

# Molekylære metoder til detektion af frøsygdomme

- PCR/real-time PCR
- Microarray/DNA-chip

Mogens Nicolaisen og Annemarie Fejer Justesen



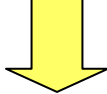
AARHUS UNIVERSITY

Faculty of Agricultural Sciences – Department of Integrated Pest Management - Flakkebjerg

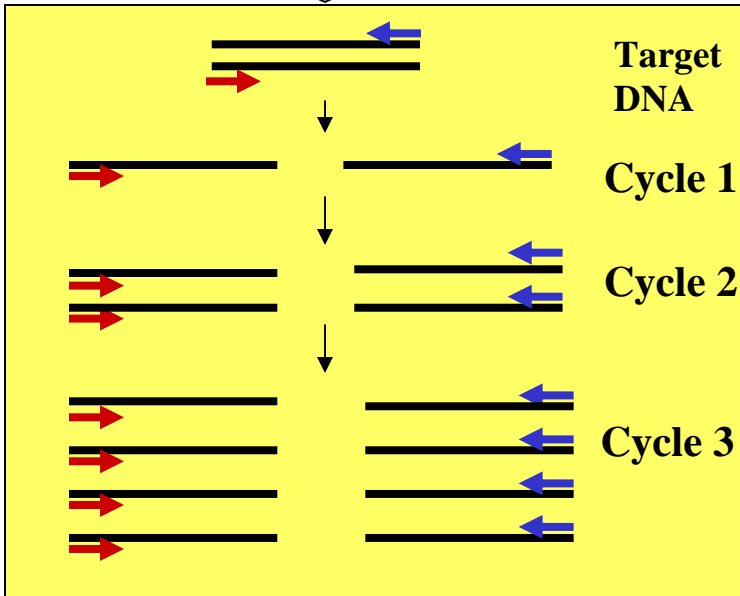
# PCR analyse



DNA-ekstraktion

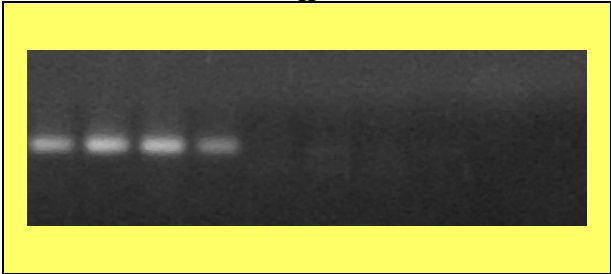


PCR

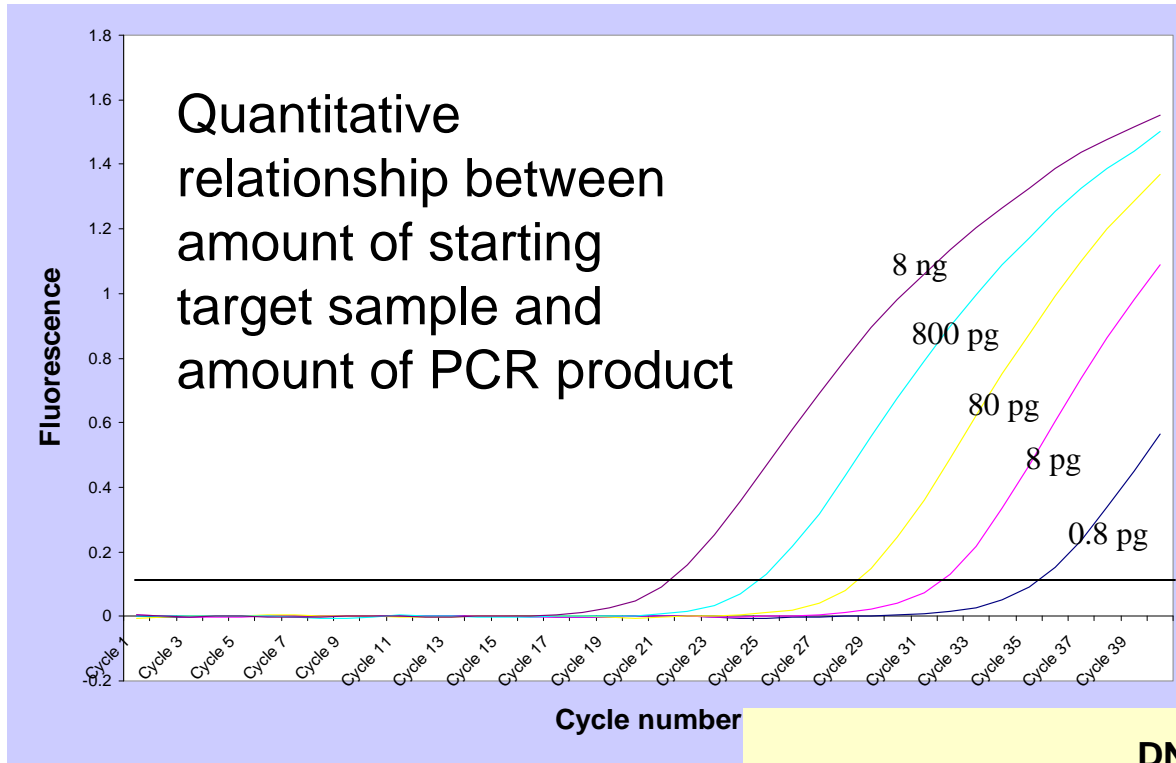


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GCTTCACATGCGACCAACCCACCCTTT  
TTCGCTTGAATGCCTGTGGCTGCCGCGT  
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GTTTTCAAAATCGTATCCGGGCTTTGT  
CGTCCTGAATCGGCTTGAAGAGCATGCC  
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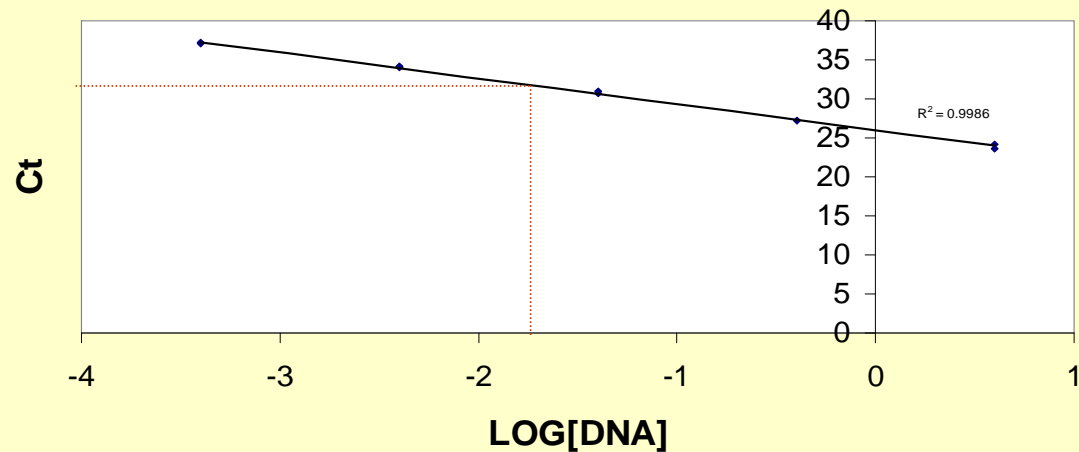
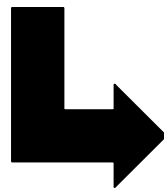
Gelkørsel



# Kvantificering vha. real-time PCR



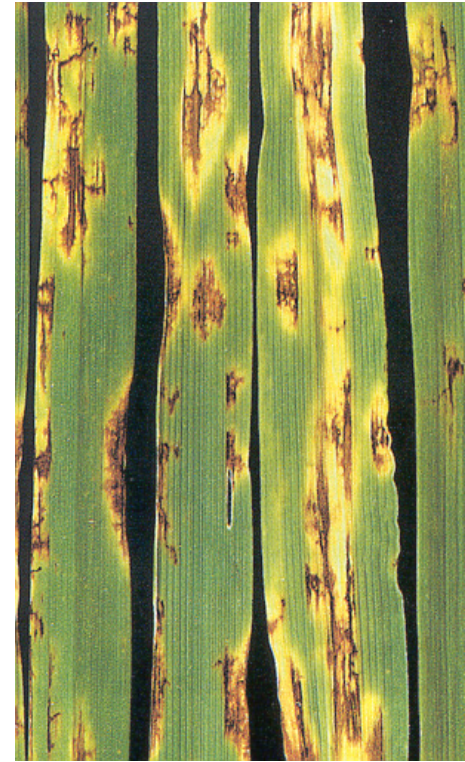
DNA standard curve



# Usædsbårne sygdomme i byg

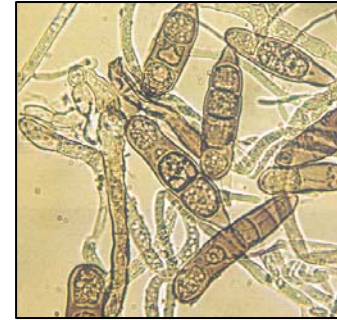
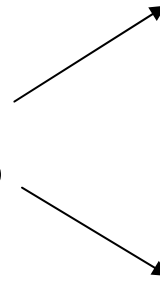
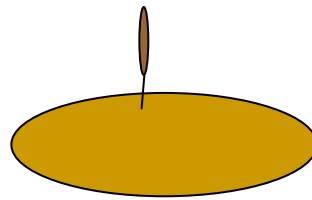
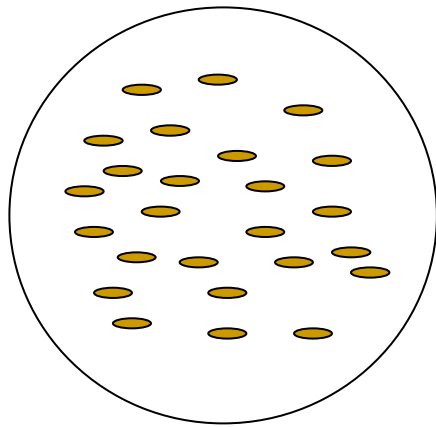


***Pyrenophora graminea***  
Imperfect: ***Drechslera graminea***

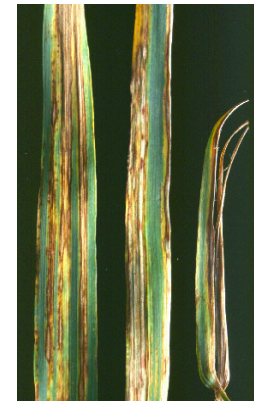
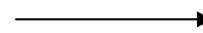


***Pyrenophora teres***  
Imperfect: ***Drechslera teres***

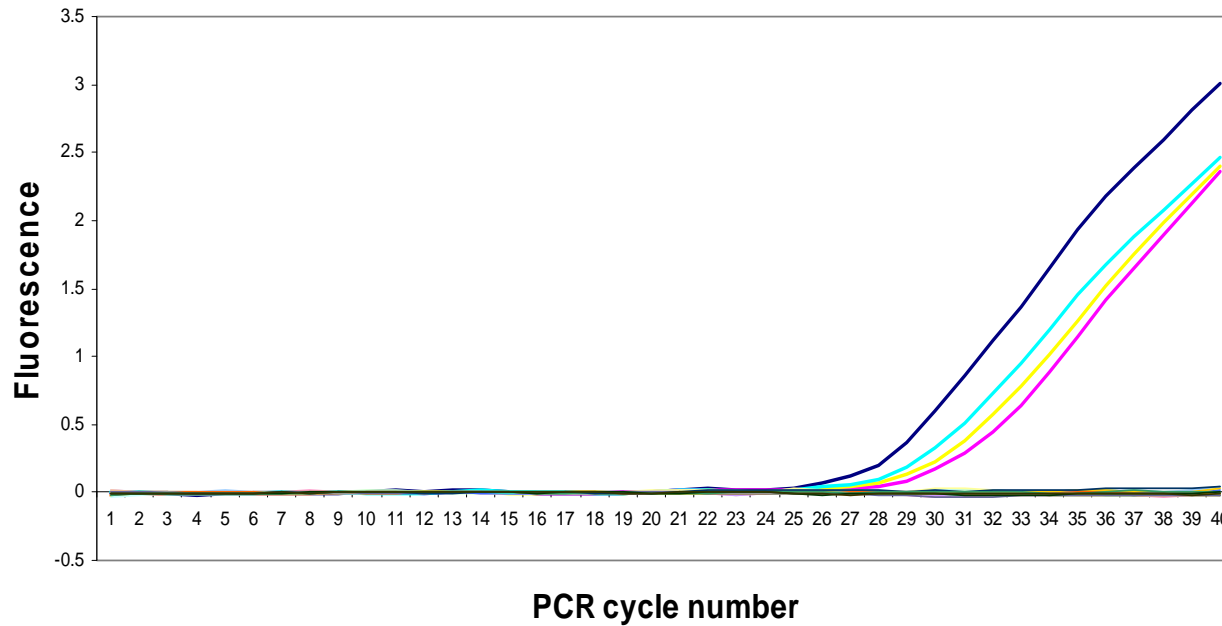
# Konventionel test for *Pyrenophora* sp.



Væksthustest 4-5 uger



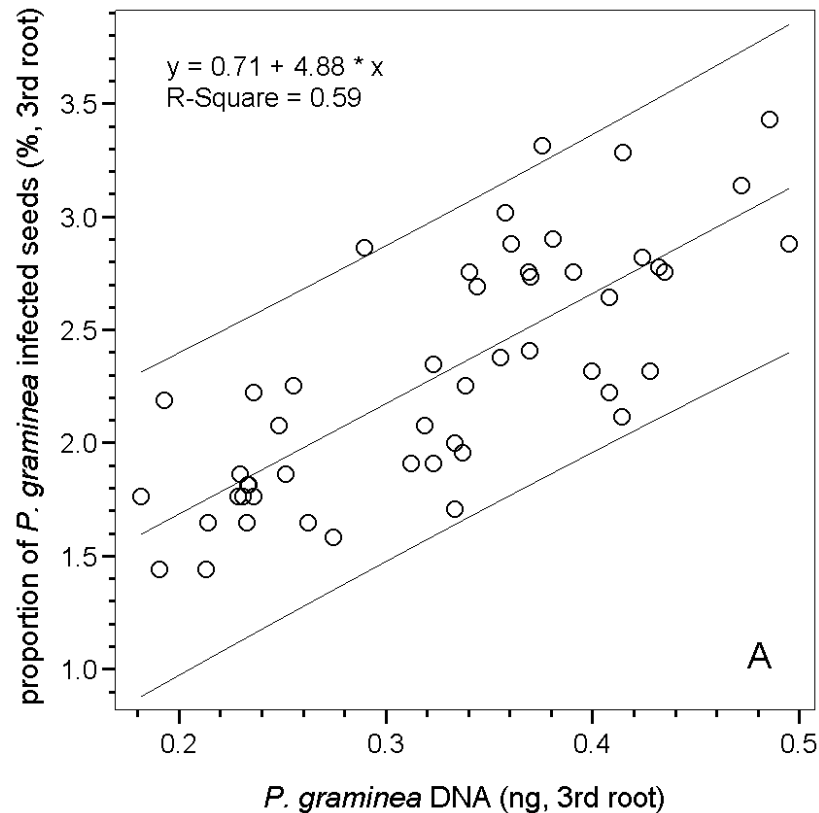
# Real-time PCR metode for sribesygge



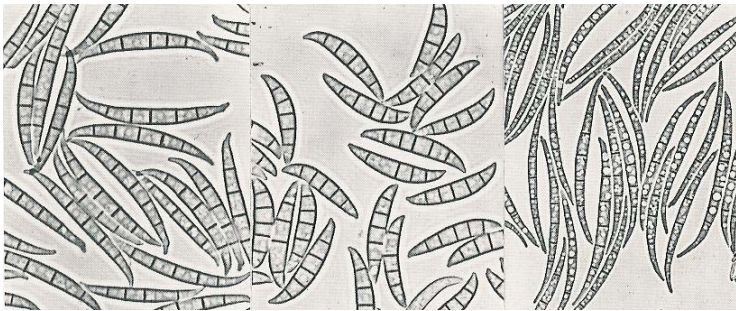
P. graminea

P. teres og  
andre

# Sammenhæng mellem mængden af DNA og antallet af inficerede kerner



# Fusarium i korn





# Fusarium i kornprøver

Real-time PCR for:



*F. graminearum*



*F. culmorum*



*F. poae*



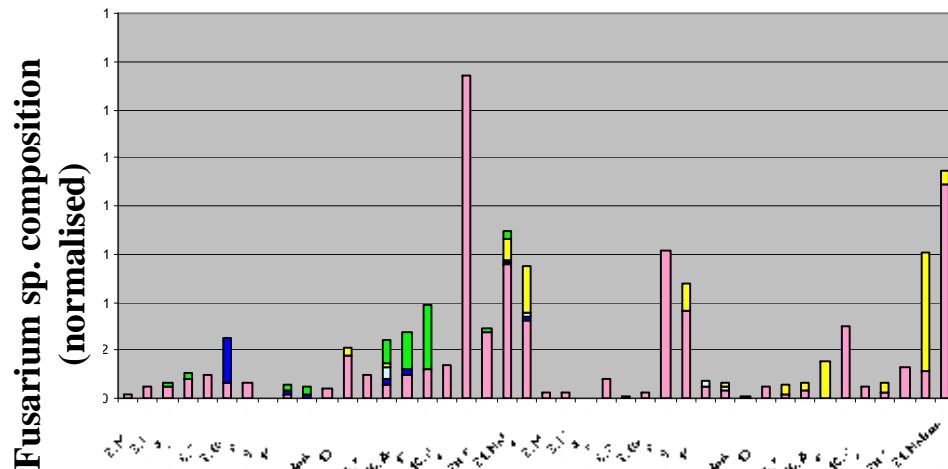
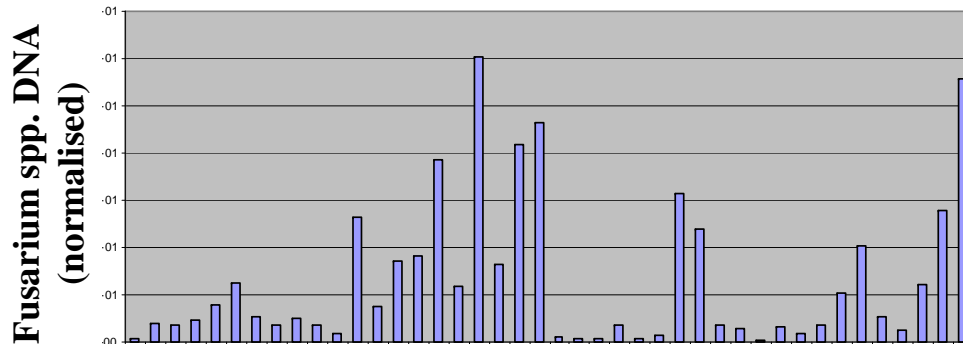
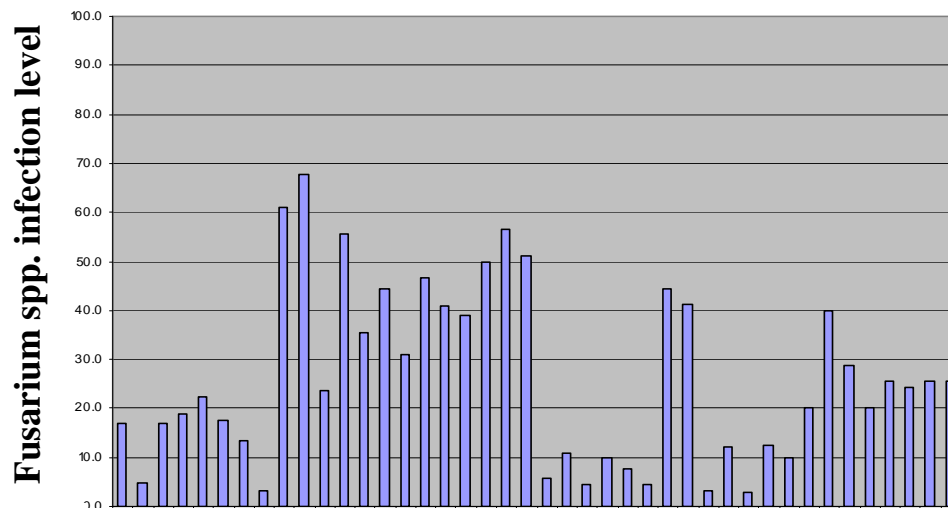
*F. langsethiae*



*F. sporotrichioides*

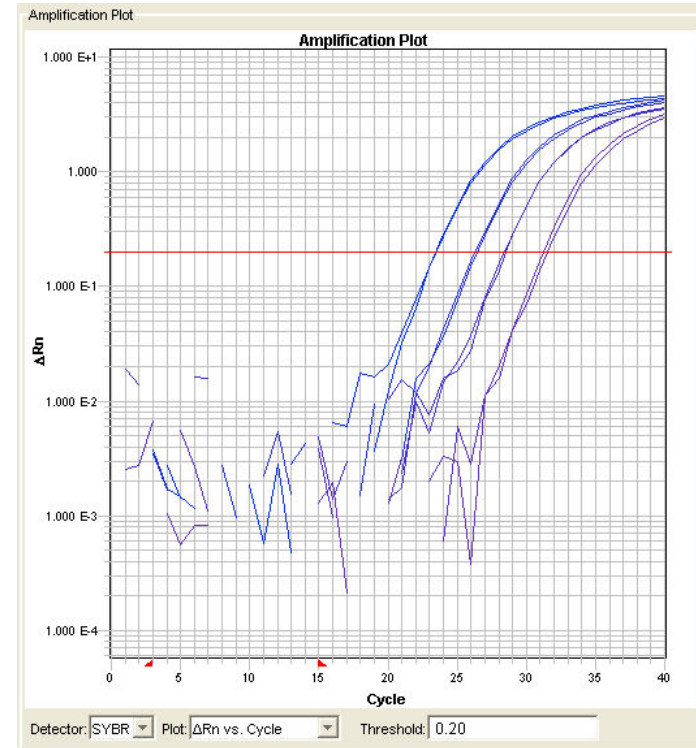


*F. equiseti*



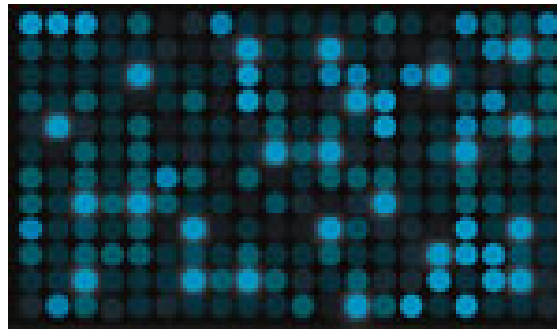
# PCR

- **Specificitet** (kan skelne arter som ikke kan skelnes morfologisk)
- **Følsomhed** (sammenlignelig med konventionelle metoder)
- **Kan automatiseres** (DNA-ekstraktion/real-time PCR)
- **Kort testtid** (1-2 dage)
- **Kvantitativ** (real-time PCR)
- **Kræver ikke speciel ekspertise**



# Multiplex detektion af plantepatogener

DNA array teknologi



Virus

Bakterier

Eukaryote patogener

# *Fusarium* chip

*F. graminearum*

*F. culmorum*

*F. pseudograminearum*

*F. equiseti*

*F. poae*

*F. langsethiae*

*F. avenaceum*

*F. tricinctum*

*F. sporotrichioides*

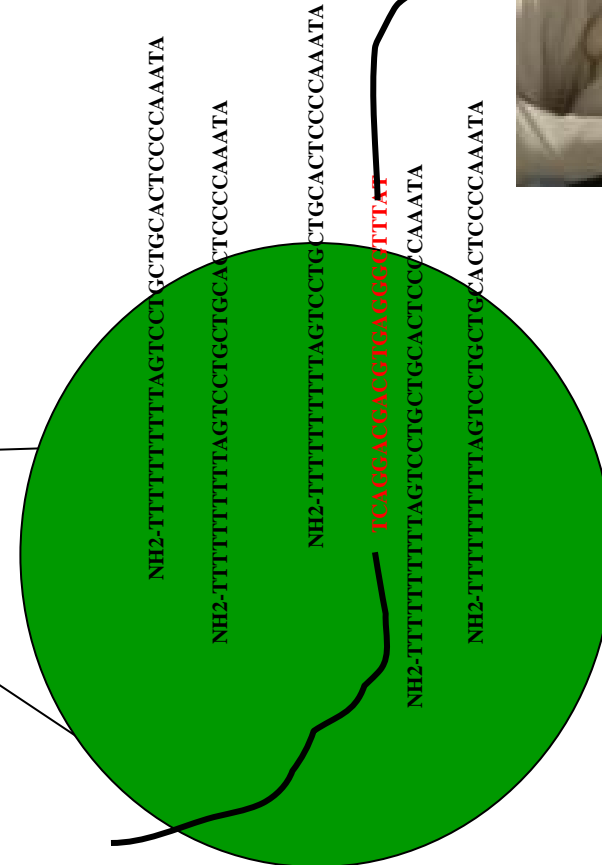
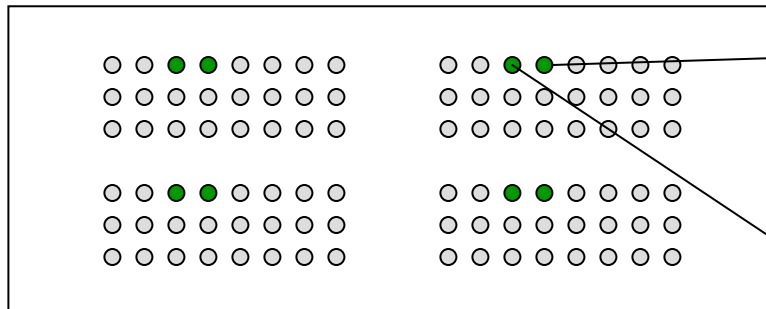
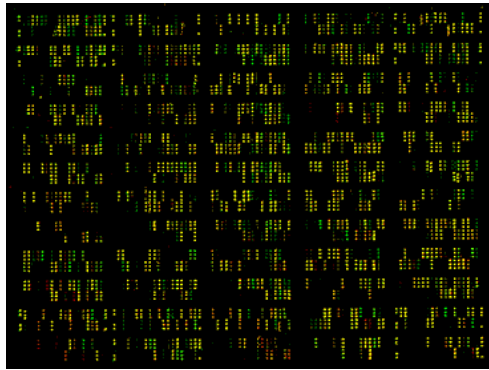
*F. subglutinans*

*F. proliferatum*

*F. verticillioides*



# Multiplex detektion med DNA-chip

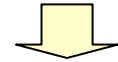
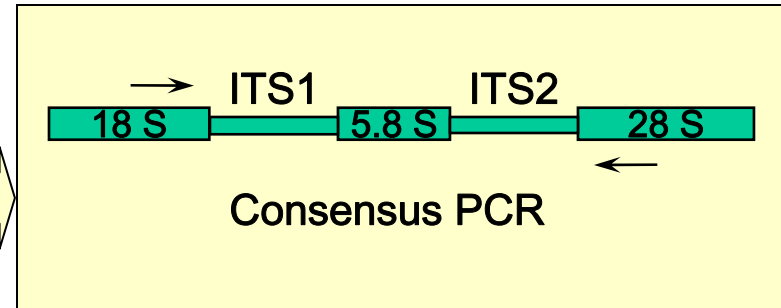


Spot ~200  $\mu$ m

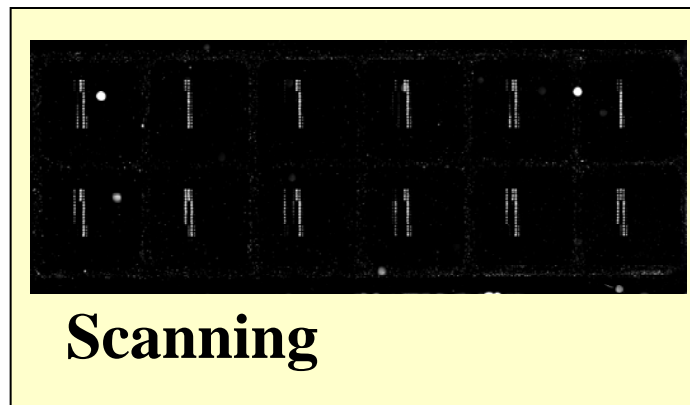
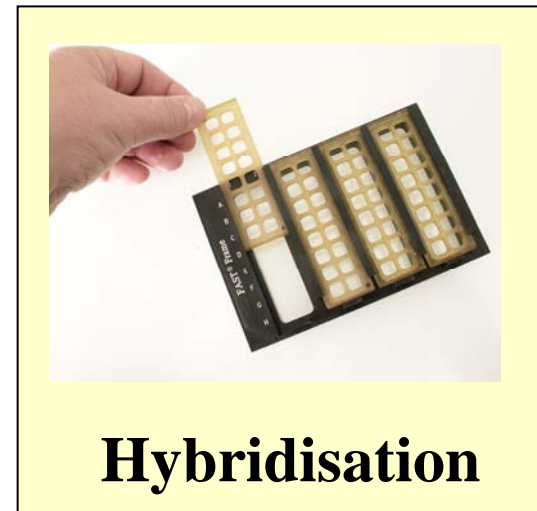
# Microarray analyse



**DNA extraction**

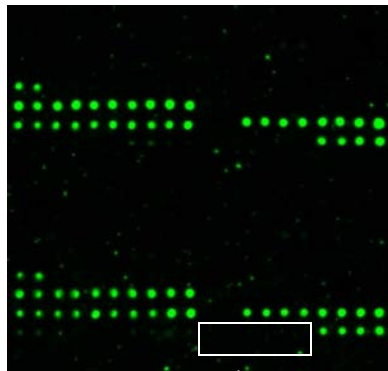


**Random primed  
Cy3 labelling**

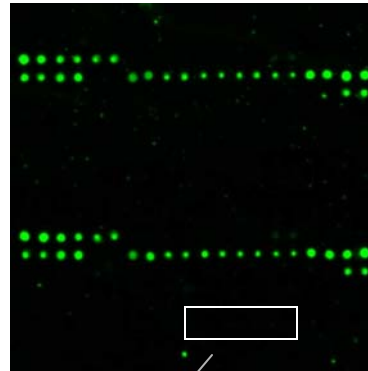


# Hybridiseringsresultater

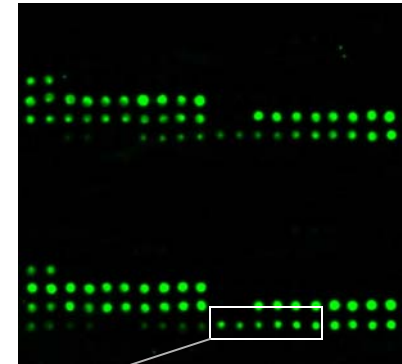
F. poae



F. avenaceum

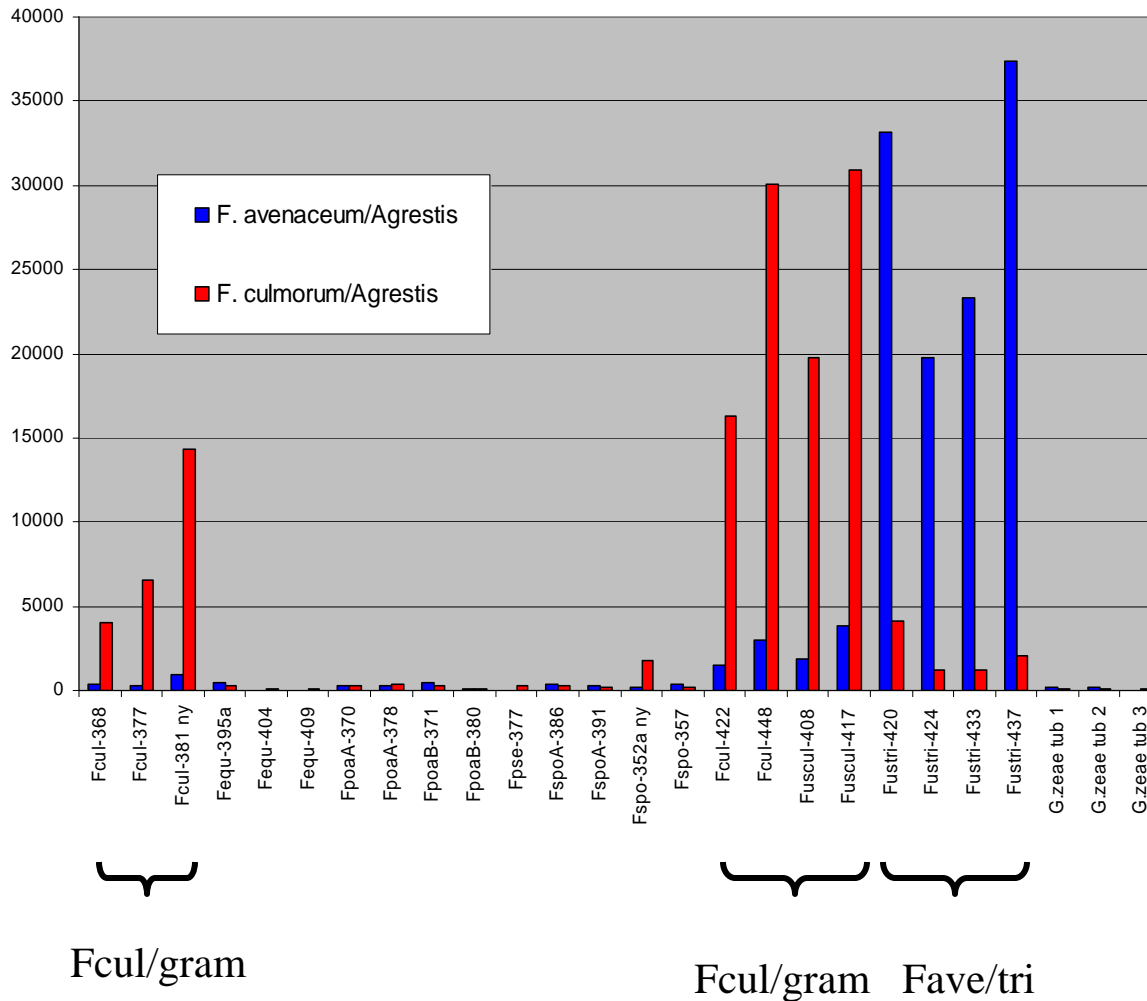


F. graminearum



F. graminearum spesifikke prober

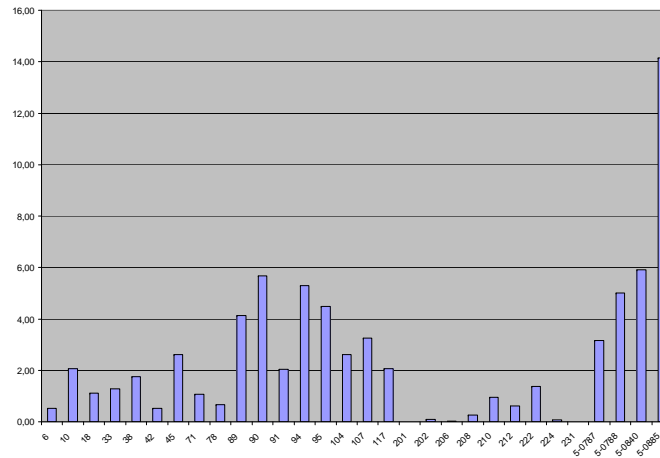
# Markprøver



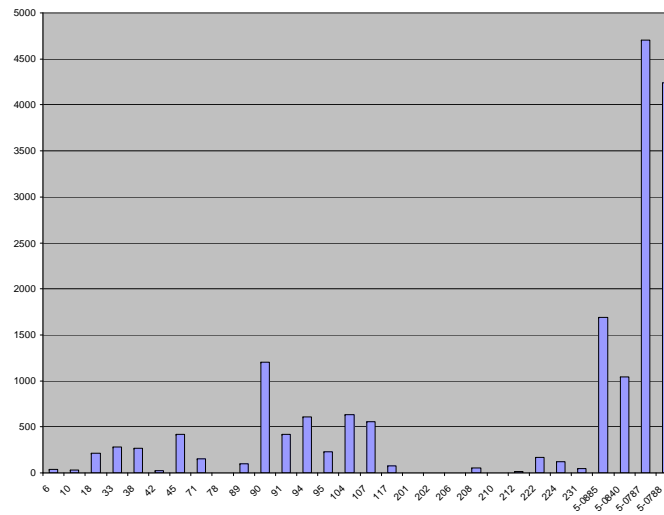
Two wheat cultivars were inoculated in the field with *F. graminearum* or *F. avenaceum* at flowering



# Microarray/ toxinanalyse af kornprøver



*F. graminearum/*  
*culmorum* microarray signal



DON indhold (ppb)

# Microarray

- Multiplex (kan teste for mange arter i én arbejds gang)
- En metode som er ”på vej”
- Følsomhed (sammenlignelig med PCR)
- Automatisering ”på vej”
- Kort testtid
- Semi-kvantitativ
- Kræver ikke speciel ekspertise

- Hans Pinnschmidt
- Skaidrė Supronienė
- Henrik Jørskov Hansen
- Henriette Nyskjold
- Lise N. Jørgensen
- Bent Nielsen
- Ulf Thrane

