

## Immunocapture Real-Time PCR to Detect Mycotoxigenic Mold Spores in Grains and Foods

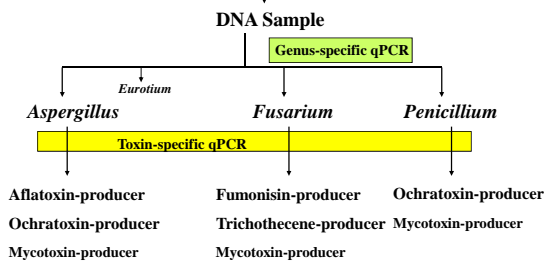
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 Department of Food Science  
 C. P. Woloshuk  
 Department of Botany and Plant Pathology  
 Purdue University

## Objectives of Research

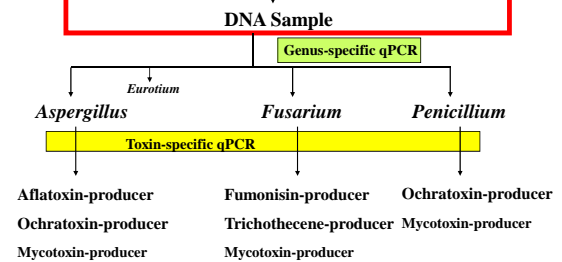
- To study methods to break mold spores to release DNA
- To incorporate the best method into immunocapture real-time PCR to detect *molds* in foods and grains
- To develop a PCR library to mycotoxigenic molds

### Detection Strategy

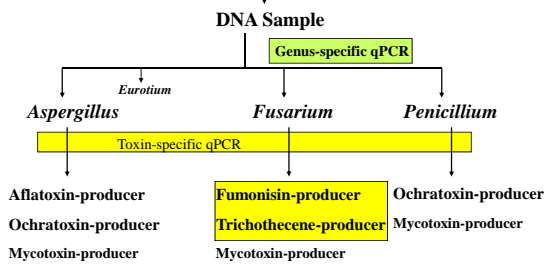
Collection of Sample with Mycotoxigenic Fungi



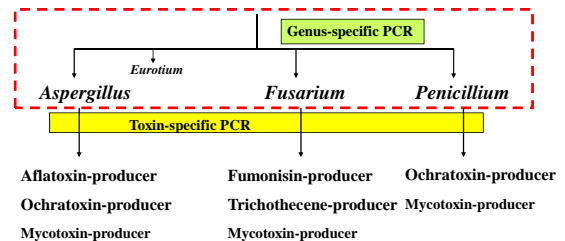
Collection of Sample with Mycotoxigenic Fungi

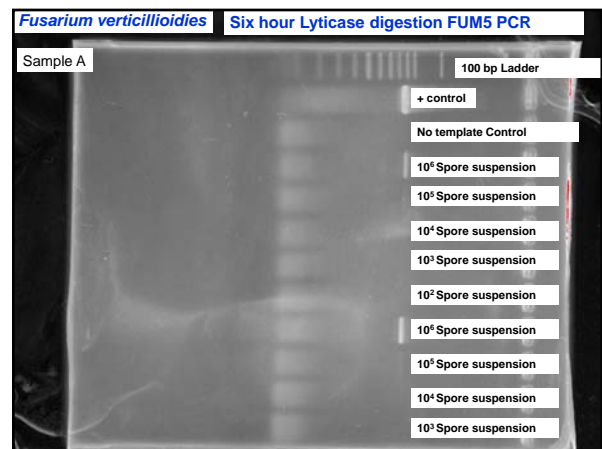
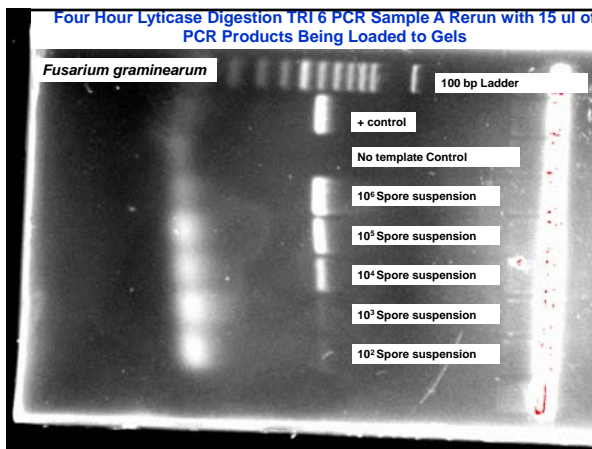
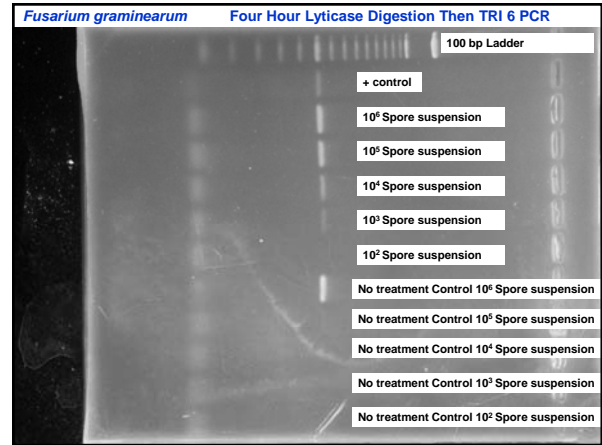
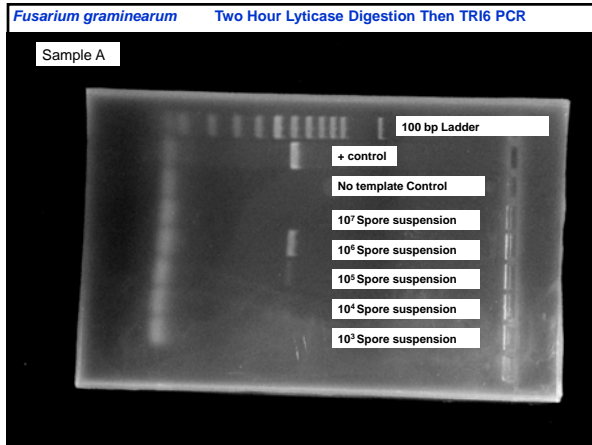
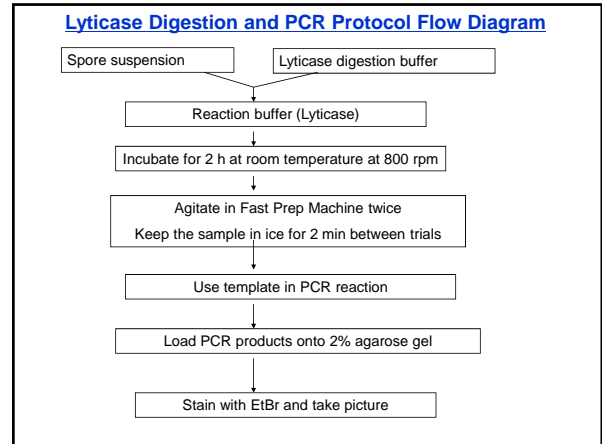
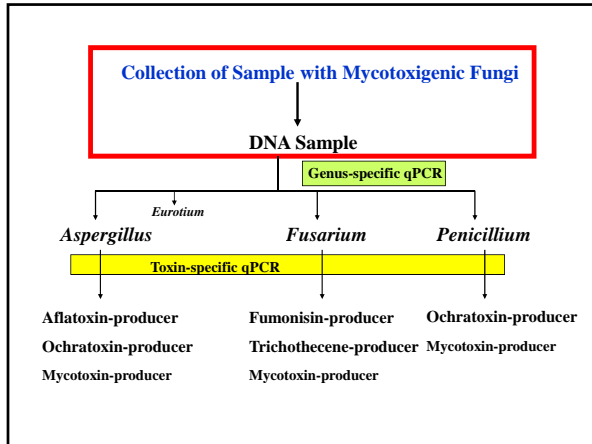


Collection of Sample with Mycotoxigenic Fungi

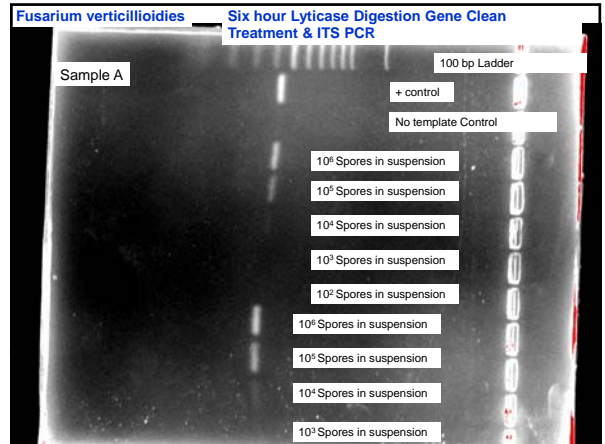
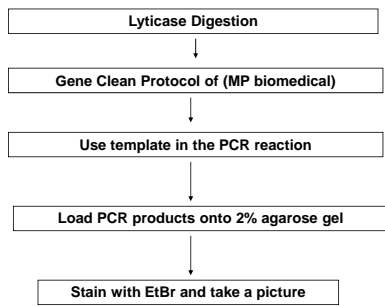


DNA Sample

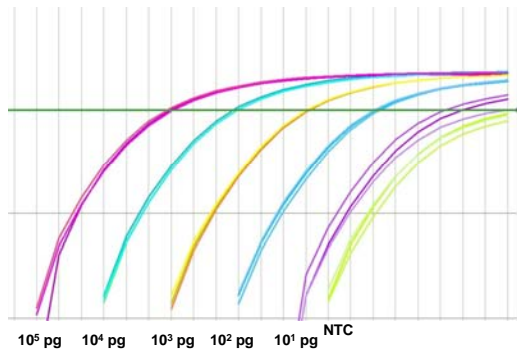




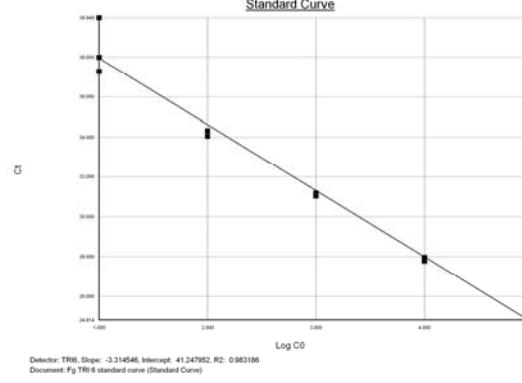
**Purification & Concentration of Lyticase Digestion Products Before Doing PCR Protocol**



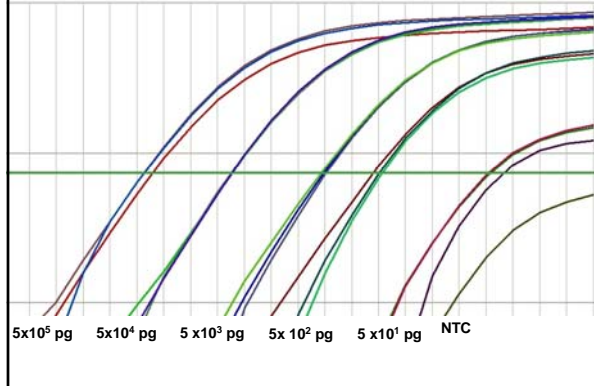
***Fusarium graminearum* Genomic DNA Dilution Series Using TRI6 Primers and Probe - Real-time PCR Amplification Plot**



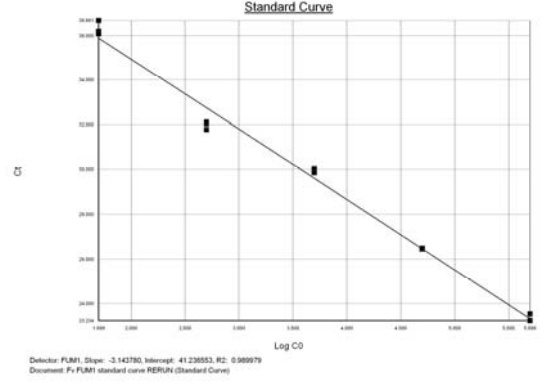
***Fusarium graminearum* Genomic DNA Dilution Series Using TRI6 Primers and Probe - Real-time PCR Standard Curve**

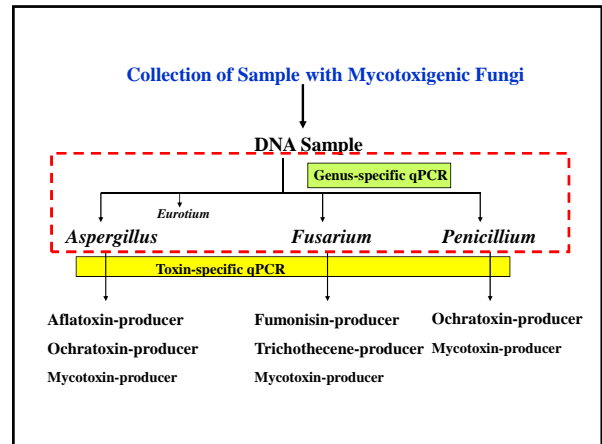
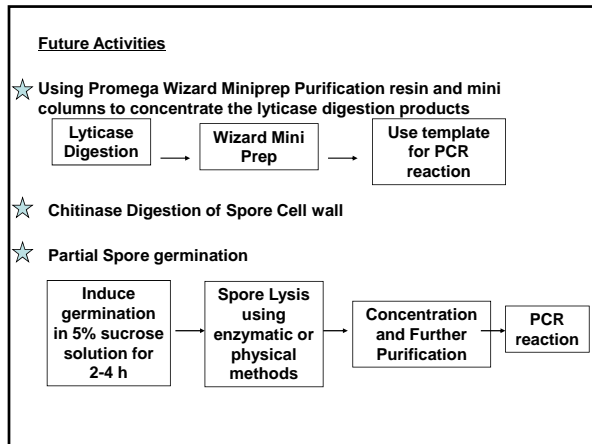


***Fusarium verticillioides* A 4643 Genomic DNA Dilution Series Using FUM1 Primers and Probe Real-time PCR Amplification Plot**



***Fusarium verticillioides* A 4643 genomic DNA dilution series using FUM1 primers and probe Real-time PCR standard curve**



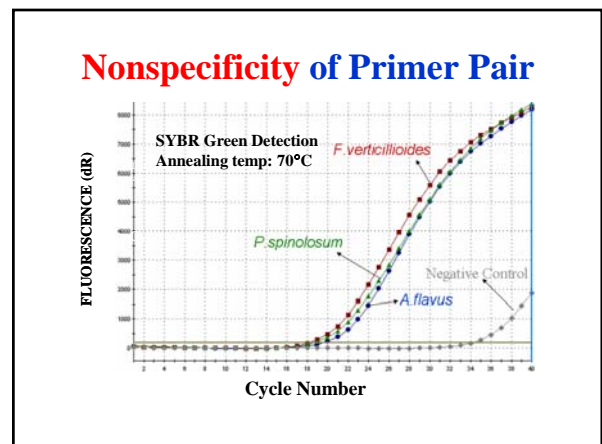
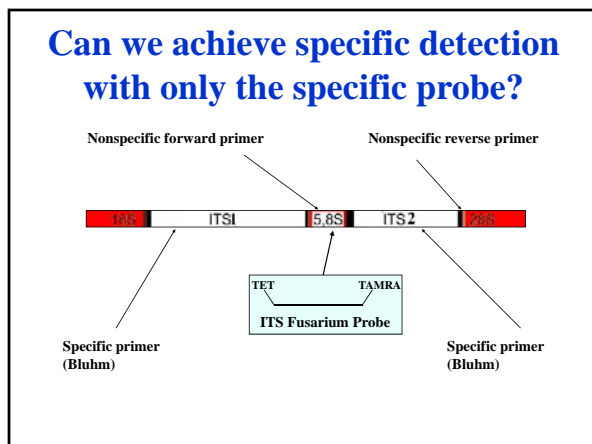


**Development of Multiplex Real-Time PCR Assay to Detect, and Quantify Three Genera of Mycotoxigenic Fungi**

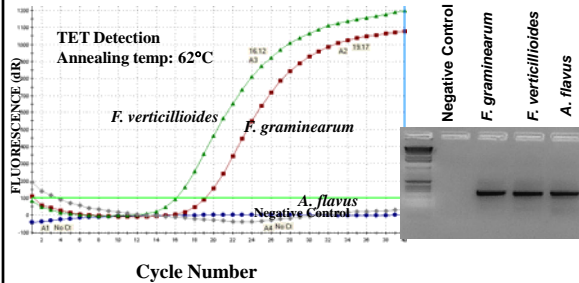
Yenny Suanthie  
Botany and Plant Pathology

**Objectives**

1. To develop genus-specific probes for mycotoxigenic species: *Aspergillus*, *Penicillium*, and *Fusarium*
2. To develop a multiplex qPCR assay
3. To validate the multiplex assay



## Can we achieve specific detection with only the specific probe?



## Objective 1: To develop genus-specific probes for mycotoxigenic species: *Aspergillus*, *Penicillium*, and *Fusarium*

### Criteria for species selection

- Based on: mycotoxins (production, importance) and occurrence (habitat, frequency)
- Very Important** = common health-related mycotoxins + often reported on grains and foodstuffs or **important in grain storage**.
  - Important** = mycotoxins that are not known to cause health problems + rare in foods
  - Less Important** = no mycotoxins production reported or known

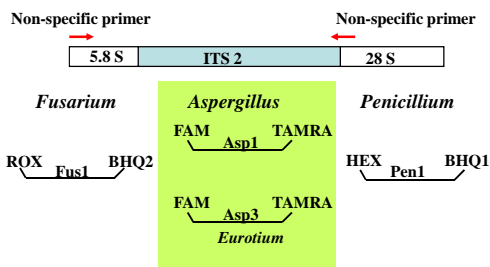
## Aspergillus

VERY IMPORTANT	IMPORTANT	LESS IMPORTANT
<i>A. nidulans</i>	<i>A. aculeatus</i>	<i>A. flavipes</i>
<i>A. candidus</i>	<i>A. fumigatus</i>	<i>A. penicillioides</i>
<i>A. clavatus</i>	<i>A. nivea</i>	<i>A. restrictus</i>
<i>A. flavus</i>	<i>A. oryzae</i>	<i>A. sydowii</i>
<i>A. parasiticus</i>	<i>A. terreus</i>	
<i>A. niger</i>	<i>A. tamaritii</i>	
<i>A. ochraceus</i>	<i>A. ustus</i>	
<i>A. versicolor</i>	<i>A. wentii</i>	
<i>A. nomius</i>		
<i>Eurotium sp.</i>		

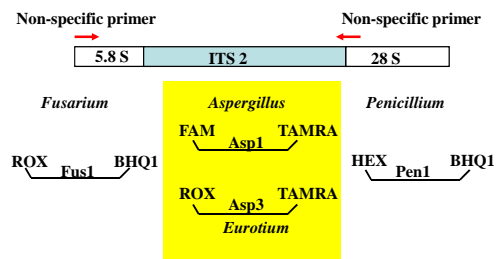
## Penicillium

VERY IMPORTANT	IMPORTANT	LESS IMPORTANT
<i>P. citrinum</i>	<i>P. crustosum</i>	<i>P. decumbens</i>
<i>P. oxalicum</i>	<i>P. citreonigrum</i>	<i>P. glabrum</i>
<i>P. aurantiogriseum</i>	<i>P. janczewskii</i>	<i>P. spinulosum</i>
<i>P. chrysogenum</i>	<i>P. canescens</i>	<i>P. implicatum</i>
<i>P. griseofulvum</i>	<i>P. janthinellum</i>	<i>P. restrictum</i>
<i>P. hordei</i>	<i>P. paxilli</i>	<i>P. sclerotiorum</i>
<i>P. verrucosum</i>	<i>P. raistrickii</i>	<i>P. thomii</i>
<i>P. viridicatum</i>	<i>P. novae-zeelandiae</i>	<i>P. phoeniceum (Eu.)</i>
<i>P. roquefortii (Epaneum)</i>	<i>P. simplicissimum</i>	<i>P. hirayamae (Eu.)</i>
<i>P. expansum</i>	<i>P. aethiopicum</i>	<i>P. corylophilum</i>
	<i>P. brevicompactum</i>	<i>P. fellutanum (Pcharlesii)</i>
	<i>P. glandicola</i>	<i>P. waksmanii</i>
	<i>P. commune (Planosum)</i>	<i>P. camemberti</i>
	<i>P. hirsutum</i>	<i>P. echinulatum</i>
	<i>P. italicum</i>	<i>P. digitatum</i>
		<i>P. allii</i>
		<i>P. ulaiense</i>
		<i>P. nalgiovense</i>
		<i>P. olsonii</i>
		<i>P. solitum</i>

## Objective 2: To develop a multiplex qPCR assay



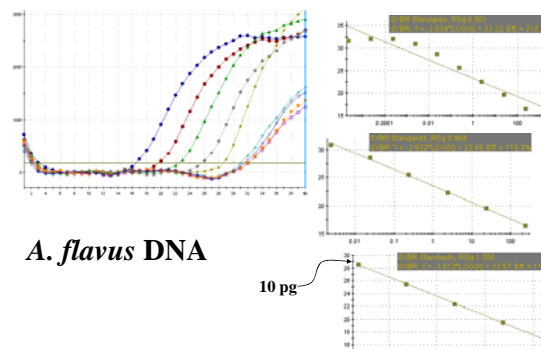
## Alternate Multiplex qPCR Assay



## Factors That Need Consideration

- Probes concentration: 1 pmole
- Primers concentration: 10 pmole
- Annealing temperature: 70 C

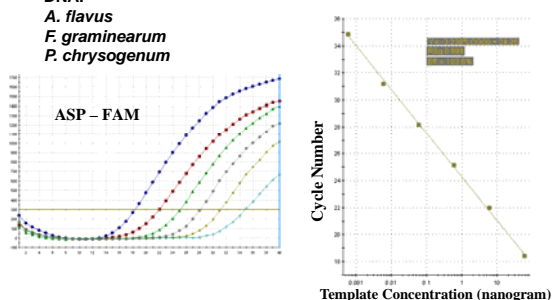
## Template Concentration



## Template Concentration

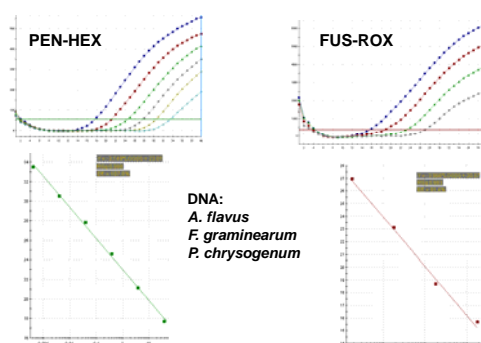
DNA:  
*A. flavus*  
*F. graminearum*  
*P. chrysogenum*

Multiplexed Assay



## Template Concentration

Multiplexed Assay



## Assay Summary

- Probes concentration: 1 pmole
- Primers concentration: 10 pmole
- Annealing temperature: 70 C
- Detection range: 10 pg - 100 ng

## OBJECTIVE 3: To Validate The Multiplex Assay

- To test various isolates
- To collect air samples in a wheat field
- To monitor storage of distillers grain

