Plasma Tip Cleaning in High Throughput Plant Genomics

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IonField SYSTEMS™

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Abstract

High throughput genomic protocols have revolutionized plant strain development. However, the sensitivity of genomic assay systems and the complexity of plant extracts have imposed the use of costly single use plastic tips for sample transfer. We at IonField Systems have developed a process utilizing an initial solvent wash in a sonicating water bath followed by the TipCharger plasma treatment system that efficiently removes residual plant matter and DNA from the plastic tips, allowing confident reuse of the tips. Our process removes DNA to undetectable levels, and allows most tips to be reused at least 10 times. This process provides a money-saving alternative to single use lab ware, without decreasing the system speed needed for high throughput plant genomic analysis.

- **Plasma, the forth state of matter, is energetically charged gas.**
- **Plasma can be generated by dielectric barrier discharge in air, using a high frequency, high voltage, low amperage electrical field.**
- **The interaction of plasma with air generates oxidizing radicals that break down all organic materials.**

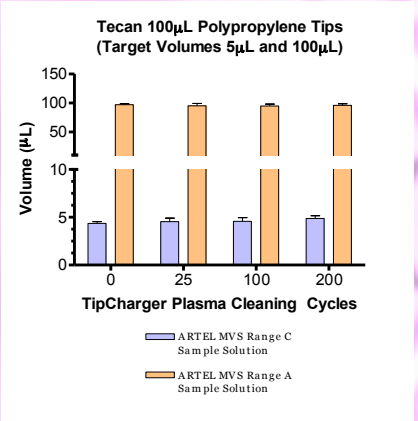
- **IonField Systems generates plasma within a module dimensioned to assay plate footprint.**
- **30 second plasma treatment breaks down organic DNA and other organics, releasing nontoxic gas byproducts.**
- **Plastic tips are routinely used 10 times without loss of assay sensitivity or robustness.**

FIGURE 1: Pipetting fidelity following TipCharger

TipCharger treatments do not impact the ability of plastic pipette tips to accurately deliver liquids to an assay plate.

Pipet procedure

- **Pipet standard reagent (5 µl and 100 µl) using tips treated with TipCharger for up to 200 cycles**
- **Quantitate volume according to manufacturers recommendation**

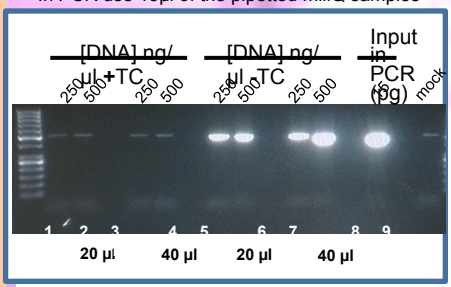


CONCLUSIONS: Cold plasma treatment of pipette tips using TipCharger does not adversely impact pipetting accuracy.

FIGURE 2: TipCharger Eliminates DNA carryover

Pipet procedure

- Pipet 20µl DNA sample up and down in needle 1
- Pipet 40µl DNA sample up and down in needle 2
- Tecan wash and TipCharger (100µl clean)
- Pipet 20µl sterile miliQ to a tube (needle 1)
- Pipet 40µl sterile miliQ to a tube (needle 2)
- Same protocol again without TipCharger
- In PCR use 15µl of the pipetted miliQ samples

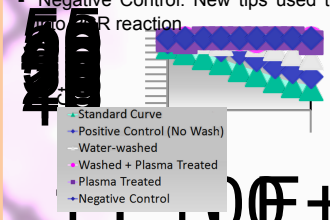


Demonstrates Cold plasma treatment can eliminate DNA from pipette tips.

FIGURE 3: PCR analysis shows TipCharger Eliminates DNA carryover

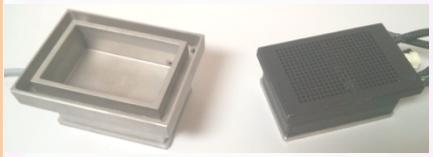
Pipet procedure

- Standard curve: New tips used to pipet plasmid DNA dose-response curve into PCR reaction
- Positive Control: Used tips used to pipet water into PCR reaction without cleaning
- Water-washed: Used tips are water-washed, then used to pipet water into PCR reaction
- Washed plus plasma treated: Used water-washed tips are treated with plasma (30 seconds), then used to pipet water into PCR reaction
- Plasma treated: Used tips are treated with plasma (30 s), then used to pipet water into PCR reaction
- Negative Control: New tips used to pipet water into PCR reaction



Plasmid DNA Sample Concentration (Log Scale)

FIGURE 4: PCR Analysis from Plant Extracts using IonField's TrUE System



In a side by side comparison carried out using 5,000 seed and leaf extracts, gene identifications derived from PCR reactions using single use tips and used tips cleaned with IonField's TrUE System (ultra sonic pre-wash followed by plasma treatment) were identical – not one sample's results would have been reported differently

CONCLUSION: IonField's TipCharger can decrease cost & improve throughput