## Development of Immunocapture Real-Time PCR to detect *Fusarium* species in Grains and Foods

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## Concern for *Fusarium* species

- Trichothecenes, zearalenone, fumonisins
- FDA advisory levels
- Regulated in other countries
- Mycotoxins survive processing
- *Fusarium* mycotoxins in foods

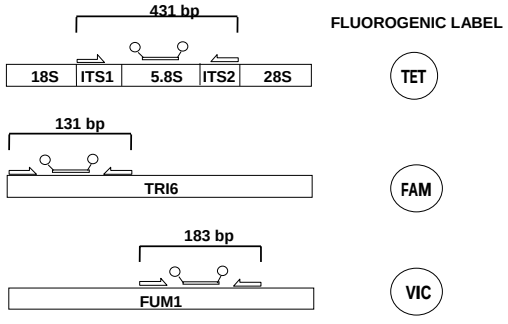
## Objective of Research

- To develop immunocature of *Fusarium* species using antibodies
- To make PCR primers specific for *F. graminearum* and *F. verticillioides*
- To use immunocapture real-time PCR to detect *Fusarium* species in foods and grains

## PCR Primers for *Fusarium* species

- General - DNA flanking the 5.8S rDNA
- *Trichothecene - Tri6* regulatory gene for biosynthesis
- Fumonisin - *Fum1* polyketide synthase gene for biosynthesis

## Primers and Probes Developed




## Immunocapture Methods

- To use immunocapture qPCR to detect *Fusarium* species in foods
- Antibodies produced to *F. graminearum and F. verticillioides*
- Developed immunocapture qPCR
- Problem – release of DNA from conidia

## Proposed Future Research from 2004

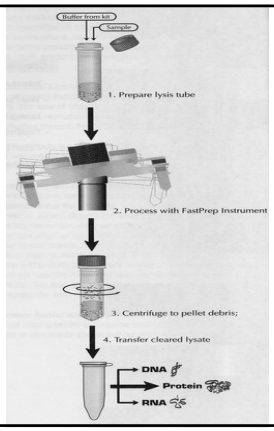
1. Procedures to break conidia for qPCR
2. Simplify immunocapture before qPCR
3. Test in industry to determine cost effectiveness

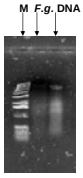
## 1. Disruption of Conidia

- Enzymes to lyse conidia
- Microwave
- Vortex with beads, sand, etc.
- FastDNA® SPIN Kit with FastPrep® Instrument

## Disruption of Conidia

*Fusarium* in broth on shaker for 2 days at room temperature → Filtered broth through 8 layers cheesecloth for pure conidia → Wash spores - sterile water → High speed disruption (FastPrep) – DNA from $10^2$ to$10^7$conidia → PCR using ITS, Tri6 and Fum1 primers



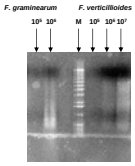



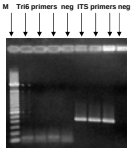

Genomic DNA from *F. graminearum* mycelia

Beat 2 times for 40s at speed setting 6 on FastPrep machine

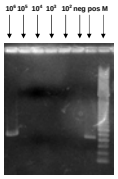
Genomic DNA from *F. graminearum* and *F. verticillioides* conidia

Beat 6 times for 40s at speed




PCR product using genomic DNA from *F. graminearum* mycelia




PCR product using DNA from *F. graminearum* conidia and ITS primers

Beat 6 times for 40s at speed

## 2. Immunocapture qPCR

- Simplify immunocapture before qPCR
- Depends on disruption of conidia

## 3. Proposed Processing Research

- **Test in industry to determine cost effectiveness**
- **Proposal submitted to USDA NRI**
- **Ranked High Priority but not funded**

## Summary of Research

- **Methods – enzymes, beads, microwave – no results**
- **FastPrep® Kit and Instrument**
- **Recommended procedure – no DNA**
- **Microscopic slides – intact conidia**
- **Too much beating destroys DNA**
- **Need to develop proper protocol**

## Future Research

- **Continue to refine FastPrep® Method**
- **Combine with qPCR**
- **Use with food and grain samples**
- **Future funding questionable**

## Genetic Detection Research

- **Monitor genetics for fungal epidemics**
- **Library of PCR primers**
- **Conventional, real-time, multiplex PCR**
- **Fungi that produce major mycotoxins**
  **- aflatoxins, fumonisins, ochratoxin, patulin, trichothecenes, zearalenone**