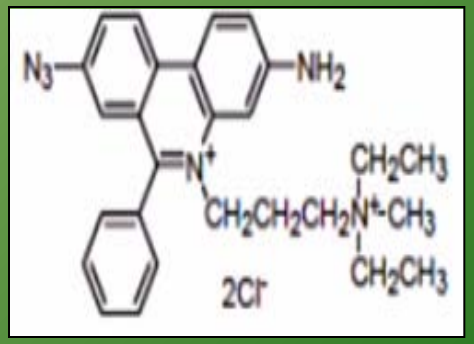




Why a need to detect viability of pathogens?

Space Exploration

Healthcare

Environment Monitoring

Bioterrorism

Methods to assess biological samples

Biological Sample

Gold Standard

Culture based methods

Determines viability

Time consuming

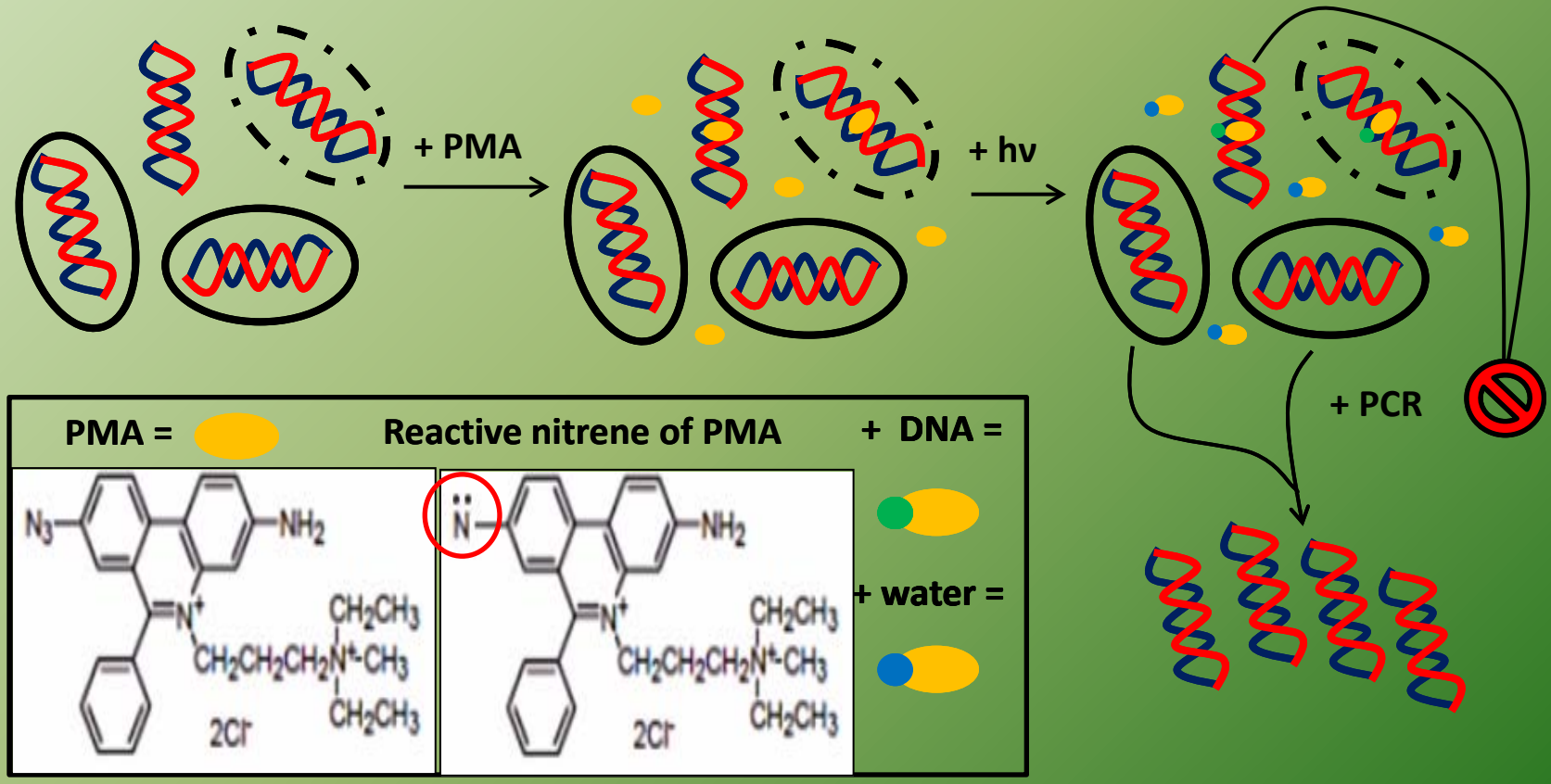
No species specificity

PCR/qPCR

Distinguishes between presence of live or dead cells

No species specificity

Propidium Monoazide (PMA) confers viability assessment to qPCR



Inconsistent Lit

Questions to Address

1. Is PMA-qPCR as *effective* as the gold standard (culturing)?

2. How does PMA *enter the cell*?

Question #1: Effectiveness of PMA-qPCR

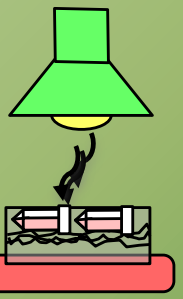
Experimental Design

Unheated, Live cells

Heated, Dead cells

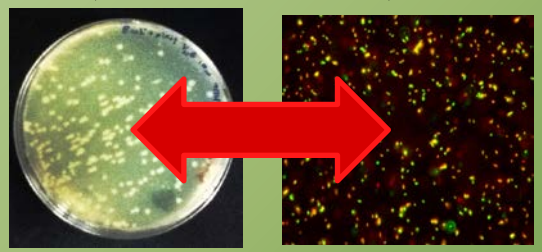
Mixed fractions (Live : Dead)
100:0, 75:25, 50:50, 25:75
10:90, 1:99, 0:100

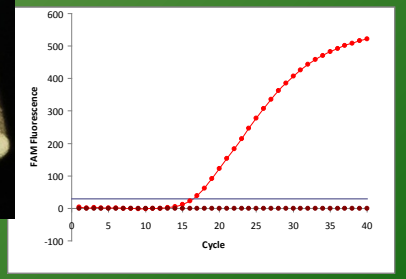
+/- PMA Treatment



Collected cells

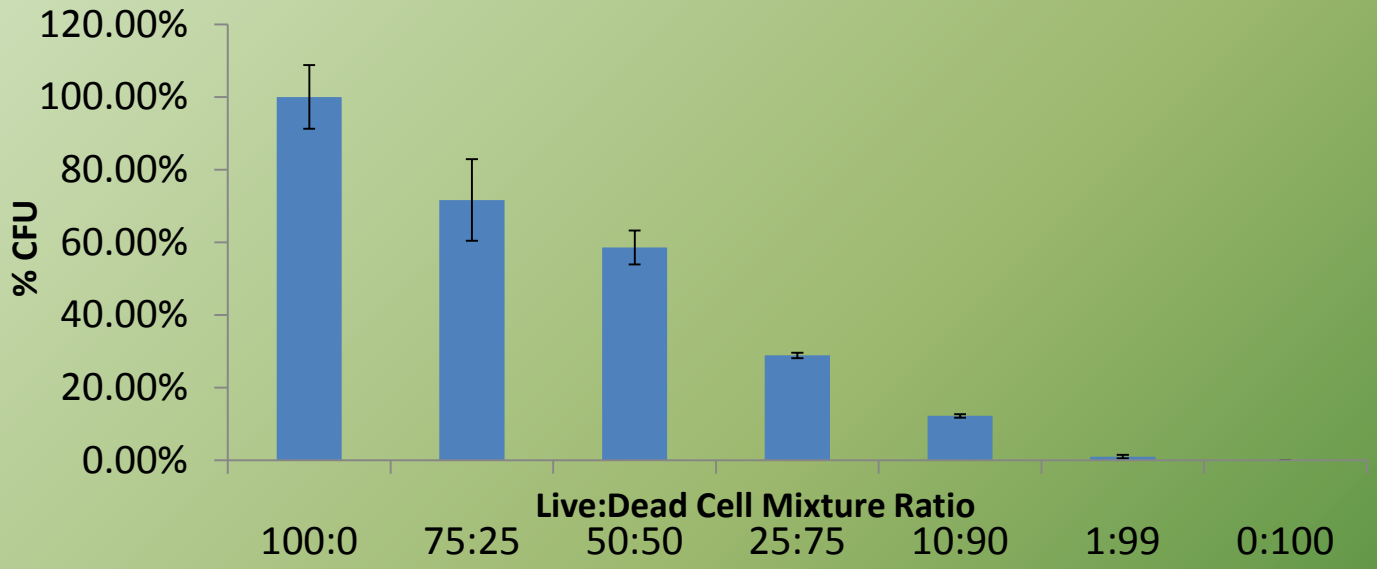
Extract DNA, qPCR analysis



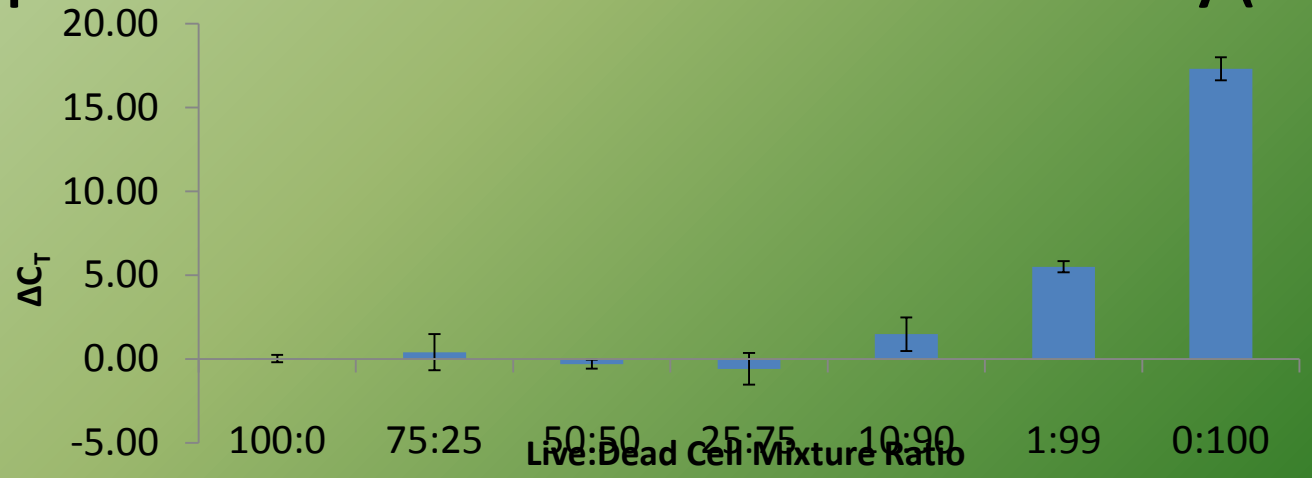


Results:

A) Comparison between Mixed Ratios and Gold Standard

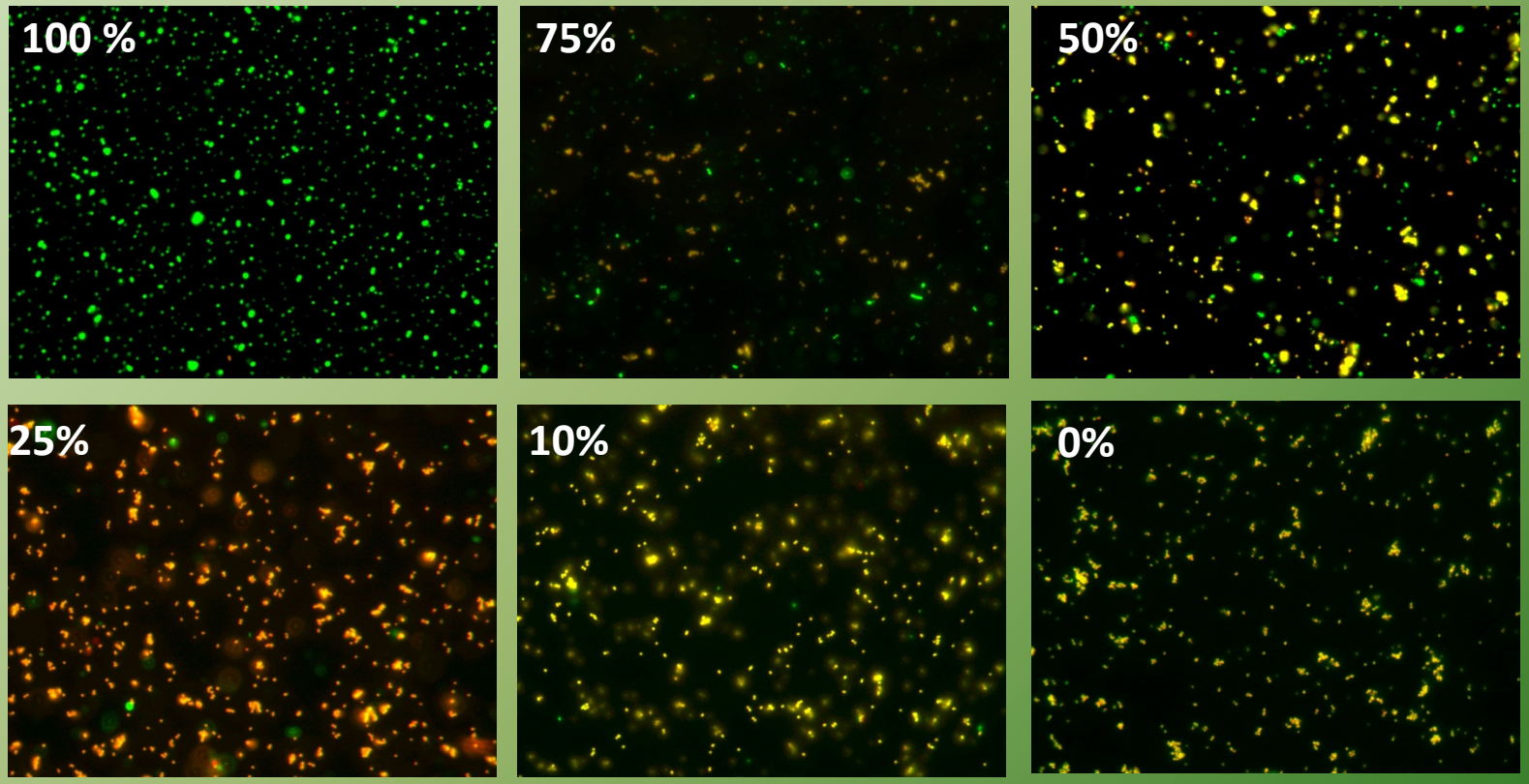


B) PMA-qPCR is effective at >10x reductions in viability (i.e., >10:90)



Results (cont):

C) PMA fluorescently labels cells quantitatively at minor reductions of viability



% Live cells

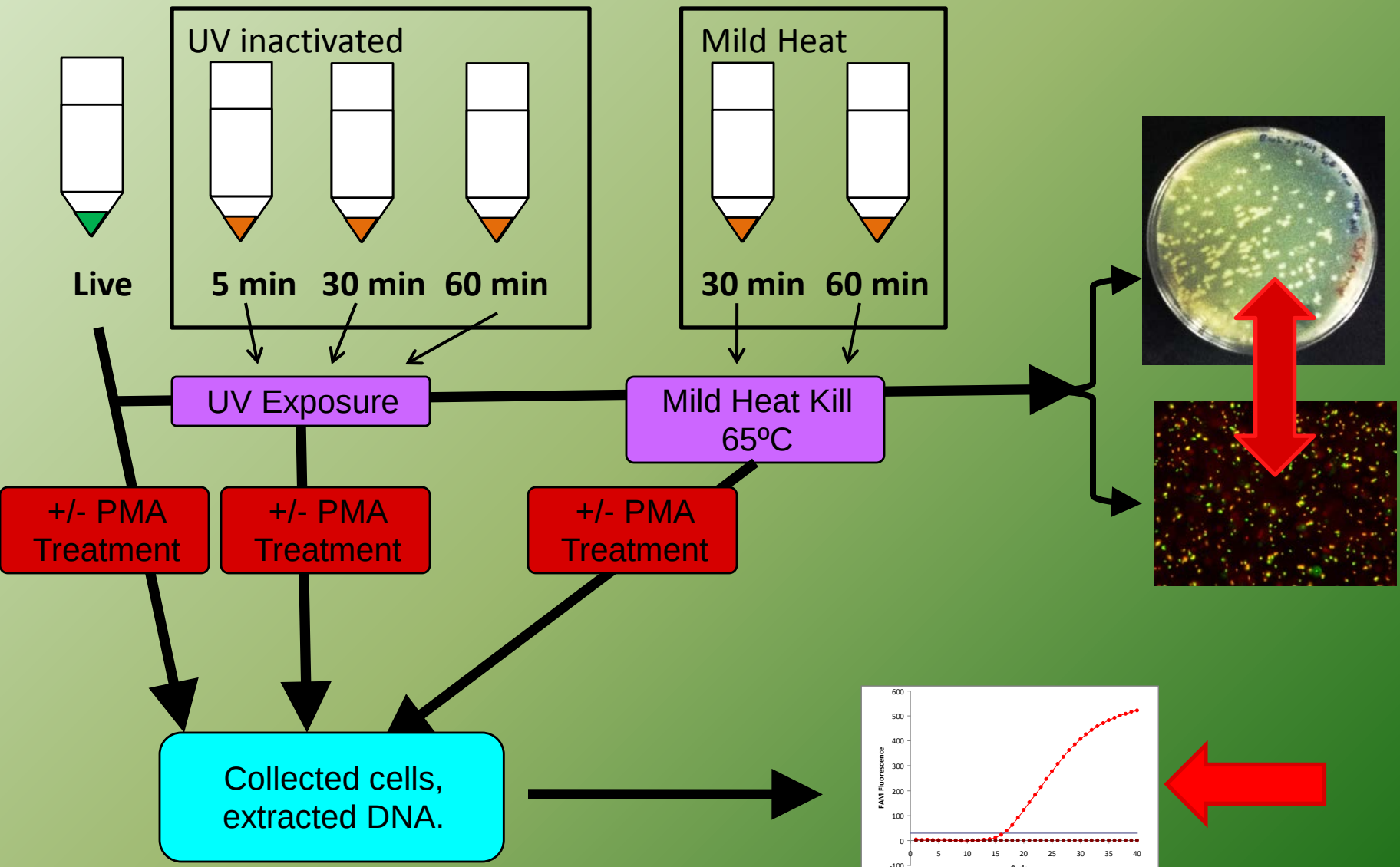
Green - Syto 9 (Total cell stain)

Red – PMA (Dead cell stain)

PMA ✓
qPCR X

Question #2: Mechanism of PMA

Experimental Design



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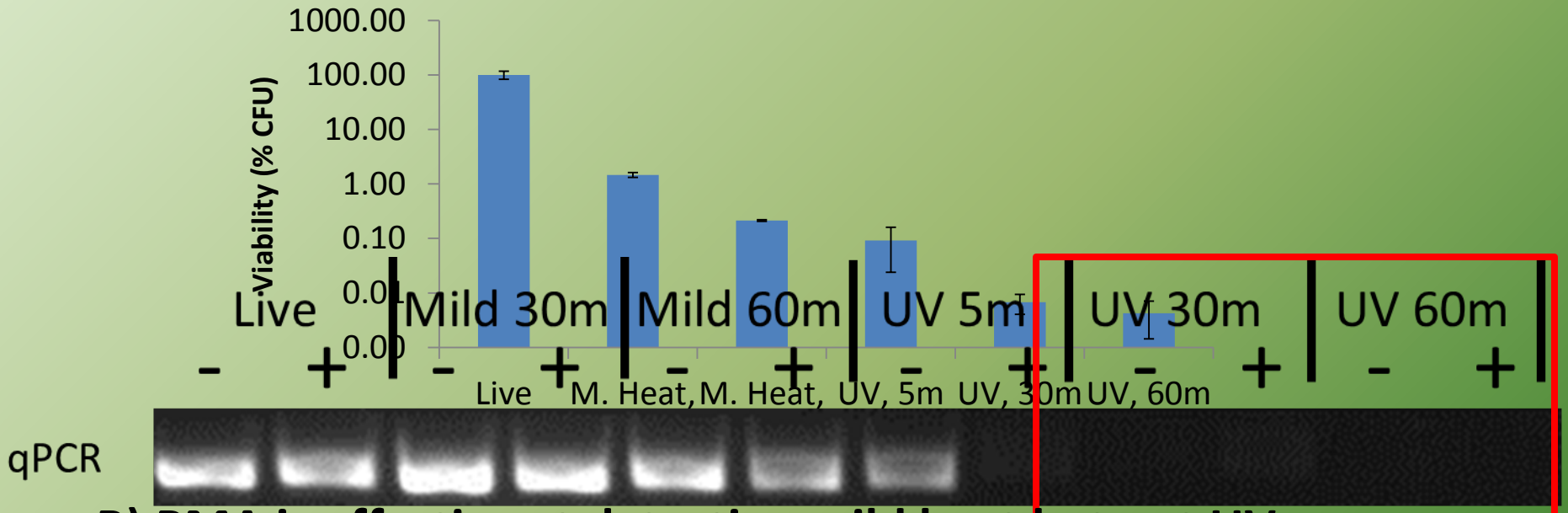
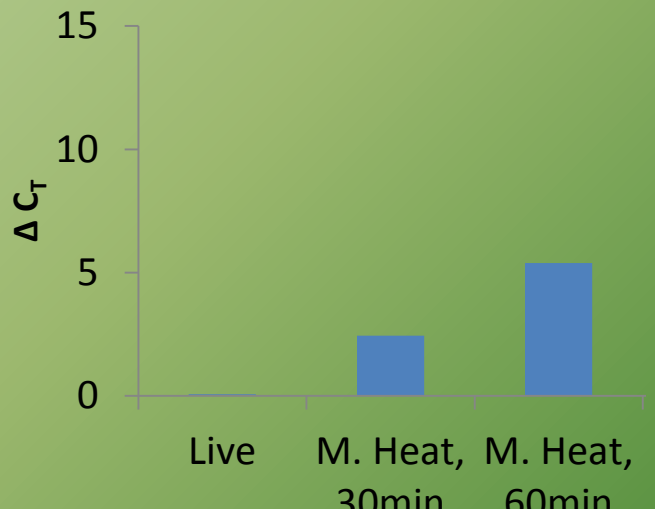
A) Comparison between Mixed Ratios and Gold Standard

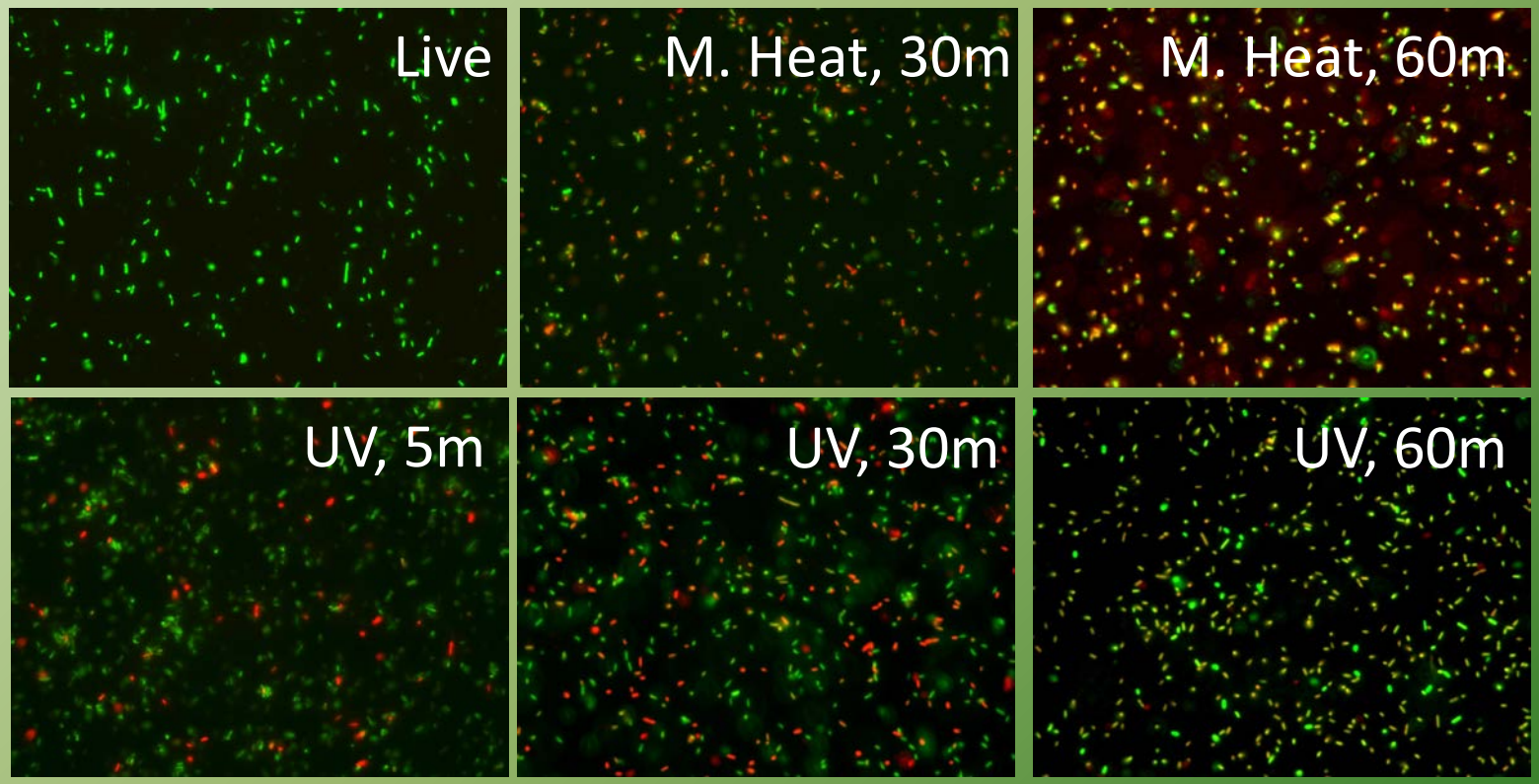


B) PMA is effective at detecting mild heat but not UV inactivated cells loss of viability



Results (cont):

C) PMA fluorescently labels mild heat and UV inactivated cells



Conclusion

- PMA-qPCR is effective at 10x and higher reductions in viability.

- PMA is effective for high and mild heat inactivations, but not UV exposure (destroy DNA)

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