MEDICINAL GENOMICS

Grim Reefer Free DNA Removal Kit

A new method for preventing dead DNA from inflating qPCR results

If the DNA is present - qPCR will detect it

One of the common objections to using qPCR for microbial testing is the fact that the method does not distinguish between live and dead DNA. qPCR primers and probes will amplify any DNA in the sample that matches the target sequence, regardless of viability. In the past, labs have had to use costly, time-consuming, and possibly hazardous methods to solve this live-dead problem.

Our proprietary Grim Reefer Free DNA Removal method is safer, faster, and better than other live-dead solutions.

Pro

qPCR primers and probes will amplify any DNA that matches the target sequence

Con

qPCR cannot distinguish between live or dead DNA

Solution

Eliminate the contaminating dead DNA

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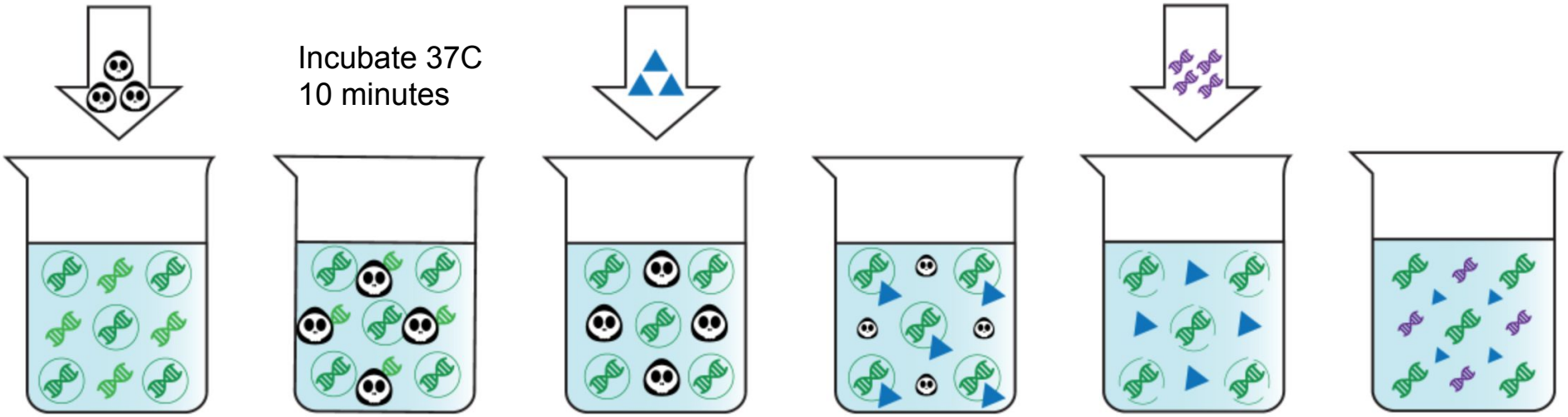
Grim Reefer Method (patent pending)



Add Grim Reefer Enzyme and Buffer to homogenized sample

Grim Reefer Enzyme eats any free DNA present in the sample

Add MGC Lysis Buffer

Lysis Buffer deactivates Grim Reefer Enzyme and lyses viable cells to expose DNA

Add Grim Reefer Positive Control

Positive Control is Free DNA used to show the Grim Reefer Enzyme has been deactivated

DNA IN LIVING CELL

FREE DNA

CELLULAR MATERIAL

GRIM REEFER ENZYME

LYSIS BUFFER

GRIM REEFER POSITIVE CONTROL

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Testing the method

Inoculate a hemp sample with cultured *E. coli* in TSB
Split resulting TSB solution into 12 tubes

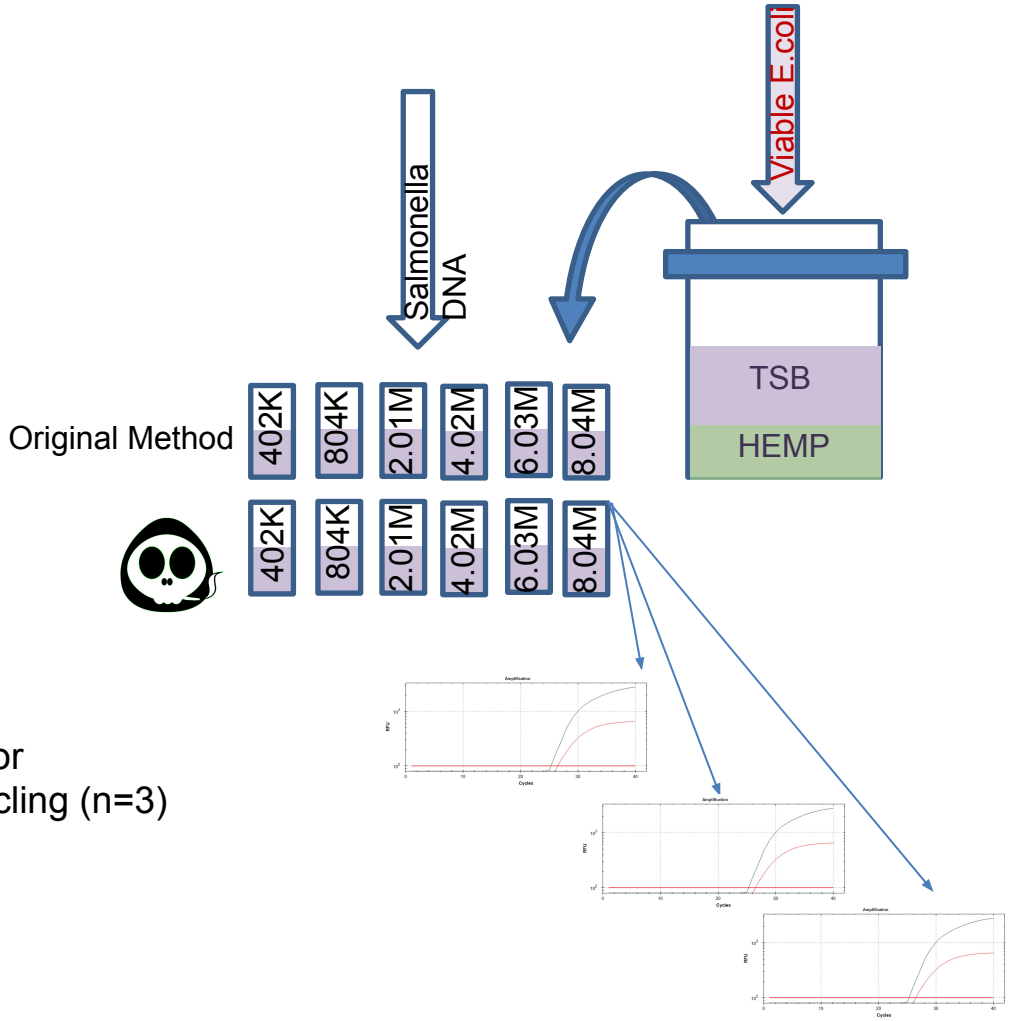
Split the 12 tubes into two sets of 6 tubes
(one Grim Reefer set, one original set)

For each set:
Spiked in 6 different levels of *salmonella* DNA (n=1)

1 = 402,000 copies
2 = 804,000 copies
3 = 2,010,000 copies
4 = 4,020,000 copies
5 = 6,030,000 copies
6 = 8,040,000 copies

Aliquot each of the 12 samples into 3 separate wells for
lysis, purification, primer/probe addition and thermocycling (n=3)

Total: 12 samples, 36 qPCR runs



E.coli Results

Hypothesis:

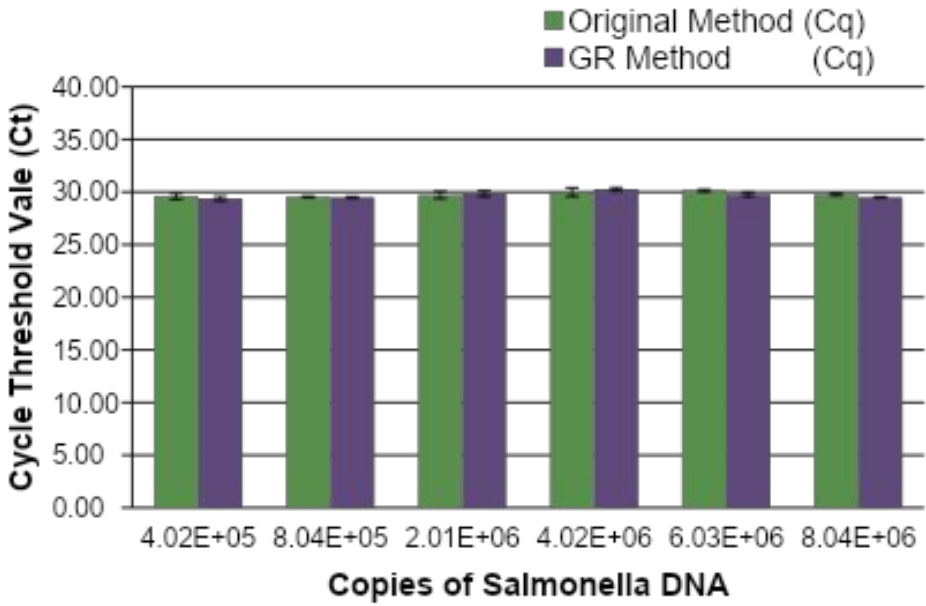
Due to inherent presence of *E. coli* DNA, E. coli results for the GR method may be lower than the Original Method, but since *E. coli* was freshly cultured the *E. coli* results will be more similar between methods than the internal control results. Note: *E.coli* cultured for 4hrs

Conclusions:

1. There is **no** statistically significantly difference between the *E. coli* results of the GR Method (avg. Cq = 29.6) and the Original Method (avg. Cq = 29.9).

2. This result is consistent with the hypothesis that the GR method does not interfere with DNA from live cells.

3. Both methods demonstrated robustness (no impact from *Salmonella* DNA concentration).



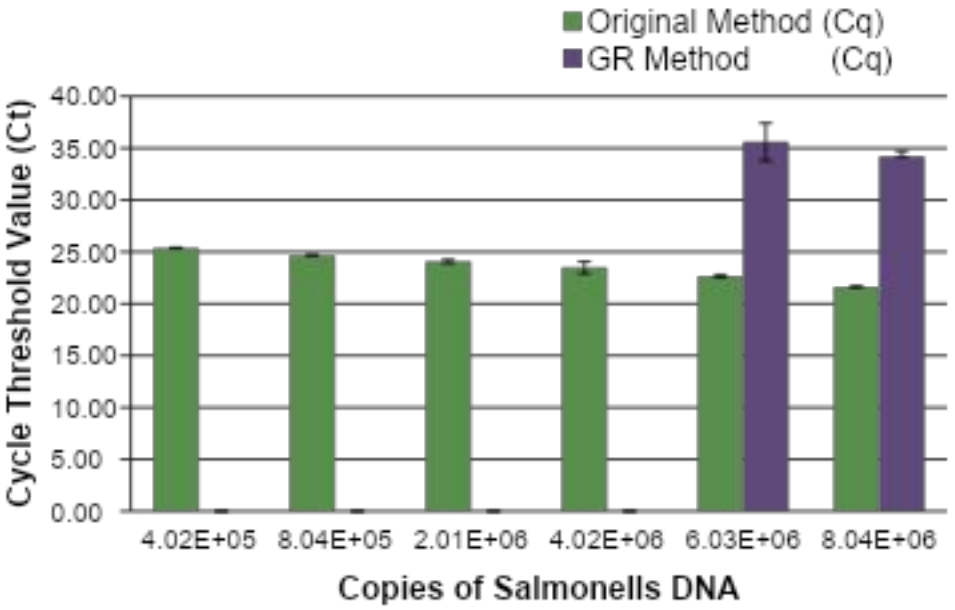
Salmonella Results

Hypothesis: Free *Salmonella* DNA will not be detected for the GR method until the amount of free DNA exhausts the enzyme.

Conclusion:

1. The GR method can consistently (n=3) deactivate 4.02 million copies of free DNA or less.

2. The results from the GR Method are exclusive of free DNA as long as free DNA ≤ 4.02 million copies. A 10 fold dilution is represented by a 3.3 Ct shift. For the 6.03 million copies there was an approximate 12.93 Ct shift which is about a 7,500 fold reduction.



Grim Reefer Positive Control Results

Hypothesis: The GR positive control DNA, is added to both GR and original method samples before DNA extraction, will be similar (at the 95% confidence interval, result in a P-value > 0.05). demonstrating GR does not impact DNA after lysis solution addition.

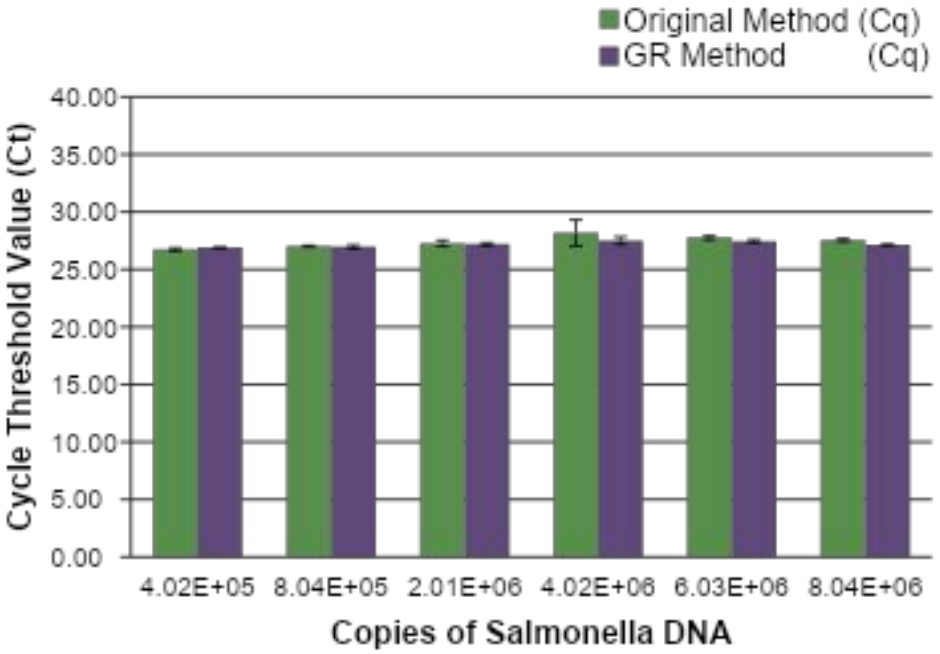
Conclusions:

1. The null hypothesis (the GR and Original Method results are the same) can **not** be rejected at the 95% confidence interval, since the resulting P-value = 0.161 > 0.05

2. There is **no** statistically significantly difference between the GR Control results of the GR Method (avg. Cq = 27.18) and the Original Method (avg. Cq = 27.42).

3. This result is consistent with the hypothesis that the GR enzyme is no longer digesting free DNA after incubation at 37C for 10 min.

4. Both methods demonstrated robustness (no impact from *Salmonella* DNA concentration).



Internal Hemp/Cannabis Control Results

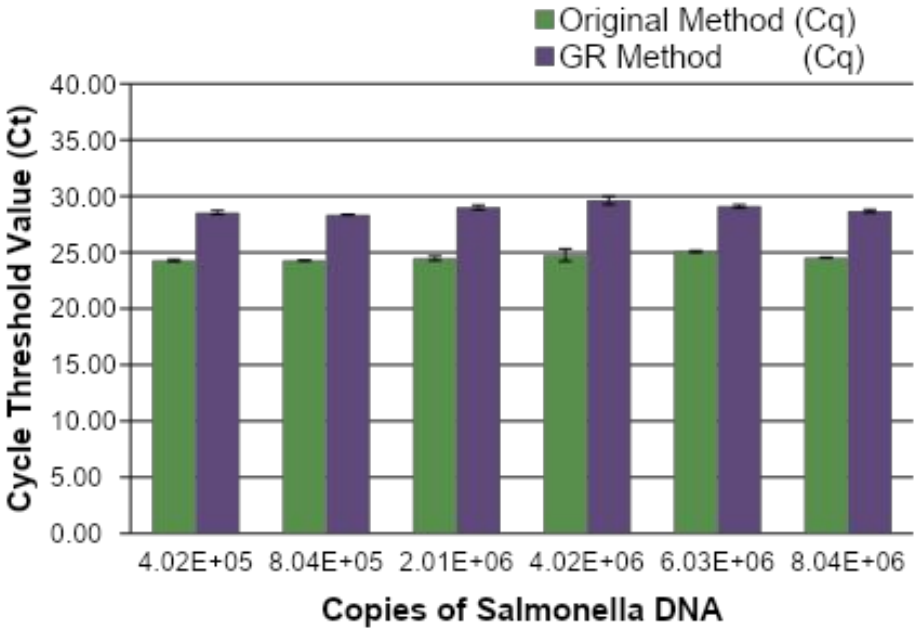
Hypothesis: Due to inherent internal control presence as free DNA, internal control results for the GR method will be significantly lower than the Original Method (at the 95% confidence interval, result in a P-value < 0.05).

Conclusions:

1. The null hypothesis (the GR and Original Method internal control results are the same) can be rejected at the 99% confidence interval, since the resulting **P-value = 2.45 x 10^{-25}**

2. There is statistically significantly less hemp DNA detected in the GR Method (avg. Cq = 28.8) than the Original Method (avg. Cq = 24.7).

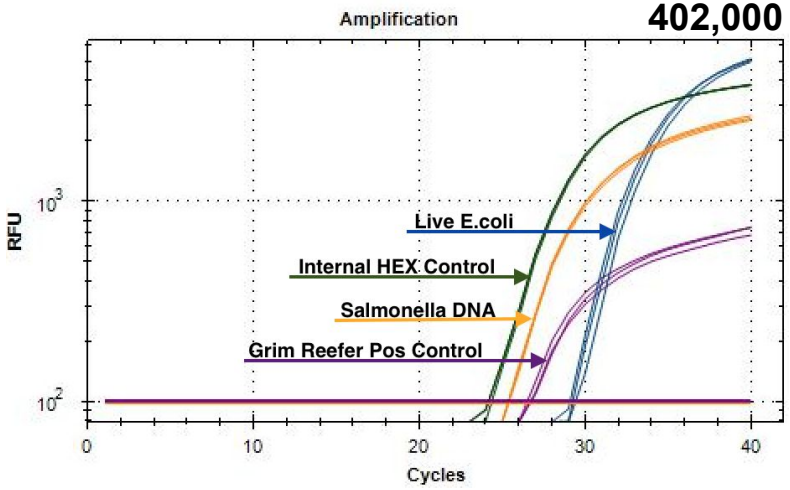
3. This result is consistent with the hypothesis that only hemp DNA inside viable cells is detected with the GR method.



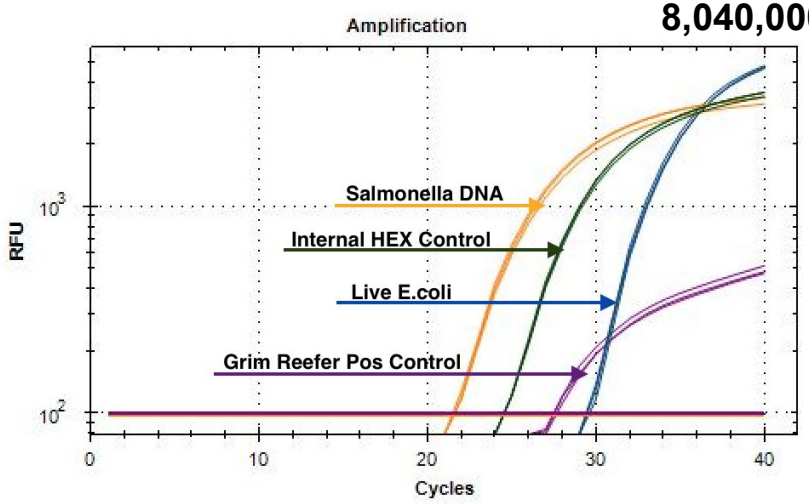
qPCR Curves

Original Method

402,000 copies *Salmonella* DNA

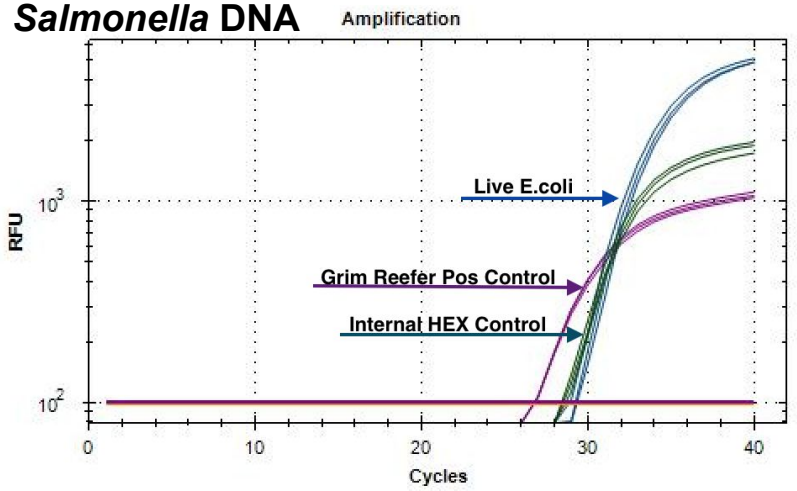


8,040,000 copies *Salmonella* DNA

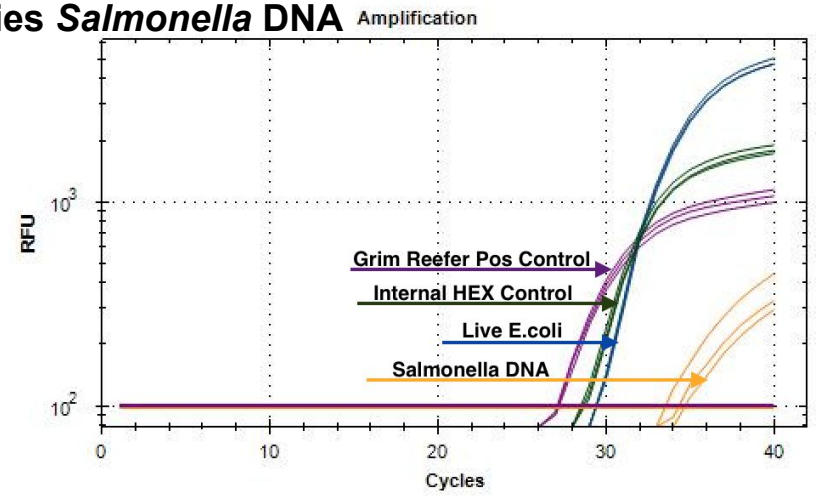


Grim Reefer Method

402,000 copies *Salmonella* DNA



8,040,000 copies *Salmonella* DNA



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Conclusion Summary

1. The GR Method is not metabolizing DNA in viable cells.
2. The GR Method is not metabolizing DNA after deactivation (addition of lysis buffer).
3. The GR Method is capable of metabolizing free DNA ≤ 4.02 million copies.
4. Both the Original and GR methods demonstrate a high level of precision (RSD < 2%).
5. Both methods demonstrated robustness for all analytes observed (none were impacted from *Salmonella* DNA addition).
6. The Original Method results are proportional to the amount of free DNA + DNA in viable cells.

Beta Testing

4 independent labs for beta testing

- 77 flower samples - Total Aerobic
- 70 flower samples - Total Yeast and Mold
- 14 flower samples - Aspergillus multiplex
- 7 flower samples - Entero/Coliform

Snap shot of beta results

Sample	Target (FAM)	Ct (FAM)	Target (HEX)	Ct (HEX)	Target (Cy5)	Ct (Cy5)
Lab 1 Original Method	TAC	25.20	SCCG	26.21	GR Positive Control	26.01
Lab 1 Grim Reefer	TAC	27.95	SCCG	32.36	GR Positive Control	26.47
Lab 2 Original Method	TAC	25.87	SCCG	27.24	GR Positive Control	30.26
Lab 2 Grim Reefer	TAC	ND	SCCG	36.57	GR Positive Control	30.92
Lab 3 Origianl Method	TAC	29.94	SCCG	30.86	GR Positive Control	30.72
Lab 3 Grim Reefer	TAC	29.89	SCCG	33.76	GR Positive Control	29.87
Lab 4 Original Method	TAC	26.24	SCCG	24.34	GR Positive Control	26.78
Lab 4 Grim Reefer	TAC	37.79	SCCG	27.67	GR Positive Control	26.81

Lab 1: Shift in TAC signal, large shift in SCCG signal, GR Positive control similar

Lab 2: TAC signal disappears, large shift in SCCG signal, GR Positive control similar

Lab 3: No shift in TAC signal, shift in SCCG signal, GR Positive control similar

Lab 4: Large shift in TAC signal, shift in SCCG signal, GR Positive control similar

Other Free DNA Removal Products

- PMA (Propidium monoazide) is a high-affinity photoreactive DNA intercalating dye. Upon photolysis, the dye covalently reacts with DNA. This results in permanent DNA modification which renders the DNA insoluble and results in its loss during DNA extraction. The dye is cell membrane impermeable and therefore can be used to modify only exposed DNA from dead cells.
    - Requires incubation in the dark followed by exposure to visible light (high power halogen lamps or specific LED devices)
    - PMA does not work equally across all microorganisms. Different organisms require different PMA concentrations and light exposure times.
    - PMA is toxic and creates biohazard waste.
- 15-30 min enzymatic treatment which requires a 15min inactivation step at 95-100 °C
    - Product literature claims an average signal reduction of 2 logs or 6 Ct's
    - Heating a sample to 95 °C for enzyme inactivation will result in the lysis of live organisms

In Summary...

- Grim Reefer offers a simple 10 minute 37 °C upfront incubation, that is non toxic, and easily deactivated without causing harm to viable organisms.
- Grim Reefer results in 3-4 log (12-13 Ct) reduction in signal from free DNA
- Grim Reefer method includes an added positive control to monitor the deactivation of the enzyme.
- Grim Reefer can be easily added into the current MGC DNA extraction protocol and provide qPCR results that are void of dead DNA.
- This can be used to guide sterilization protocols at grows

How to Buy?

The Grim Reefer Free DNA Removal Kit contains the GR enzyme and buffer reagent. There is enough reagent to process 250 tests (0.25 gram sampling) or 125 tests (1 gram sampling).

The Grim Reefer Assay and Control are also required to run the protocol but are sold separately.

The Grim Reefer products can be found on the Medicinal Genomics webstore or by contacting Medicinal Genomics at 877-574-3582
or by emailing us at sales@medicinalgenomics.com