Experimental Tools and Resources Available in Arabidopsis

Manish Raizada, University of Guelph, Canada

Community website: The Arabidopsis Information Resource (TAIR) at http://www.Arabidopsis.org

Can order DNA stocks, seeds, mutants, etc. from TAIR; seeds and mutants come from Arabidopsis Biological Resource Centre (ABRC) in Ohio

Can commercially purchase seeds from Lehle Seeds in USA (http://www.Arabidopsis.org) (e.g. 5000-20,000 seeds for ~$100 of wild-types)

Growth and Life Cycle-

-6 week life cycle (seed to seed)

-each plant naturally self-pollinates – produces thousands of seeds

-grow seedlings on agar media (MS salts and nutrients) in Petri Dishes with indoor fluorescent light (indoors) or in soil pots in greenhouse/plant growth chambers

-one greenhouse can fit 5-10,000 plants easily

-seeds are healthy for ~3 years

-require low light (100-300 uM m2 sec), 23C daytime is typical, low fertilizer requirements

-major problem is disease/pests

Wild plant – geographic isolates are called ecotypes

Ecotypes are a source of genetic variation

Two main ecotypes used by community: Columbia (Col), Landsberg erecta (Ler): most mutants, DNA mapping markers exist in these ecotypes, so best to propose most experiments in these

Col genome has been fully sequenced: ~25,000 genes, 125 million bp, 5 chromosomes, lots of genome duplication (genetic redundancy) caused by ancient genome fusions – implication is that sometimes difficult to reveal a phenotype by a knock-out mutation – might have to create a double knock-out with the redundant homolog
Genome Sequence: http://www.arabidopsis.org/servlets/sv
TIGR: http://www.tigr.org/tdb/e2k1/ath1/
ENSEMBL: http://atensembl.arabidopsis.info/Arabidopsis_thaliana_TIGR/

Selfing – naturally homozygous

Outcrossing feasible, but not thousands of plants

Transformation

Transformation extremely easy and rapid via Agrobacterium-mediated transformation (link to website);

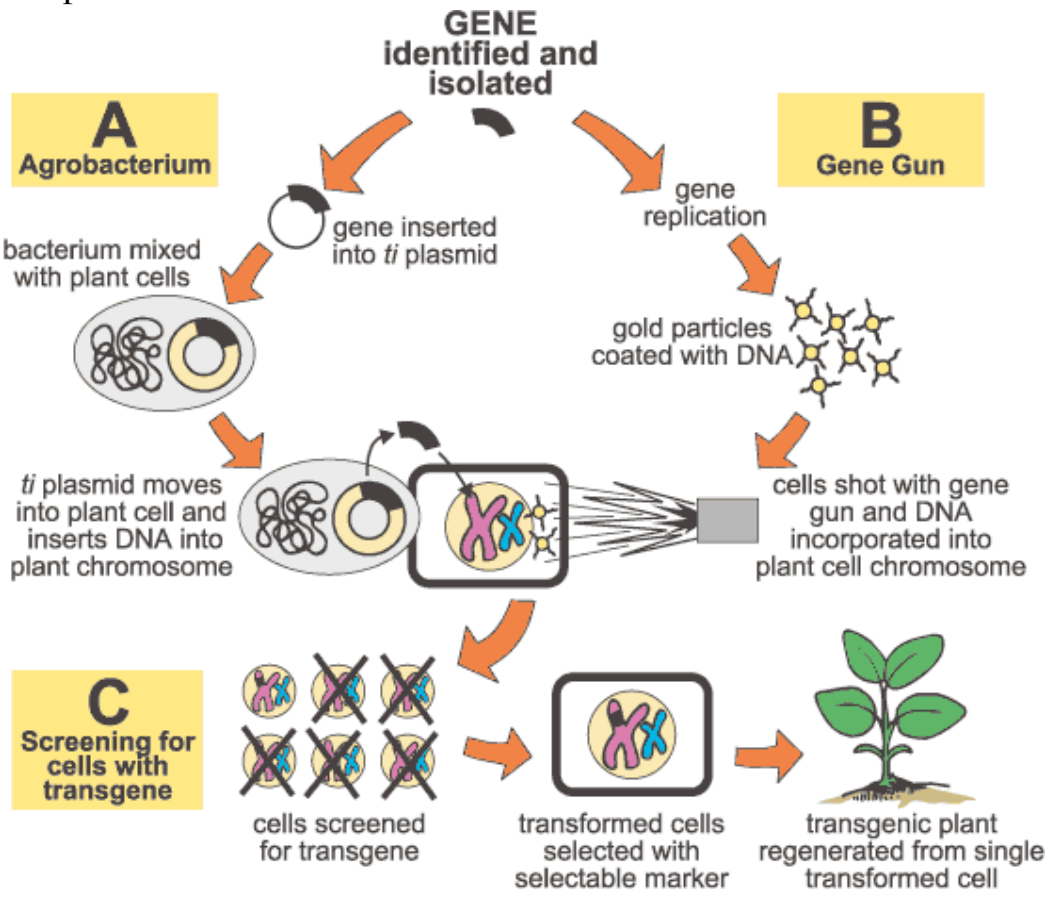
Need to select transformants via herbicide/antibiotic resistance (typically Kanamycin or Basta).

Floral Dip

TDNA

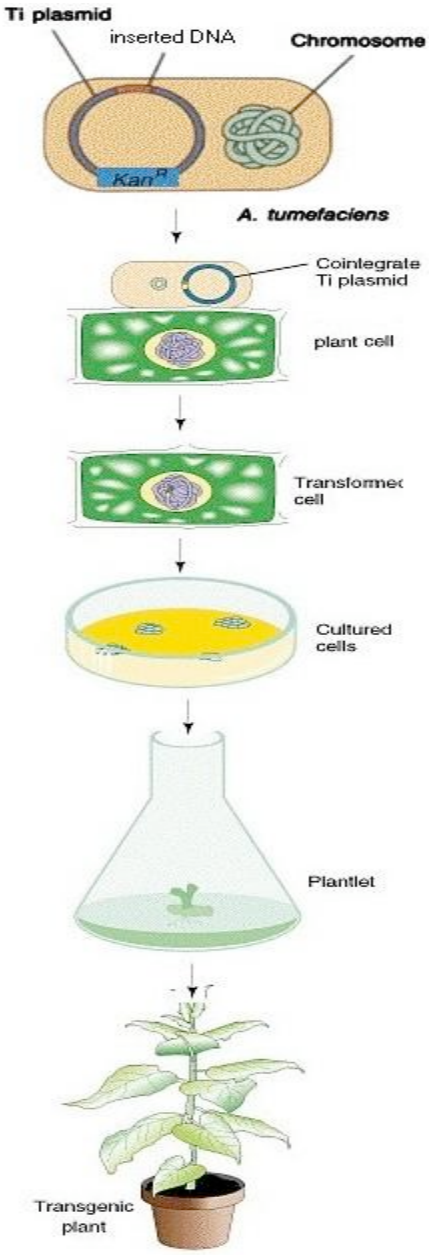
Binary vector

Many constitutive (CaMV 35S viral promoter, Ubiquitini, Actin promoters) and tissue-specific promoters exist



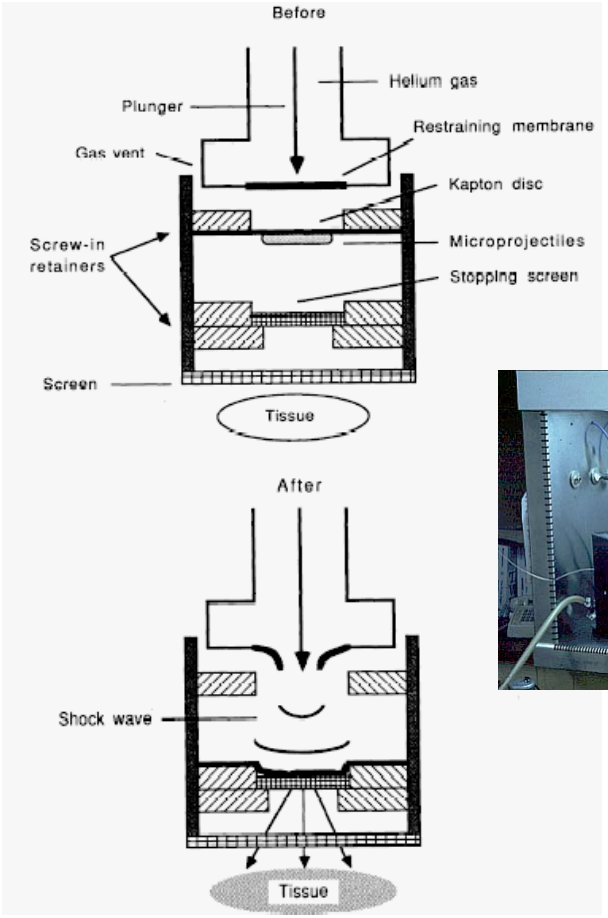
Agrobacterium-mediated transformation

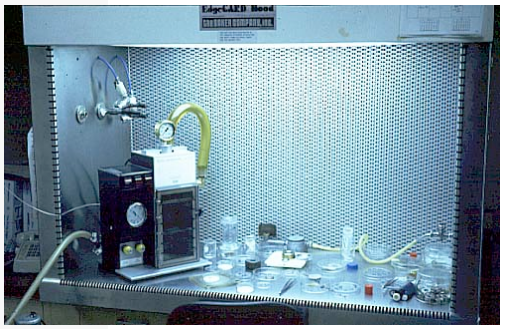
Arabidopsis
Select
For
Transformed
Cells with
A marker
Such as
Antibiotic
Or herbicide
Resistance
e.g. Kanamycin
Or Basta



Biolistics (gene gun)

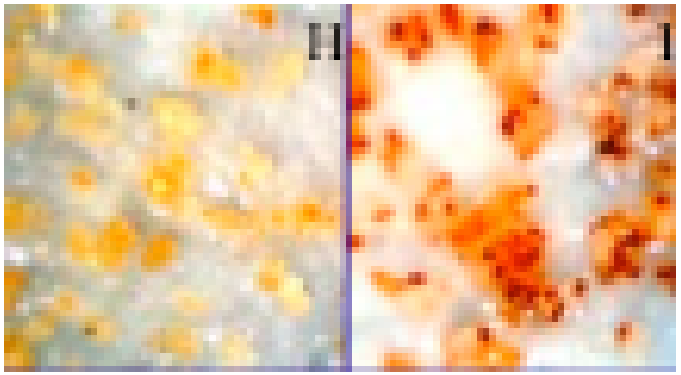
Method for transient (or stable) transformation of plant cells: BioRad PDS/HE Biolistic Gun to shoot plasmid DNA, coated onto 1 uM gold particles across the tough plant cell wall:



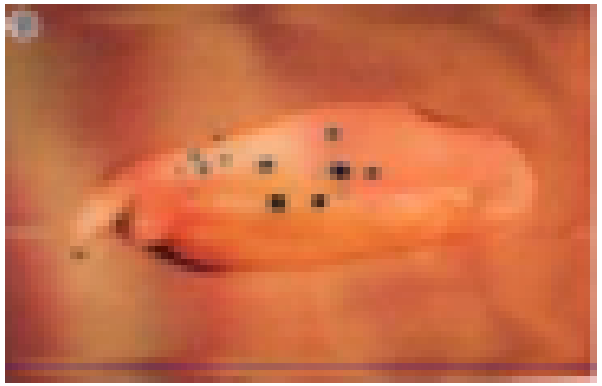


Visible Reporter Genes – to visualize/identify specific cell types or when genes turned/off by connecting promoters to Green Fluorescent Protein, firefly luciferase or beta-glucuronidase (GUS); advantages and disadvantages of each:
GFP
Luciferase
GUS
(show pics of each)
Can request reporters from other labs – if material is published, must make available "in a reasonable time" as DNA and/or seeds

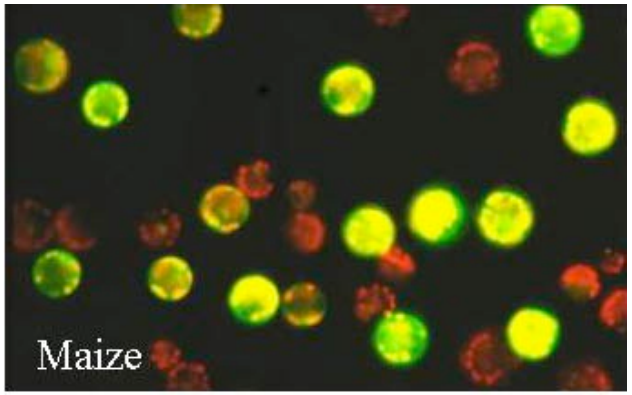
Anthocyanin Transcription Factor Shot into plant tissue (red spots)



Ubiq promoter-GUS shot into Corn embryo (blue spots)



GFP expressed in corn cells



Mutants and Genetics

Lots of mutants available – request from original lab or ABRC (if deposited)
http://www.arabidopsis.org -- then Search Seed Stocks

Forward Genetics:

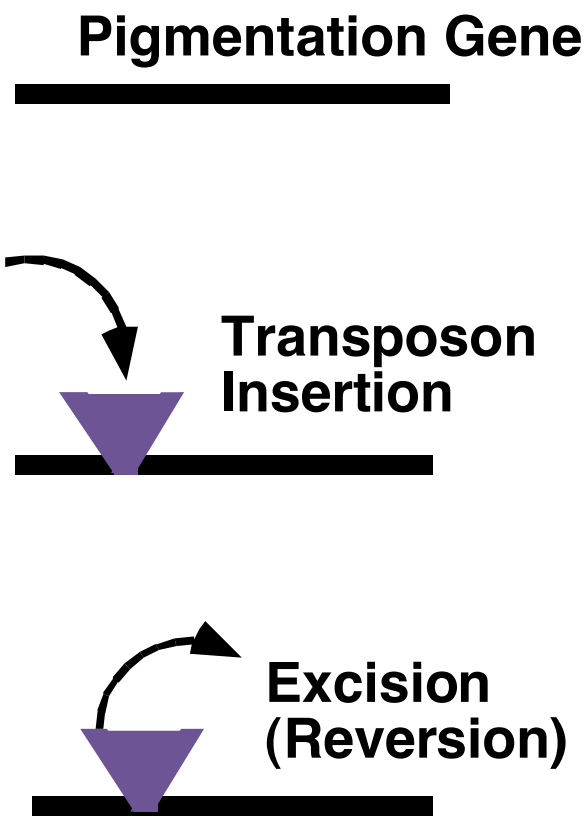
Create mutations, screen for phenotype, then find mutation (gene)
Mutagens:
-for point mutations – EMS (chemical) (can purchase mutagenized seeds from Lehle Seed Company, at http://www.Arabidopsis.com)
-for deletions – Xrays/gamma irradiation – also from Lehle Seeds (http://www.arabidopsis.com)

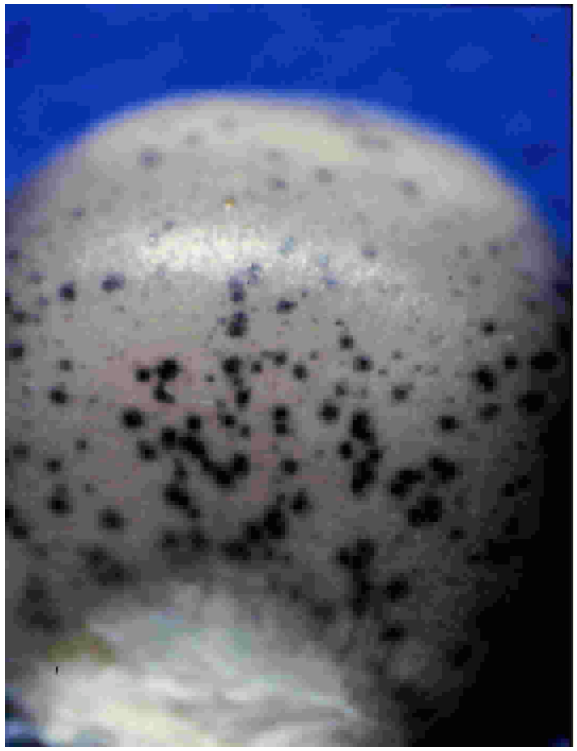
-transposon insertion knockouts– primarily Ac/Ds, also En/Spm transposons
-TDNA insertion knockouts

Reverse Genetics – because genome is sequenced, can start with a gene of interest (e.g. kinase of unknown function, perhaps upregulated in microarray after pathogen infection), and determine its function by ordering a knock-out insertion:
-Collections of transposon or TDNA insertions that have been pre-mapped:
-see http://www.Arabidopsis.org
-SALK institute mapped TDNA insertion line:
http://signal.salk.edu/cgi-bin/tdnaexpress

-Can also knock-down expression of a single gene or gene family using antisense RNA (RNAi) transgenes
-can also overexpress or misexpress a gene of interest to help identify function

Transposons are parasitic DNA that physically move around the genome, inserting and excising from host chromosomes.

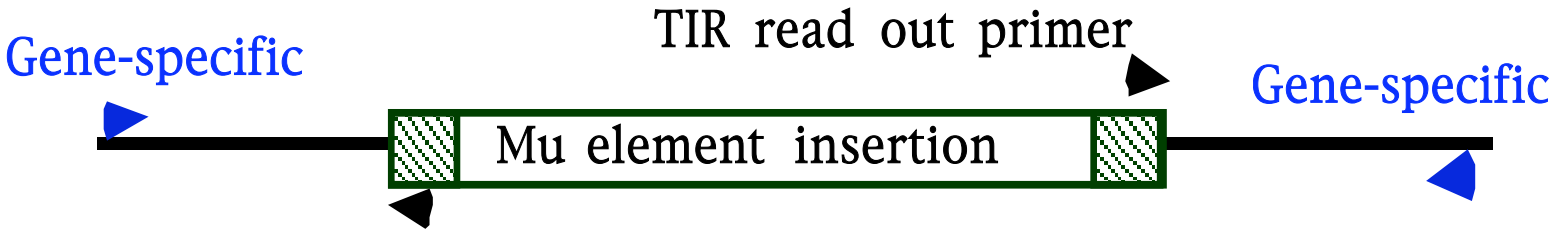




Transposons can be used for gene discovery in 4 ways:

Demo on board.

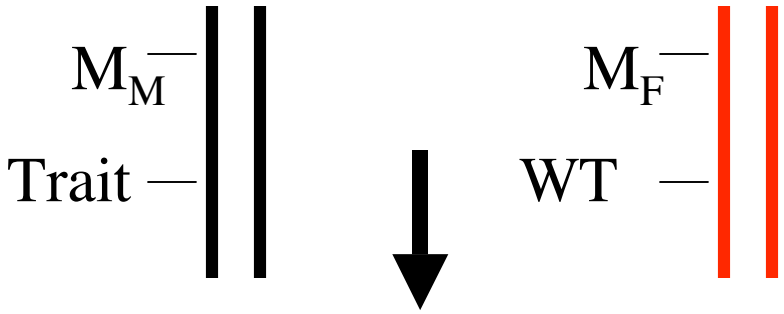
1. Forward Genetics -- create mutants by insertions into genes to identify an unknown gene sequence.
2. Reverse Genetics -- start with a known gene sequence, but not a function, create a large population of transposon insertions, and screen to find the transposon insertion into the gene sequence of interest



3. Activation Tagging -- insert a strong promoter into a transposon, then randomly insert next to plant genes.
4. Enhancer trapping -- place a visible pigment/fluorescent gene reporter into a transposon, and then insert randomly next to plant regulatory regions to help identify genes according to when/how they become expressed.

Genetics and Mapping in Arabidopsis: start with a point mutation and attempt to isolate underlying gene (=positional or map-based cloning)

($M^{M/F}$- Male/Female Markers)



Mate

Let the children undergo meiosis (Random Assortment, Recombination)
Examine grandchildren or later

Genotype using male and female molecular markers:
--to find frequency of association between Trait and Molecular Marker:

- **If Trait and M_F co-segregate frequently,then lots of interval recombination = large map distance = far away from the gene…cold!!**
- **If Trait and M_F almost never cosegregate,then low interval recombination = short map distance = nearing gene….hot!!**

Genetics and Mapping in Arabidopsis:

How are recombinants detected today?

Isolate DNA from thousands of progeny randomly, genotype male and female markers and compare their co-segregation with the trait of interest.

Genetics and Gene Mapping in Arabidopsis

-embryonic diploid

-if have an unknown mutation (e.g. EMS point mutation) and wish to map the gene and isolate it via positional cloning/map-based cloning, it is feasible, but expect 1-3 years of effort:

-genetic mapping in Arabidopsis: Ler x Col (male x female parent ecotypes)

-need to isolate genomic DNA from 1000-3000 F2 progeny (lots of work)

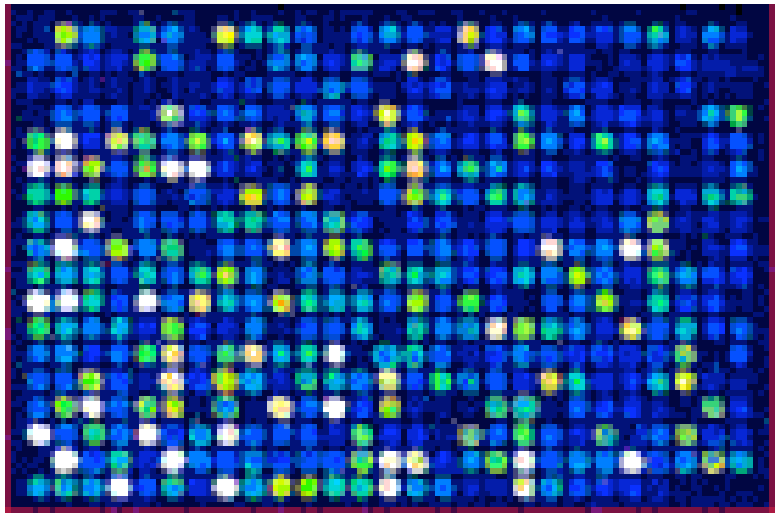
-molecular markers – CAPS markers (PCR-based)

Microarray Resources for mRNA detection

-high-throughput Northern to detect RNA expression of thousands of genes on a small silicon chip

-cost is $500-$1000 per array to analyze all Arabidopsis genes (but need 3 replicates per treatment per tissue, so very expensive, but efficient)

-commercial (do Google search)



Yellow-red = more RNA-chip hybridization = more RNA Expressed in tissue

Standard Molecular Methods

Genomic DNA isolation

RNA isolation

RTPCR – mRNA detection

Westerns – involves producing an antibody in mice

Northerns – RNA detection

Southerns – DNA detection

Protein-Protein Interaction Methods

-proteins do their work usually in complexes with other proteins

-Yeast 2-hybrid

-Immunoprecipitation/pull-down experiments

Yeast 2-Hybrid: A method to find proteins that interact in the nucleus (of yeast) with a specific (bait) plant protein.

1. Fusion plant bait protein to yeast transcription factor (GAL4) DNA-binding domain.
2. Express library of plant cDNAs in yeast, fused to yeast transcription factor activation domain. One yeast cell expresses one plant cDNA.
3. When two plant proteins interact, then the two parts of the yeast transcription factor also interact (BD and AD), making it functional -- turns on a nutrient-selectable or color marker gene.
4. Select yeast cells and determine which plant cDNA is causing protein interaction.

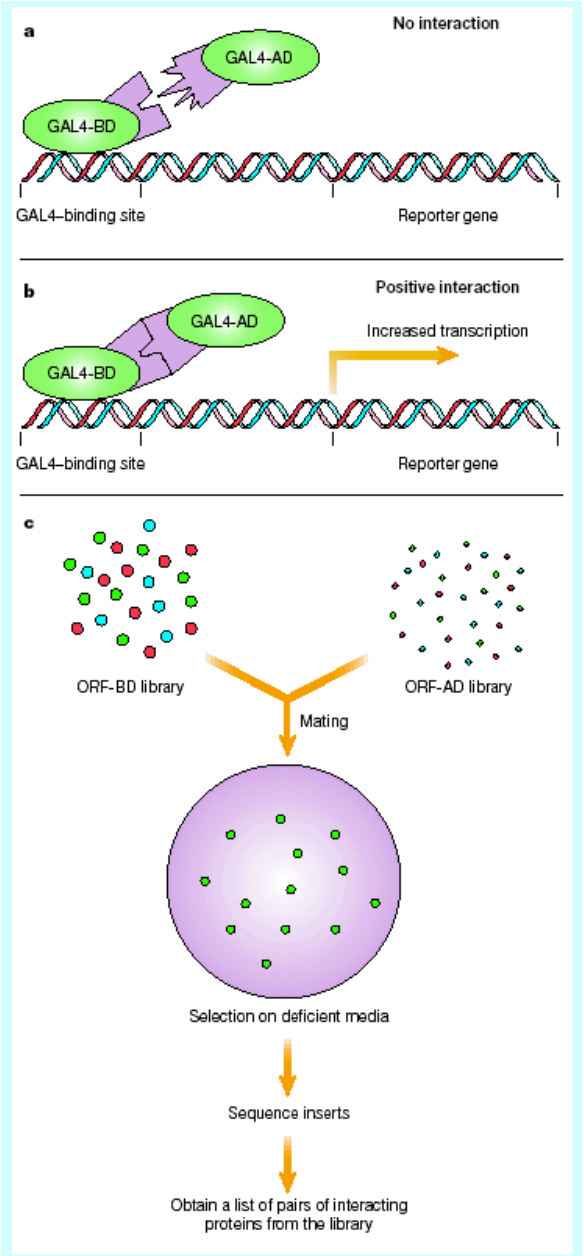


Figure 7 The yeast two-hybrid system. **a**, Different ORFs are expressed as fusion proteins to either the GAL4 DNA-binding domain (GAL4-BD) or its activation domain (GAL4-AD). If the proteins encoded by the ORFs do not interact with each other, the fusion proteins are not brought into close proximity and there is no activation of transcription of the reporter gene containing the upstream GAL4-binding sites. **b**, If the ORFs encode proteins that interact with each other, the fusion proteins are assembled at the GAL4-binding site of the reporter gene, which leads to activation

Detecting interacting Proteins

Affinity Purification

Attach protein of interest to a column and then pass whole protein extract through column and see what sticks

(difficult)

Also: immunoprecipitation

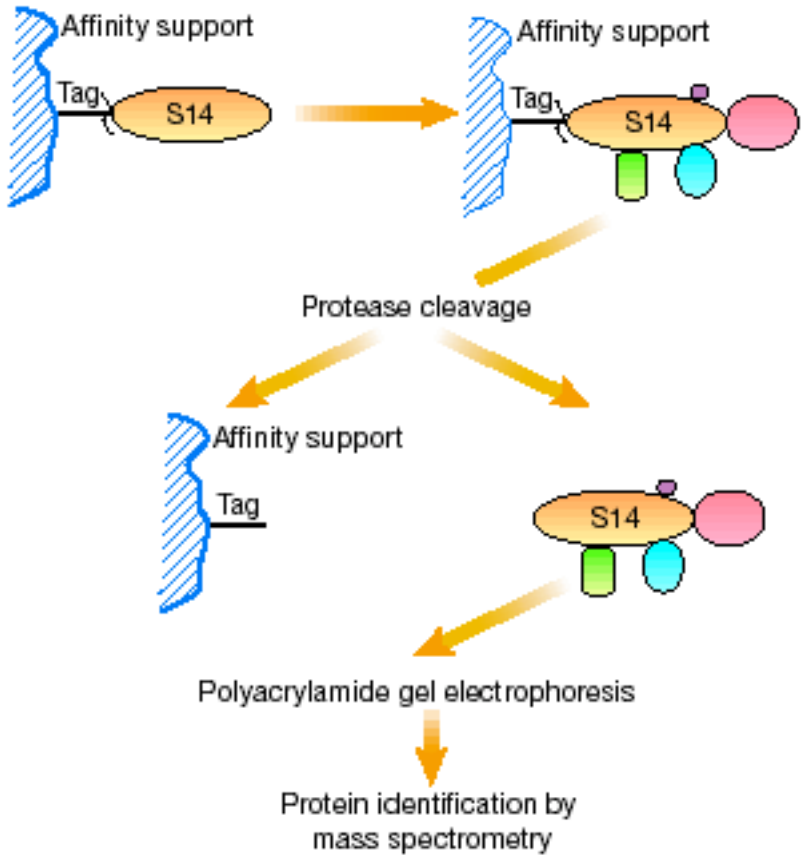
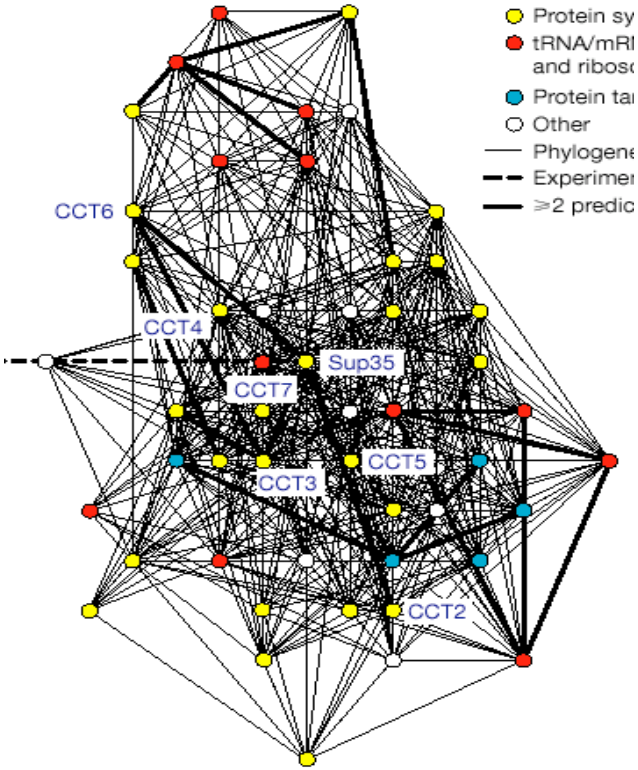


Figure 5 A generic strategy to isolate interacting proteins. The protein of interest is expressed as a fusion protein with a cleavable affinity tag to identify interacting proteins. In this case, S14 protein (spot S14 identified from gel shown in Fig. 6a) is immobilized onto agarose beads using a GST tag. Nuclear cell extracts are incubated with the beads and the beads washed extensively. Thrombin is used to cleave between the GST and the S14 protein, which results in elution of all proteins that are specifically bound to S14. The advantage of this method is that the proteins that are nonspecifically bound to the matrix or the tag itself are not eluted. The eluted proteins are resolved by one- or two-dimensional gel electrophoresis and compared to GST alone. The bands or spots corresponding to proteins specifically bound to the tagged proteins are excised and analysed by mass spectrometry. (Figure courtesy of A. King)

Protein-Protein interaction Map



DNA-Protein Interaction techniques (e.g. transcription factor-promoter binding)
-Yeast 1-hybrid
-EMSA/gel shift experiments

In vitro protein/enzyme assays
-involves over-expressing the plant gene in bacteria or yeast cells and purifying the protein to use in an in vitro assay