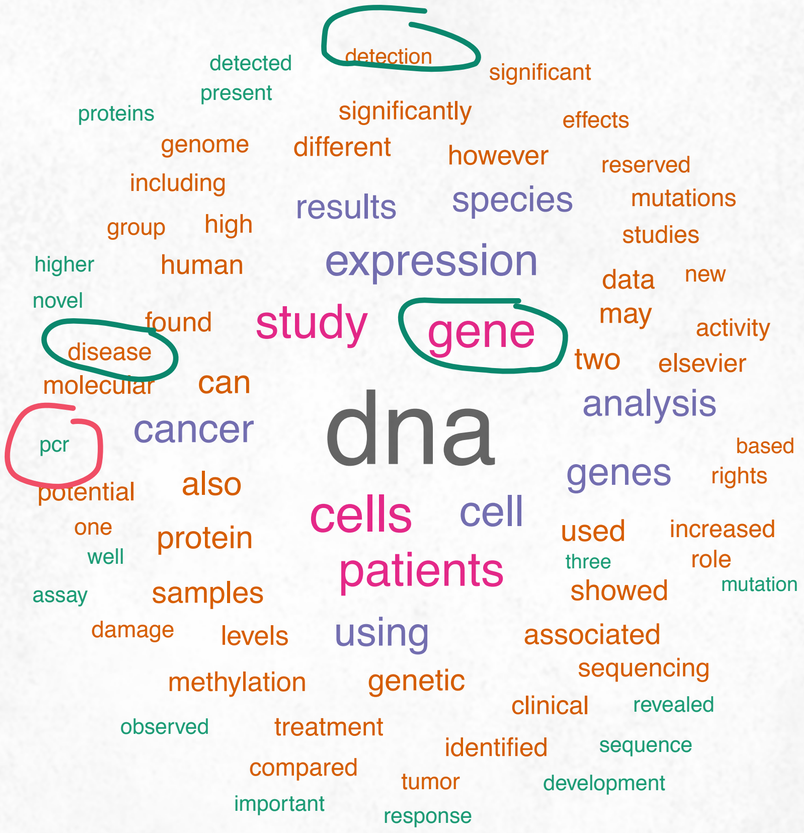
Using polymerase chain reaction to diagnose threats to food supplies

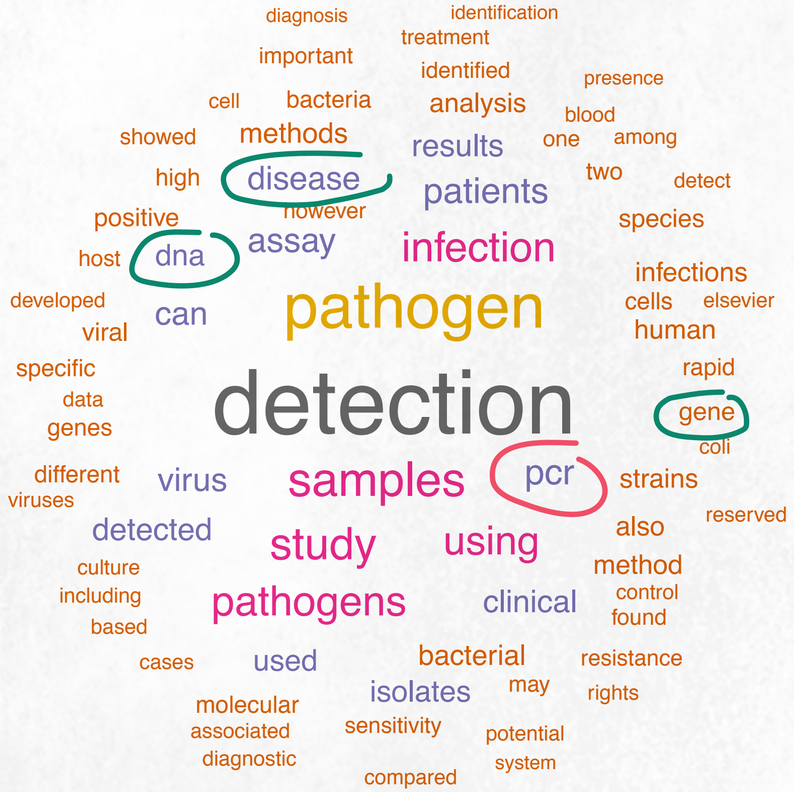
NOURISH THE FUTURE

Tomorrow's science is looking for leaders

PCR in the top 100 terms of science abstracts with ‘DNA’ or ‘Pathogen Detection’ as key words



10,000 papers analyzed
(years: 2015–18)



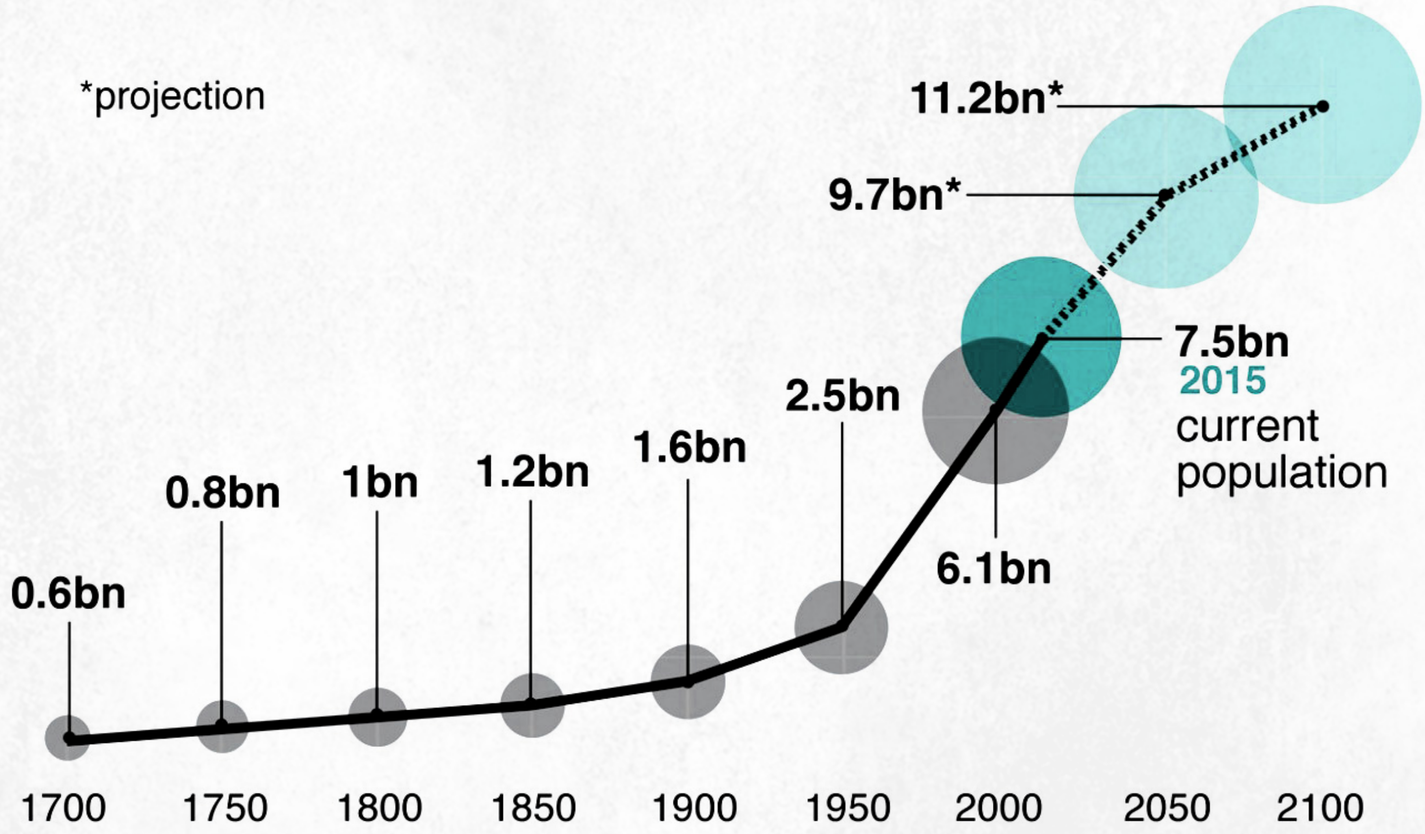
4,800 papers analyzed
(years: 2015–18)

Pathogen farm story

Two farmers, Bill B. and Deandra D., each grow 1500 acres of corn in North Dakota. They sample their soils and collect data on yield from each field to help them make planting decisions for the following year. In one of each of their fields they noticed a drop in yield after harvest this year. There is a new corn variety that is available to help resist two common pathogens and they want to see if they have that pathogen in their soil.

Your job is to help them determine if they have either or both pathogens.

Population growth and food demand



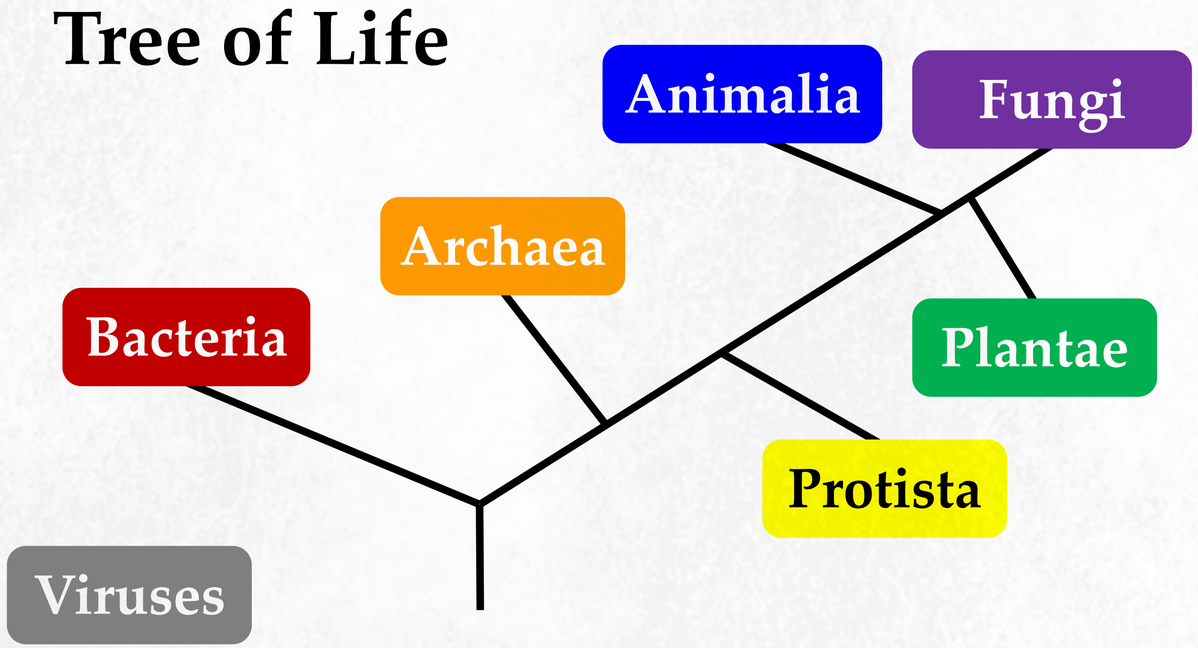
Satisfying food demand in just 30 years requires production to increase by 70%.

news.mn/r/2110145

What is a major obstacle to future food security and crop sustainability?

Plant pathogens and pests

- Organisms that limit crops from their maximum potential
  - Loss of yield (amount of seed produced)
- Parasitism is the most common lifestyle for organisms across the tree of life



Pathogens and pests contribute to dramatic worldwide yield losses

These 5 crops account for *half* of global human calorie intake!

Rice
30.0% yield loss

Wheat
21.5% yield loss

Corn
22.6% yield loss

Soybean
21.4% yield loss

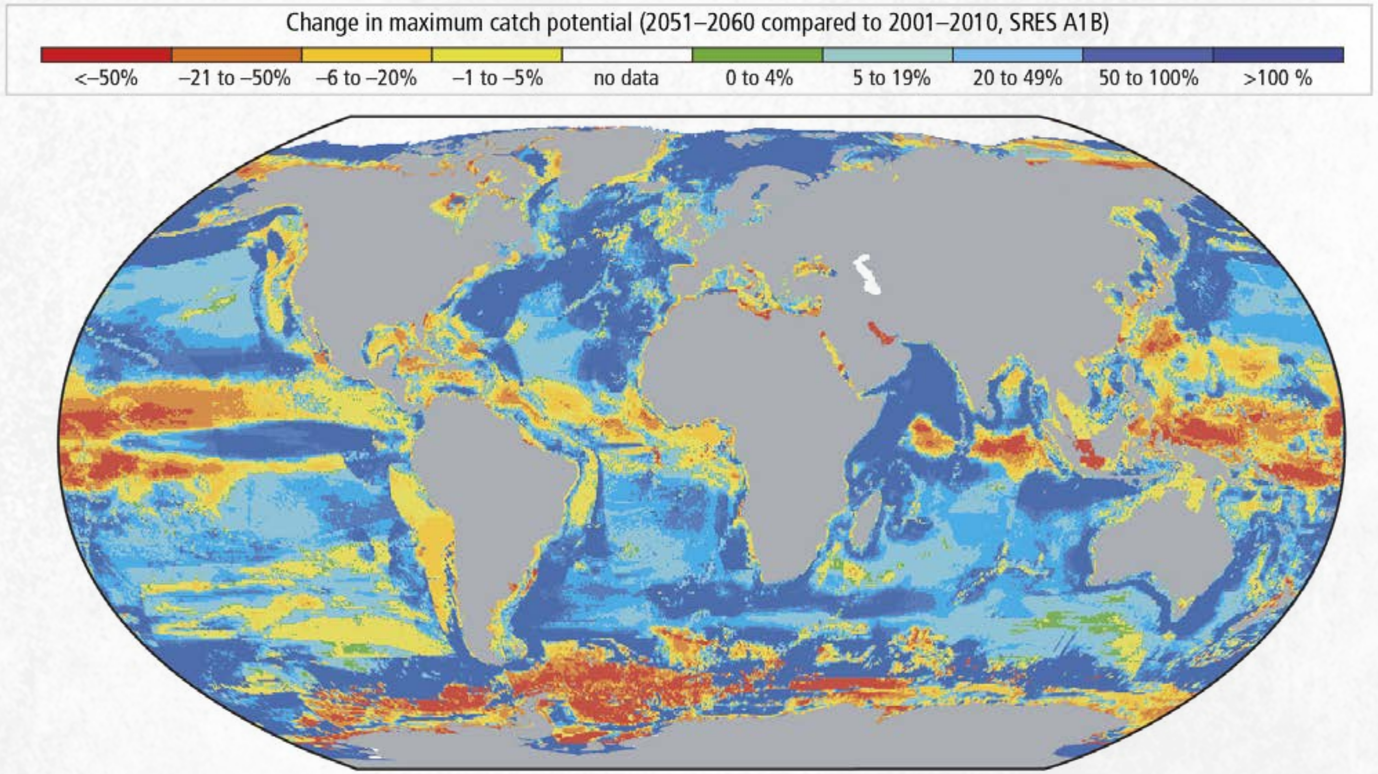
Potato
17.2% yield loss

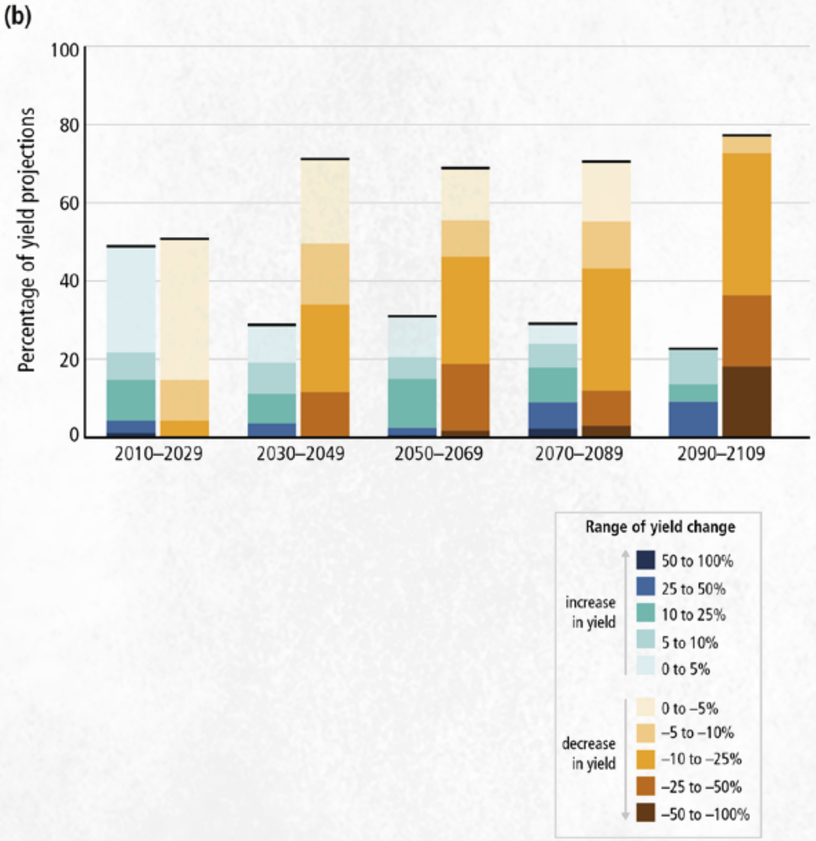
Savary et al. 2019. Nature Ecol & Evol, 3, 430-439

What are some factors contributing to disease spread?

Global trade

What are some factors contributing to disease spread?





Changing climate

What are some issues with disease management?

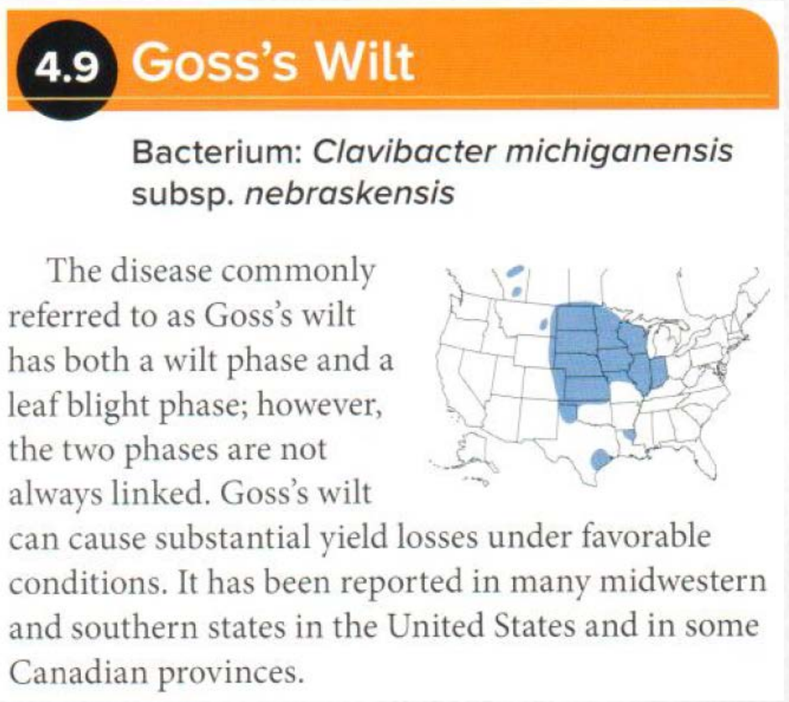
Symptoms of different diseases can look the same visually, but have differences in effective control

https://extension.udel.edu/weeklycropupdate/?p=10800

https://cropwatch.unl.edu/2017/update-bacterial-leaf-streak-corn-nebraska

What are some issues with disease management?

Assumptions on the distributions of pathogens

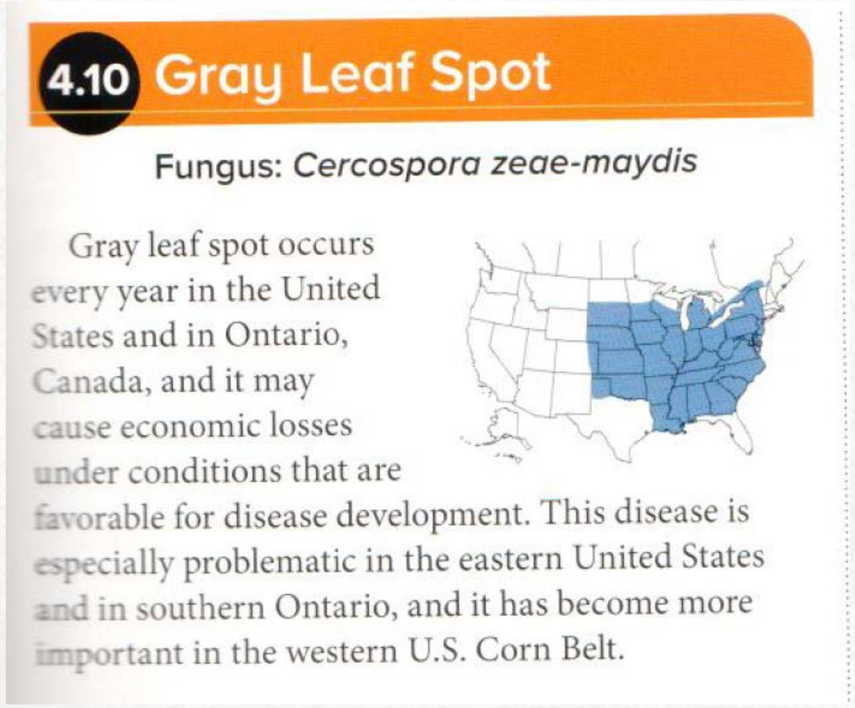


4.9 Goss's Wilt

Bacterium: *Clavibacter michiganensis* subsp. *nebraskensis*

The disease commonly referred to as Goss's wilt has both a wilt phase and a leaf blight phase; however, the two phases are not always linked. Goss's wilt can cause substantial yield losses under favorable conditions. It has been reported in many midwestern and southern states in the United States and in some Canadian provinces.

Patchy records



4.10 Gray Leaf Spot

Fungus: *Cercospora zeae-maydis*

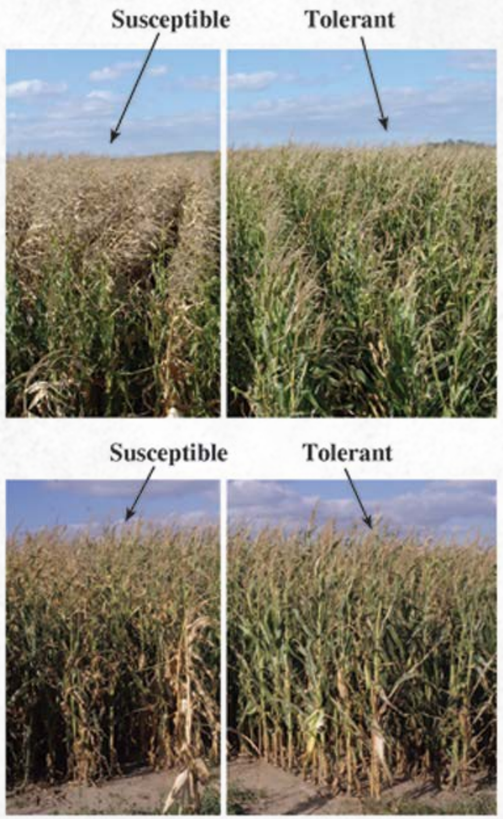
Gray leaf spot occurs every year in the United States and in Ontario, Canada, and it may cause economic losses under conditions that are favorable for disease development. This disease is especially problematic in the eastern United States and in southern Ontario, and it has become more important in the western U.S. Corn Belt.

Political boundaries

Wise et al. 2016 - A Farmer's Guide to Corn Diseases

What are some issues with disease management?

Subclinical infections



extensionpublications.unl.edu/assets/html/g1675/build/g1675.htm

To summarize

- Plant pathogens and pests are a huge source of food loss
- Issues facing control:
  - Difficult to predict outbreaks
  - Difficult to visually diagnose
  - Unknown distribution

Towards a solution

- Use of biotechnology through DNA diagnostics

Overview of DNA diagnostics

Soil sample

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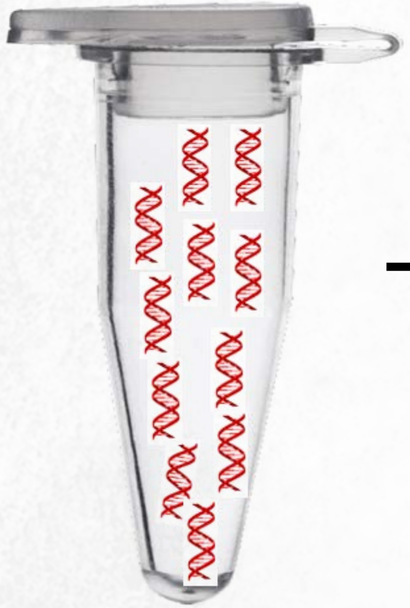
DNA extraction

→



Amplification using PCR

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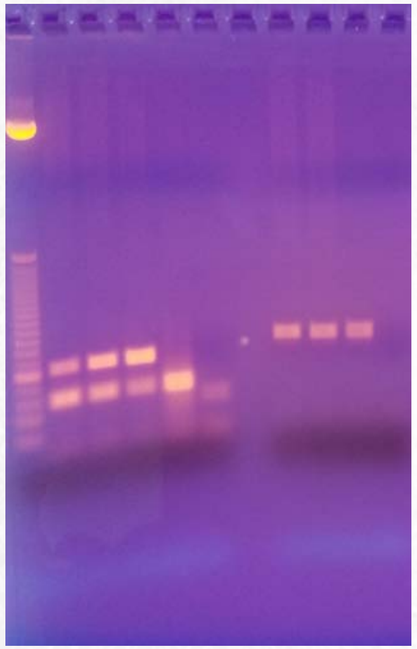


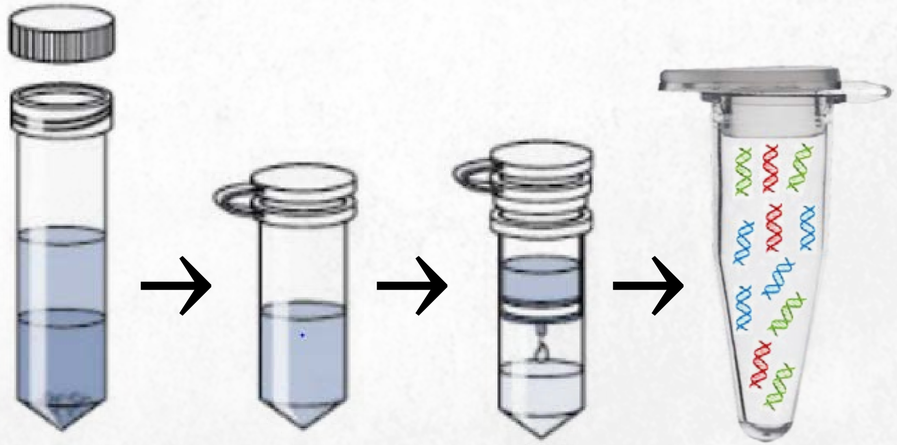
Detection via gel electrophoresis

Soil DNA extraction

Vortex disrupt

Centrifuge

Soil extraction kit



Multiple clean and filter strips

Pipettes and tips

Polymerase chain reaction (PCR): A molecular Xerox/copier technique

Process to identify and amplify specific section of DNA

Two major components:

1. Reagents for creating DNA
2. Changes in temperature

PCR classroom activity

PCR: Soil pathogens

Reagents for PCR

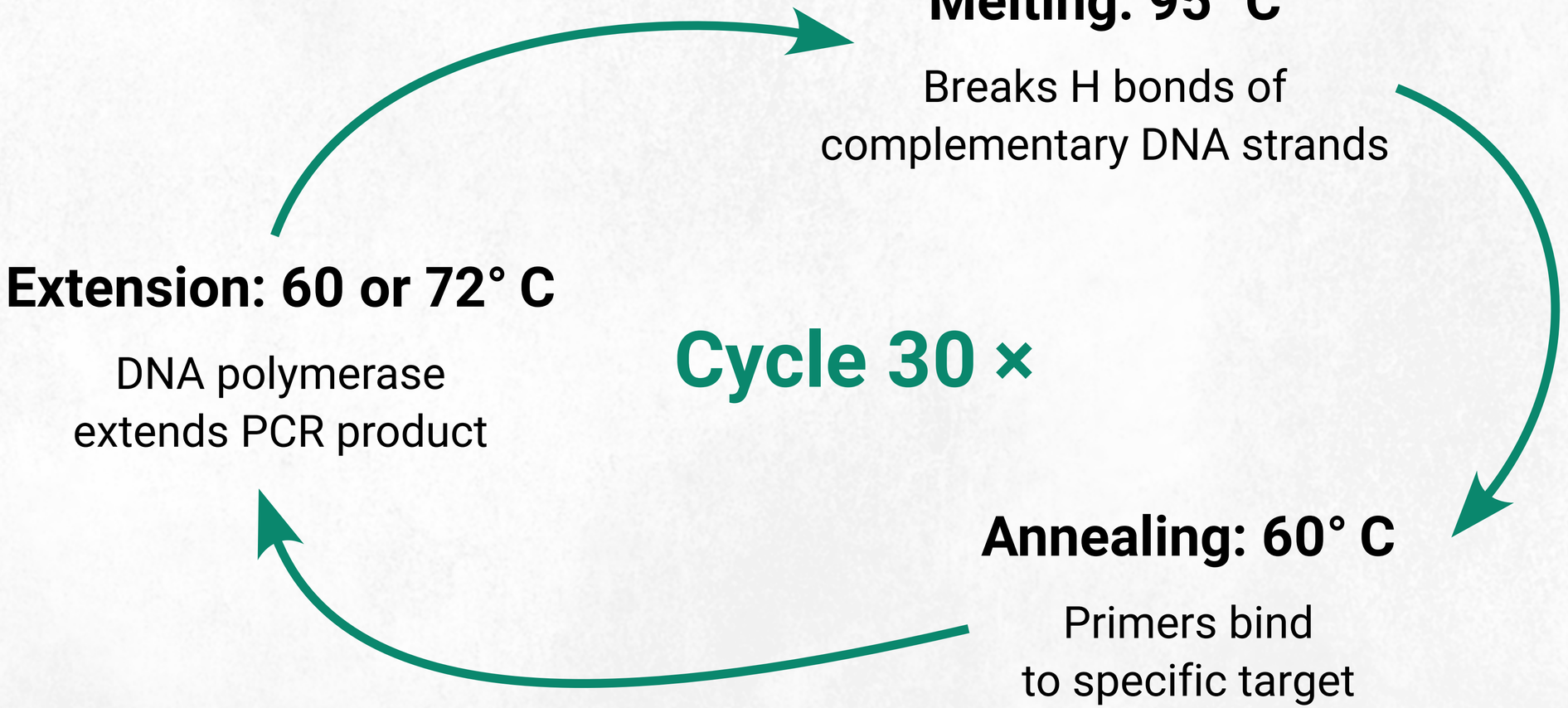
Item	Purpose
Extracted Soil DNA (Template)	Source of DNA to assess for pathogens
Primers (Pairs)	Small segments of DNA sequence that complement DNA from specific pathogen
dNTPs (deoxy-nucleoside triphosphates)	Single molecules containing A, T, G, C for making new DNA molecules
DNA polymerase	Molecule that adds the dNTPs to create the complementary strand
PCR Buffer	Contains MgCl & maintains pH for the reaction

pre-mixed at NAGC

pre-mixed various companies

PCR: Soil pathogens

Change in temperature

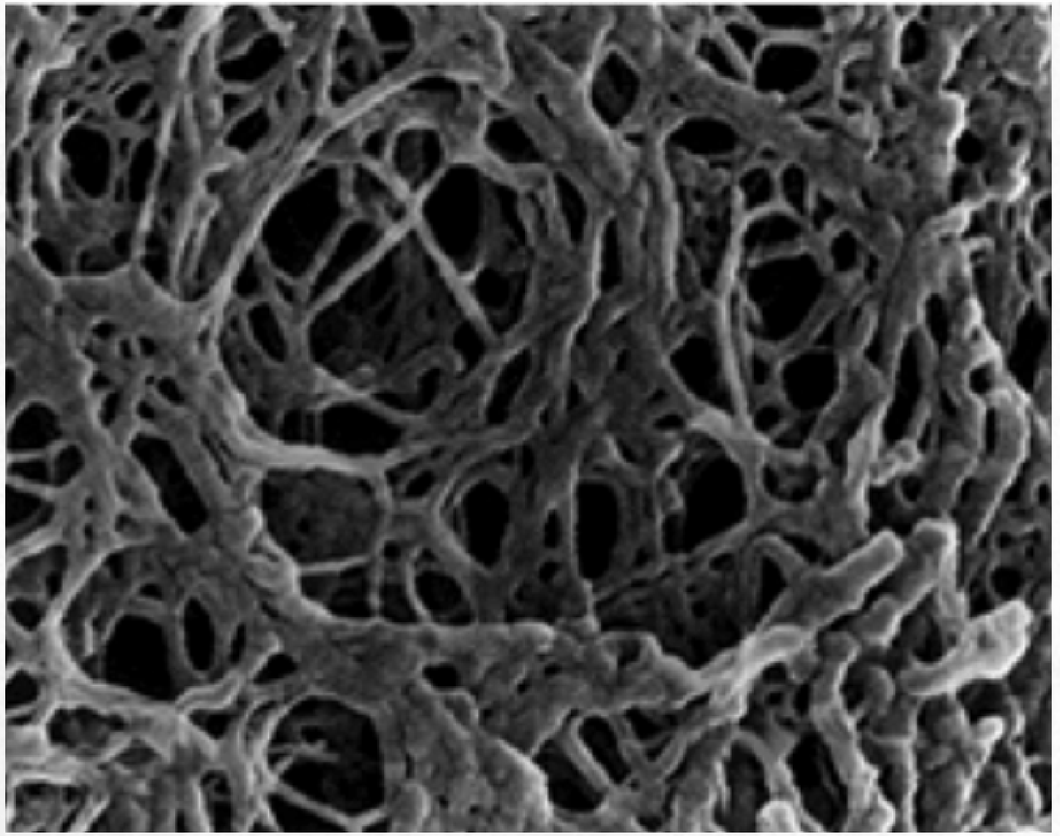


Gel electrophoresis

Purpose: Separate PCR products (DNA) for detection

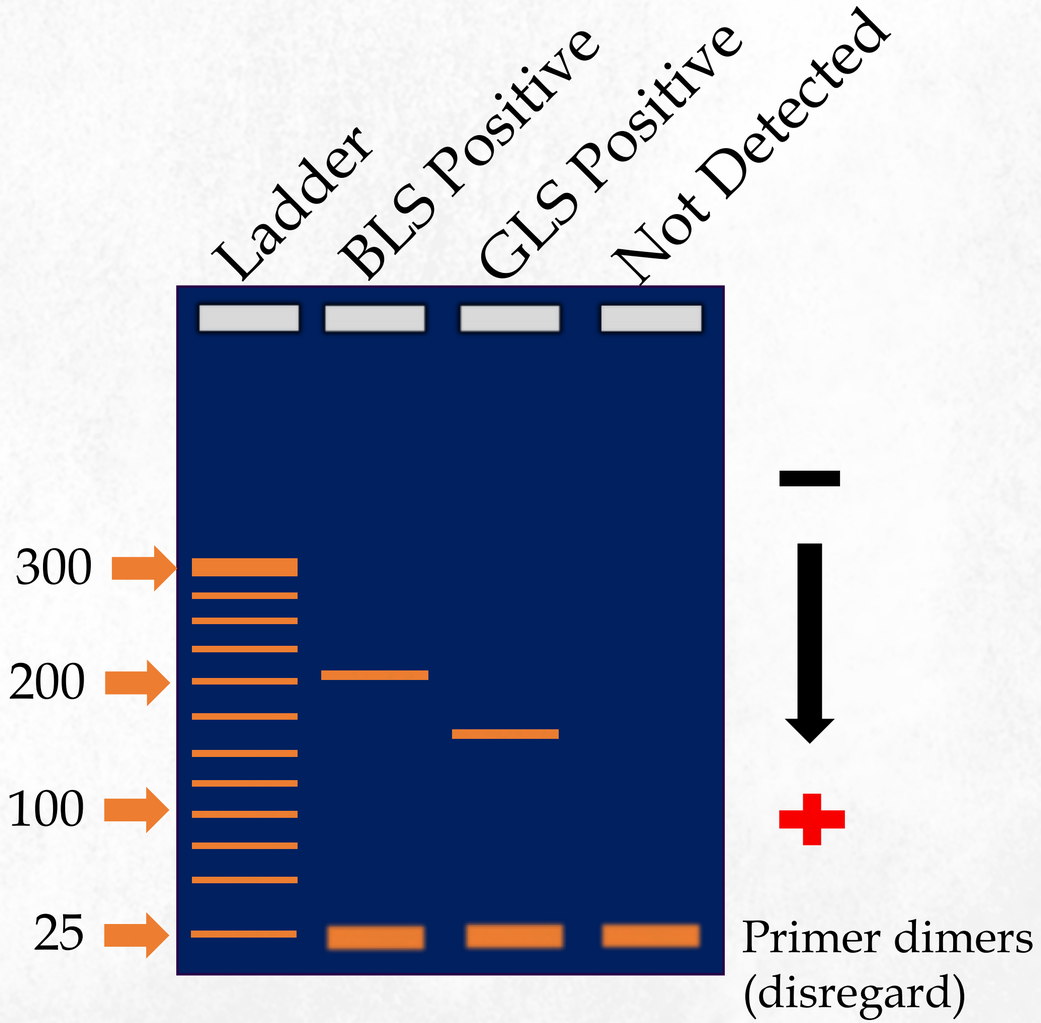
Agarose structure is porous, which allows DNA to pass through

Smaller PCR products pass through gel faster than larger products



Agarose gel image from electron microscope

Gel interpretation



Recap of DNA extraction, PCR, gel analysis

Soil sample

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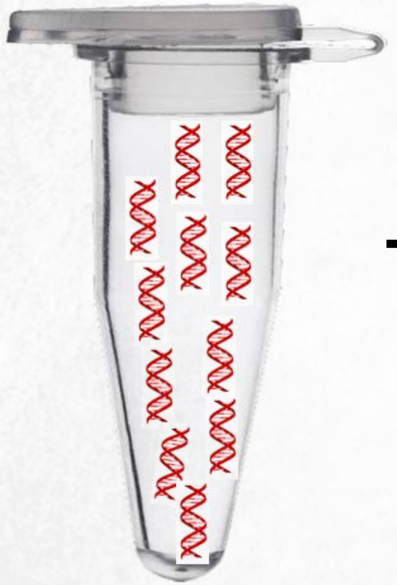
1.5 hours

DNA extraction

→

Setup: 20 min
Run time: 2 hours



Amplification using PCR

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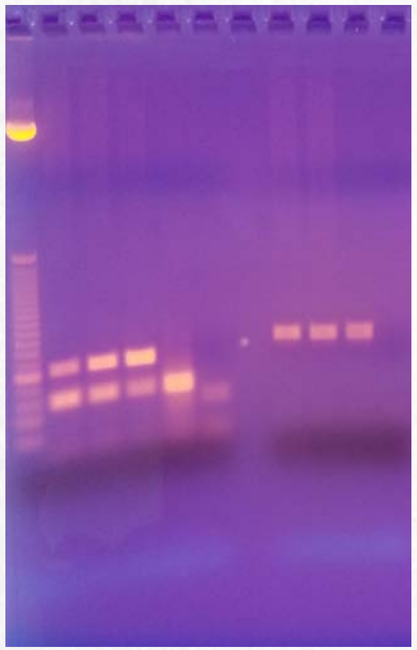
Setup: 10 min
Run time: 30 min



Detection via gel electrophoresis

Goal

- Recommended samples:
  - Community gardens
  - Raised beds
  - Potting soil
  - Topsoil