NASA Institute for Advanced Concepts

A Flexible Architecture for Plant Functional Genomics in Space Environments

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A Flexible Architecture for Plant Functional Genomics in Space Environments

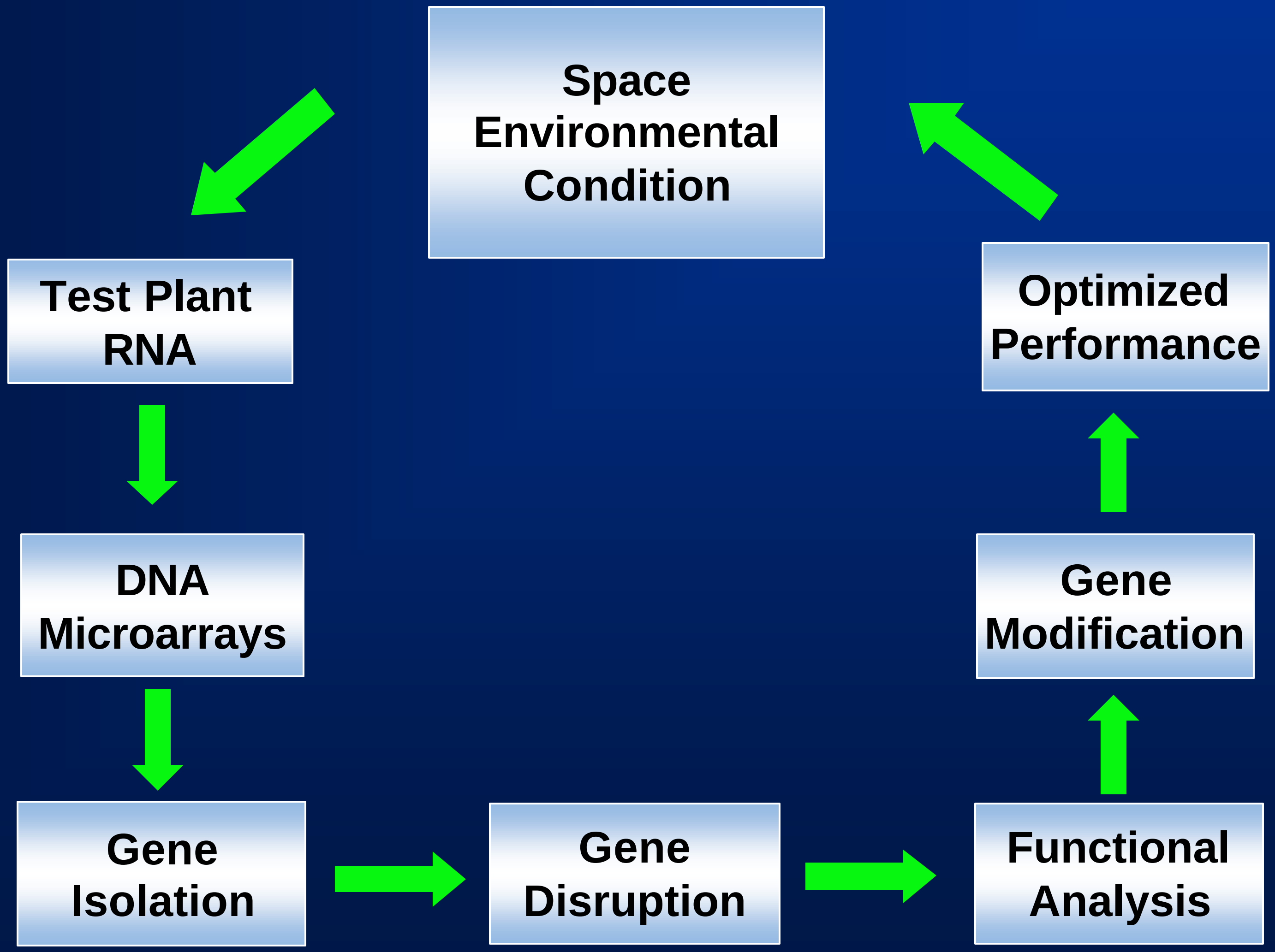
Goals:

- Remotely measure the response of pla
  to any unique space condition

- Determine gene function

- Optimize plant performance under spa
  conditions

Basic Architecture for Plant Functional Genomics

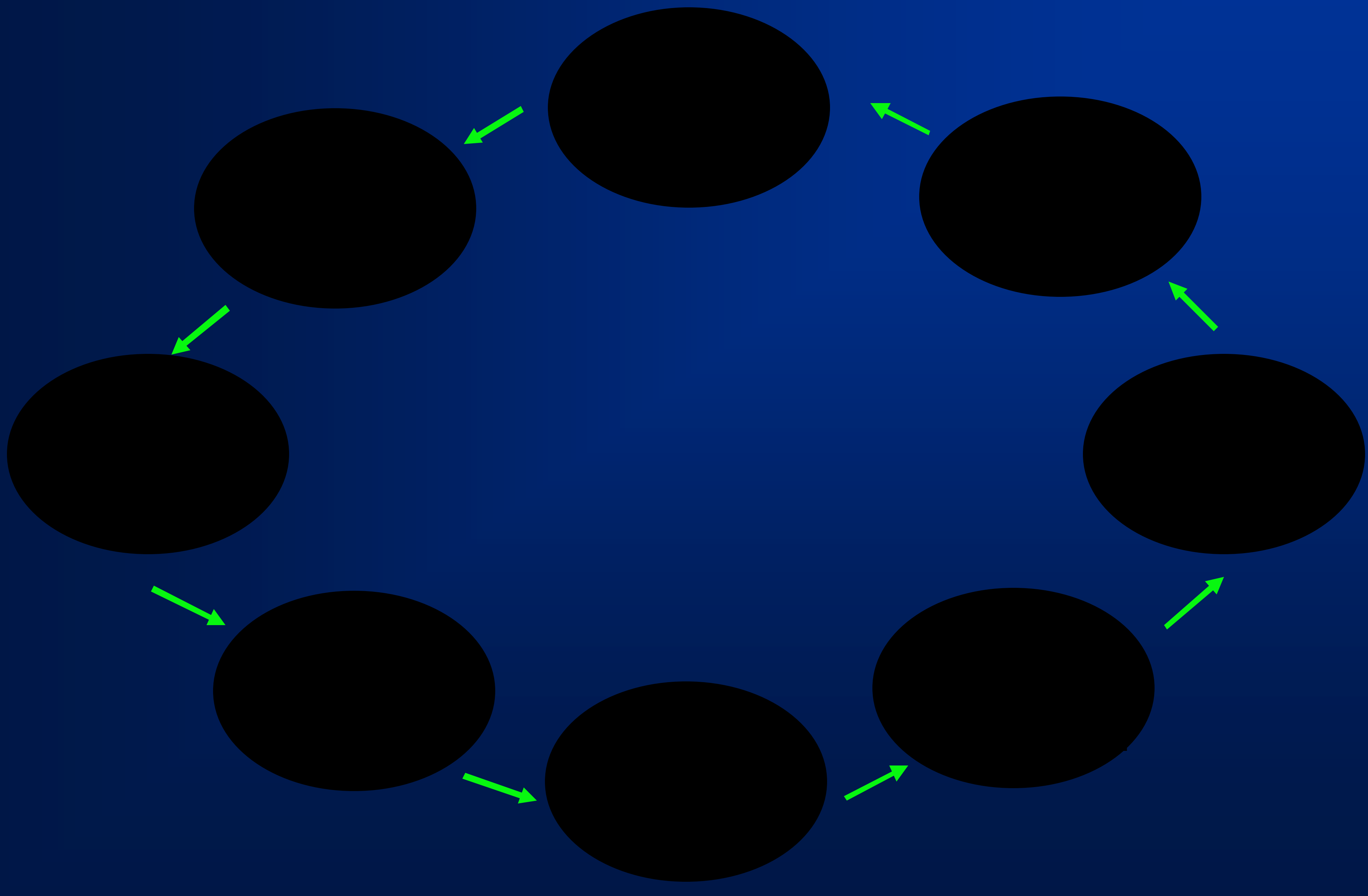


The "NIAC Dilemna"

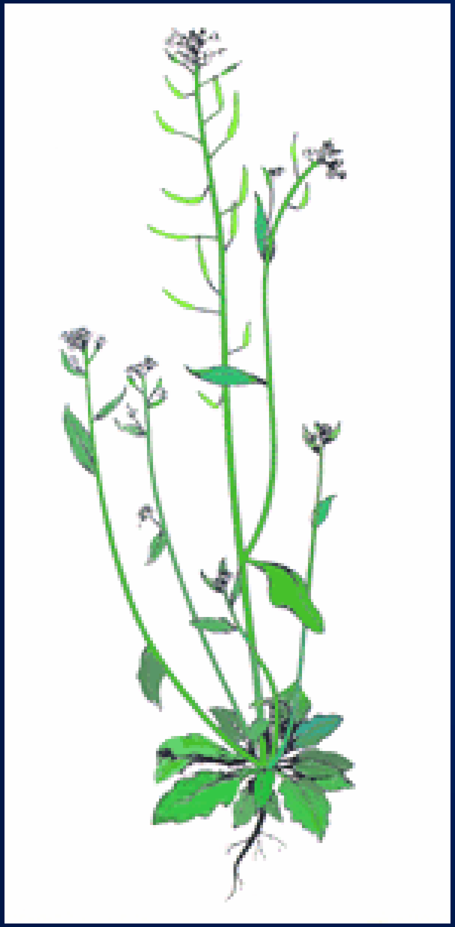
**"Planning for things that will be practical in 2010 - 2040
but needing to demonstrate practibility now"**

Dr. Steven Dubowsky, MIT
(NIAC Fellow)

Model System to Test Architecture Feasibility



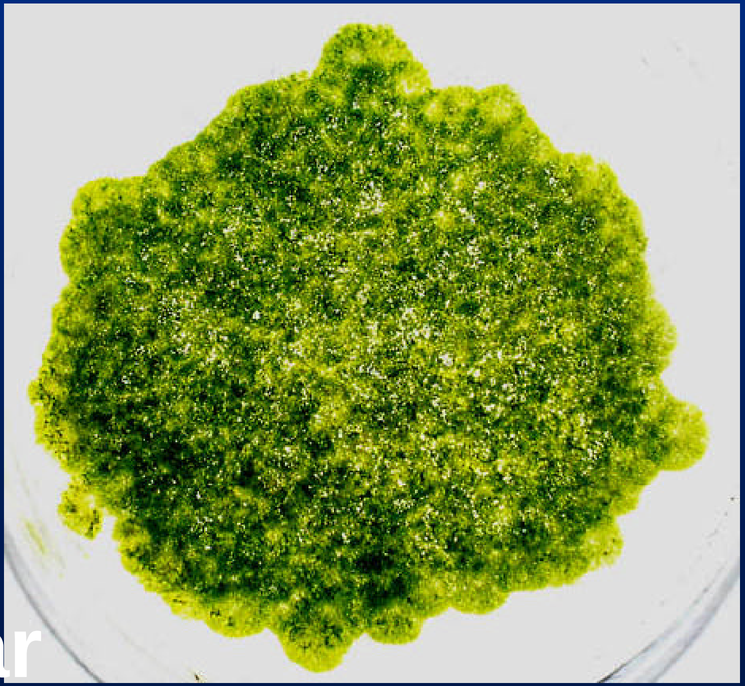
Arabidopsis thaliana:



- model plant system
- defined gravitropic response
- genome sequence complete
- targeted gene knock-outs inefficient

Physcomitrella patens :

- gene knockout efficiencies > 90% via homologous recombination
- defined gravitropic response
- more closely related to dicots than monocots
- similar DNA usage
- used to determine cellular function of an *Arabidopsis* gene



Basic Architecture for Plant Functional Genomics

Space Environmental Condition

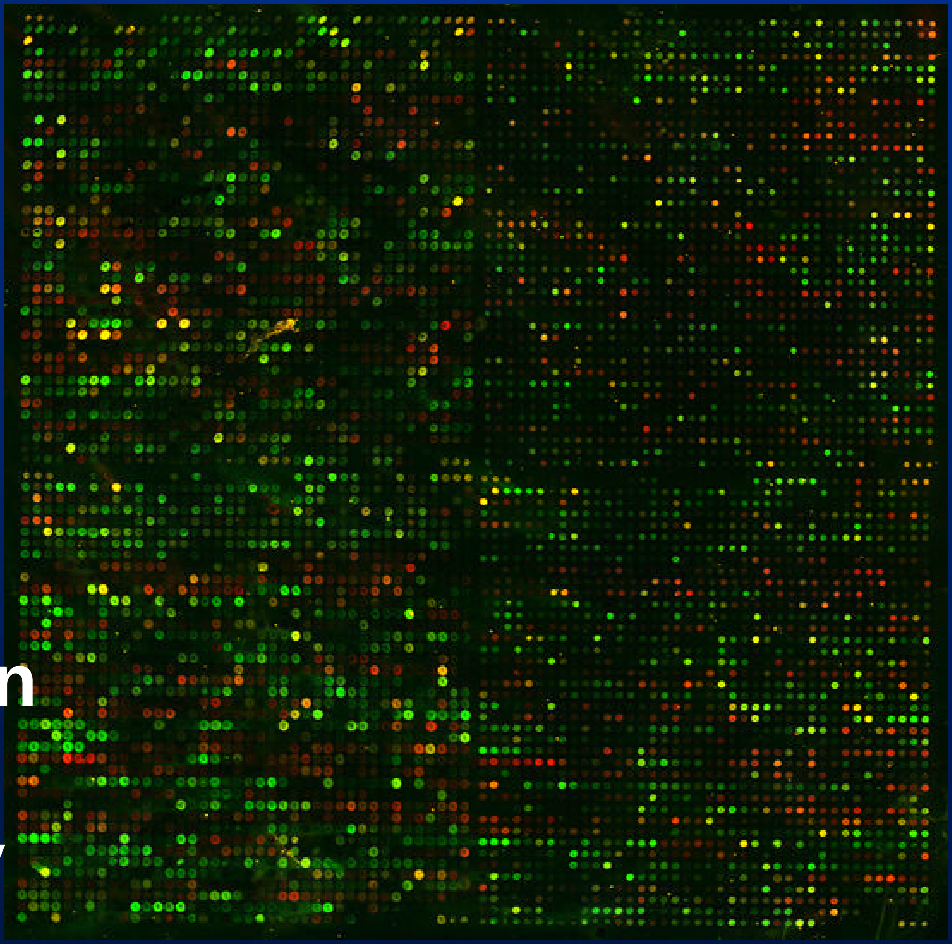
Test Plant RNA

DNA Microarrays

A Flexible Architecture for Plant Functional Genomics in Space Environments

Microarray Technology

- temporal and spatial gene expression
- Affymetrix *Arabidopsis* gene chips with over 8200 genes
- provides information on gene involvement in a process or pathway



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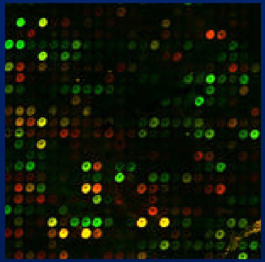

Gene Isolation

Data Mining:
Plant Genome Sequence Databases

The Arabidopsis Information Resource
tair

TIGR
THE INSTITUTE FOR GENOMIC RESEARCH

Moss EST Sequence Databases

The Physcomitrella EST Program

University of Leeds, UK/ Washington University, St. Louis, MO

Goal: 30,000 EST sequences

To Date: 14,410 moss EST sequences on GenBank

***Physcomitrella* Genomics Program**

University of Freiburg/BASF

To Date: 120,000 EST sequences representing 22,000 different genes

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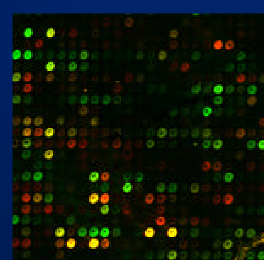
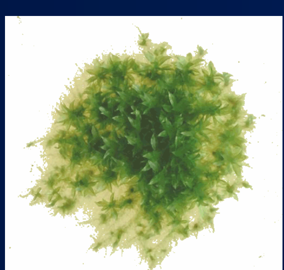




Gene Isolation

Gene Disruption

Functional Genomics

Gene function - analysis of gene malfunction

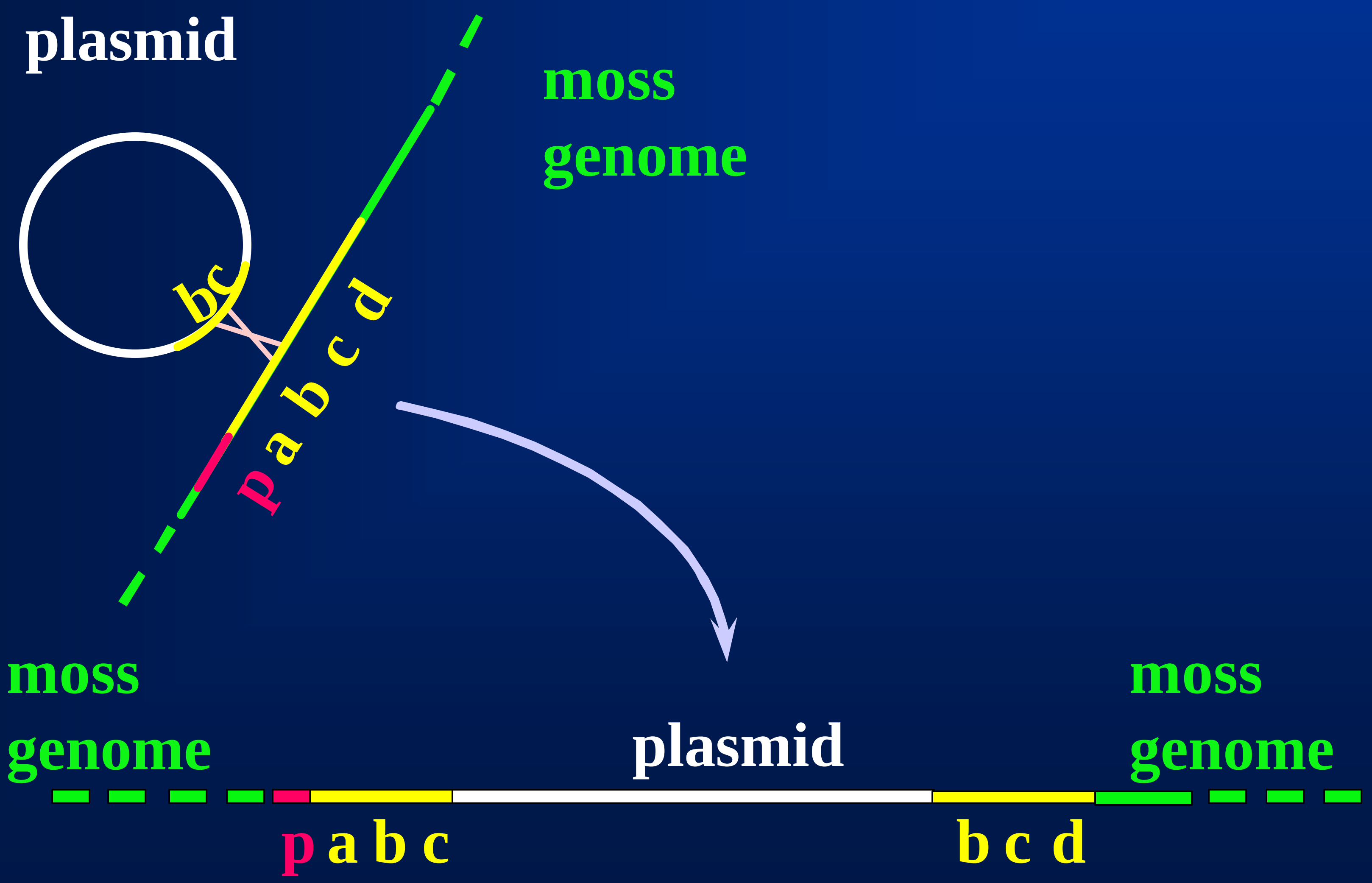
Conventional Approaches:

- manipulate the level of gene expression
- block the expression of a gene

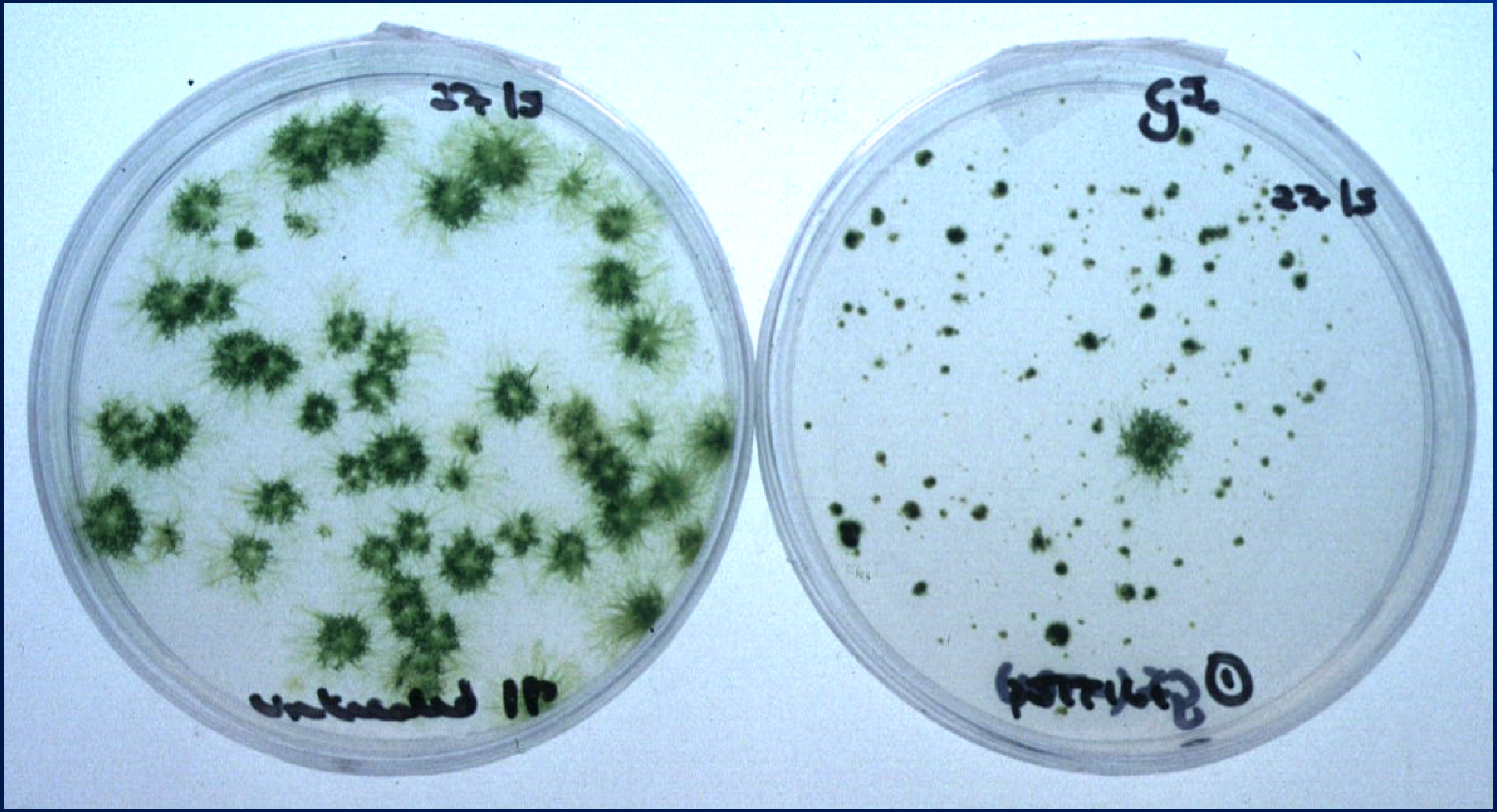
Problems:

- non-directed integration of transgenes
- positional effects
- gene silencing due to co-suppression
- incorrect spatial and temporal expressio

Targeted Gene Knock-out through Homologous Recombination



Regeneration following transformation of protoplasts



No selection Antibiotic selection

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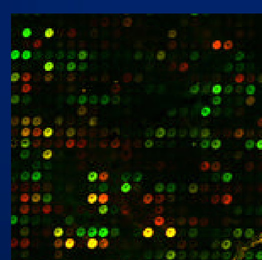
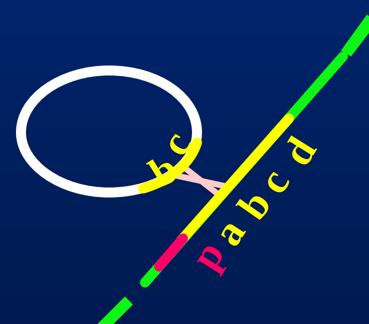
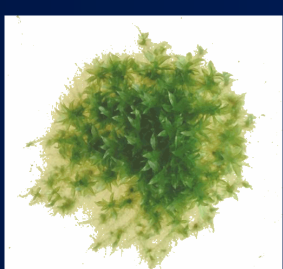






Gene Isolation

Gene Disruption

Functional Analysis

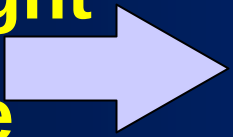
Transfer strips of mutagenized tissue and allow to regenerate

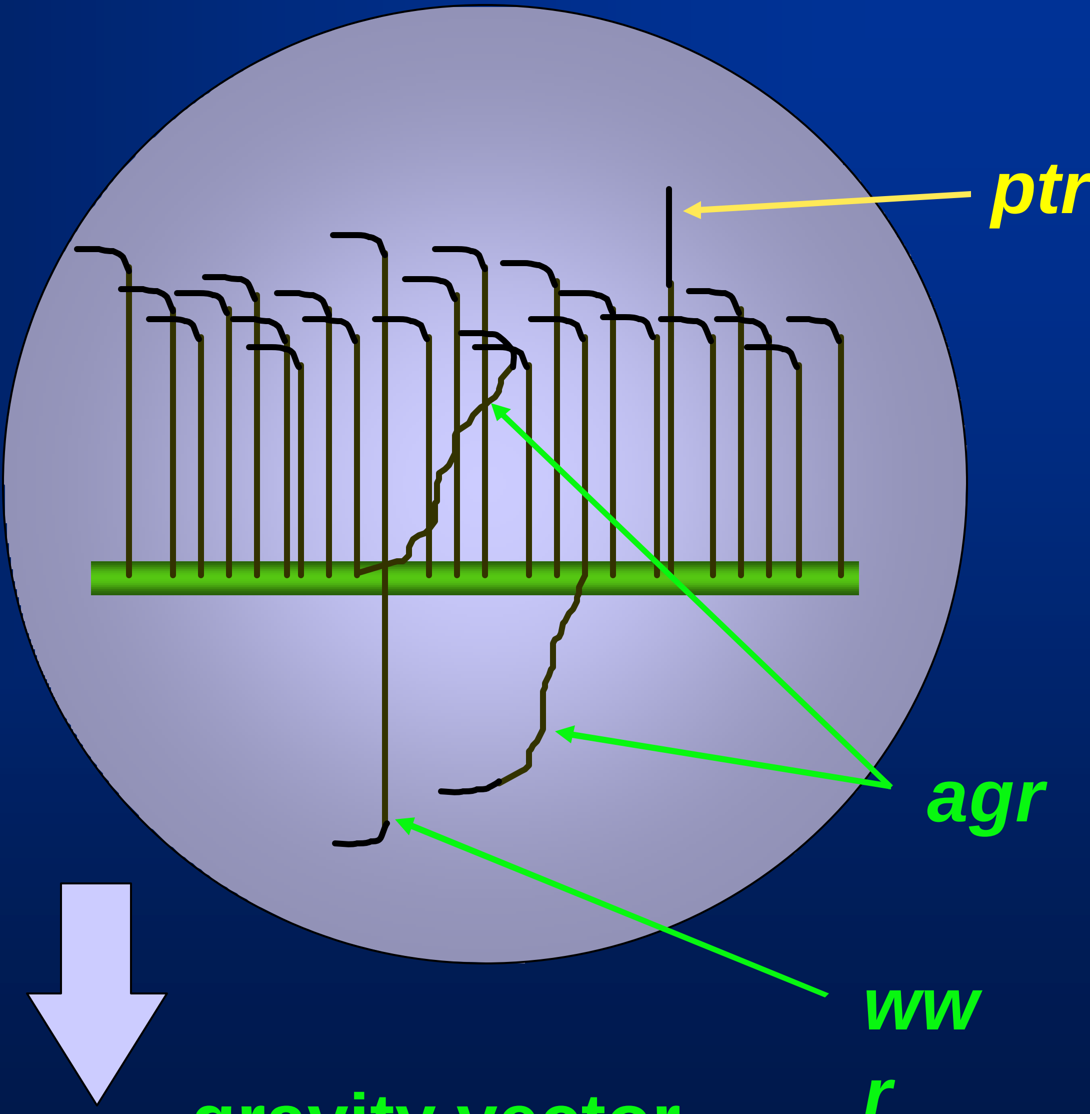
Transfer to unilateral light

Grow in darkness

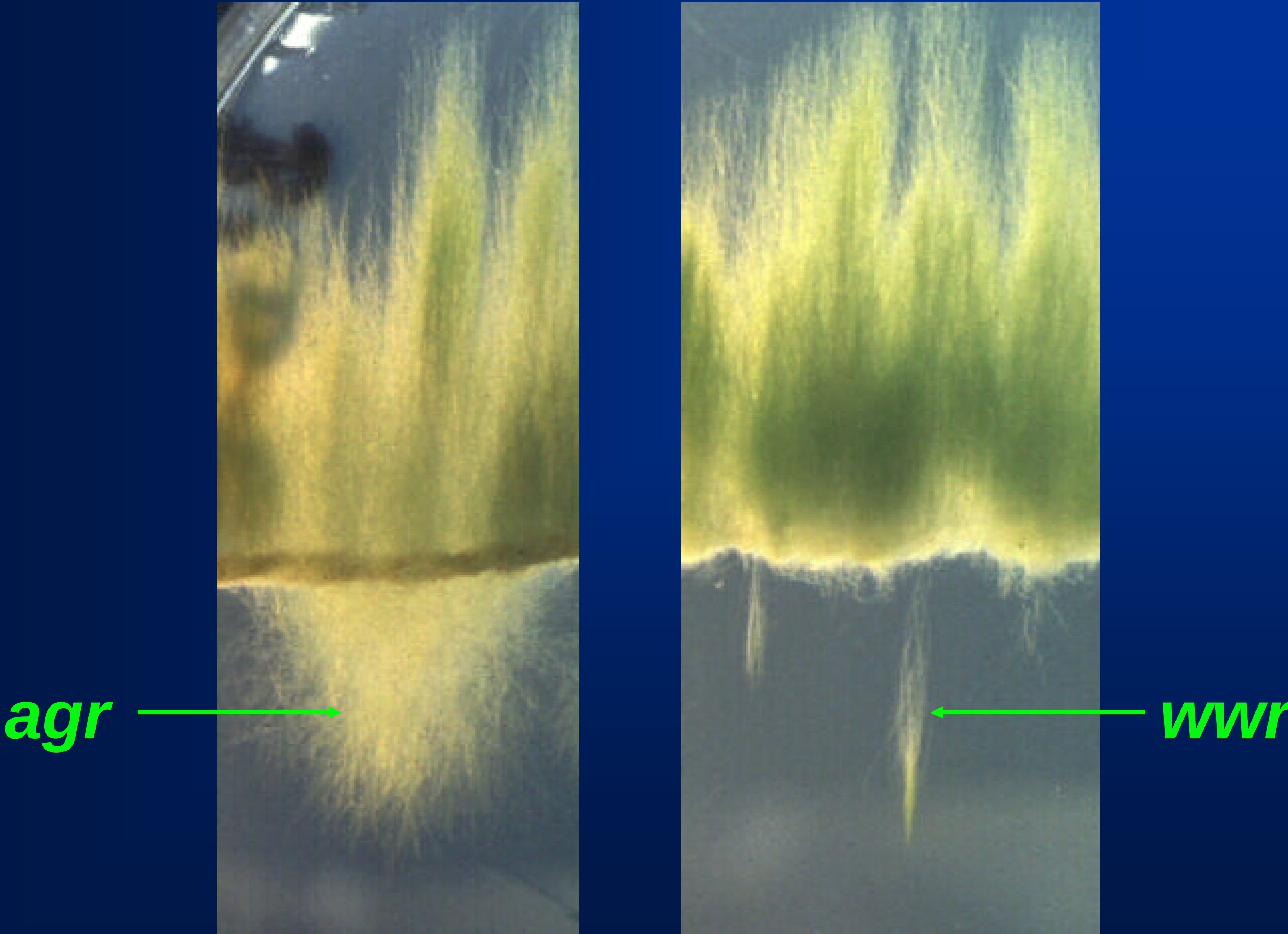
light

light source





gravity vector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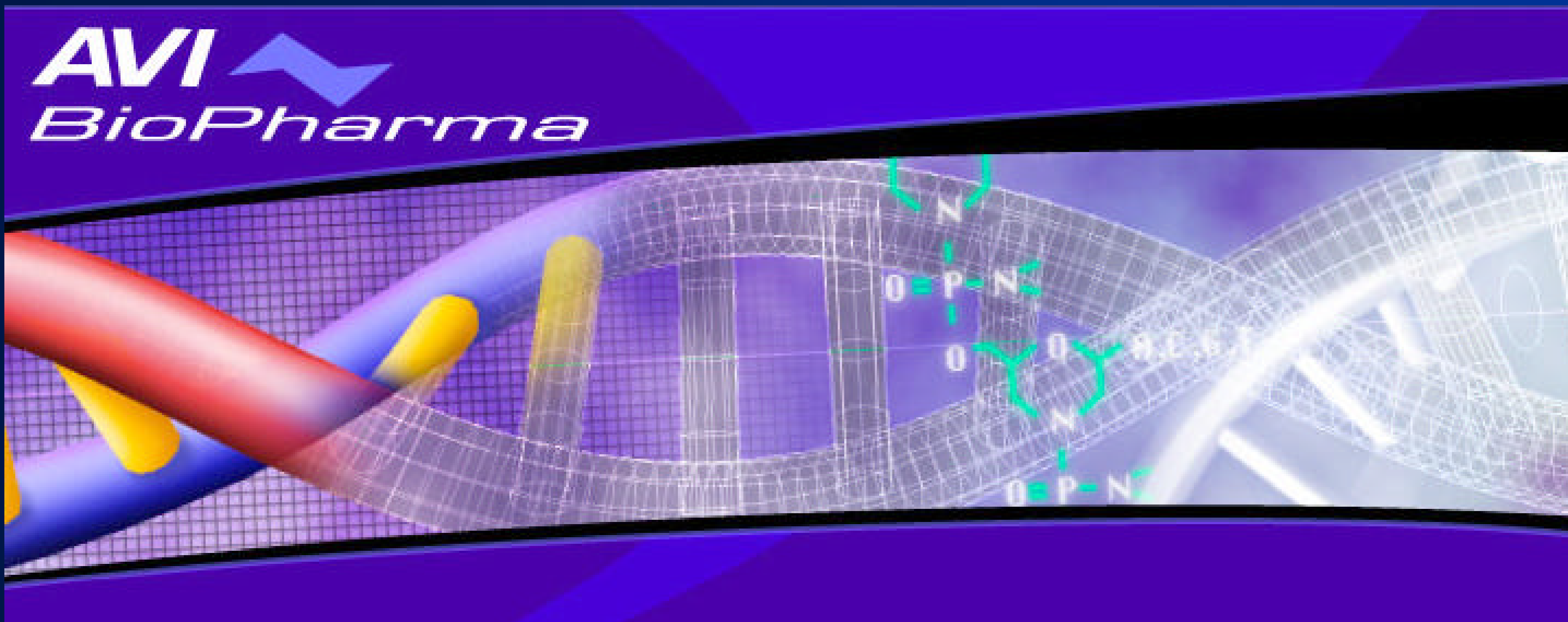
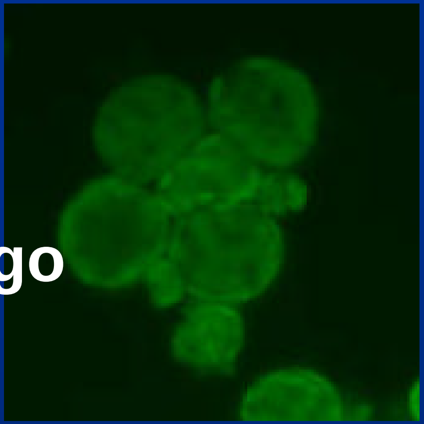
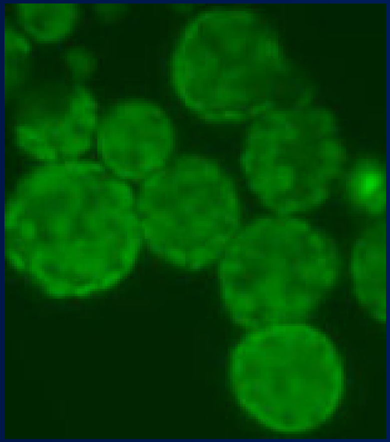
wwr


AVI
BioPharma

Feasibility of Antisense Knockouts: Can we get the oligos in?



Untransformed moss protoplasts with no fluorescein-tagged oligo



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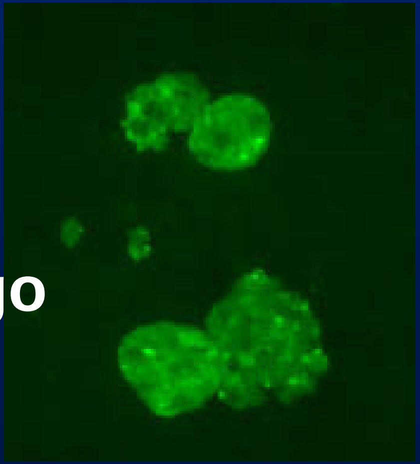

Moss protoplasts transformed with fluorescien-tagged oligo

Architecture Issues & Accomplishmen

Ö **Test plant *Arabidopsis thaliana* as model system to identify genes involved in the gravitropic response**

Ö **Optimize sample preparation for differential expression analysis**

Ö **Affymetrix Microarray analysis**

Ö **Identify genes whose expression increases**

Architecture Issues & Accomplishment

- Ö Establish *Physcomitrella patens* model sys
  to determine function of genes
  - Ö propagation and maintenance
  - Ö protoplast isolation, transformation, and
    regeneration
- ÖCollaborative agreements with two sources
  moss gene databases
- ÖAssemble molecular tools for gene knock-ou
  - Ö cDNA and genomic libraries
  - Ö two types of vectors

What’s Next?

Near Future:

- Increase repetitions for microarray analysis to confirm differential gene expression
- Construct replacement and knock-out vecto using available genomic and cDNA seque
- Generate transformed moss and score for phenotype

The Future: 2002-2020

- Introduce additional environmen conditions into architecture
  - space-flown plant material

- Expand collaborations with Affymetrix and AVI Biopharma to develop remote technologies

- Adapt architecture to accommodate additional plant systems as data and technologies evolve

- Work with engineers to optimize architecture for space flight/habitat