



Frontiers of Modern Genetics

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University of the Third Age (U3A)
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What will you learn? (hopefully)

- 1) Quick 'catch-up' on cells, genetics and DNA (central dogma)
- 2) Special advances in modern genetics
- 3) Impact and future needs

A microscopic cross-section of a plant stem, likely a dicot, showing various tissue layers. The image is overlaid with fluorescence, with a bright green layer along the outer edge (epidermis and cortex) and a deep red layer in the inner tissues (vascular bundles and pith). The vascular bundles are arranged in a ring, and the pith is in the center. The overall appearance is that of a young stem section.

Fundamental Questions:

How does GENETICS function?

How does it affect us directly?

***Examples and approximations will
be given***

A microscopic cross-section of a plant stem, likely a dicot, showing various tissue layers. The image is enhanced with fluorescence, with the outer cortex and epidermis appearing bright green and the inner vascular tissues appearing in shades of red and orange. The central pith is visible as a darker, more uniform area.

What is GENETICS:

The science / study of inheritance of TRAITS in both higher organisms (humans, other animals, plants, fungi, algae), viruses and bacteria

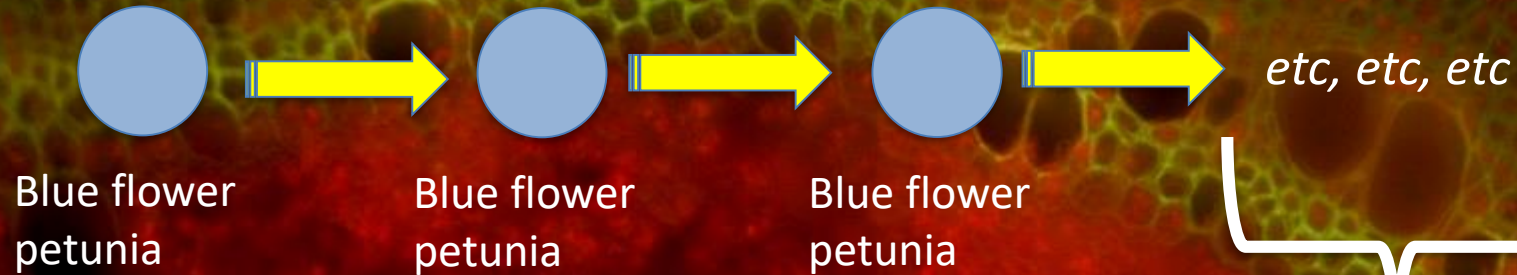
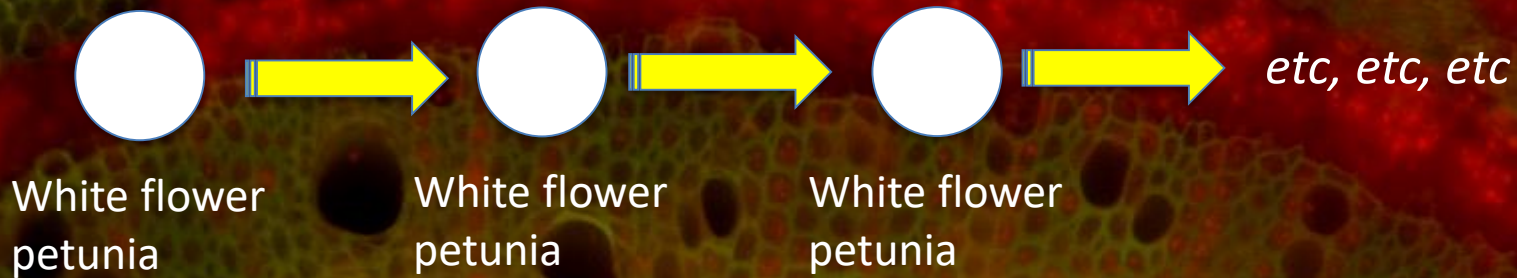
A fluorescence micrograph of a plant tissue section, likely a leaf cross-section. The image shows a network of green fluorescent structures, possibly cell walls or specific organelles, against a dark red background. The green structures form a complex, interconnected pattern, with some larger, more circular structures visible in the lower right quadrant. The overall appearance is that of a highly detailed biological structure.

What is MODERN GENETICS:

Analysis of inheritance and gene expression viewed from either a WHOLISTIC or MOLECULAR perspective (or their combination)

Quickly some BASIC GENETICS:

2) Traits of a species are inherited

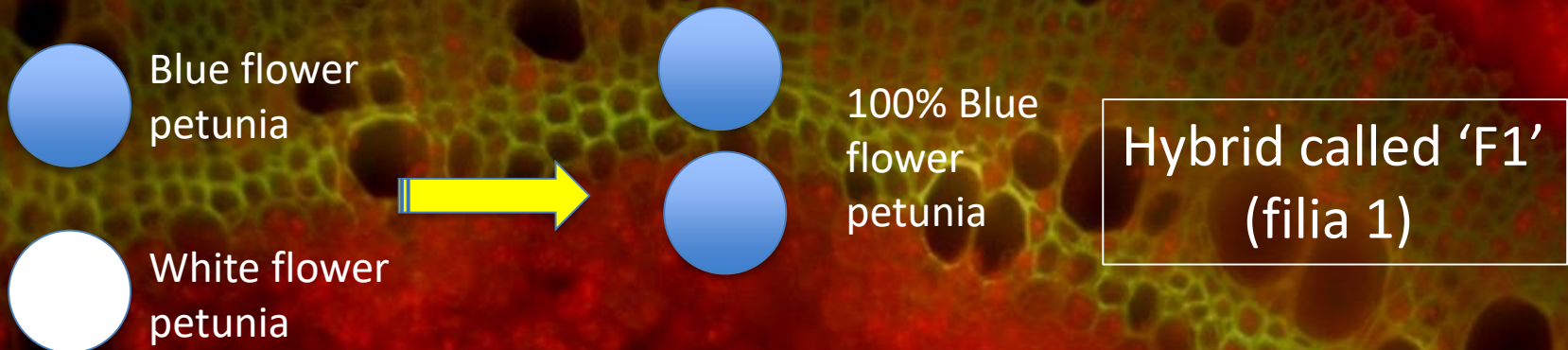



Bar random and rare mutations

Quickly some BASIC GENETICS:

2) Traits of a species are inherited

-- what, if HYBRIDISED/crossed ????

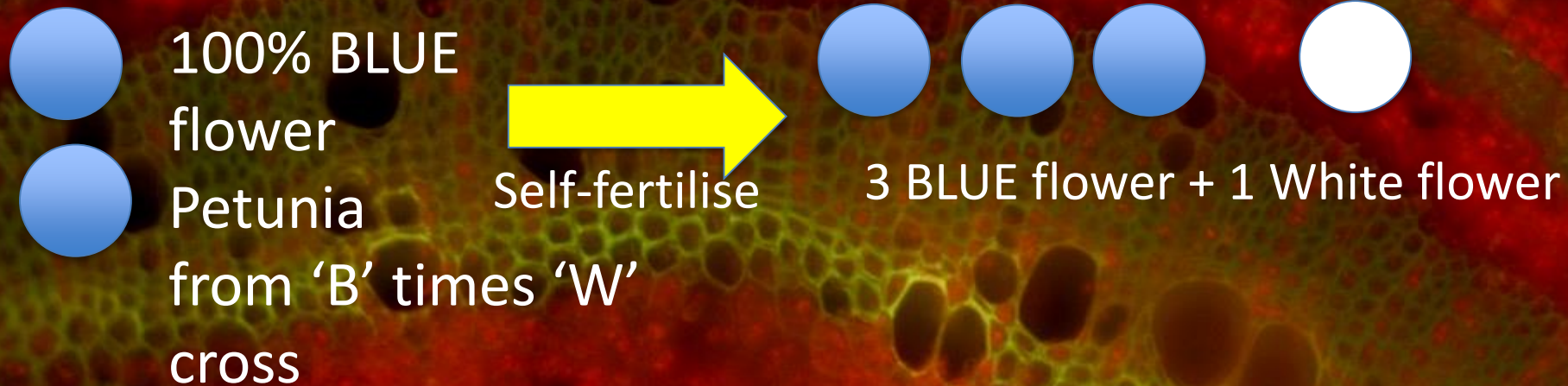


*The 'blue' trait is dominant; 'white' is recessive

*Non-function in 'white' is suggested

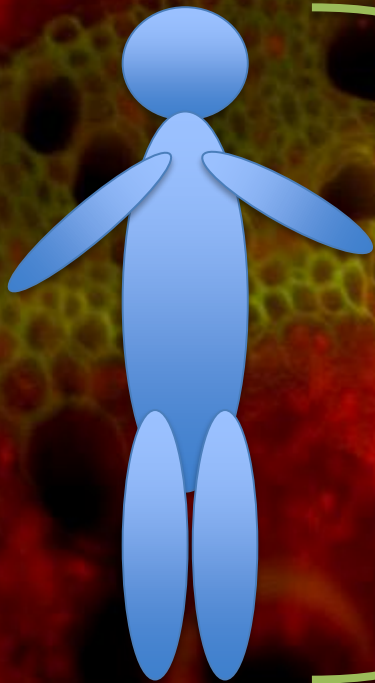
Quickly some BASIC GENETICS:

2) Traits of a species are inherited
and segregate, as if **'PARTICULAR'**



Gregor Mendel Law of Inheritance

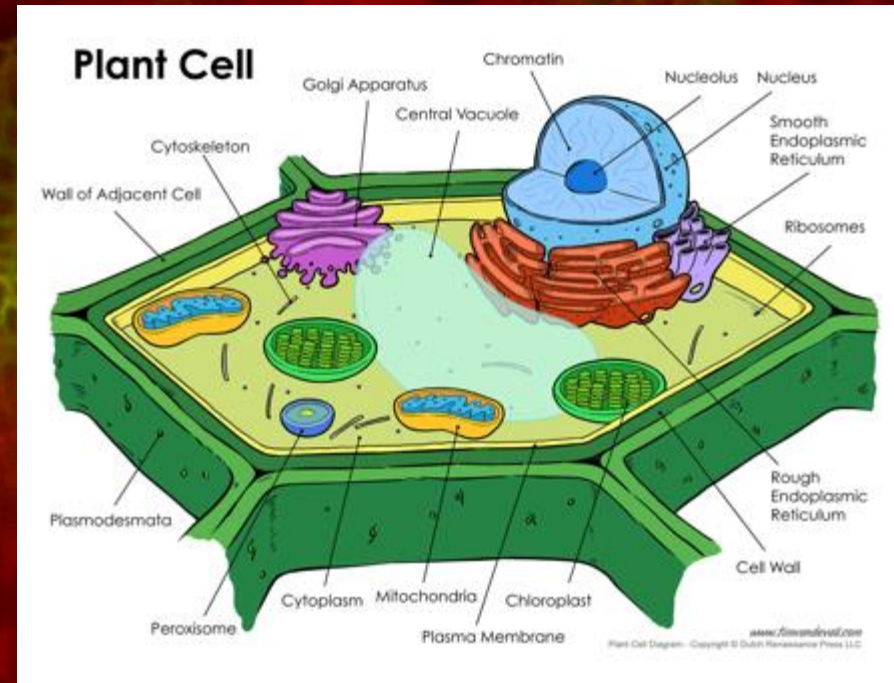
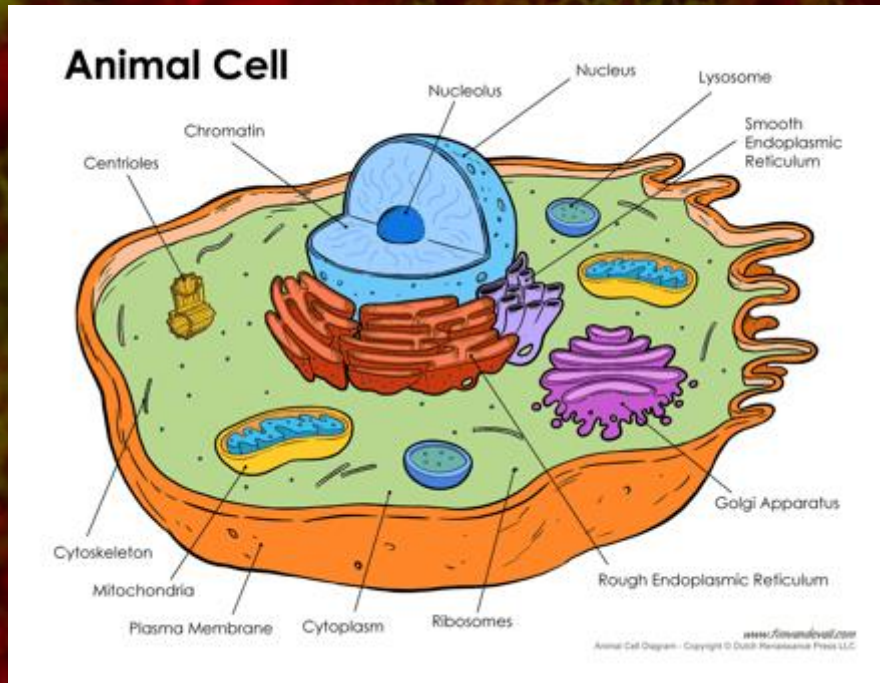
3) Humans, animals and plants are
made up of CELLS
Bacteria are commonly SINGLE cells



About
10 trillion
cells
(10,000,000,000,000)

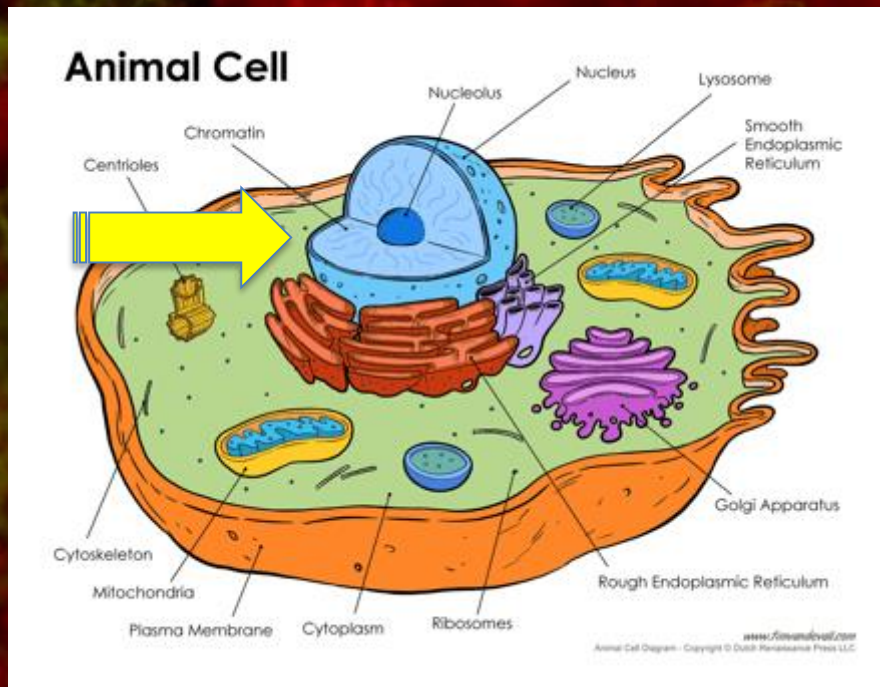
and some CELL BIOLOGY:

4) Cells have a common design
(some minor differences depending on function and origin)

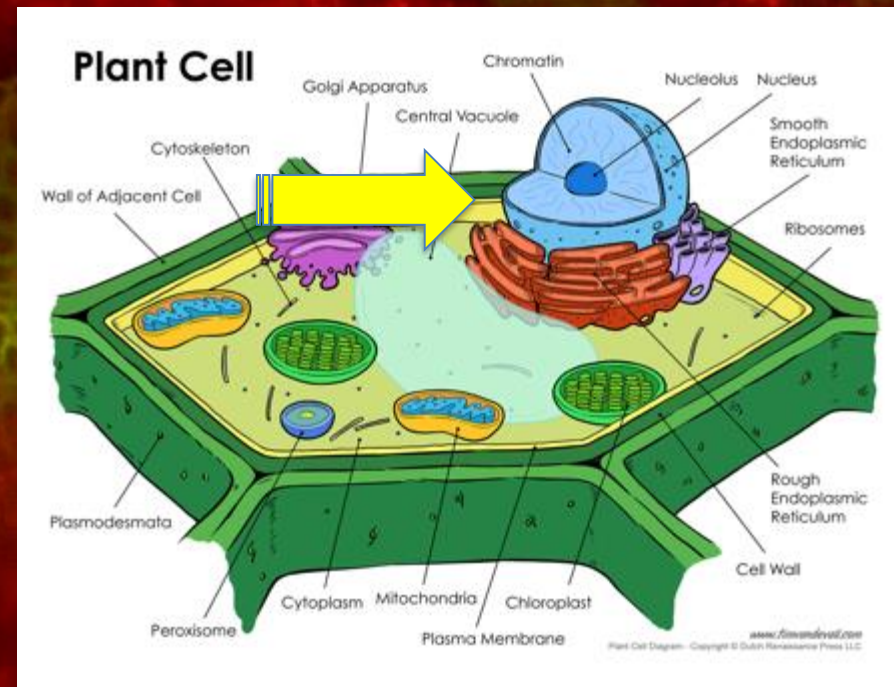


4) the nucleus stores genetic material (DNA)

(note: small DNA also found in mitochondria and chloroplasts)



About 3 to 12 microns long



About 20 to 50 microns long

4) Bacterial cells are much smaller

“General”
Plant
Cell

50 micron long

1 micron long

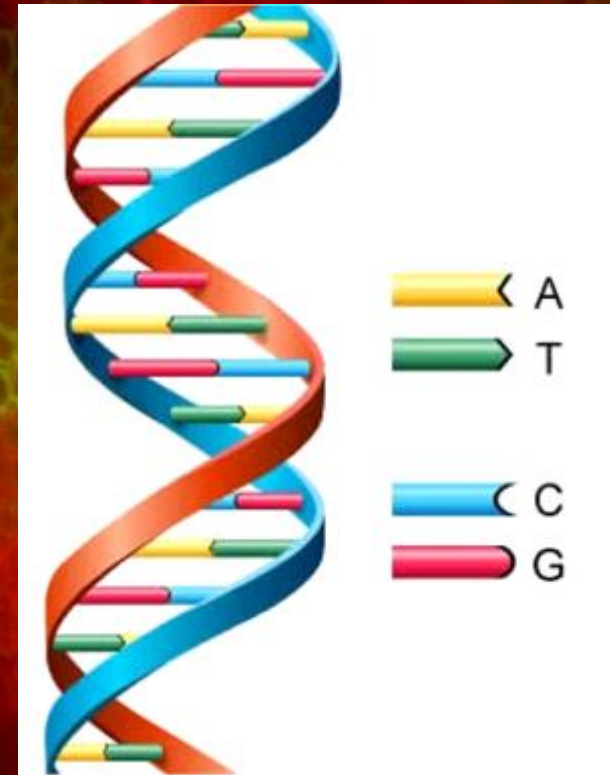
Each bacterial cell contains DNA but not in a nucleus (PROKARYOTE)
Bacteria have about 3000-4000 genes; in contrast humans have 25,000 genes

The molecular basis of inheritance:

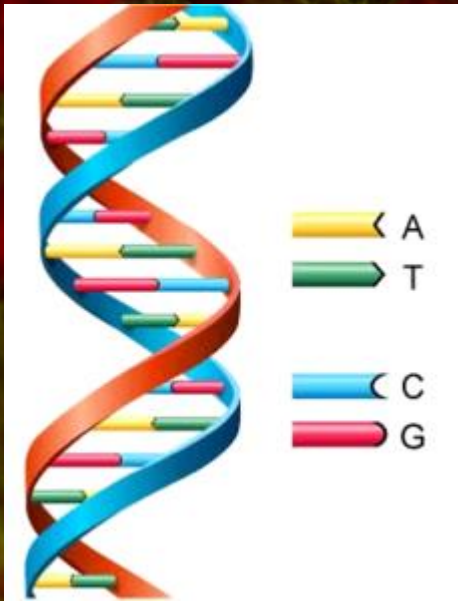
5) DNA: deoxy-ribo-nucleic acid (double helix)
(note: chemically the same in ALL organisms; bacteria to humans!!)



Structure discovered by
James Watson and Francis Crick (1953)



5) DNA: deoxy-ribo-nucleic acid make-up



Sugar: deoxy-ribose

Phosphate: PO_4^-

These make the sugar-phosphate backbones
(**strong** coupling (covalent))

Nucleotides:

Adenine A

Guanine G

Thymine T

Cytosine C

- Double Helix

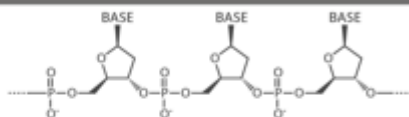
- A and T bind, G and C bind (ionic; **weak**)

5) DNA: deoxy-ribo-nucleic acid make-up

THE CHEMICAL STRUCTURE OF DNA

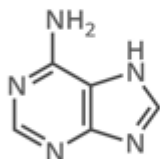
DNA (deoxyribonucleic acid) carries genetic information in all multicellular forms of life. It carries instructions for the creation of proteins, which carry out a wide range of roles in the body.

THE SUGAR PHOSPHATE 'BACKBONE'

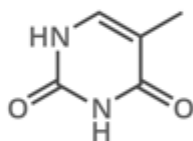


DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine & cytosine.

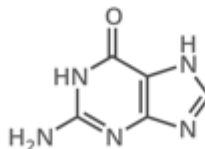
A ADENINE



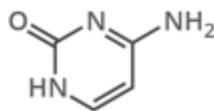
T THYMINE



G GUANINE

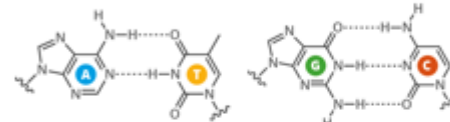


C CYTOSINE



WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), whilst guanine (G) always pairs with cytosine (C).



FROM DNA TO PROTEINS



The bases along a single strand of DNA act as a code. The letters form three letter 'words', or codons, which code for different amino acids - the building blocks of proteins.

An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It does this by splitting apart the two strands that form the double helix, then reading a strand and copying the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

DNA SEQUENCE **T T C C T G A A C C C G T T A**

mRNA SEQUENCE **U U G C C U G A A C C C G U U A**

AMINO ACID Phenylalanine Leucine Asparagine Proline Leucine

In multicellular organisms, the mRNA carries genetic code out of the nucleus, to the cell's cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of converting turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.



5) DNA: deoxy-ribo-nucleic acid

THE CHEMICAL STRUCTURE OF DNA

DNA (deoxyribonucleic acid) carries genetic information in all multicellular forms of life. It carries instructions for the creation of proteins, which carry out a wide range of roles in the body.

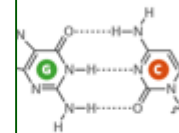
THE SUGAR PHOSPHATE 'BACKBONE'

WHAT HOLDS DNA STRANDS TOGETHER?

DNA self-replicates

- by unzipping,
- re-synthesis of new strands,
- and
- separation of new strands

ids between bases on adjacent
whilst guanine (G) always pairs



PROTEINS

PROTEIN

ide. The letters form three letter
acids - the building blocks of

s mRNA (messenger ribonucleic
that form the double helix, then
nucleotides. The only difference
the place of thymine (T), another



etic code out of the nucleus, to
place. 'Translation' is the process
ins. Molecules called ribosomes
the amino acids coded for.



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6) DNA is kept in CHROMOSOMES

(note: different sizes and DNA per chromosome.

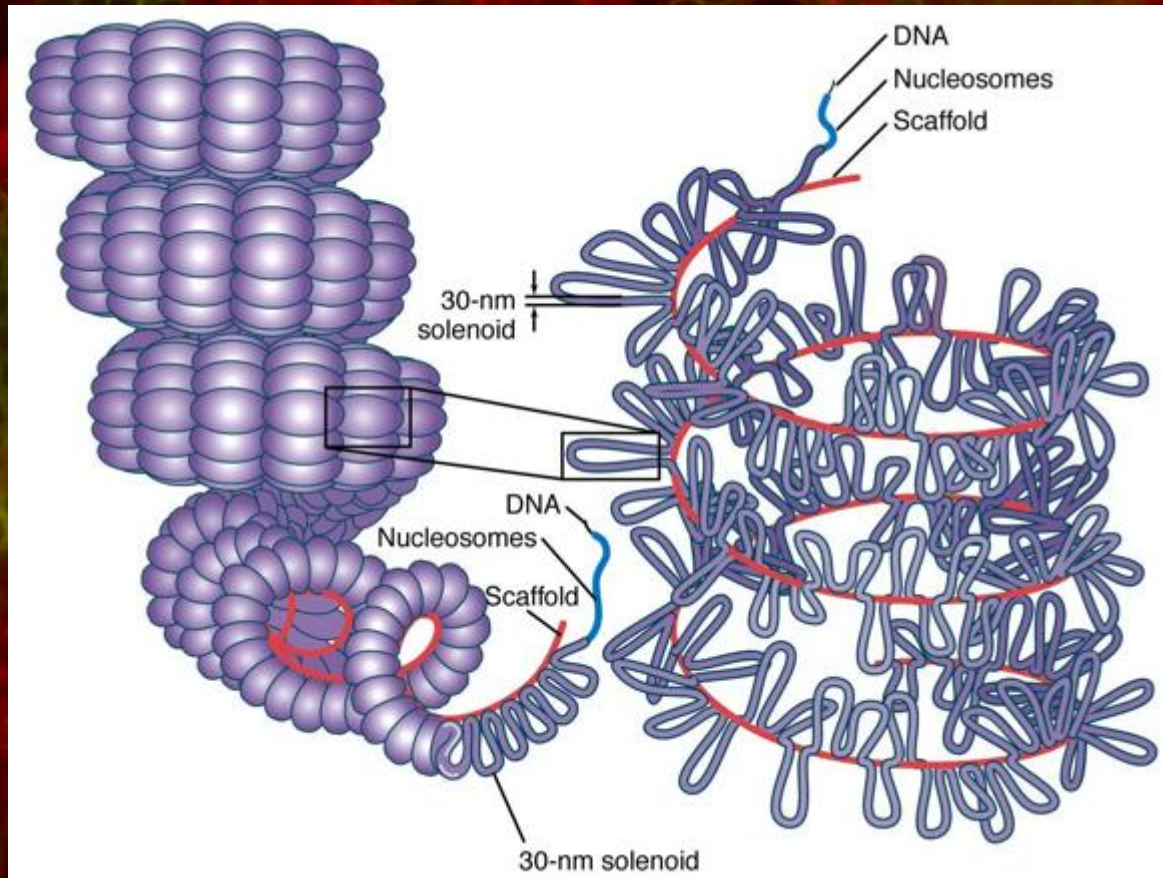
'Chromo'-'some' means: 'coloured' 'body')



Metaphase
chromosome;
coiled DNA

Human cells each have 46 chromosomes*; 23 from mum, 23 from dad
Soybean has 40 chromosomes; 20 from the pollen, 20 from egg

7) VERY large amounts of DNA are coiled up as solenoid structures



8) The CENTRAL DOGMA

DNA sequence (..ATGGGGGCCCTTATAG...)
is read in TRIPLET fashion (*e.g.*, ATG x n).

The product is RNA (ribonucleic acid)

RNA is functional by itself (rRNA, tRNA, miRNA)
or

is TRANSLATED to make PROTEINS

DNA → RNA → protein → function in cell



Expressed DNA is called a **'GENE'**

Changes in DNA sequence are **'MUTATIONS'**

Mutations may or may not alter function

Mutations are dominant, or recessive, or "0"

DNA sequencing:

- developed in early 1970
- (2 D electrophoresis)

(Aussie example: Professor John Shine)

'Shine-Dalgarno' sequence in bacterial promoters' AUG start codon

ACCUCCUUA 3'

These 9 nucleotides took over
3 years (one PhD study) to discover

John Shine (ANU, Genentech, ANU, Sydney):



Genentech USA) made profits from synthetic genes and their products. Prof. Shine (now retired) donated \$10 million to the Australian Academy

DNA sequencing:



DNA sequencing gel



Professor Fred Sanger

DNA sequencing machines



DNA sequencing instruments:

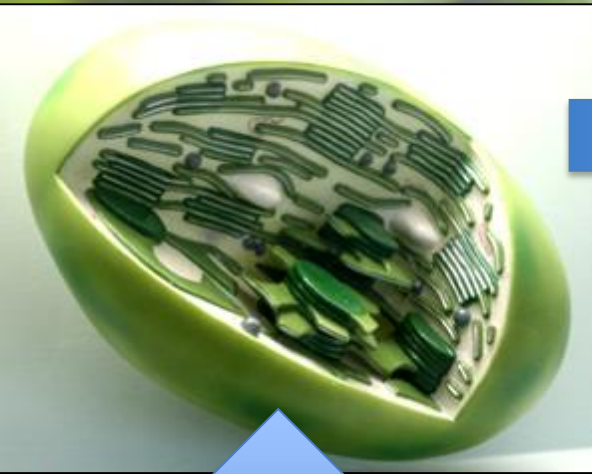


ILLUMINA sequencer



PACBio sequencer

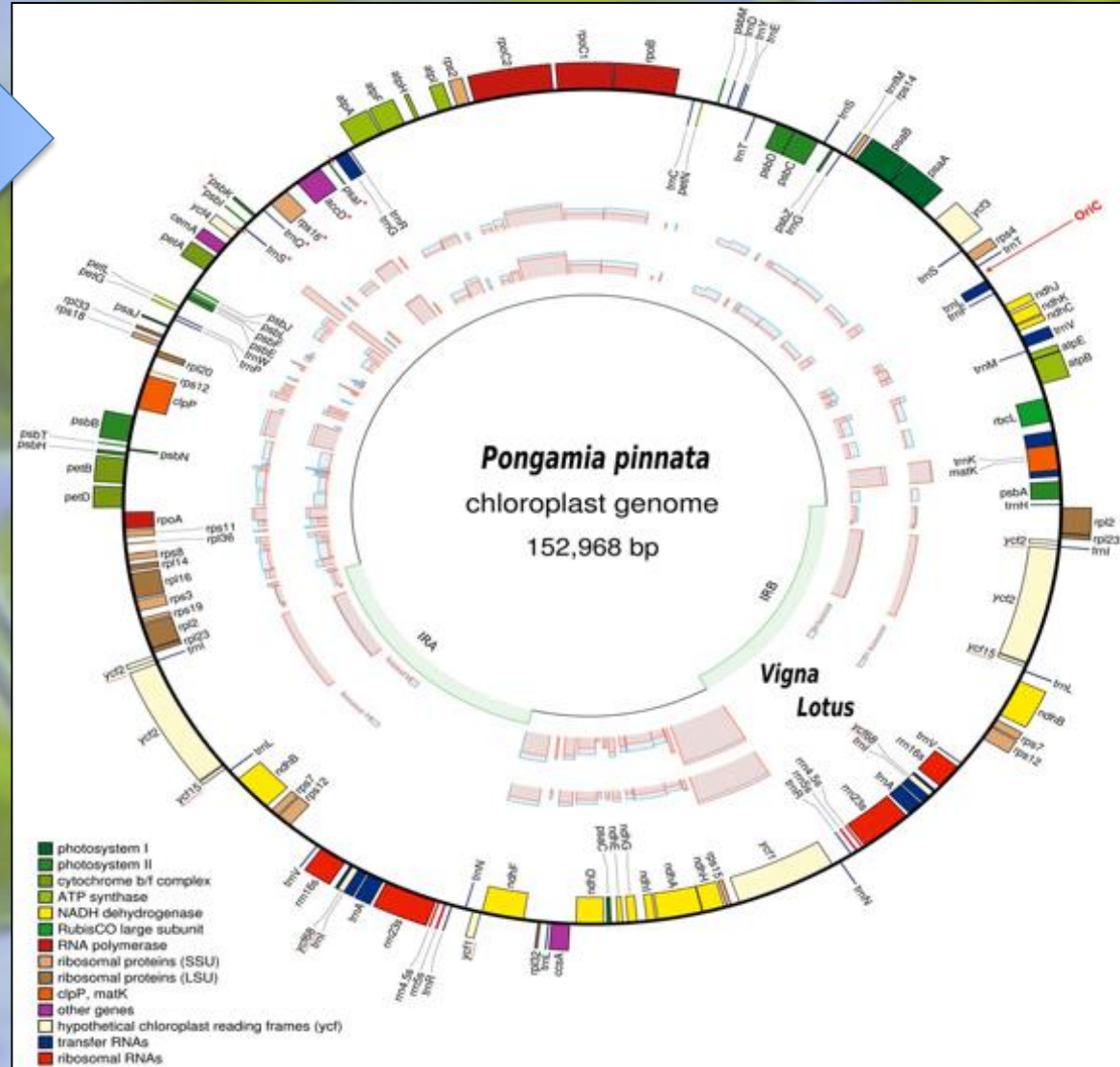
Specific example: tree chloroplast (only small; 153,000 base pairs)



about
50

about 100

Chloroplasts are the site
of PHOTOSYNTHESIS
(capture of CO₂ gas to
make sugar and O₂ gas)



DNA sequencing:

Total DNA (called 'GENOME') of 1000s of species has been obtained.

Animals, humans, plants, viruses, bacteria, fungi

Lays the basis for DNA Profiling, DNA fingerprinting, DNA forensics

Now: about \$1-2,000 to have YOUR OWN DNA sequenced. Disease diagnosis.

DNA sequencing:

- *Genomes are large (millions of base pairs)
- *Most DNA is 'non-functional'
- *Some is repeated 100,000 times (e.g., ATAATn)
- *Higher organisms have about 15,000 to 40,000 genes
- *Some species are polyploid (multiple sets of chromosomes, and thus extra genes (duplicated))

Idealised Human genome: 6,469,660,000 bp (diploid)

Soybean genome: 2,300,000,000 bp (diploid)

Wheat genome: 160,000,000,000 bp (hexaploid)

Escherichia coli genome: 4,600,000 bp (4288 genes)

A fluorescence microscopy image of a tissue section. The image shows a cross-section of tissue with a prominent red signal in the upper and middle layers, and a green signal outlining the tissue structure and some internal components. The background is dark, making the red and green signals stand out.

RNA sequencing (RNAseq):

- **Gives a 'TRANSCRIPTOME'**
- **Near complete sequence of ALL RNA**
- **Identifies, via BIOINFORMATICS, the expression of 30 - 100,000 genes in a tissue**



Reverse Genetics:

Forwards Genetics goes from altered phenotype to DNA analysis

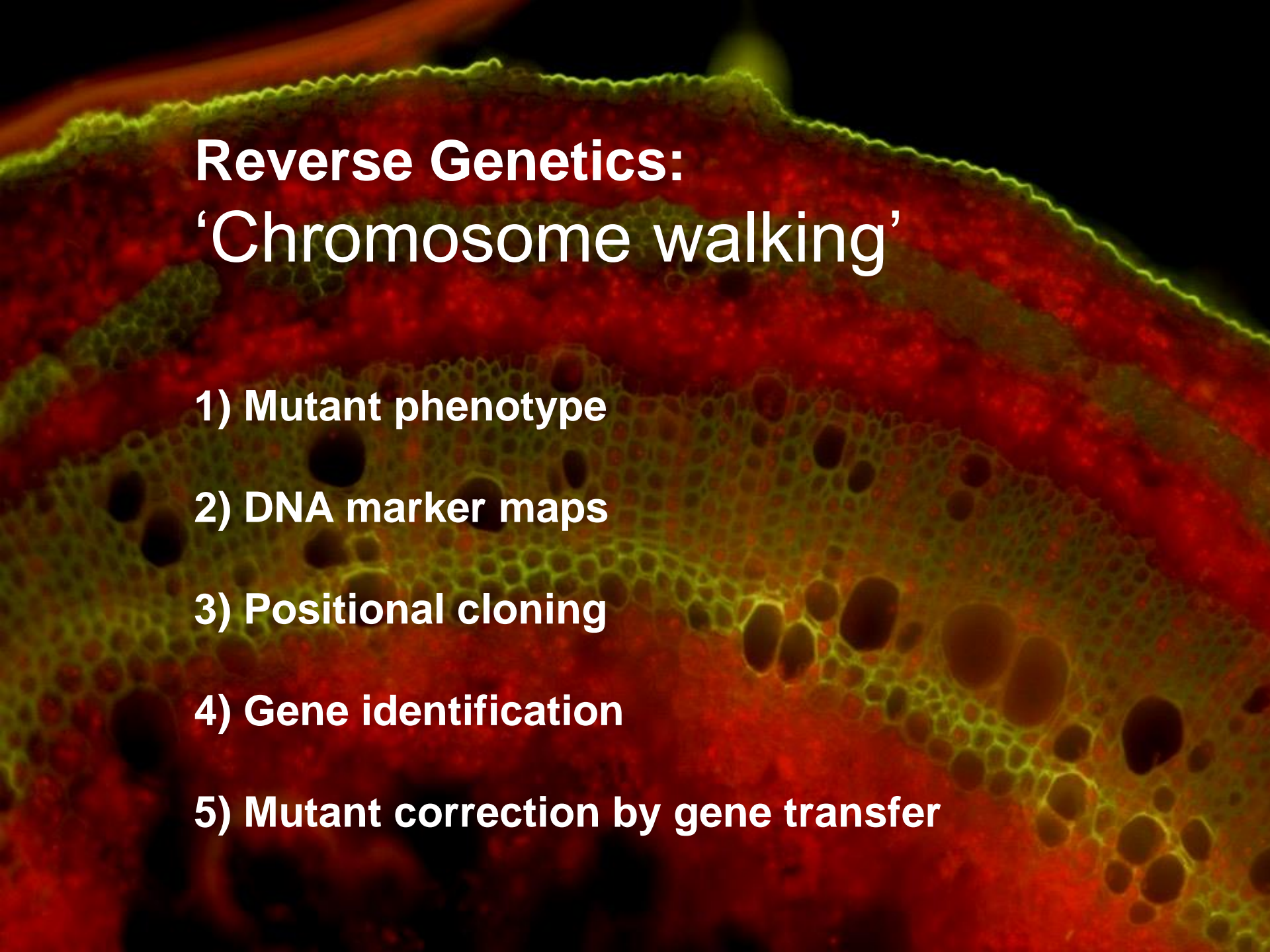
Reverse Genetics is the 'reverse', *i.e.*, DNA to phenotype

A fluorescence micrograph of a plant tissue section, likely a leaf cross-section. The image shows a complex network of cells with green and red fluorescent signals. The green signal highlights the cell walls and some internal structures, while the red signal is more diffuse, filling the cytoplasm and some larger structures. The overall appearance is that of a highly organized biological system.

Reverse Genetics:

To do so, we need to isolate DNA that is responsible for a phenotype.

This involves 'positional cloning' also called 'Chromosome walking'



Reverse Genetics: 'Chromosome walking'

1) Mutant phenotype

2) DNA marker maps

3) Positional cloning

4) Gene identification

5) Mutant correction by gene transfer

A fluorescence micrograph of a soybean leaf cross-section. The image shows several layers of cells. The outermost layer is a thin, wavy green line representing the cuticle and epidermis. Below this is a thick, dense layer of red, representing the palisade mesophyll. The next layer is a more loosely packed green layer, likely the spongy mesophyll, containing large, dark, circular structures that are stomata. The bottom-most layer is another red layer, representing the lower epidermis and possibly the vascular bundle sheath. The overall appearance is that of a cross-section of a leaf with distinct layers of tissue.

**Example of chromosome walking
from plants
(Soybean)**

Root Systems with Nodules



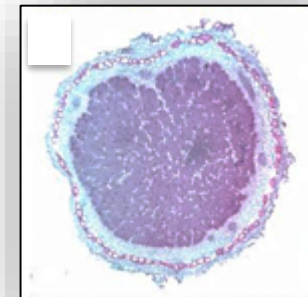
Pea
(*Pisum sativum*)



Soybean
(*Glycine max*)



A soybean nodule
(determinate)

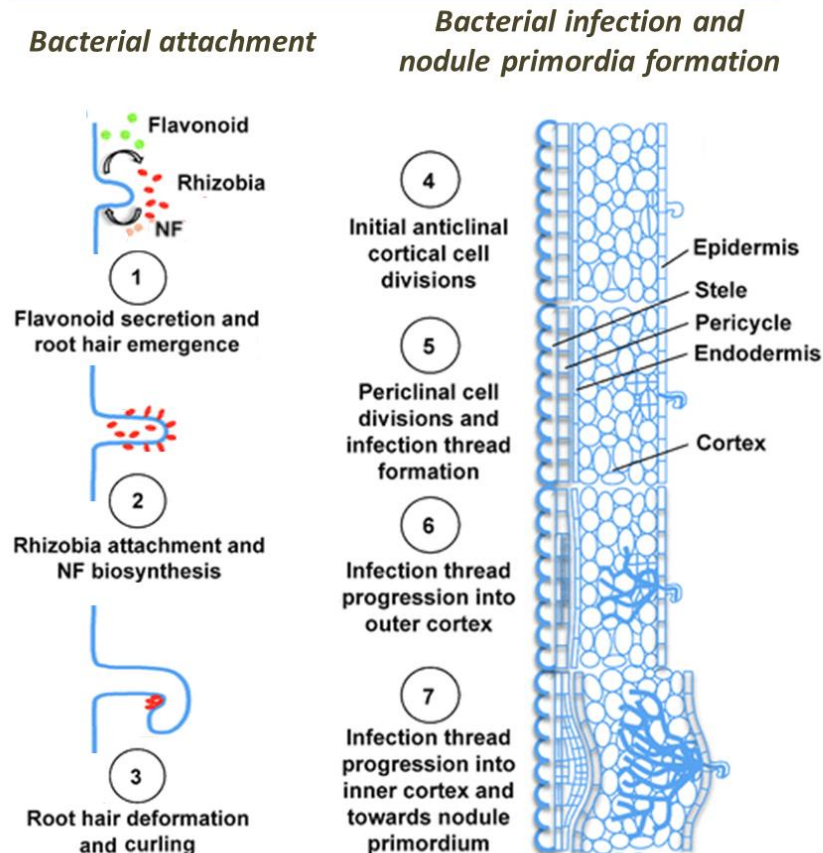


95% of legumes
nodulate.
18,000 species in total

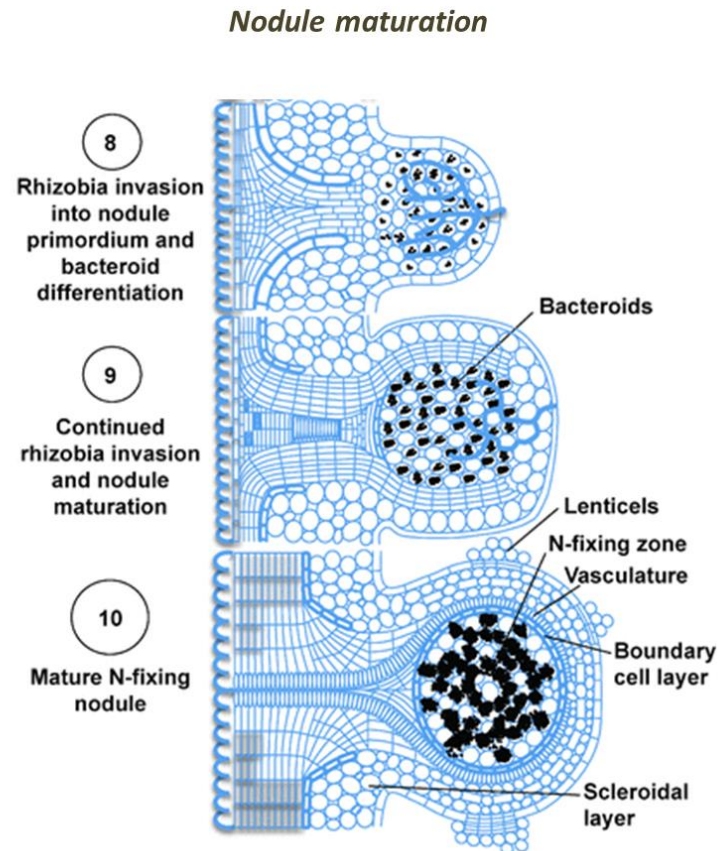
SOYBEAN Nodule DEVELOPMENT

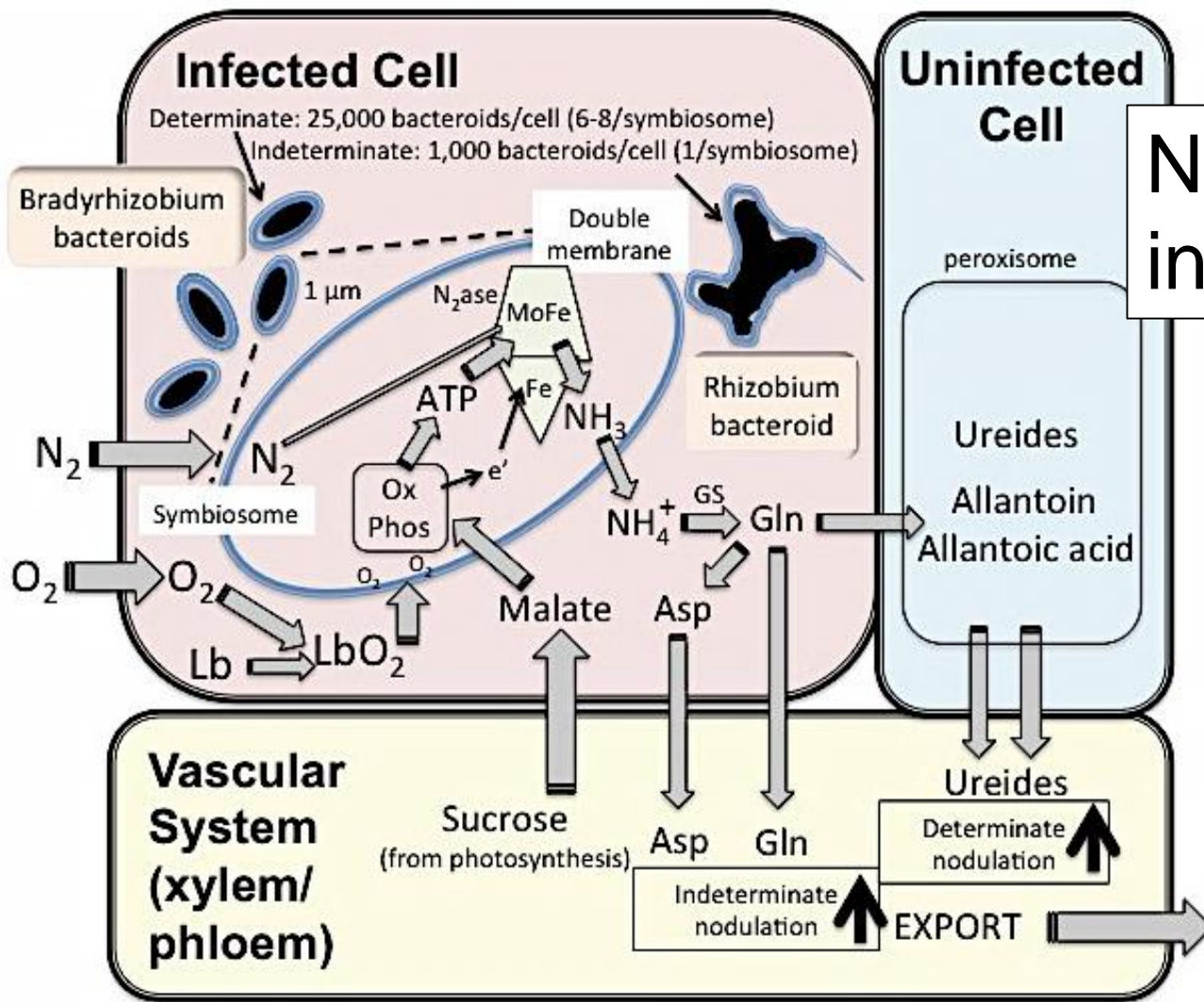
**** morphological changes associated with nodulation ****

Early Stages of Nodulation



Later Stages of Nodulation



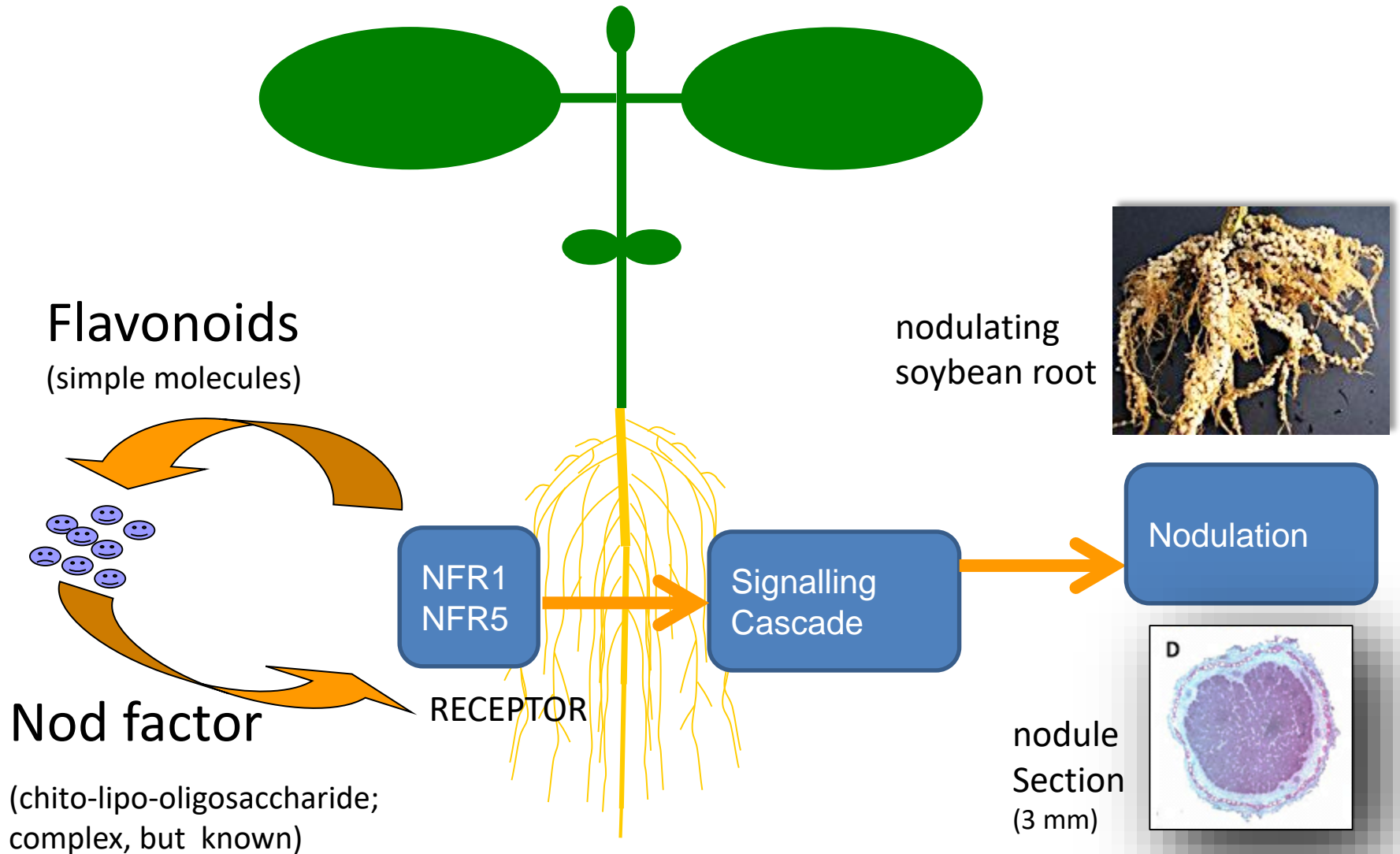


Nitrogen fixation
inside the nodule

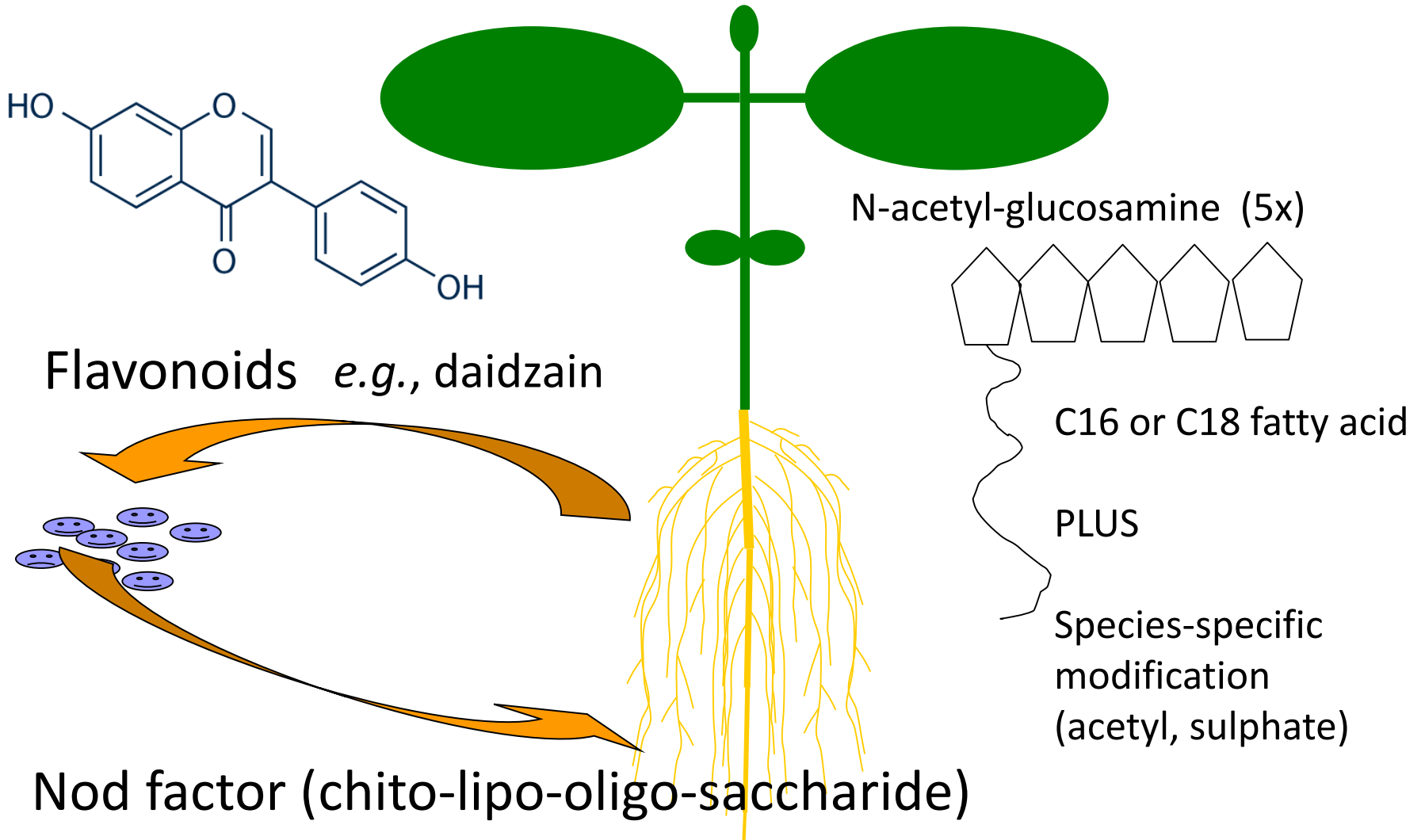
Leghemoglobin
 Ammonia assimilation
 Iron protein (Fe)
 Molybdenum-Iron
 (Fe-Mo) protein
 16 ATP per mole N

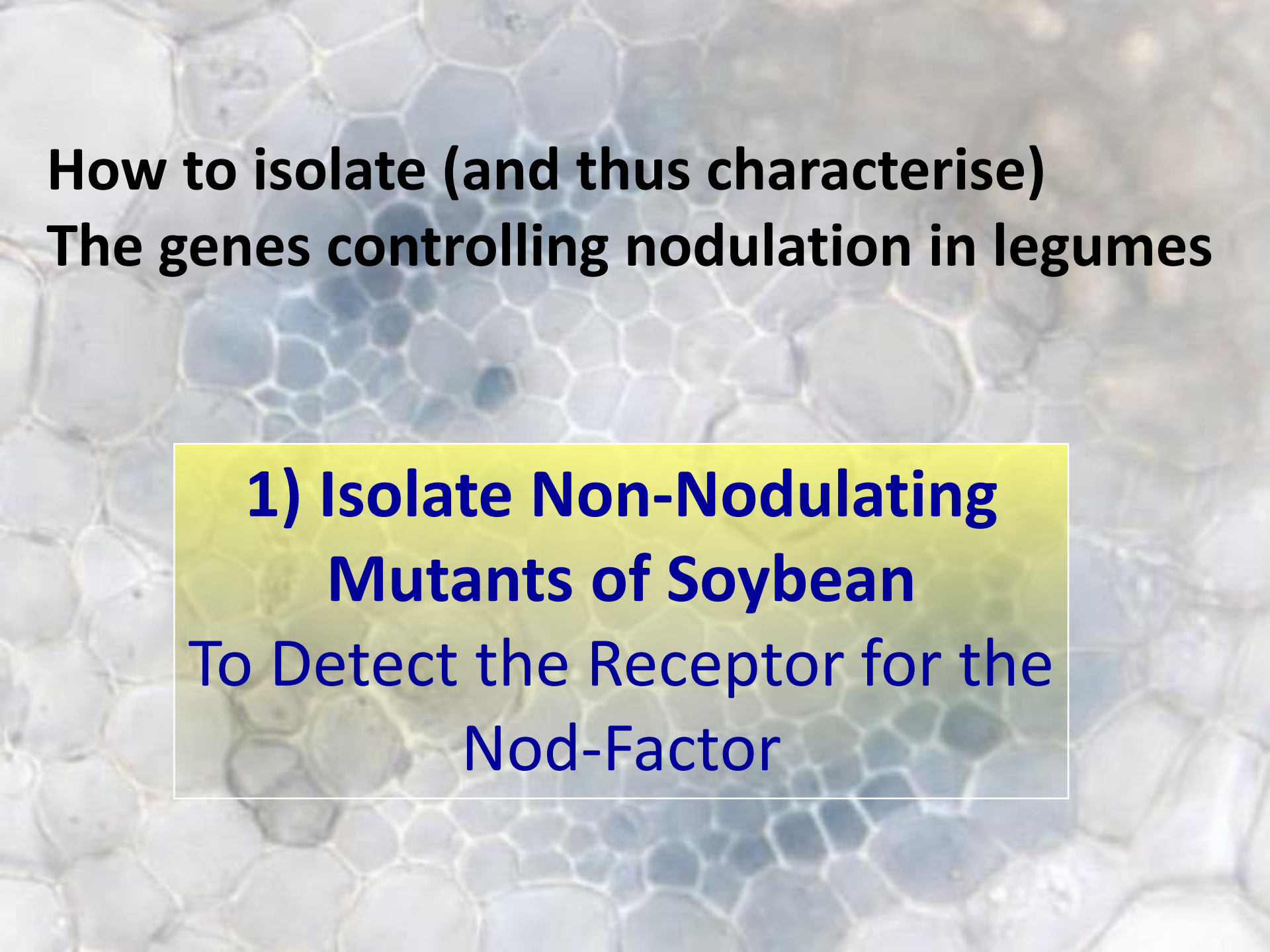


Nodule Development Pathway



Nodule Development Pathway

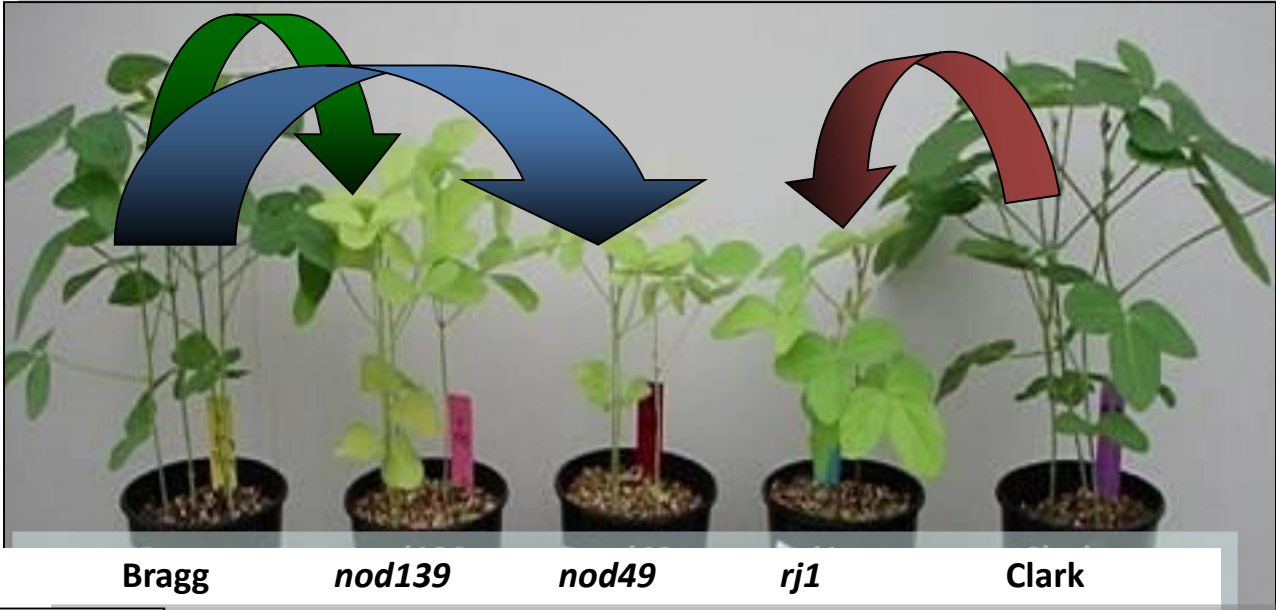


A microscopic image of plant cells, likely from a legume, showing a network of cell walls and large, clear cytoplasmic vacuoles. The cells are roughly polygonal in shape and arranged in a somewhat regular pattern.

How to isolate (and thus characterise) The genes controlling nodulation in legumes

**1) Isolate Non-Nodulating
Mutants of Soybean
To Detect the Receptor for the
Nod-Factor**

Non-Nodulation Mutants of Soybean



Non-Nodulation mutants made with EMS



Nod⁺ Nod⁻ Nod⁻ Nod⁻ Nod⁺

Non-Nodulation Mutants of Soybean



- 1) *nod49* (*rj1*) and *nod139* (*nn5*) lack nodules
- 2) *nod139* has no 'Rhizobium'-response
- 3) *nod49* develops subepidermal CCDs
- 4) *nod49* and *nod139* are root-controlled
- 5) mutant phenotype determined by recessive alleles



Nod⁺ Nod⁻ Nod⁻ Nod⁻ Nod⁺

Cloning of *nod49* in Soybean

the plant journal

 **SEB**
Society for
Experimental Biology

The Plant Journal (2011) 65, 39–50

doi: 10.1111/j.1365-313X.2010.04398.x

Nodulation factor receptor kinase 1 α controls nodule organ number in soybean (*Glycine max* L. Merr)

Arief Indrasumunar^{1,2}, Iain Searle^{3,4}, Meng-Han Lin¹, Attila Kereszt^{1,5}, Artem Men^{1,6}, Bernard J. Carroll^{1,3}
and Peter M. Gresshoff^{1,*}

¹ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, Brisbane St Lucia, QLD 4072, Australia,

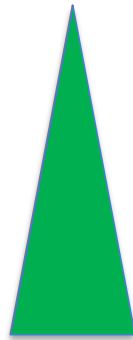
Cloning of '*Nodulation*' trait in Soybean

(Indrasumunar et al (2011) Plant Journal 65: 39-50)

Mutant nod49



Wild type *Glycine soja*



Wild type *G. soja*:

-Nodulates

-has slightly different DNA in places

(called **MOLECULAR MARKERS**

or **Polymorphisms**)

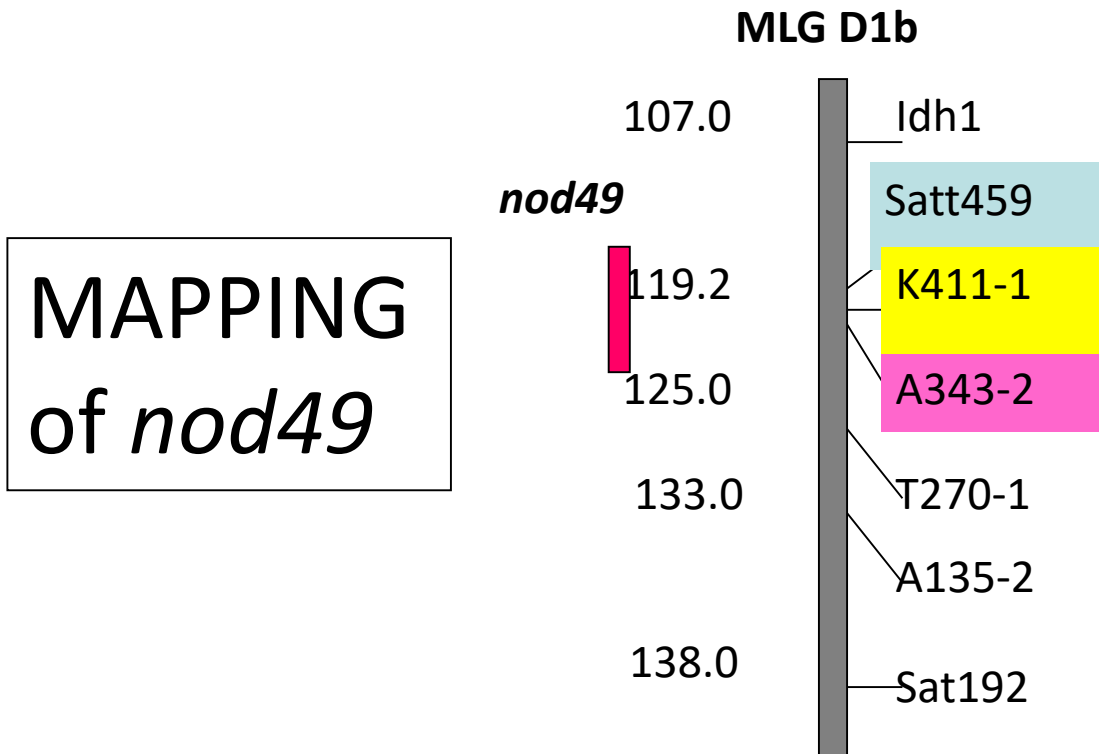
Crossed and then selfed: Non-nodulation segregates at 1 to 3 in F2 (Mendel ratio !!!)

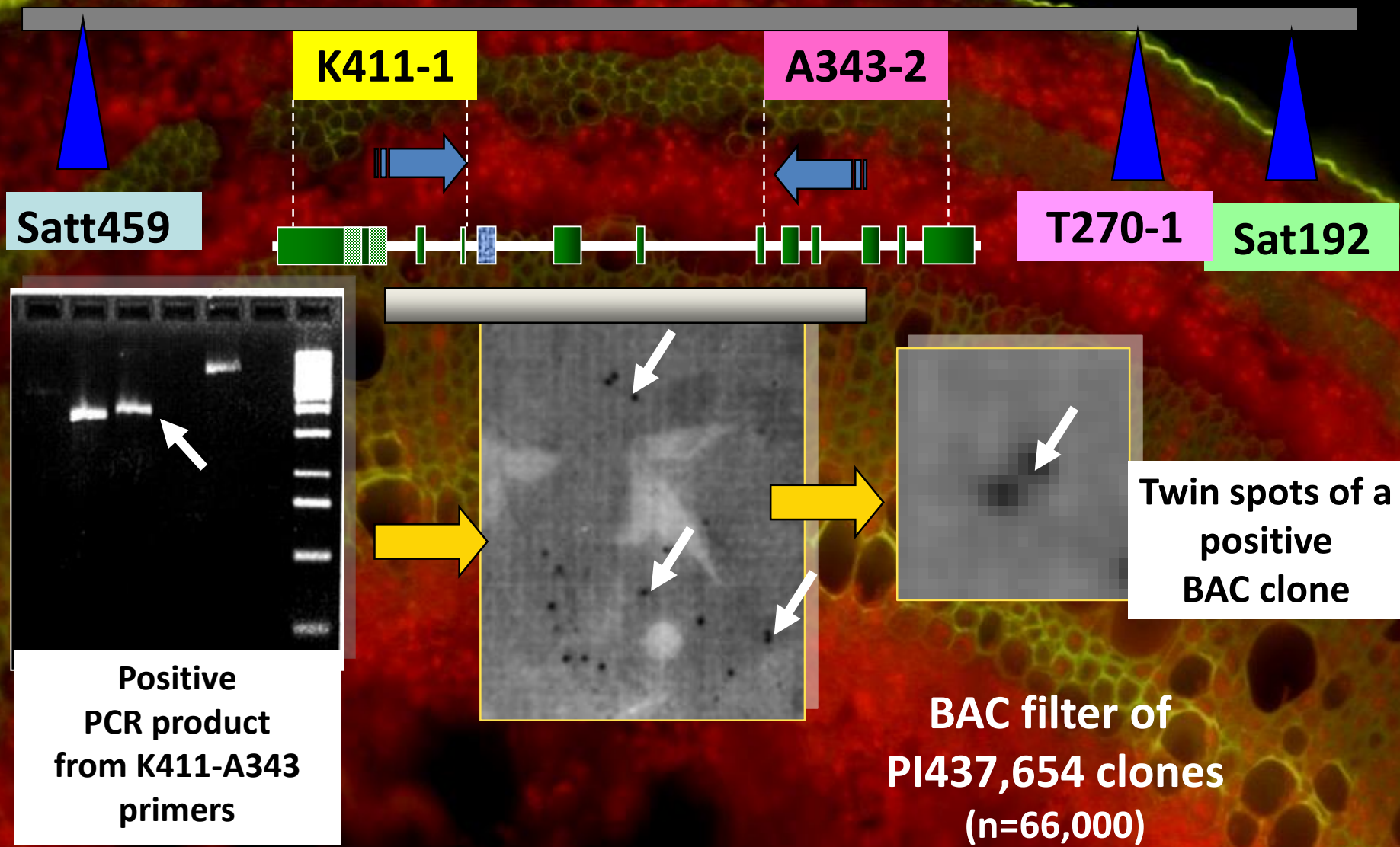
Look at segregation of Molecular Markers and you get a map of each chromosome

Look which Molecular Markers moved along with the non-nodulation trait

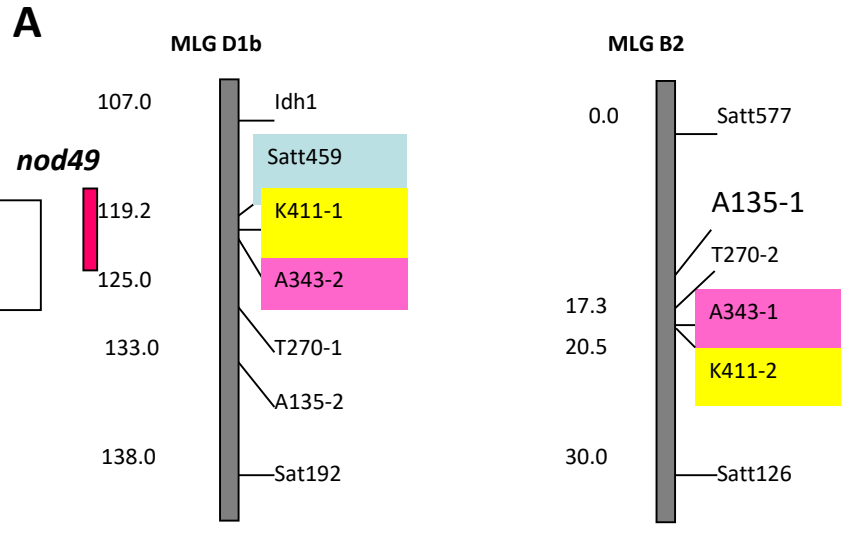
Cloning of *nod49* in Soybean

(Indrasumunar et al (2011) Plant Journal 65: 39-50)



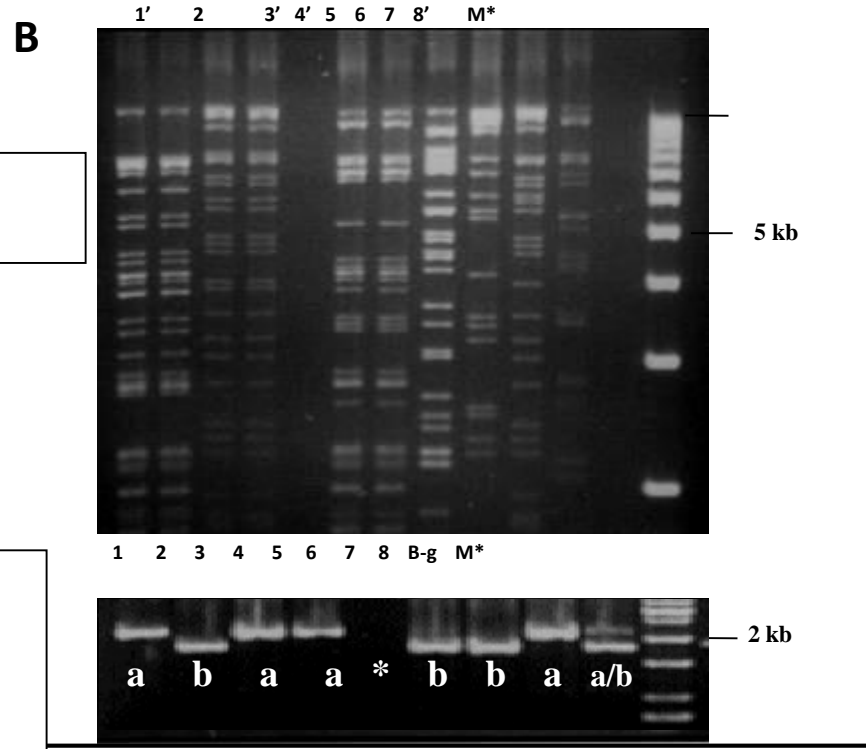


MAPPING



Identified two regions (*i.e.*, two genes)

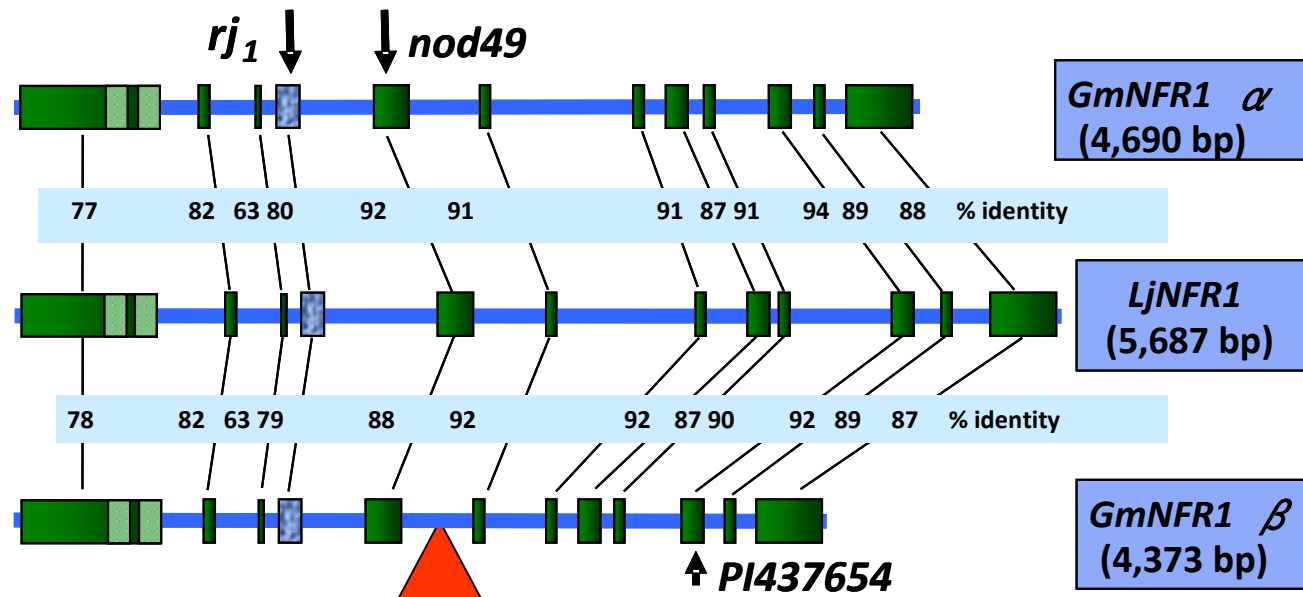
BAC CLONING



Identified a small (α) and a large (β) gene

GENE ISOLATION

Performed Chromosome Walking



- TWO genes sequenced: *GmNFR1* α and *GmNFR1* β
- Two mutant alleles identified in *GmNFR1* α (*nod49* and *rj1*)
- *GmNFR1* β has an insertion in intron & is non-functional
- *GmNFR1* β makes a normal transcript (ORF)
- mRNA for *GmNFR1* β about 1-5% of *GmNFR1* α

Complementation using chimeric plants

PROTOCOL

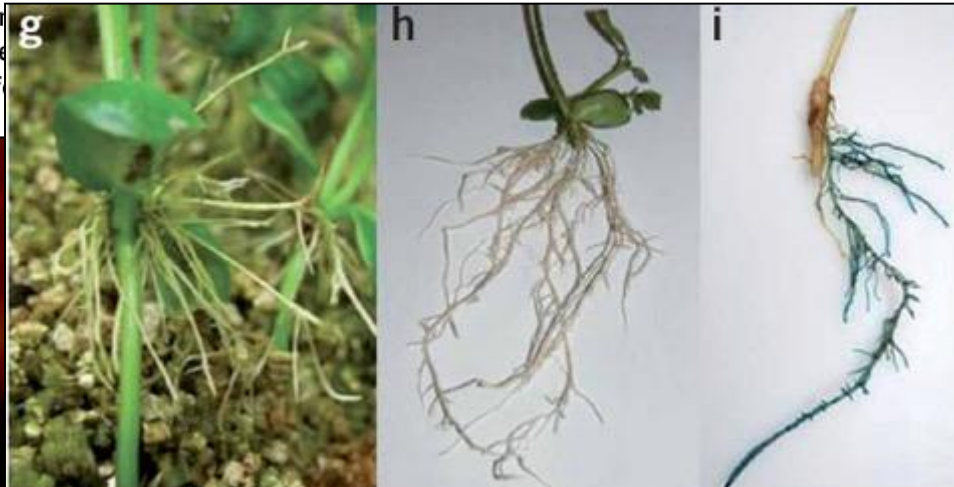
Agrobacterium rhizogenes-mediated transformation of soybean to study root biology

Attila Kereszt^{1,2}, Dongxue Li^{1,2}, Arief Indrasumunar¹, Cuc DT Nguyen¹, Sureporn Nontachaiyapoom¹, Mark Kinkema¹ & Peter M Gresshoff¹

¹ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, St Lucia, Queensland 4072, Australia. ²These authors contributed equally to this work. Correspondence should be addressed to P.M.G. (p.gresshoff@uq.edu.au).

Published online 19 April 2007; doi:10.1038/nprot.2007.141

This protocol is used to induce transgenic roots on soybean to study the function of genes required in biological processes of the root. Young seedlings with unfolded cotyledons are infected at the cotyledonary node and/or hypocotyl with *Agrobacterium rhizogenes* carrying the gene of interest. When the emerged hairy roots can support the growth of the plant, the infected plants are transferred to soil. When the emerged hairy roots can support the growth of the plant, almost 100% of the infected plants form hairy roots.



Gene Discovery 'Everywhere'

- 1) The "Positional Cloning" approach works in all higher organisms
- 2) Diverse variations exist (synthetic gene probes from protein)
- 3) Candidate genes from one species help to detect genes in others

NB: genes in humans are nearly the same in all other mammals, even plants. Many physiological/biochemical functions are shared across species groups, even biological kingdoms !!!

Take-home messages

So much more needs to be discovered.

Sadly so many problems are not close to being solved.

Detailed knowledge of gene function and expression in specific cells or tissues, and their networking, is helping to dissect the complexity of biology ('macro' and 'micro')

A fluorescence micrograph of a plant stem cross-section. The image shows distinct layers of tissue. The outermost layer is a thin, bright green line. Below it is a thick, dark red layer. The central part of the stem is composed of several layers of green cells, with some larger, more prominent cells visible. The overall appearance is that of a vascular bundle or a similar plant structure. The text "Thank you" is overlaid in the center in a white, sans-serif font.

Thank you

Modern Genetics: gene discovery

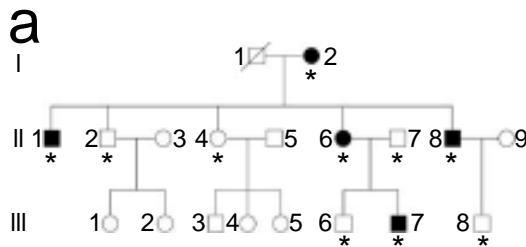
Mutations in *TRPV4* cause an inherited arthropathy of hands and feet

Shireen R Lamandé^{1,2}, Yuan Yuan³, Irma L Gresshoff^{1,2}, Lynn Rowley¹, Daniele Belluocci¹, Kumara Kaluarachchi¹, Christopher B Little⁴, Elke Botzenhart⁵, Klaus Zerres⁵, David J Amor^{1,2,6}, William G Cole⁷, Ravi Savarirayan^{1,2,6}, Peter McIntyre³ & John F Bateman^{1,8}

Nature Genetics

(University of Melbourne research team)

nature
genetics



Inheritance (dominant)

The PHENOTYPE

The protein structure

