

Divergence, Inc.

Developing
Safe and Effective Products
for the
Control of Plant Parasites

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August 20, 2003



DIVERGENCE
The Nematode Genomics Company

Divergence Background

- > R&D company formed in 1999
 - *Dr. Jim McCarter; former leader of the parasitic nematode sequencing group at the Genome Sequencing Center*
- > Genome-based approach to the Discovery of Novel Nematicides and Nematode Resistant Plants
 - *Identifying genes essential to the nematode not present in humans or non-target species*
- > Focused on effective, safe and environmentally sound solutions
 - *Nematode specific targets allows for the design of controls which minimize the likelihood of toxicity to non-target species*



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Divergence in a Snapshot

Divergence Validated Genome-Based Discovery Platform

- Two Novel Nematicidal Chemistries advanced to development since 2000
- Efficacy of Chemistry Across a Range of Nematodes; Novel MOA
- Chemistry Based on Known Targets Selected To Avoid Toxicity
- Pre-Selects for Risk Reduction & Cost Reduction in Product Development
- Parallel Transgenic Development

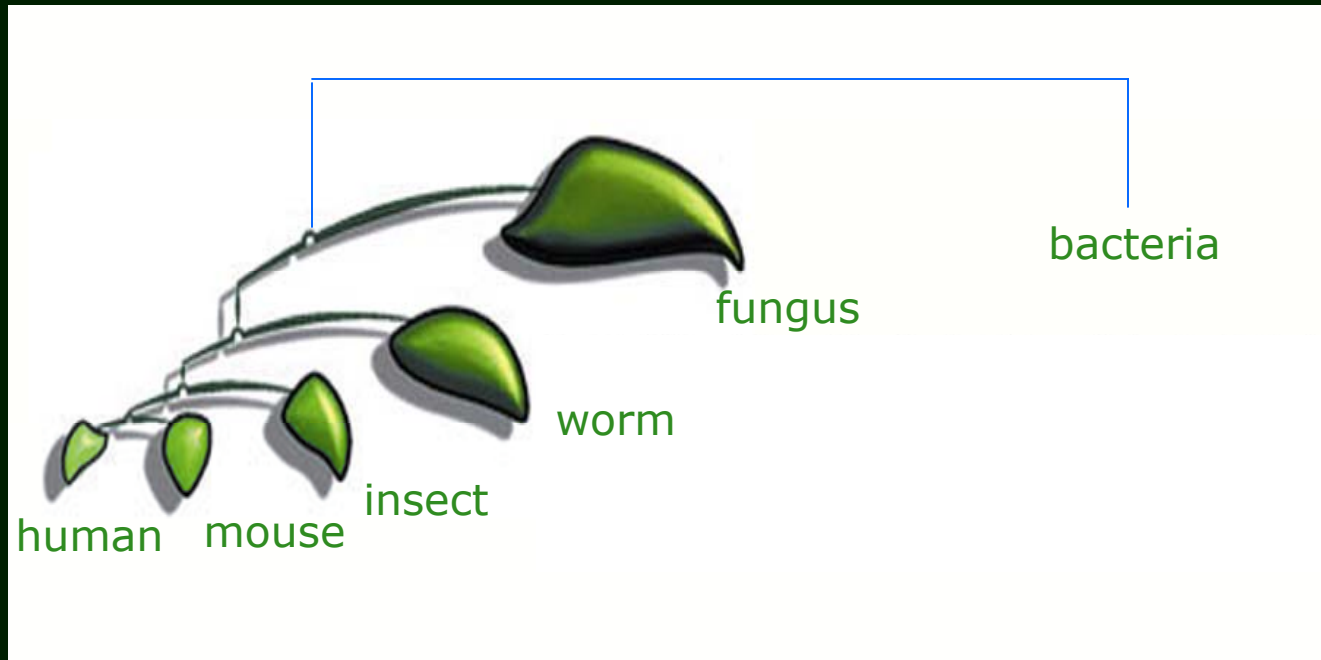
Funding, Grants, Awards

- Investors Include Industry Insiders - Vagelos, Shapiro, Gantz, etc.
- NIH Phase I & II SBIRs, 2 NSF Phase I SBIRs, National Corn Growers
- 4 Regional Awards in 2002 Recognizing Company & Founders



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Evolutionary Relationships Make Nematodes a Challenging Target



- Humans and Worms Share Nearly 1/2 Their Genes
 - Essential Genes Tend to Be Conserved Genes
- Finding Nematode specificity Requires Complete Genomes

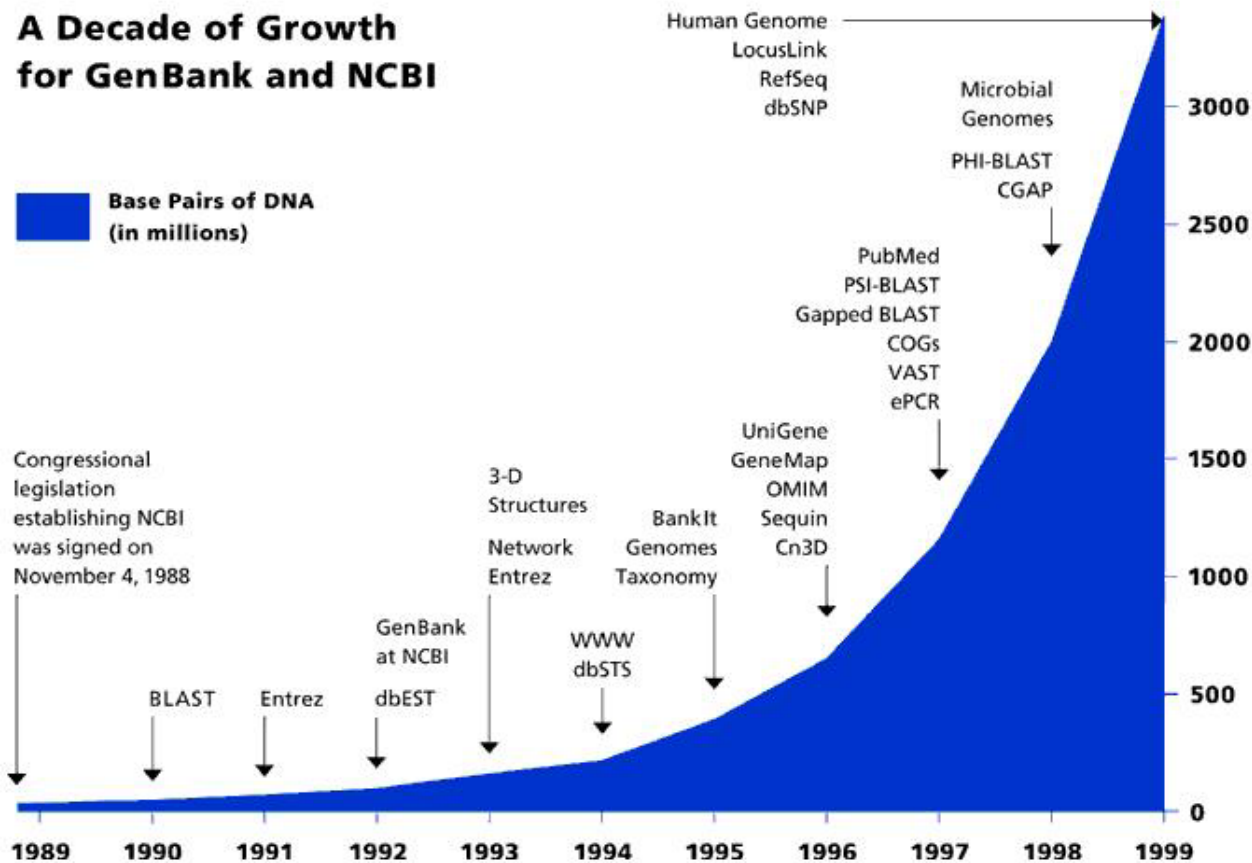


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Genomics: Explosion of Data in the late 1990's

A Decade of Growth for GenBank and NCBI

Base Pairs of DNA
(in millions)



3.3 billion



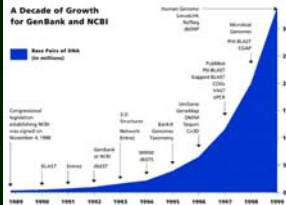
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Accelerates Today with GSC a Key Contributor

2003

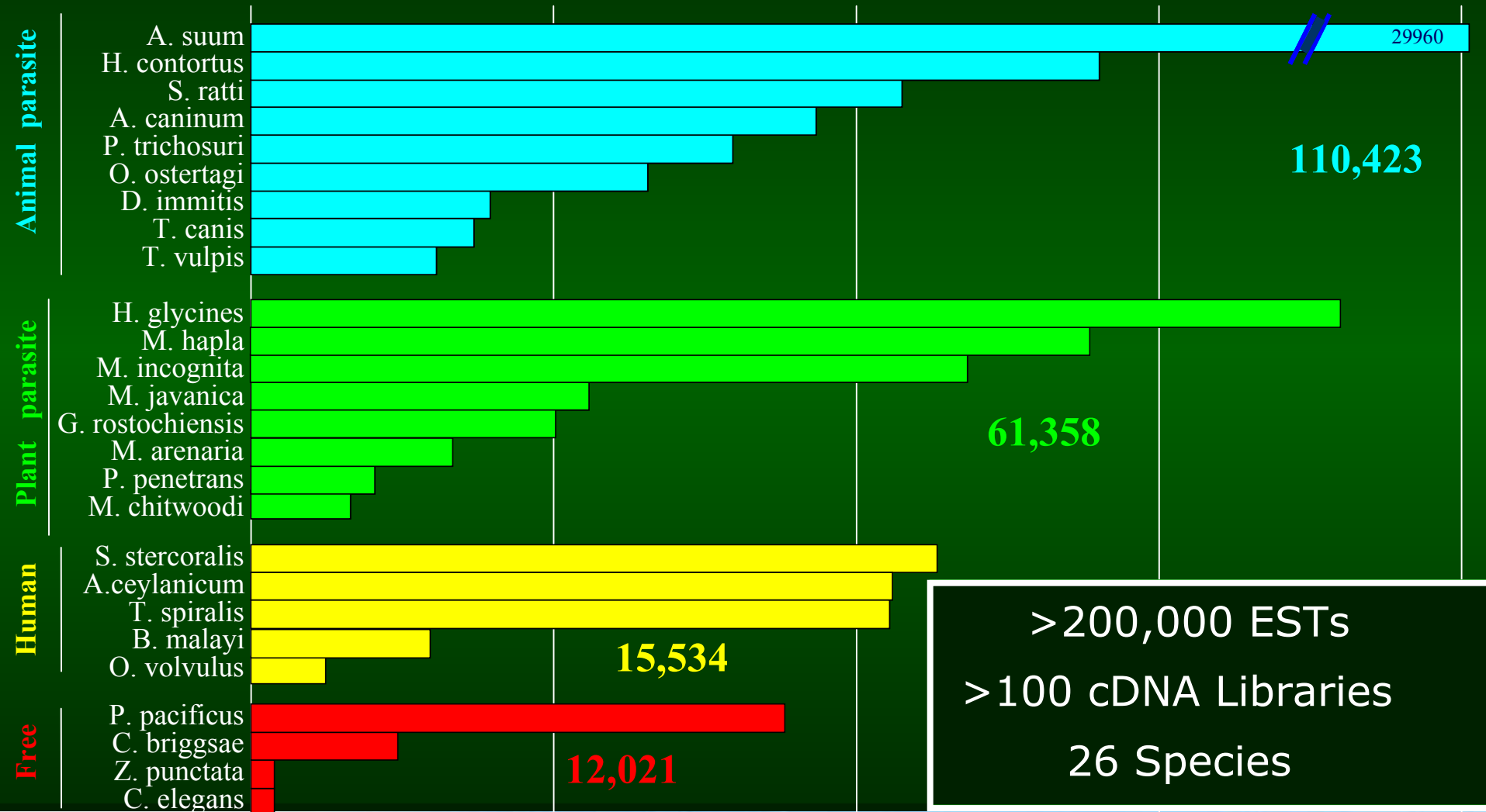
20+ billion

- St. Louis based GSC led first finished sequence of an animal, the nematode *C. elegans*, with Sanger Centre
- Sequenced significant portions of mouse/human genome
- World's Largest Effort in Parasitic Nematode Sequencing (235K ESTs)
- 26 Species; including 10 parasites of plants
- Extensive network of collaborators including leading academic labs
- \$2M+ in Support from NIH, NSF, Whitney Foundation, Merck – all data publicly available



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GSC Nematode EST Totals, 4/03

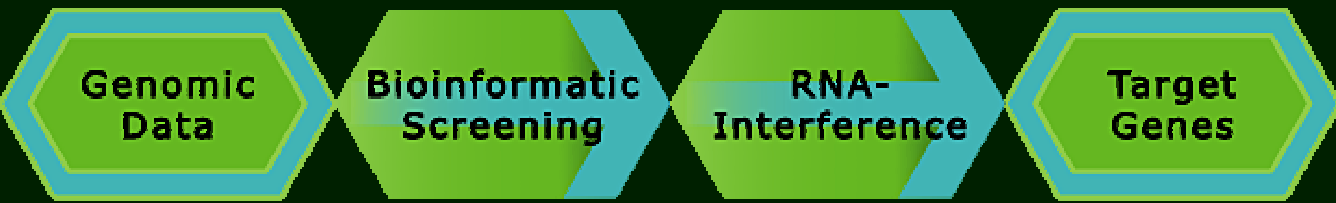


>200,000 ESTs
 >100 cDNA Libraries
 26 Species

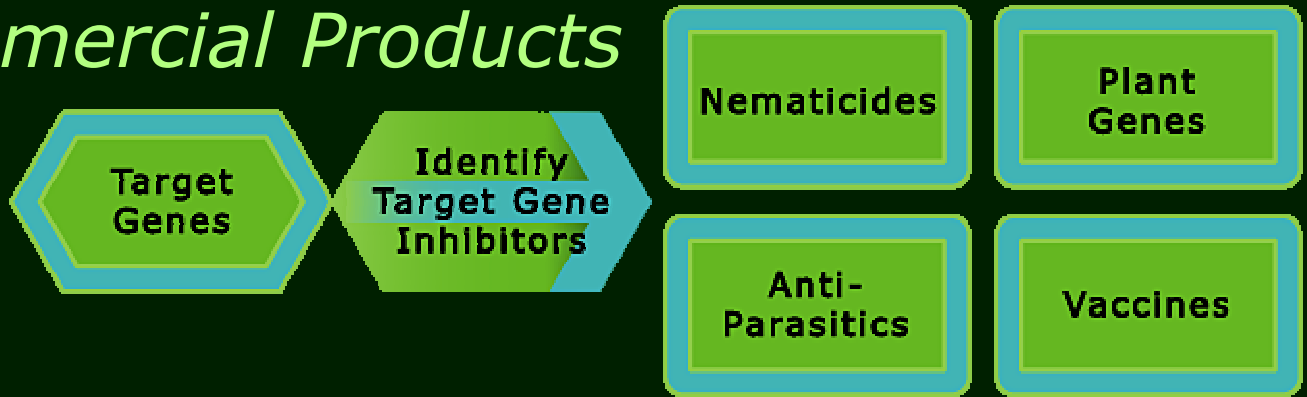


Divergence - Successful Discovery Platform

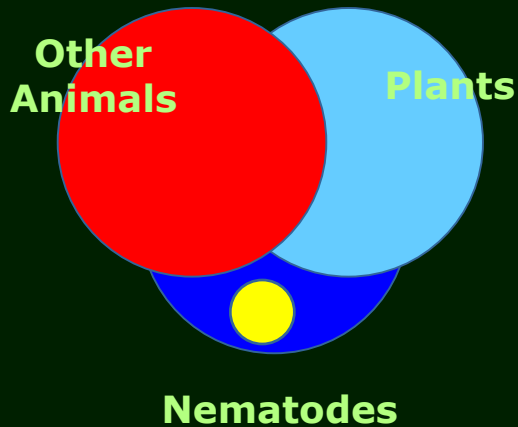
First Target Genes...



... Then Commercial Products



Rational and Sequential Process for Harvesting Promising Leads from the Deluge of Data



Not in human, required for survival

Bioinformatics Filter
20,000 > 1,000

Functional RNAi Filter
1,000 > 100

Curation
>100 Prioritized Gene Leads



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Divergence Validated Target Genes Fall into Distinct Classes

I. Enzymes of known function with defined small molecule substrates
DIV8338, DIV3202, DIV3441



**Design & Test
Inhibitors**

II. Enzymes, channels, receptors with known function
DIV6916, DIV5704, DIV3876, etc.



**Hi-Thruput
Screens**

III. Secreted & transmembrane proteins on accessible surfaces
(intestine, etc.)
DIV7429, DIV9015, DIV8907, etc.



**Transgenic &
Vaccine Approaches**

IV. Nematode Specific Proteins of unknown function
DIV5225, DIV0129, DIV9276



**Monitor
Literature**



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Two Approaches Toward Transgenic Control of Crop Parasitic Nematodes

1. Engineering plants to express small-molecule metabolic inhibitors that target nematode-specific biochemical pathways (Class I targets).
2. Engineering plants to produce small polypeptide inhibitors that specifically target essential proteins in the nematode intestine (Class III targets).



Class I Targets Allow Rapid Creation of Candidate Small-Molecule Inhibitors

I. Enzymes of known function with defined small molecule substrates
DIV8338, DIV3202, DIV3441



Design & Test
Inhibitors



**DIV3202/
3441**

DIV8338



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Class I Example: DIV3202 and DIV3441

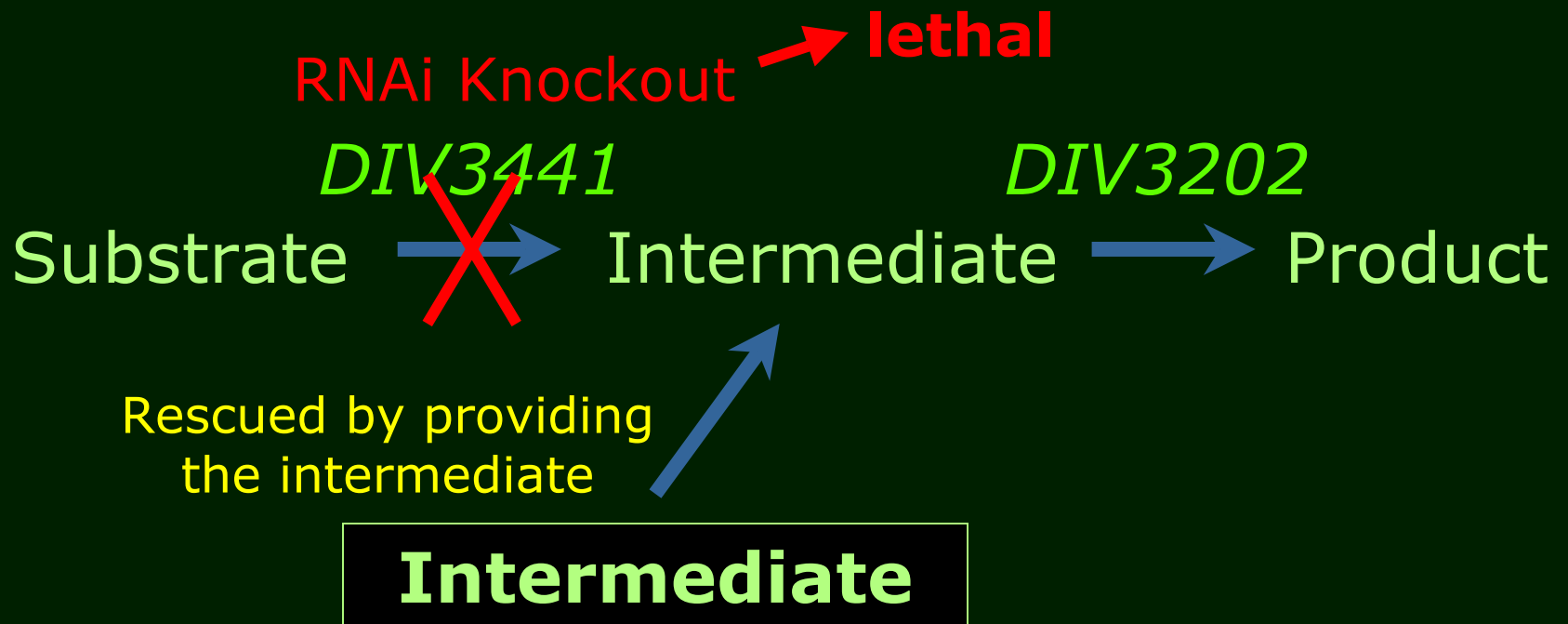


- Targets Work Sequentially in Enzymatic Pathway Not Found in Vertebrates
- Both Targets Essential in Multiple Steps During *C. elegans* Lifecycle
- Both Targets Present and Conserved in Many Parasitic Nematodes



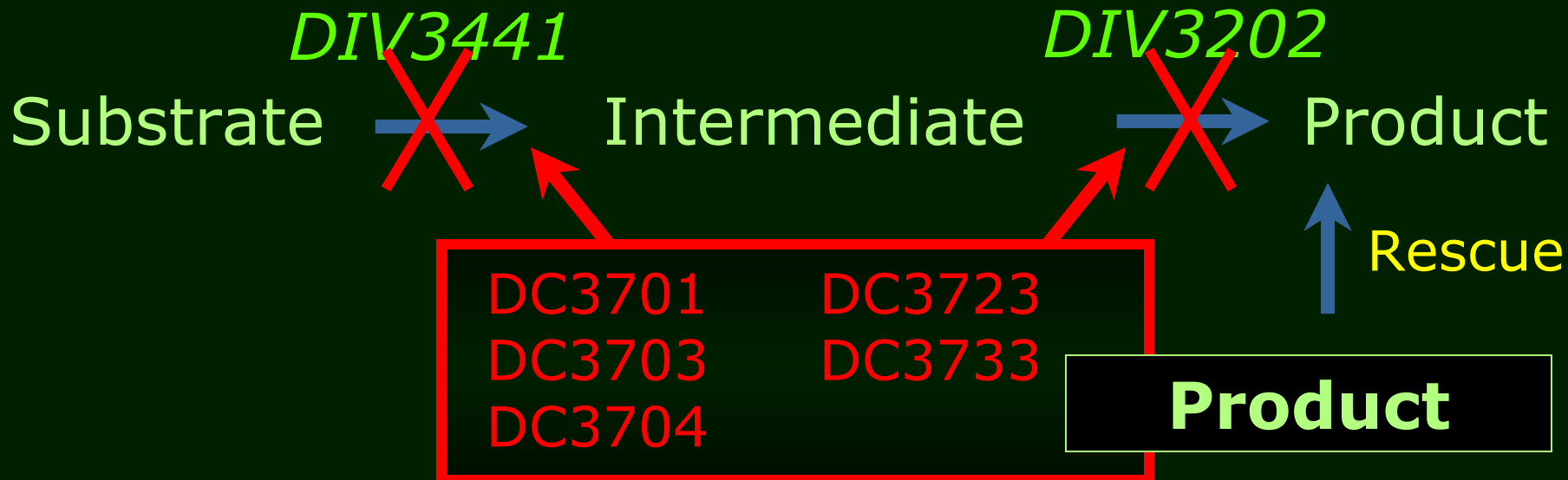
DIV3202 and DIV3441

- RNAi Knockouts of Either Target Can Be Rescued by a High Dose of Intermediate in Pathway



DIV3202 and DIV3441 Are Excellent Nematicide Targets

- In All 5 Cases, Chemical Knockout Can Be Rescued by a High Dose of Downstream Product in the Pathway
- Product Rescue Provides Basis for a In Vivo High Throughput Screen



Class I Example: DIV8338

DIV8338

Substrate → Product

- Target Works in Enzymatic Pathway Not Found in Vertebrates
- Target Essential in Multiple Steps During *C. elegans* Lifecycle
 - Target Present and Conserved in Many Parasitic Nematodes



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DIV 8338 is an Excellent Target for both Chemical and Transgenic Products

- 10 Chemicals Selected Based on Structural Similarity to Substrates and Products in the Pathway
- In 5 Cases, Chemicals Were Nematicidal



DC7651	DC7647
DC7646	DC7653
DC3772	



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Nematicidal Example



No treatment



DC7647/DC7651



Transgenic Example: DIV8338

- Ideal for Chemical as well as Transgenic Development
- Several Inhibitors are Derivatives of Human-Consumed Plant-Derived Natural Products
- Plant Genes Controlling Production of These Products Have Been Cloned and are Being Tested in Transgenic "Hairy Roots" in the Lab

Control hairy roots infected With Root Knot Nematode



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Class III Example: Intestinal Lumen Target Validation

- Of 5 Initial Targets Chosen, 4 Localize to the Intestine
- 2 Targets Are Known to Play Roles in Protein-Protein Interactions and Overexpression of these Peptides is Lethal to the Nematode
- Generation of Transgenic Plants Expressing these Peptides is in Progress



Divergence Alliances & Relationships



Focus of Transgenic Development

- > Transgenic Nematode Control Solutions for Corn, Cotton and Soybeans
 - Research collaboration with NCGA
- > Capabilities for in-house development in model crops and academic partnered development in commercial germplasm
 - Gene or multiple genes to provide control of most economically significant nematodes (e.g. root-knot, reniform)
 - Stacked with other traits of interest
 - Backup genes to ensure resistance management/spectrum
 - Germplasm introduction based on geographical need



Summary

- > Excellent Safety Profile as targets unique to nematodes
- > Focused on transgenic solutions for row crop nematode control
- > Currently, two distinct approaches under development
 - Additional targets prioritized for advancement
- > Progressing through transgenic developmental milestones



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