Plant Pathology 602
Plant-Microbe Interactions

Lecture 2
Molecular methods for studying host-pathogen interactions I

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Plant Pathology 602
Plant-Microbe Interactions

Outline - lecture 2

- Refresher on gene expression
- Strategies for molecular study of plant pathogens and their hosts

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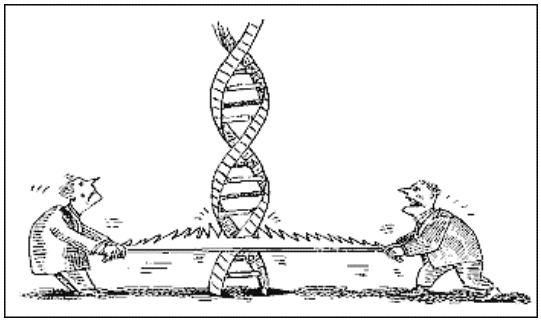
Refresher on gene expression -
DNA: The stuff of life



Refresher on gene expression

Gene

Refresher on gene expression

Gene

⇩

Transcript

Refresher on gene expression

Gene

⇩

Transcript

⇩

Product

Refresher on gene expression

Gene DNA

⇩

Transcript

⇩

Product

Refresher on gene expression

Gene DNA

⇩ ⇩

Transcript RNA

⇩

Product

Refresher on gene expression

Gene DNA

⇩ ⇩

Transcript RNA

⇩ ⇩

Product Protein

Refresher on gene expression

Gene DNA

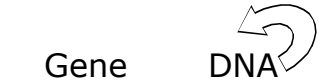
⇩ ⇩ Transcription

Transcript RNA

⇩ ⇩ Translation

Product Protein

Central dogma of molecular biology



Gene DNA

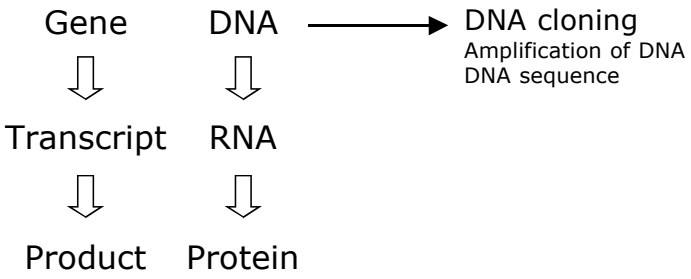
⇩ ⇩ Transcription

Transcript RNA

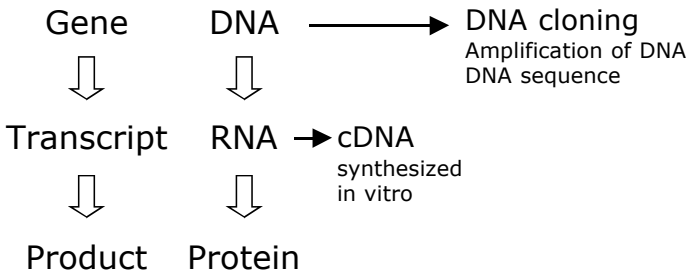
⇩ ⇩ Translation

Product Protein

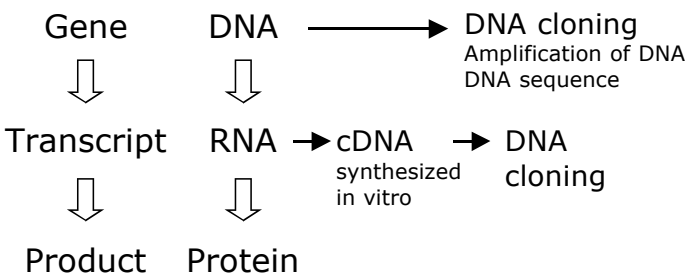
Refresher on gene expression



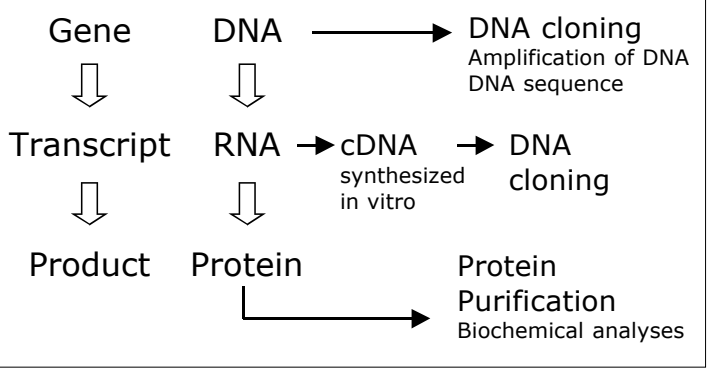
Refresher on gene expression



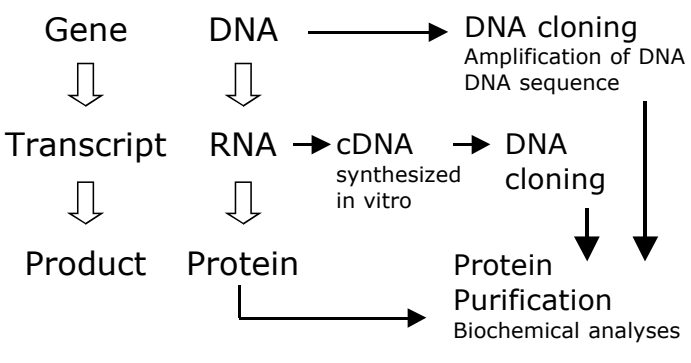
Refresher on gene expression



Refresher on gene expression



Refresher on gene expression



Refresher on gene expression

prokaryotes	eukaryotes
■ Structurally simpler	■ Structurally more complex
■ Cells do not contain nucleus	■ Cells contain a true nucleus
■ Genomic DNA generally naked and circular	■ DNA contained in chromosomes in nuclei
■ Genes are generally present in single sets (haploidy)	■ Cell organelles (mitochondria, chloroplasts etc…)
	■ Genes are present in two or more sets (diploidy)

Refresher on gene expression
Technical challenges in the molecular genetic study of eukaryotes

- Structurally more complex
- DNA transformation can be limiting (cell wall etc…)
- Large genomes (lots of noncoding sequences, gene families...)
- Diploid organisms (more than one gene to disrupt)

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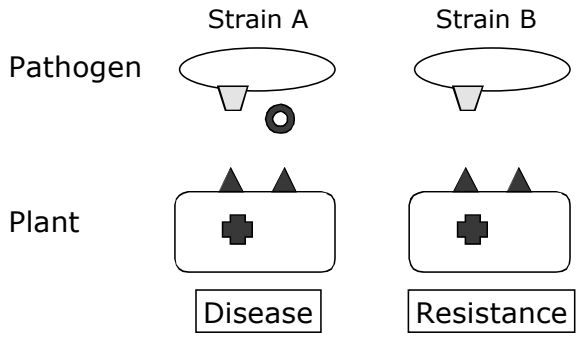
- Strategies for molecular study of plant pathogens and their hosts

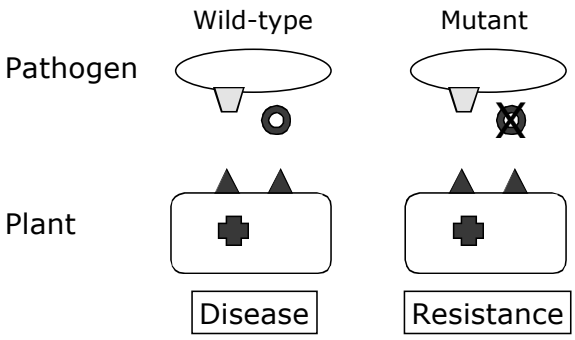
Strategies for molecular study of plant pathogens and their hosts
Genetic approach

- Many methods are centered around a (molecular) genetic approach
- Basis of the genetic approach is to start with two individuals/populations that differ in phenotype and identify the genes that determine the difference
- Variation could be natural or artificially generated by mutagenesis

Genetic approach- Natural variation



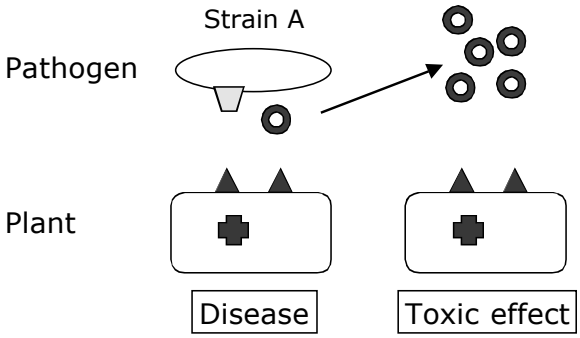
Genetic approach- Mutagenesis



Strategies for molecular study of plant pathogens and their hosts
Biochemical approach

- A protein/secondary metabolite with a particular property can be purified from the pathogen/plant
- The amino-acid sequence of the protein can then be determined and the corresponding gene isolated
- Example: toxin or plant cell wall degrading enzymes isolated from pathogens

Biochemical approach



Strategies to isolate pathogen genes involved in disease (virulence/avirulence)

- Gene disruption
- Gene silencing
- Map-based cloning
- Differential gene expression
- Biochemical approach
- Genomics (random sequencing of DNA)

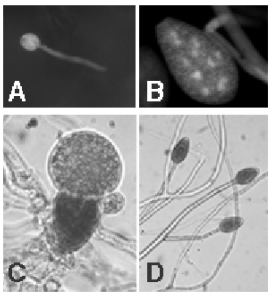
Strategies to isolate plant genes involved in disease (resistance/susceptibility)

- Mutagenesis (transposon and others)
- Map-based cloning
- Differential gene expression
- Biochemical approach
- Candidate genes (similarity based)
- Model plants (Arabidopsis, rice)

Gene transfer = DNA transformation

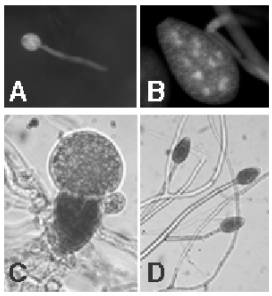
Fungi/oomycetes

- Proroplasts or zoospores
- Liposome mediated
- Electroporation
- Particle bombardment
- *Agrobacterium*-mediated



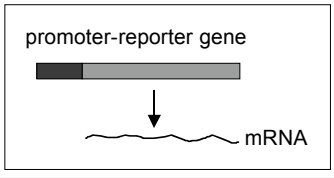
Reporter genes (ex: GFP or GUS)

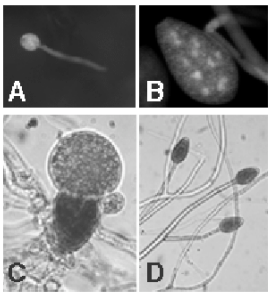
- GFP: green fluorescent protein
- GUS: beta glucuronidase



Reporter genes (ex: GFP or GUS)

- GFP: green fluorescent protein
- GUS: beta glucuronidase
- Constitutive promoters
- Inducible promoters





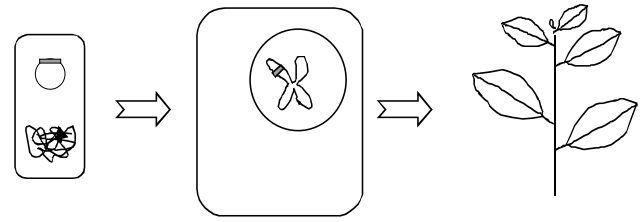
Gene transfer = DNA transformation
Plants

- Stable or transient
- *Agrobacterium*-mediated
- Particle bombardment

Gene transfer into plant cells

- Transgene - exogenous recombinant DNA fragment(s) introduced into plants
- Transgenic plants - plants that contain exogenous recombinant DNA, also commonly called Genetically Modified Organisms or GMOs

How are transgenic plants generated?



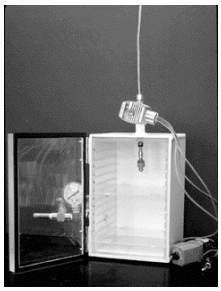
BACTERIA | PLANT CELL WITH TRANSGENE | TRANGENIC PLANT

Gene transfer into plant cells

- Agrobacterium - The natural genetic engineer!!!!
- A bacterium that can naturally transfer genes into plants

Gene transfer into plant cells

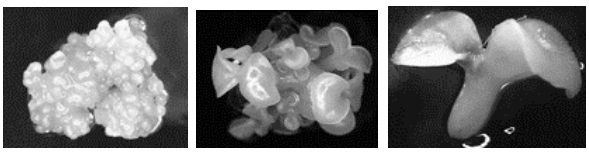
- Particle bombardment - A method for introducing DNA into plant cells using a gene gun



http://www2.oardc.ohio-state.edu/plantranslab/

Plant regeneration

- Totipotency - unique ability of isolated plant cells to regenerate into a whole plant

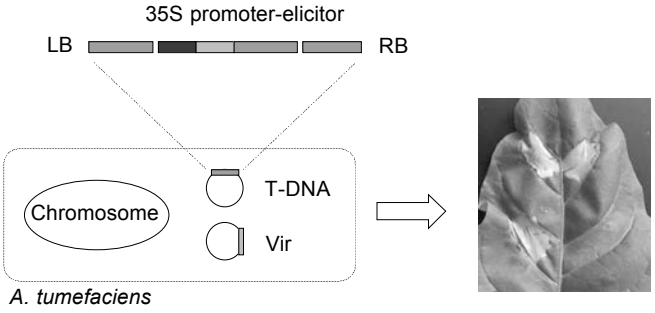


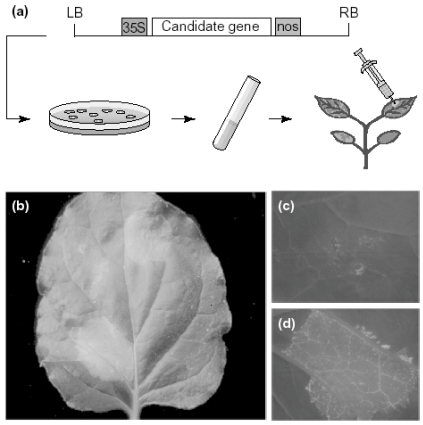
http://www2.oardc.ohio-state.edu/plantranslab/

Transient transformation assays in plants

- Agroinfiltration
- Virus based (example Potato Virus X)
- Transformation of protoplasts (electroporation)

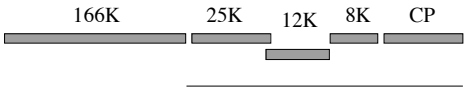
Agroinfiltration Assay
Example: HR elicitors



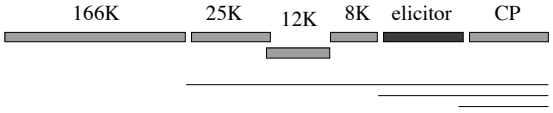


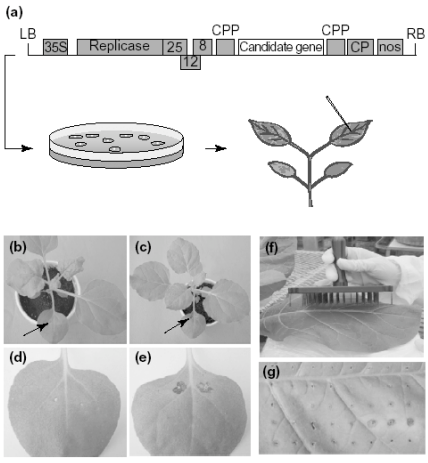
Expression of HR elicitor genes from the Potato Virus X genome

PVX



PVX-INF

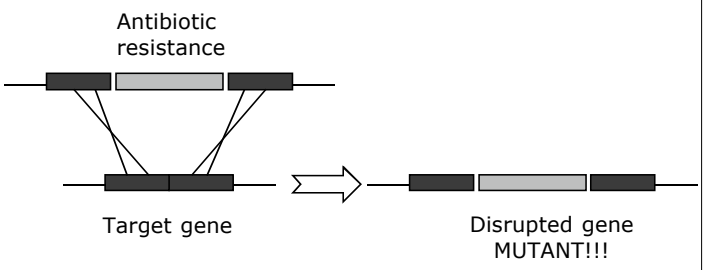




Gene disruption or gene knockout

- Gene disruption can be obtained by inserting a piece of DNA inside the target gene creating a “disruption” or mutation
- Inserted DNA is generally an artificially engineered fragment and usually contains a gene for antibiotic resistance for selection
- Insertion can be random or can be targeted through the process of homologous recombination

Gene disruption or gene knockout



Gene disruption or gene knockout
Challenges for eukaryotes

- Most fungal species are haploid but in diploid species disruption of the two copies is necessary to obtain a null mutant
- DNA transformation can be limiting
- Rates of homologous recombination vary between organisms (example: very low frequency in *Phytophthora*)

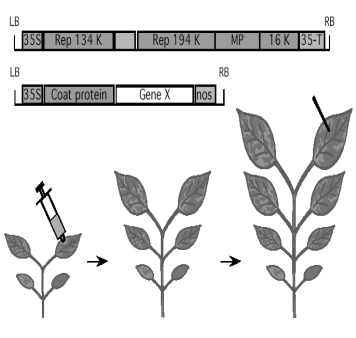
Gene silencing or RNAi (RNA intereference)

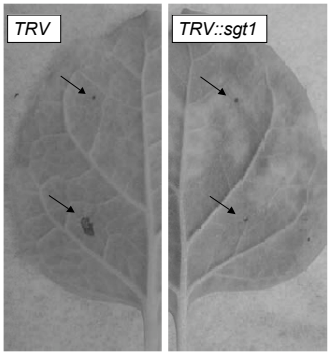
- Introduction of a gene in an antisense (wrong) orientation or sometimes even in a sense orientation triggers a gene silencing response in the eukaryotic cell
- "Foreign" DNA is silenced that is the gene is not expressed anymore or the RNA gets degraded
- If the "foreign" DNA is similar to a gene of the transformed organism, silencing will affect both the transgene and the endogenous genes thus resulting in a mutant phenotype

Gene silencing - Example: Virus induced Gene Silencing (VIGS)
phytoene desaturase in tobacco results in bleaching

Source: D. Baulcombe lab

Virus Induced Gene Silencing to characterize plant response to elicitors

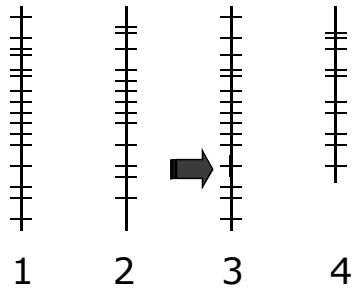




Map-based cloning

- If individuals with different phenotypes are available then genetic crosses can be set up and segregation of the differing genes can be noted
- Molecular markers (mutation scattered throughout the genome and identified using molecular tools such as restriction enzyme digests or PCR) can be identified in this segregating population
- Markers that are genetically linked to the segregating gene can be used to identify the target gene

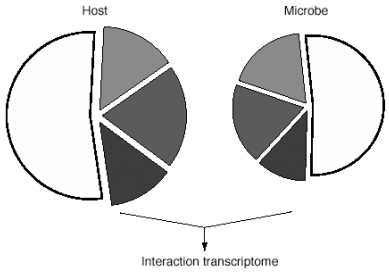
Map-based cloning - genetic map

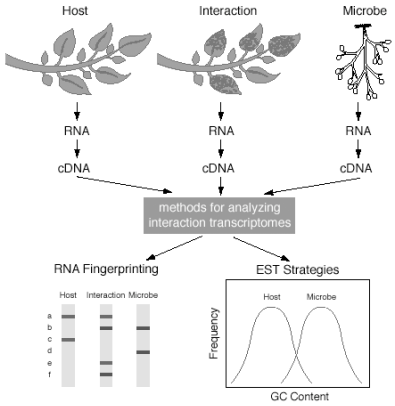


Differential gene expression

- Pathogen and plant genes that are highly expressed during infection are likely to be involved in the infection process
- Patterns of gene expression are revealed by the populations of mRNA present in the cell
- Methods to compare and subtract different RNA (cDNA) populations exist and can be applied to the identification of infection induced genes

The interaction transcriptome

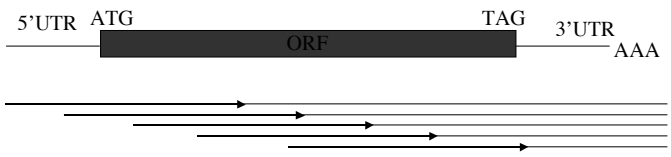




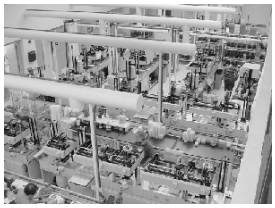
Genomics (random sequencing of DNA)

- Genomics or random sequencing of genomic DNA and/or cDNA can be applied to the study of plant-microbe interactions
- Various computer analyses of the sequenced genes can be used to identify candidate genes (bioinformatics)
- Functional genetic assays need to be performed to test the function of the candidate genes (functional genomics)

Example: cDNA sequencing (Expressed Sequence Tags)



The functional genomics paradigm



Sequence → Candidate genes → Phenotype

bioinformatics Functional assay

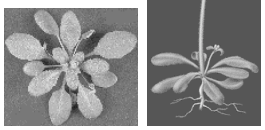
Paradigm shifts in biological research

Adapted from Peltonen and McKusick, Science 291:1224

Structural genomics	-->	Functional genomics
Genomics	-->	Proteomics
Map-based	-->	Sequence-based gene discovery
Monogenic traits	-->	Multifactorial traits
Analysis of one gene	-->	Analysis of multiple genes in gene families, pathways, or systems
Gene action	-->	Gene regulation
Etiology (mutation)	-->	Pathogenesis (mechanism)
One species	-->	Several species

Model plants (Arabidopsis, rice)

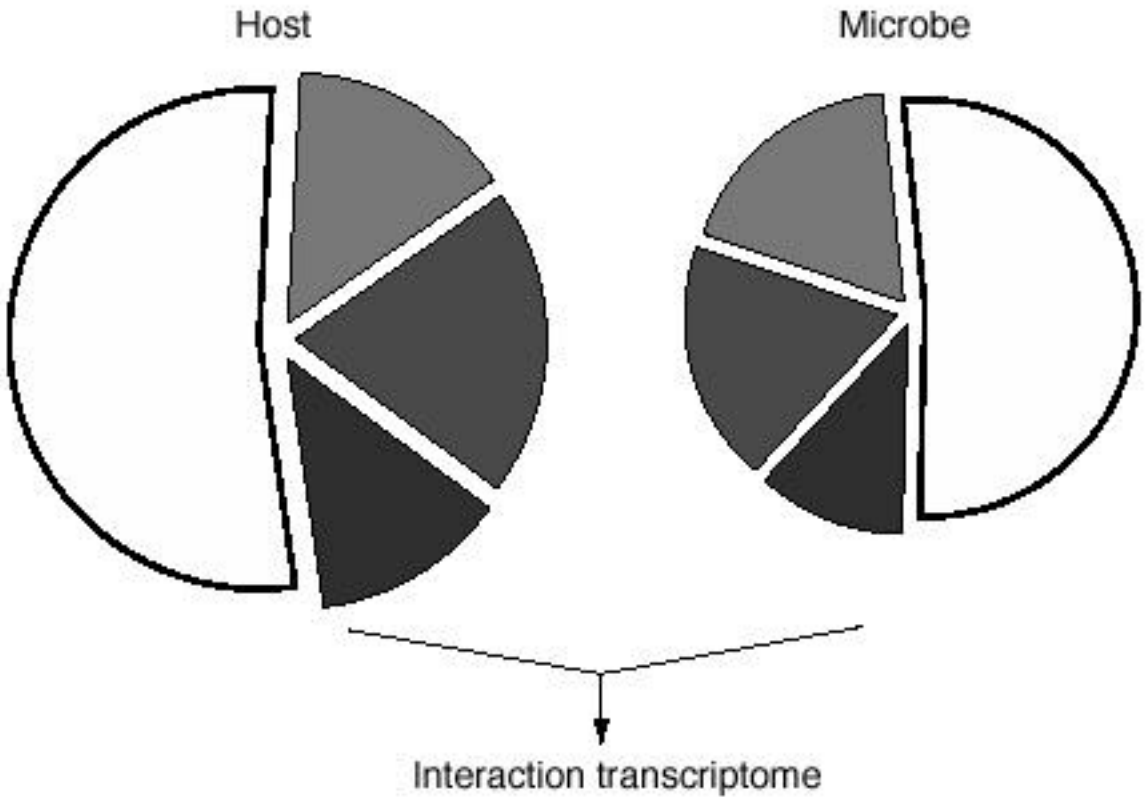
- Short generation time
- Small genomes
- Genome sequences available
- Powerful mutagenesis tools
- DNA transformation and functional assays available
- Resistant to diverse pathogens

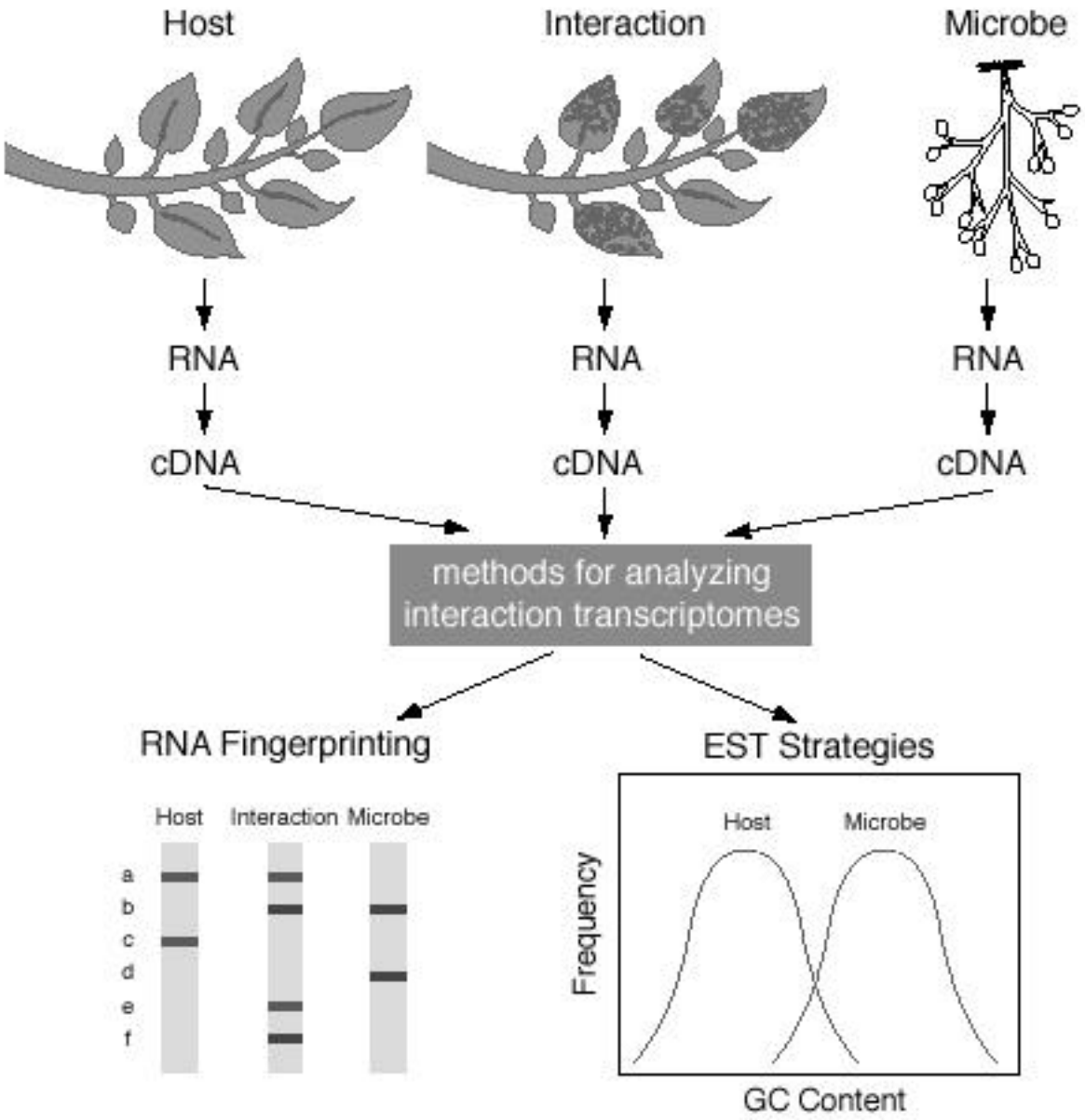


Glossary of genetic terms

- http://www.weihenstephan.de/~schlind/genglos.html
- http://www.genome.gov/glossary.cfm

The interaction transcriptome

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