



Invasion of common reed  
(*Phragmites australis*) into North  
American Wetlands



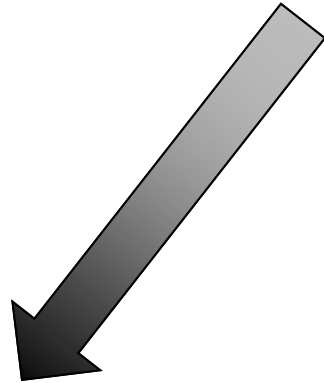
# Introduced Species (Nonnative)

- Organisms released into an environment outside of their native geographic range

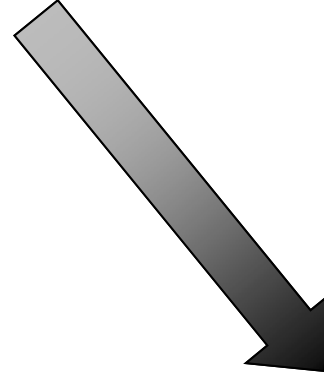
How are they introduced?

- Accidental
- Intentional

# What happens after a nonnative species is introduced?



OR



- Never a threat to native species & never establish in the new environment

- Establish themselves & spread throughout an environment – called **invasive species**

# Common Traits of Invasive Species

- High **fecundity** – production of many offspring each reproductive event
- Capable of **Asexual reproduction**
- Tolerant of broad range of environments

# Common Environmental Conditions Facilitating Species Invasion

- Organisms associated with human activity (e.g., ornamental plants)
- Disturbance (natural or human induced)
  - Examples: Fire, volcanic eruption, flood, clear cutting forest habitat, etc.
- Lack of biotic limitations
  - Examples: No predators, no competition, no disease

# Negative Effects of Invasive Species

- Loss of native species due to introduced pathogens, predators, competitors
  - Reduced **Species diversity** – the number of different types of species in an environment
- Modification of habitat structure – loss of function for native species
- Economic loss
  - Costs associated with eradication, damage to water flow structures (e.g., Zebra Mussels clog sewer pipes in many cities)

# Case Study: *Phragmites australis*

- *Phragmites australis* (common reed) native species present in southwest U.S. for at least 40,000 years
- Records from 1800s indicate the plant was rare, but it has spread dramatically in past 150 years
- Now it is found throughout the U.S. and is recognized as an invasive or nuisance species







<http://www.scienceteacherprogram.org/envsci/Vincent2006.html>





<http://badluckcity.wordpress.com/2011/08/10/phragmites/>









Asexual Reproduction  
can occur via  
**rhizomes** –  
underground lateral  
stems that produce  
new plants  
genetically identical  
to parent plant

<http://www.ksl.com/index.php?nid=148&sid=6754314>



# Recent explosion of *Phragmites* in N.A.

- Two varieties of *Phragmites* now co-occur in N.A.
  - Native and Nonnative
- Increase in *Phragmites* stands attributed to the nonnative variety (Saltonstall 2002)

# Negative Effects of Nonnative *Phragmites* in N.A.

- Altered water flow along waterways
- Increased sedimentation and channelization in waterways
- Reduction in plant diversity
- Alters habitat used by variety of organisms: fish, birds, small mammals

# Recent explosion of *Phragmites* in N.A.



Nonnative

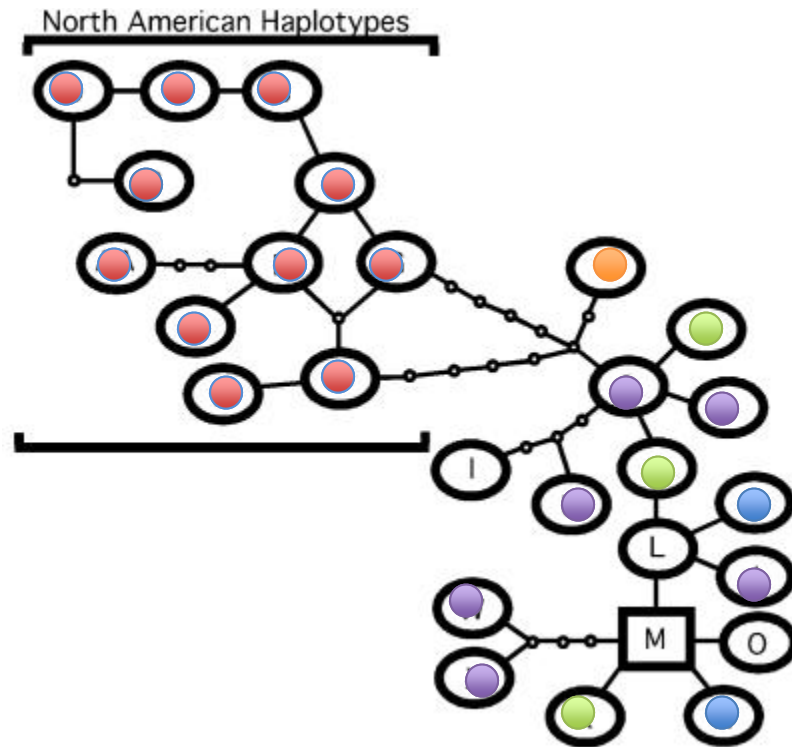
Native

- Two varieties are difficult to distinguish by morphology



# Recent explosion of *Phragmites* in N.A.

- Two N.A. varieties distinguishable by genetic analysis
- **Haplotype**: combination of gene variants (alleles) that are inherited together



## Haplotypes

North America:

A-H, S, Z, AA, I, M

Native

Nonnative

South America: I, Y

Europe: L, M, N, O, T

Asia/Australia: I, J, L, M, O, P, Q, U, W, X

Africa: K, M, R, V

Figure 1 (Saltonstall 2002)

a) Native Haplotypes Before 1910



b) Invasive Haplotype Before 1910



▲ native haplotypes

■ haplotype I

● nonnative haplotype M

c) Native Haplotypes After 1960



d) Invasive Haplotype After 1960



- Primary nonnative haplotype is M

Figure 2, Saltonstall (2002)

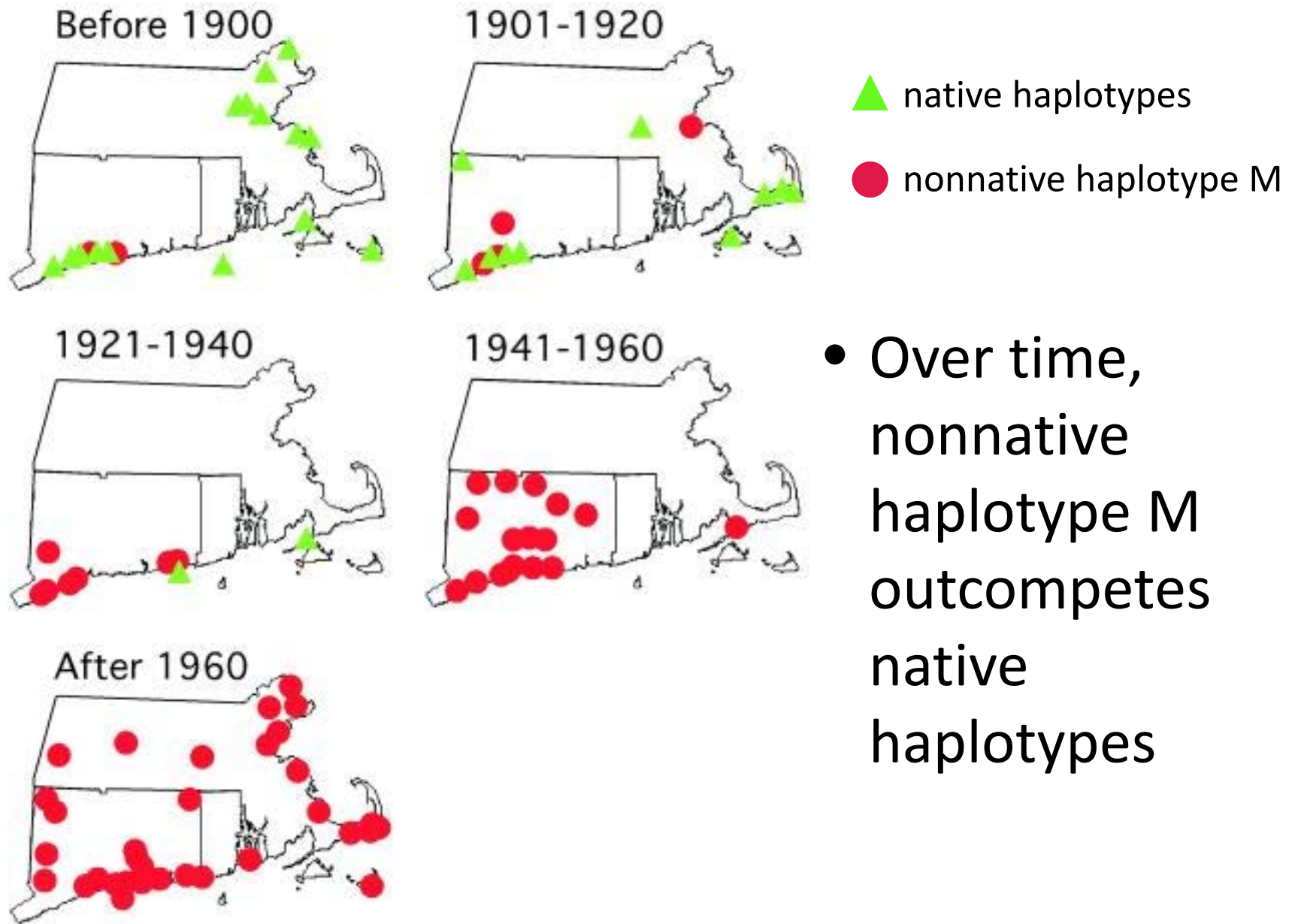


Figure 3, Saltonstall (2002)



# Genetic Analyses to Distinguish Haplotypes

## Two Methods

- DNA sequencing
- Restriction Fragment Length Polymorphism (RFLP)

# DNA Sequencing

Fresh or Herbarium samples of *Phragmites*



Extract DNA



Use polymerase chain reaction (PCR) to amplify  
two non-coding chloroplast regions



Sequence non-coding chloroplast regions



Analyze the sequences

# DNA Sequencing

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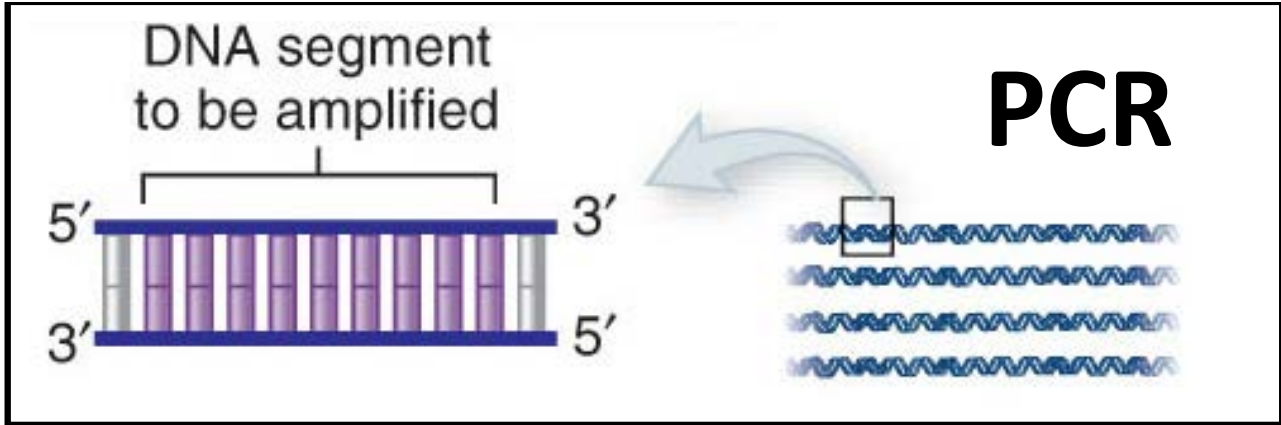


Analyze the sequences

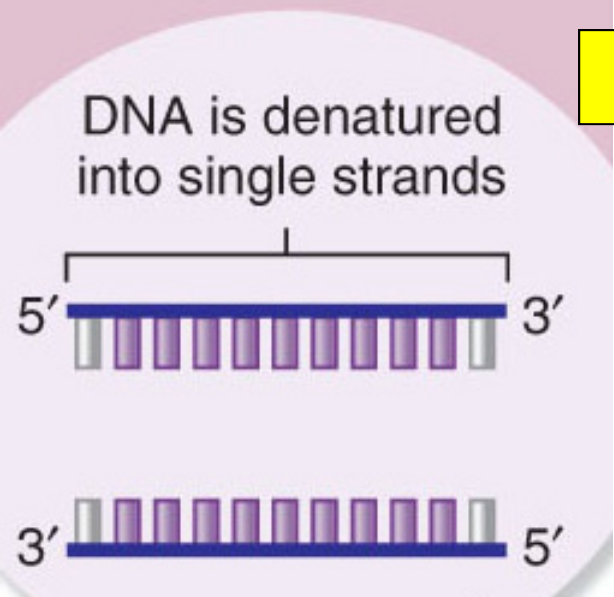


# DNA Sequencing

- **DNA extraction** – lyse cells from organisms to isolate genetic information
- **Polymerase chain reaction (PCR)**
  - Allows amplification of a small DNA fragment from a desired **genetic locus** (specific region or sequence of a chromosome)



1. Sample is first heated to denature DNA.



2. DNA is cooled to a lower temperature to allow annealing of primers.

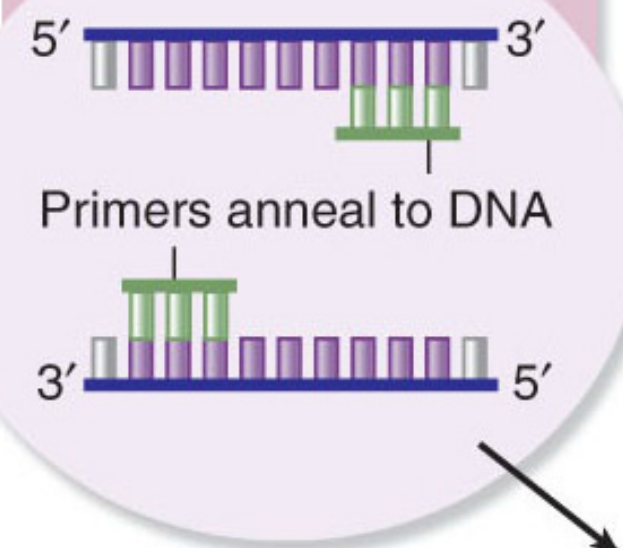
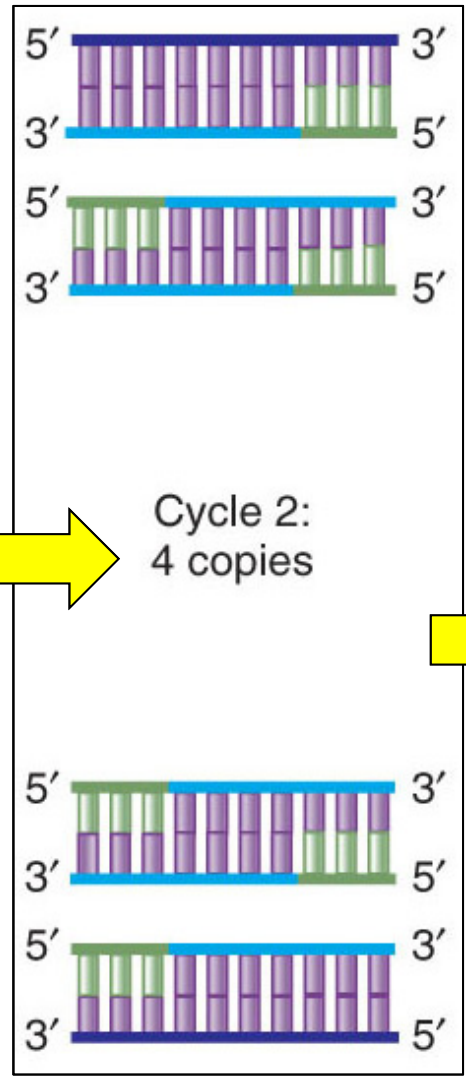
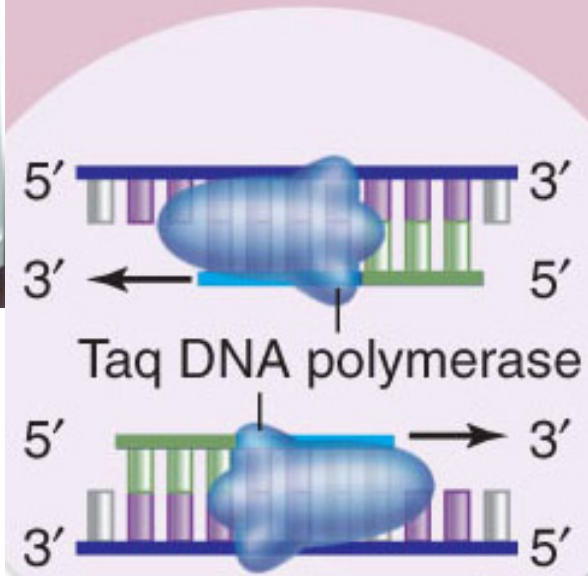


Figure: McGraw-Hill Companies, Inc.

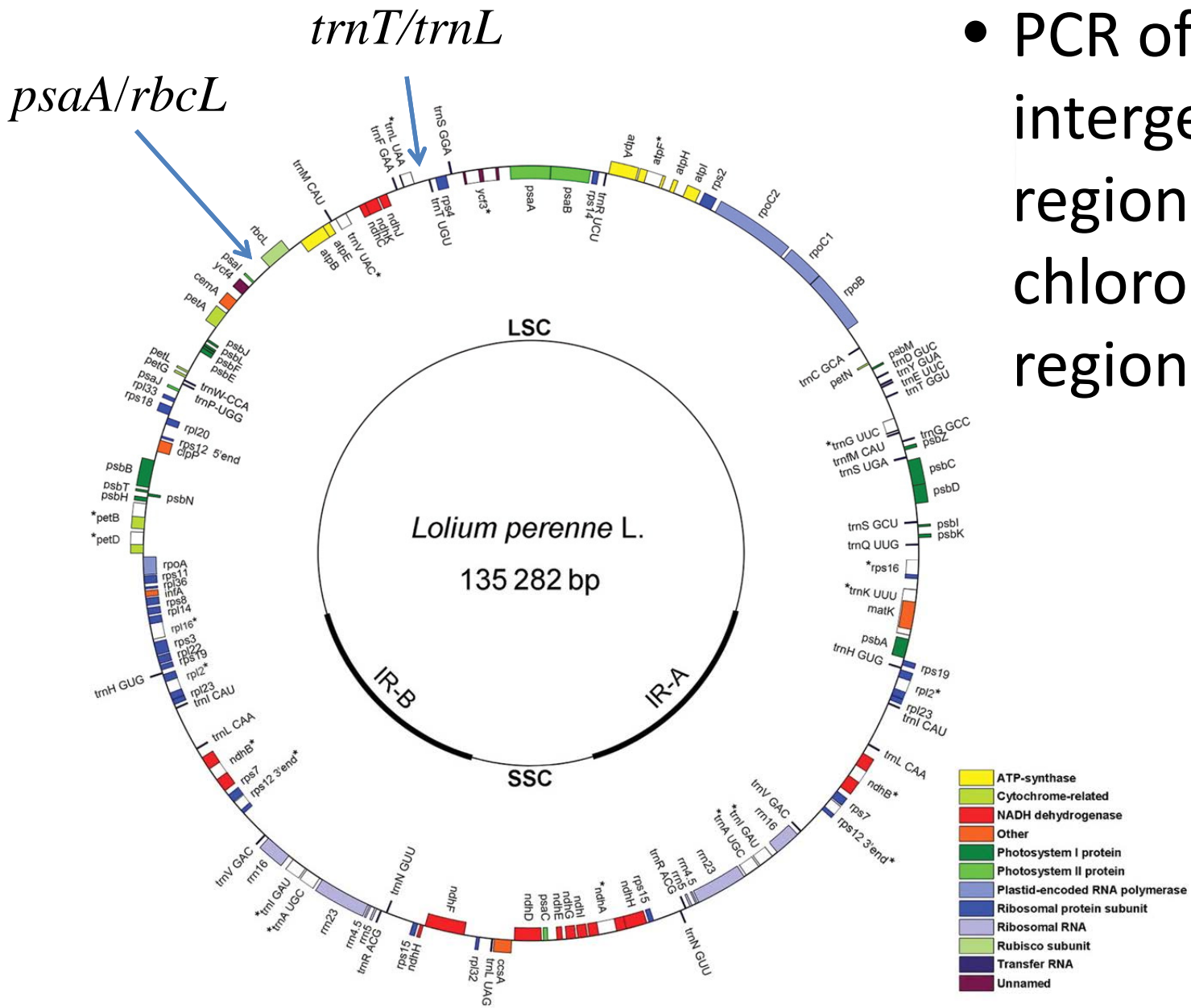


3. DNA is heated to 72°C, the optimal temperature for Taq DNA polymerase to extend primers.



**After 20 cycles, a single fragment produces over one million ( $2^{20}$ ) copies!**

Figure: McGraw-Hill Companies, Inc.



- PCR of two intergenic regions of chloroplast region



# DNA Sequencing

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Sequence non-coding chloroplast regions



Analyze the sequences

# Sequencing Basics

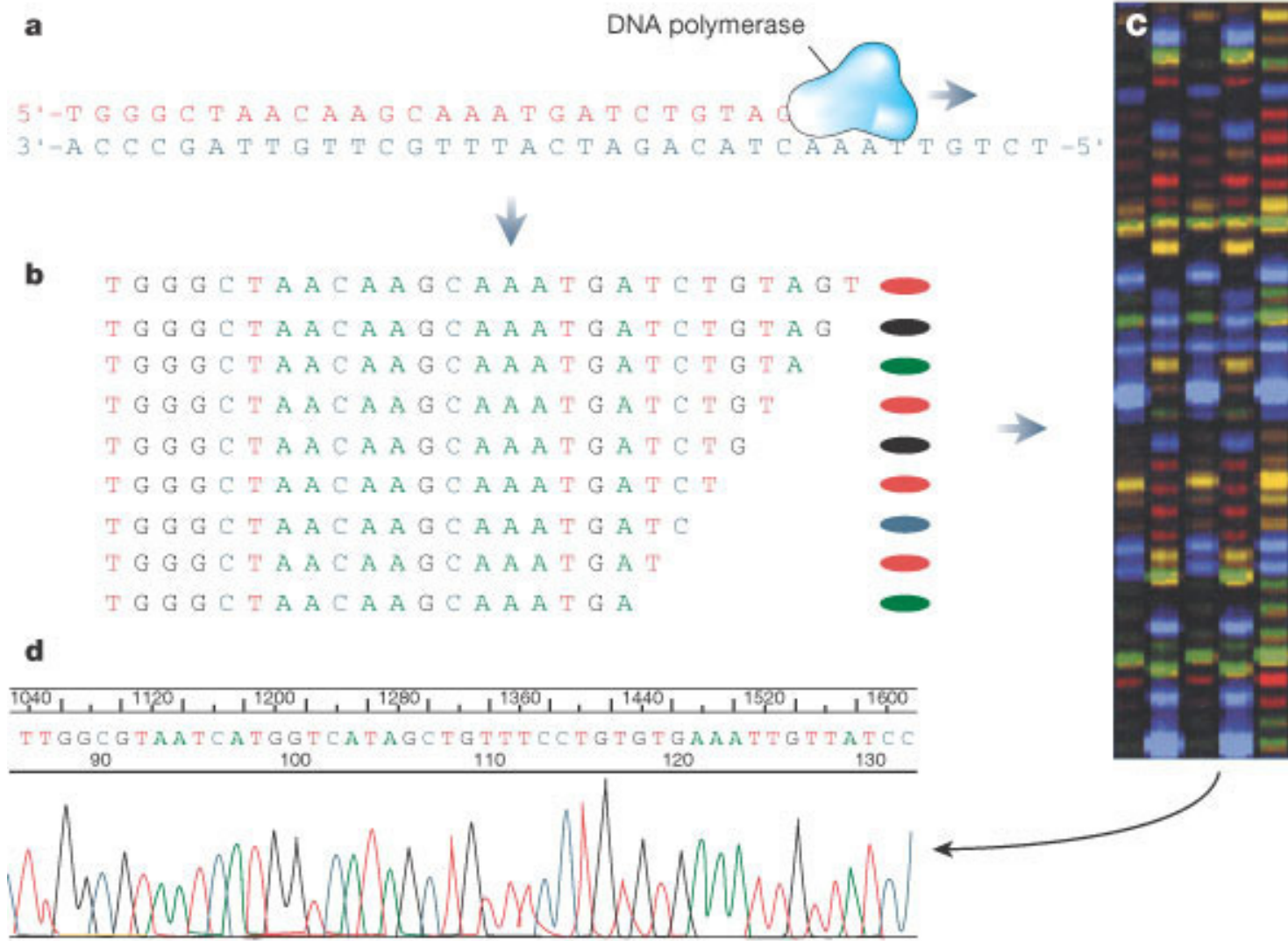


Figure 6; Hood and Galas; Nature: 421 (2003)

[MORE](#)

# DNA Sequencing

Fresh or Herbarium samples of *Phragmites*



Extract DNA



Use polymerase chain reaction (PCR) to amplify  
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Sequence non-coding chloroplast regions



Analyze the sequences

# Sequence Analysis

- Compare new sequences with DNA sequences from known haplotypes
  - to identify a possible matching haplotype for sequences of unknown identity



# Genetic Analyses to Distinguish Haplotypes

## Two Methods

- DNA sequencing
- Restriction Fragment Length Polymorphism (RFLP)

# RFLP

- Use restriction enzymes to cleave DNA at specific sequences

# RFLP

Fresh or Herbarium samples of *Phragmites*



Extract DNA



Use polymerase chain reaction (PCR) to amplify two non-coding chloroplast regions



Use restriction enzymes to cut PCR products from non-coding chloroplast regions



Visualize PCR products after cutting with enzymes

# RFLP: Example Restriction Enzyme

trnL region PCR product

11 native North American haplotypes



One cut site –  
Native  
Haplotypes

invasive haplotype M



No cut sites –  
Nonnative  
Haplotype M



# RFLP

Fresh or Herbarium samples of *Phragmites*



Extract DNA



Use polymerase chain reaction (PCR) to amplify two non-coding chloroplast regions



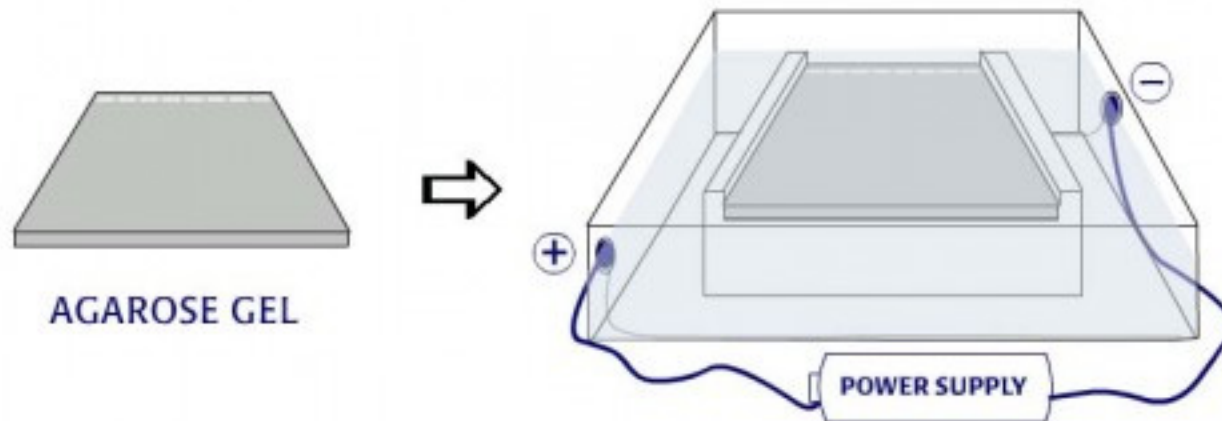
Use restriction enzymes to cut PCR products from non-coding chloroplast regions



Visualize PCR products after cutting with enzymes

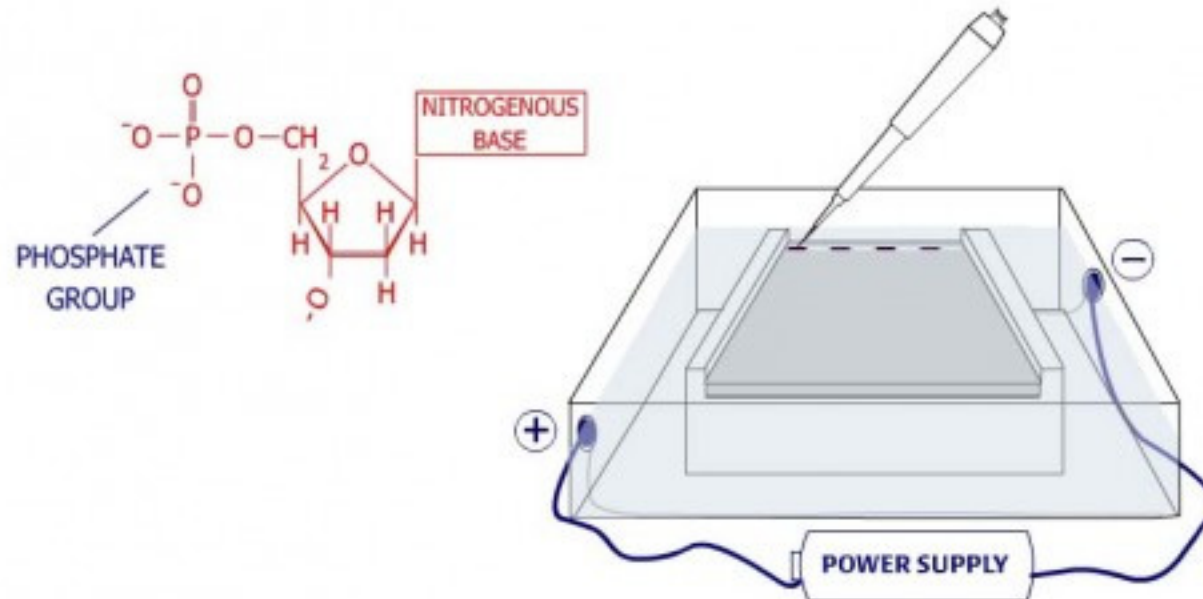
# Analysis of PCR Products

- Gel electrophoresis



# Analysis of PCR Products

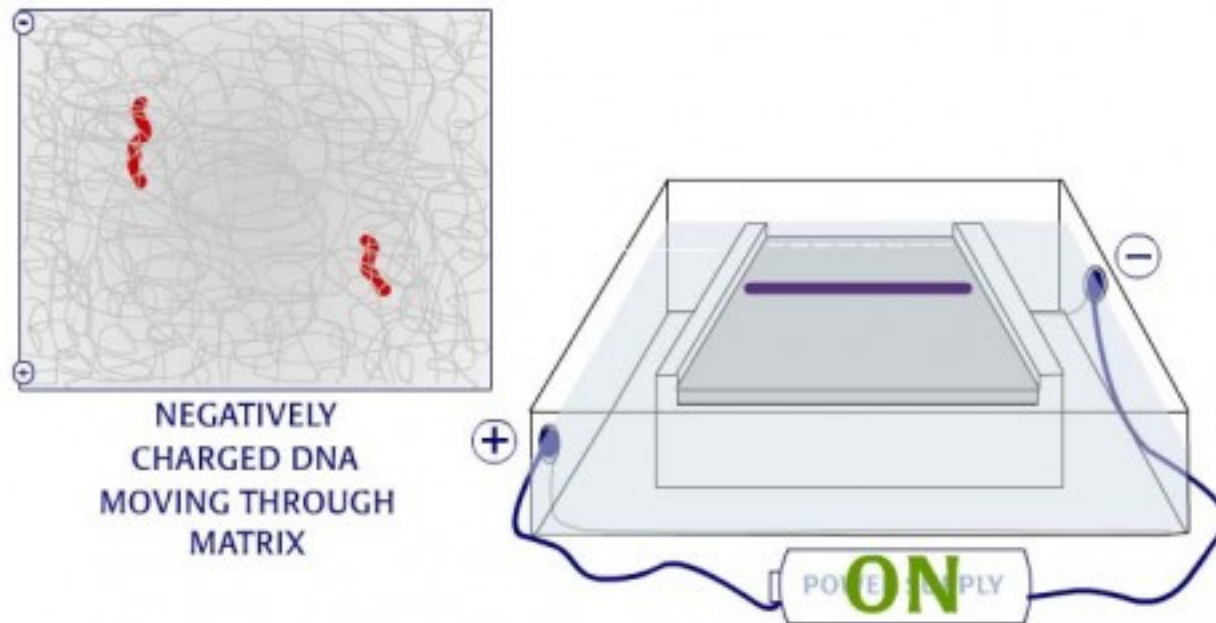
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# Analysis of PCR Products

- Gel electrophoresis



# RFLP: Example Gel Electrophoresis

