

Lawrence Berkeley National Laboratory

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Title

Roya de la Soja: Una Nueva Amenaza

Permalink

<https://escholarship.org/uc/item/3bc8649z>

Author

Posada-Buitrago, Martha Lucia

Publication Date

2005-06-09



DOE JOINT GENOME INSTITUTE

US DEPARTMENT OF ENERGY

OFFICE OF SCIENCE

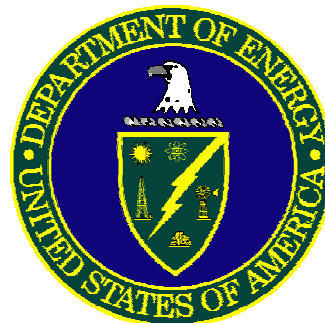
LBNL-58995 Spanish

Presentación del DOE-Joint Genome Institute

**Martha Lucia Posada-Buitrago, Ph.D.
Molecular Biologist**



Opened in 1999
~240 UC Employees
60,000 sf
~\$66M Annual Budget

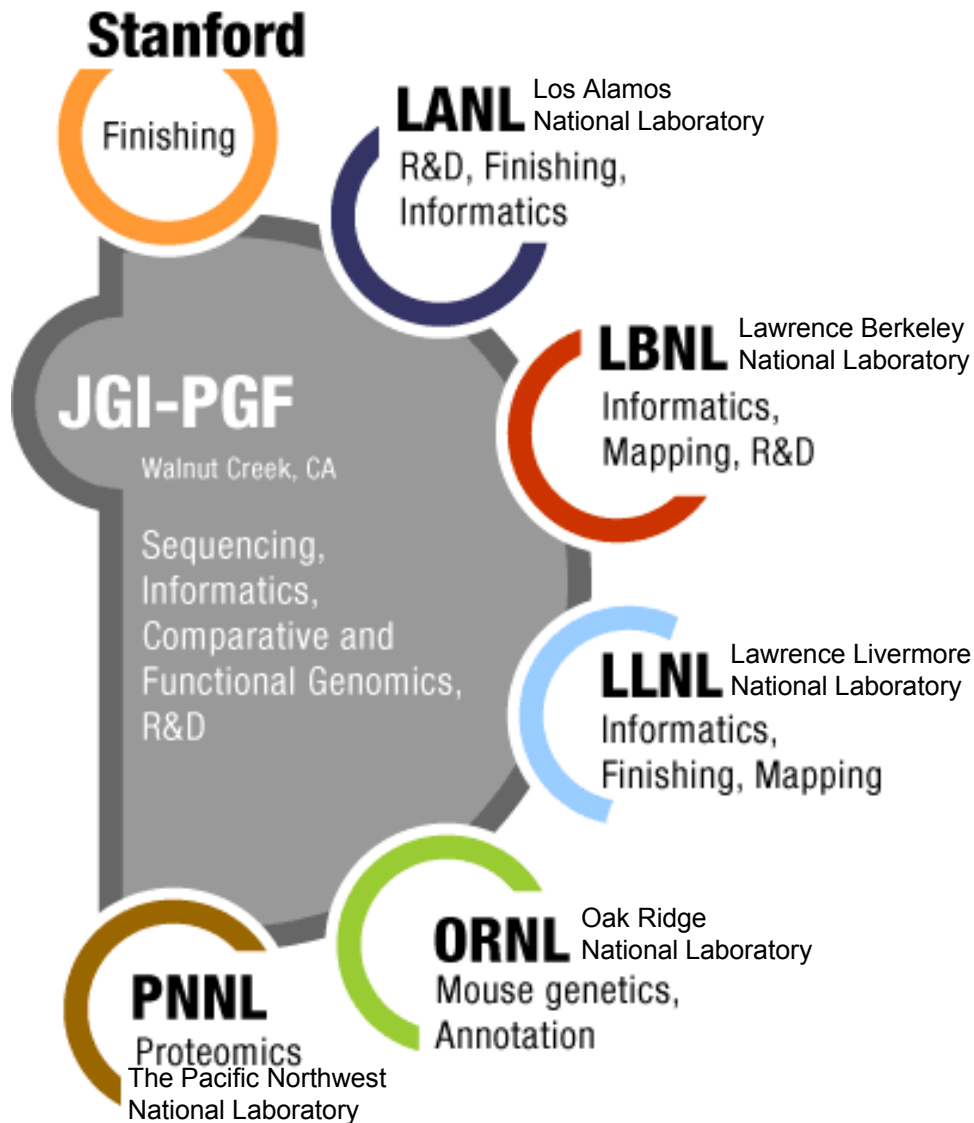


Mission:

To develop and exploit new sequencing and other high-throughput, genome-scale and computational technologies as a means for discovering and characterizing the basic principles and relationships underlying the organization, function, and evolution of living systems.

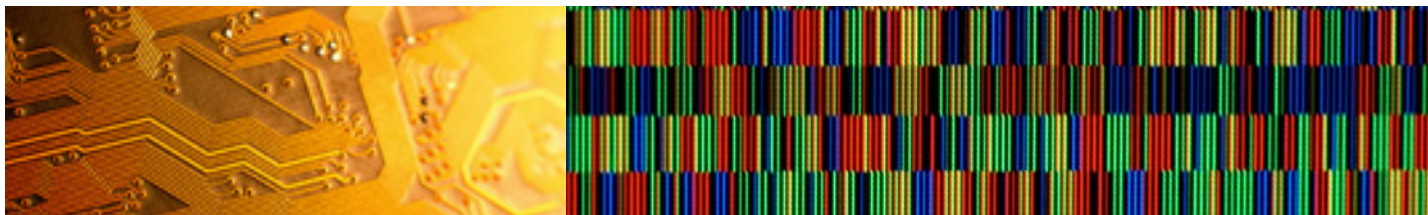
JGI Partnerships

U.S. D.O.E. JOINT GENOME INSTITUTE

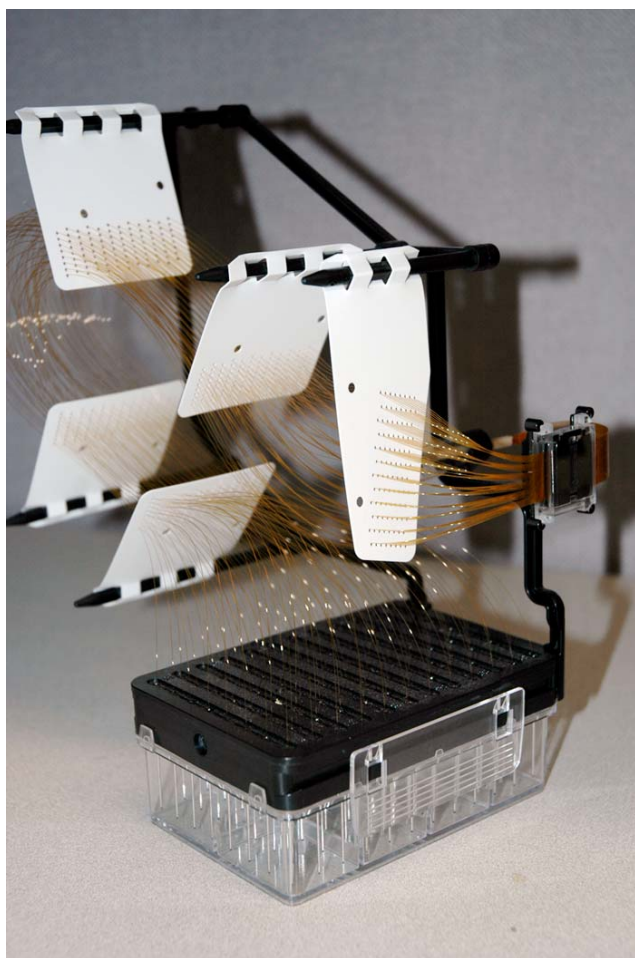


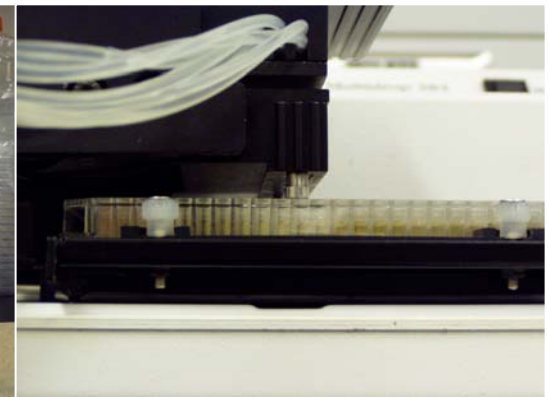
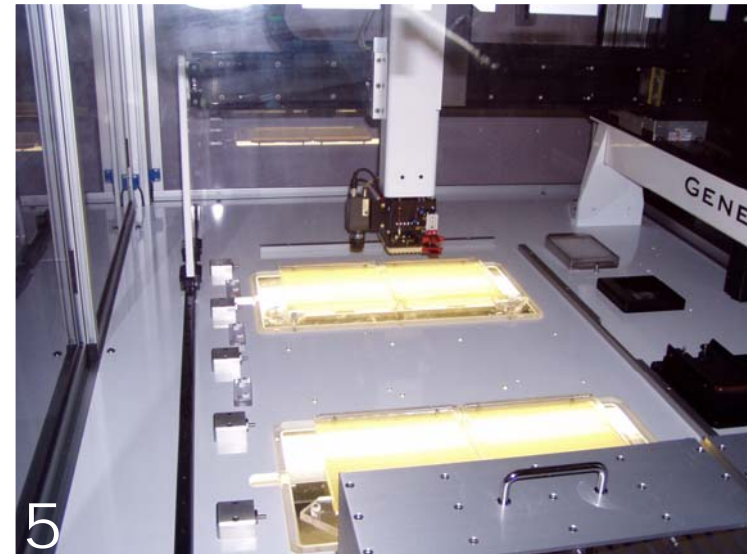
- April 2002: 1 gb/month
- January 2004: 2 gb/month
- July 2004: 2.5 gb/month
- March 2005: 3.1 gb/month
(equivalent to 1 human genome/month)

Total (3/99-4/05) 82.893 gb
(equivalent of sequencing 27 human genomes)



Automated DNA Sequencing





1. Shear DNA
2. Ligate into pUC18
3. Transform
4. Plate
5. Pick colonies
6. Grow overnight

DNA sequencing process

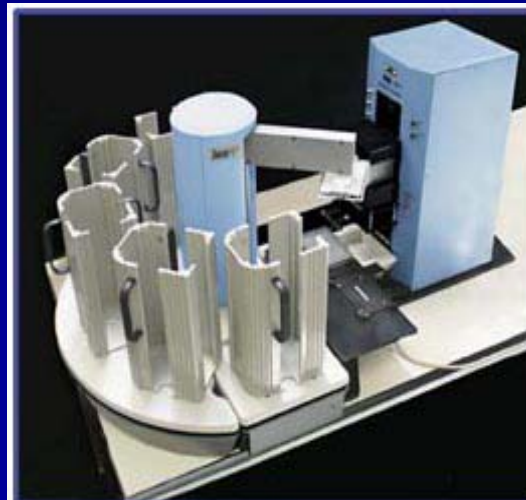
Rolling circle amplification of plasmid clones for sequencing template

1. PlateMate adds lysis buffer to small amount of culture



2. Cells are heat-lysed

3. Hydra 384's with Twister arms add RCA reagents.

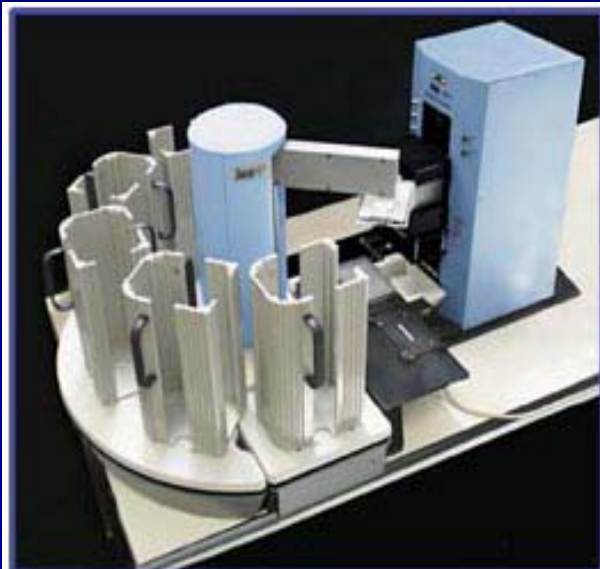


4. ON incubation

DNA sequencing process

Sequencing Chemistry

F and R reactions
are separated
with hydra 384's
with twister arms



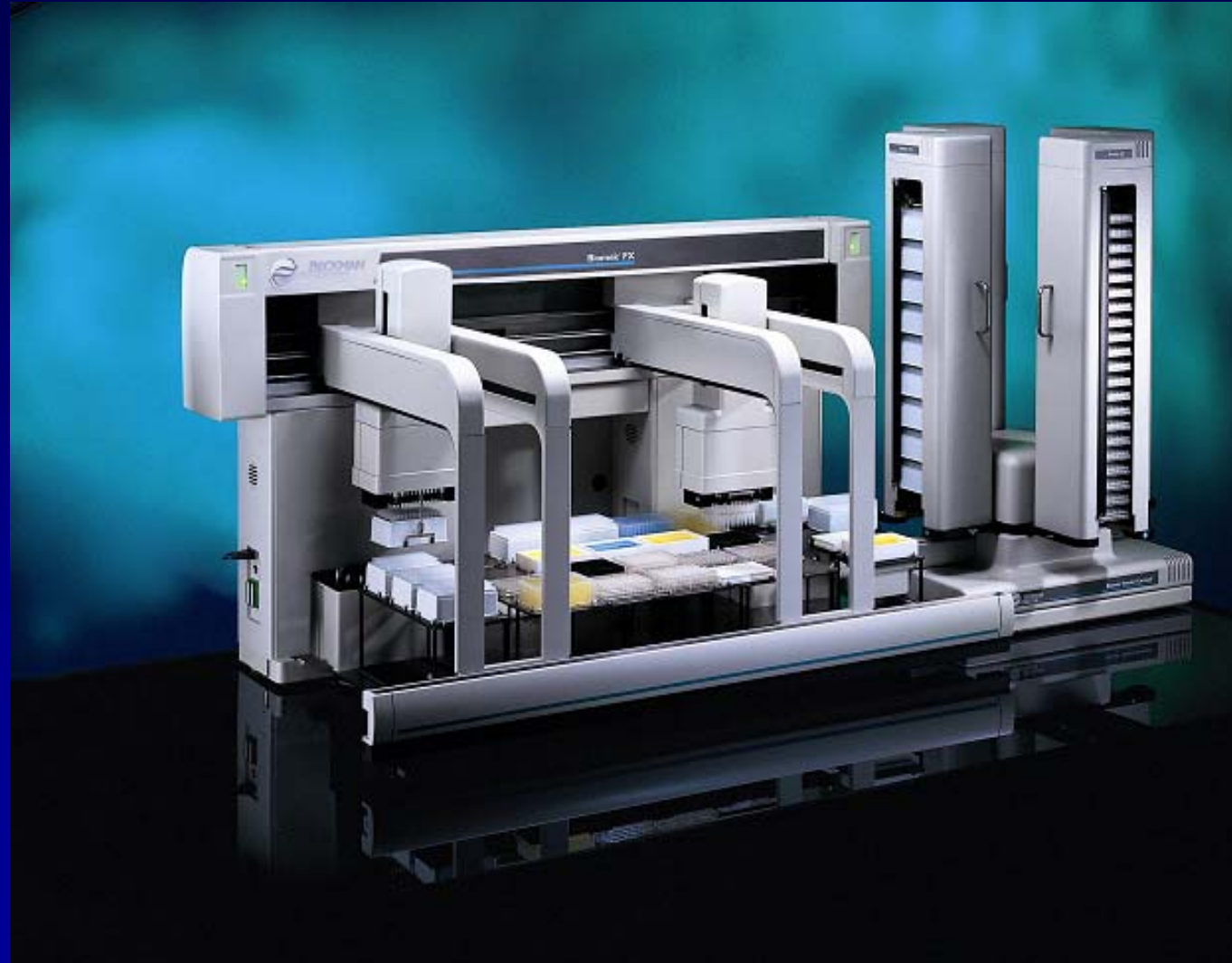
Sequencing
reagents are
added with Cavro
Dispense System



... more sequencing chemistry

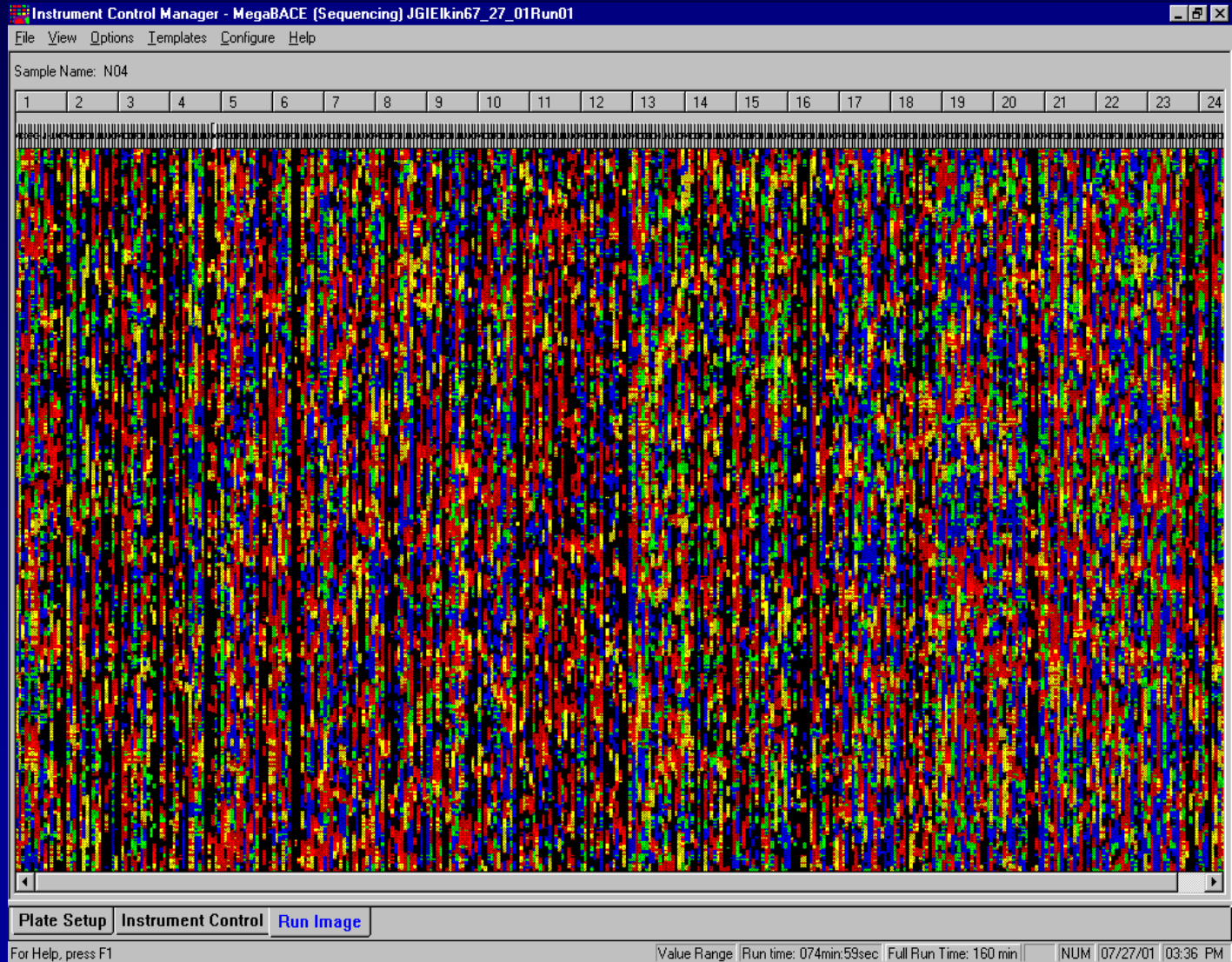


Sequence reactions with Quad-head PCR machines, then clean-up using BioMek robots and SPRI



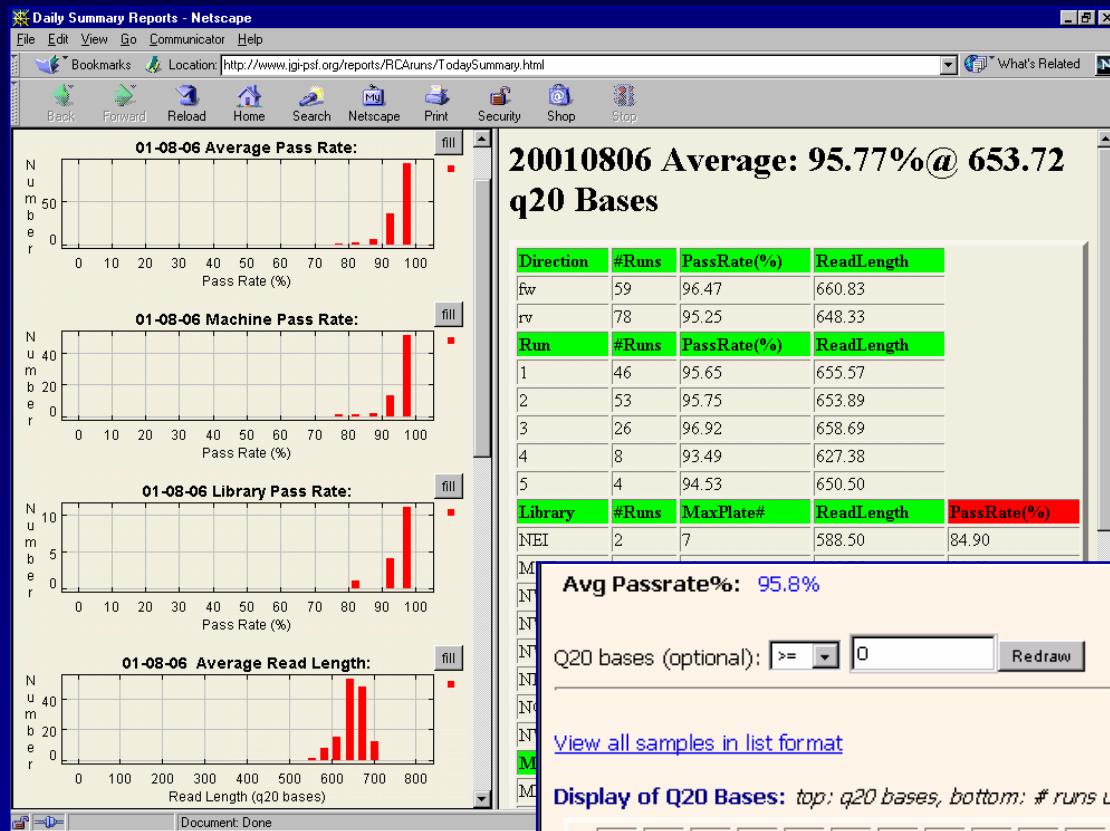
DNA sequencing process Capillary Group

**35 MegaBACE
4000
60 ABI 3730
Q20 / month =
3.1 Gb**



Online tracking of progress

LIMS uses bar code readers at every step and allows real time tracking of all reagents, personnel, and processes



Avg Passrate%: 95.8%

Q20 bases (optional):

[View all samples in list format](#)

Display of Q20 Bases: top: q20 bases, bottom: # runs used in calculation*

	1	2	3	4	5	6	7	8	9	10	11	12
a	746	756	768	468	760	726	732	748	766	775	619	597
b	774	604	677	746	756	749	584	744	744	783	780	730
c	736	746	747	779	743	648	600	591	764	758	509	761
d	716	725	672	712	736	759	738	555	591	751	718	748
e	749	742	746	748	746	724	719	655	746	644	740	736
f	681	714	734	732	729	757	737	399	769	734	717	739
g	708	567	0	707	694	601	700	0	0	727	621	36
h	721	728	734	741	684	720	725	667	512	734	733	729

Additional Analysis:

[View array change history](#)
[Plot FW vs. RV](#)
[Graph Q20 readlengths by well](#)

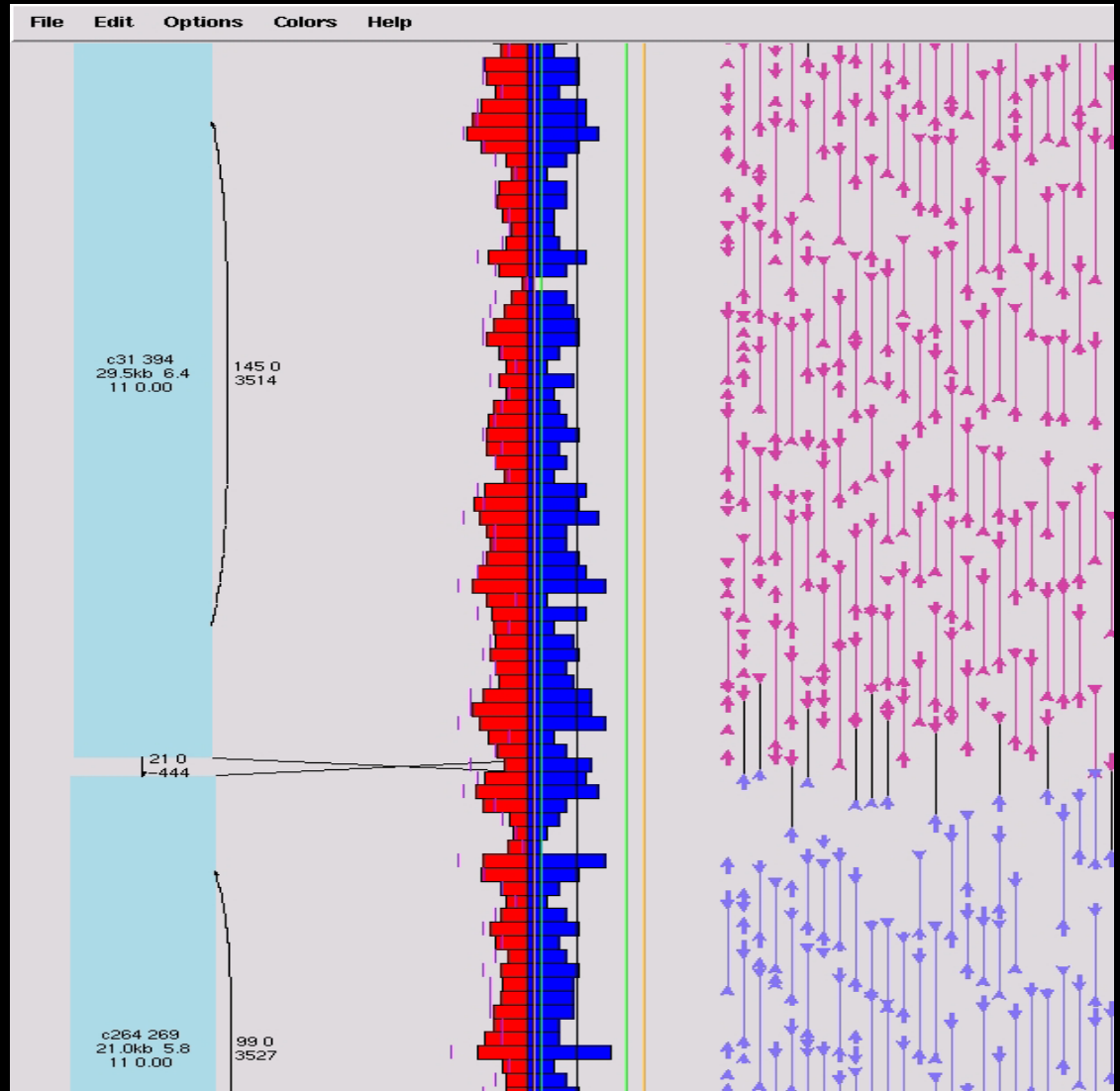
Color Scheme: 1 2-250 251-350 351-450 451-525 526-600 >600

DNA Sequence Assembly

Informatics team
assembles, verifies,
annotates genomes

Best assemblies
come from end
sequences from a
mixture of clone
sizes.

Typically, the JGI
makes 3 libraries:
3-4 Kb in plasmids
8-10 Kb in plasmids
40 Kb in fosmids




Genome annotation and visualization tools

Netscape: JGI Genome Browser

Navigation icons: Back, Forward, Reload, Home, Search, Guide, Images, Print, Security, Stop, Netscape. LBNL 4.75

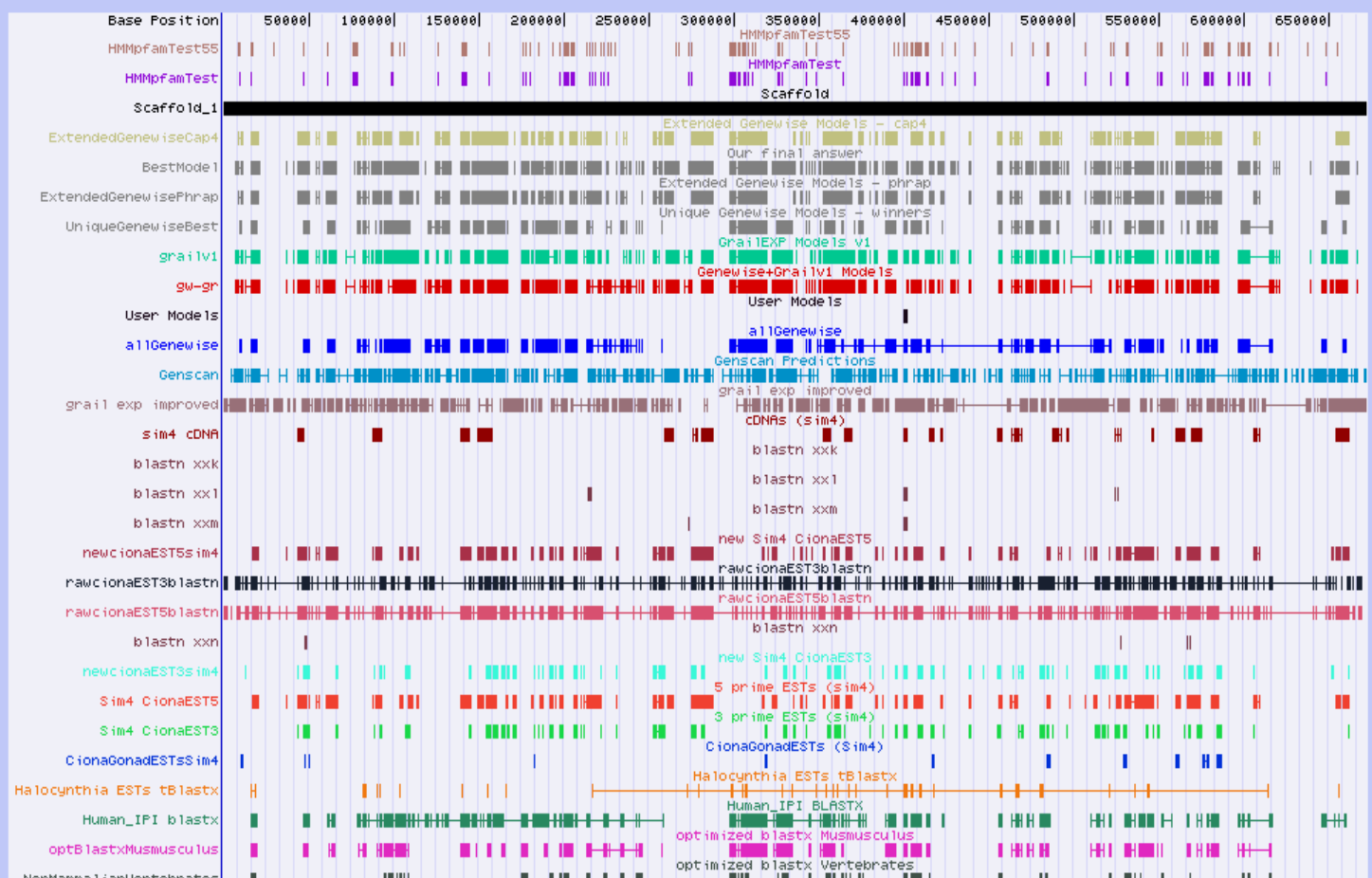
Location: http://grolithe.jgi-psf.org/cgi-bin/splat?db=ciona3&position=Scaffold_1 What's Related

UCB LBL Profusion Google NCBI JGI Production db

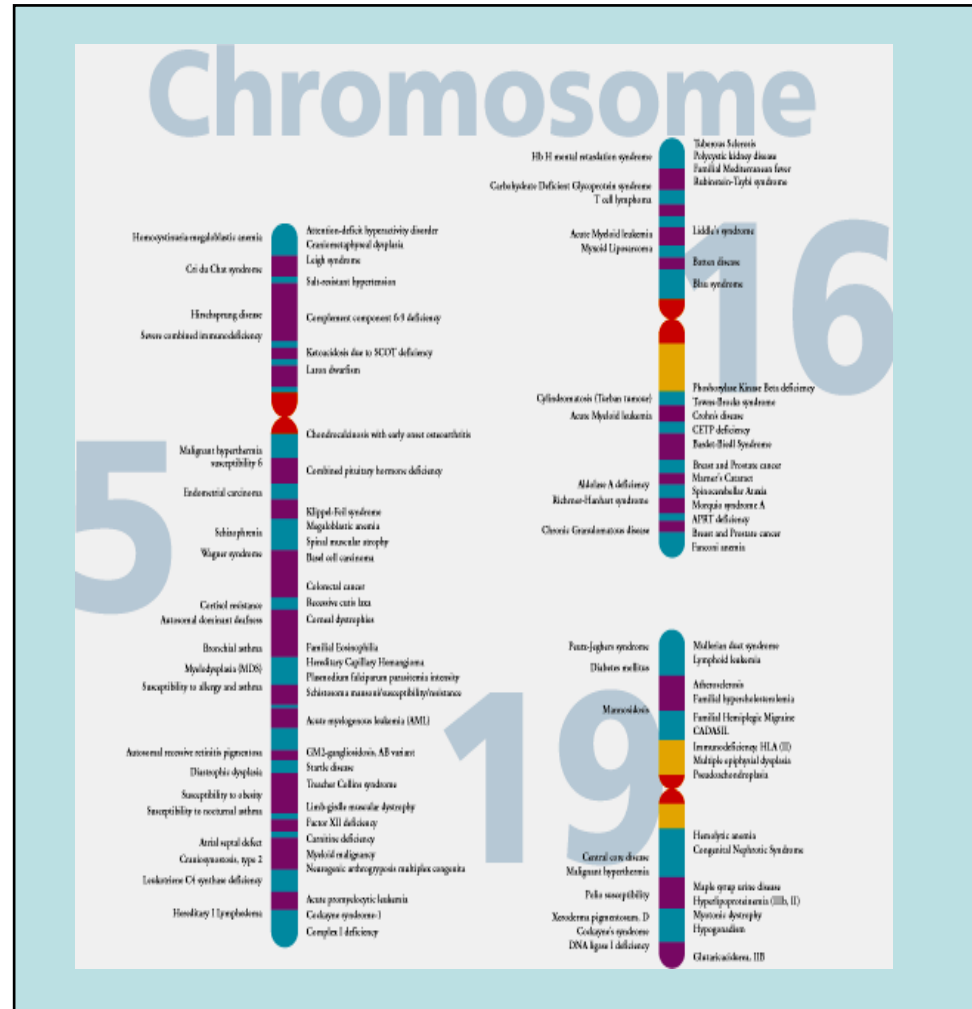
JGI DOE JOINT GENOME INSTITUTE Clona Genome Project (internal) 

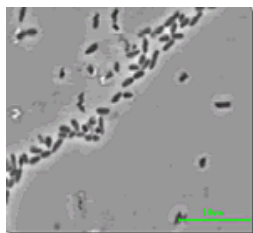
JGI Home Search Blast GO Browse Annotation Clona Home

move <<< << < > >> >>> zoom in 1.5x 3x 10x zoom out 1.5x 3x 10x
position Scaffold_1:1-672427 size 672427, image width (pixels) 960 jump/refresh

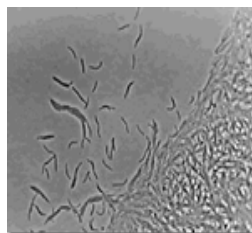


11% of Human Genome Sequenced by JGI

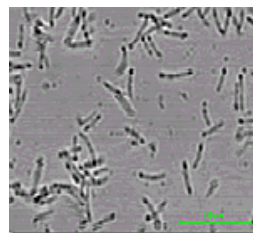




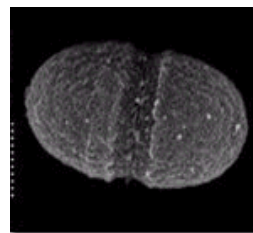
Burkholderia cepacia



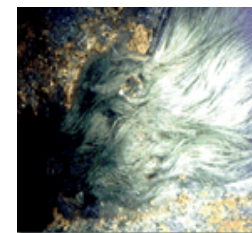
Cytophaga hutchinsonii



Desulfitobacterium halfniense



Enterococcus faecium



Ferroplasma acidarmanus



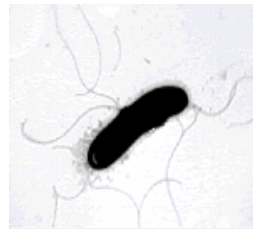
Magnetospirillum magnetotacticum



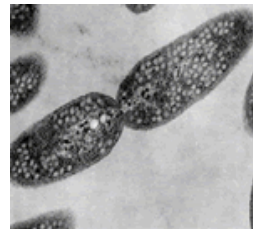
Nitrosomonas europaea



Prochlorococcus marinus



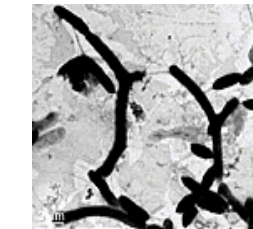
Pseudomonas fluorescens



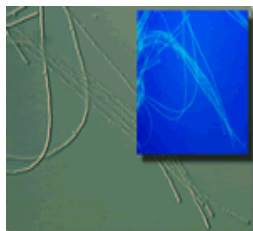
Rhodobacter sphaeroides



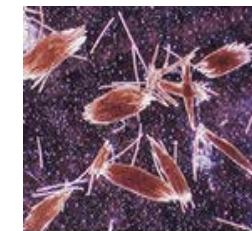
Rhodospseudomonas palustris



Spingomonas aromaticivorans



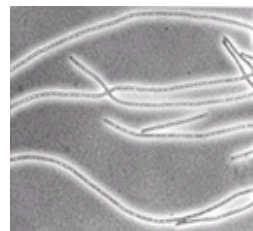
Thermomonospora fusca



Trichodesmium erythraeum



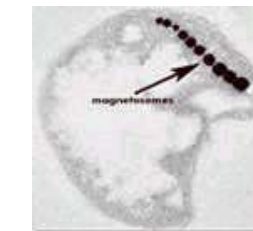
Xylella fastidiosa



Nostoc punctiforme



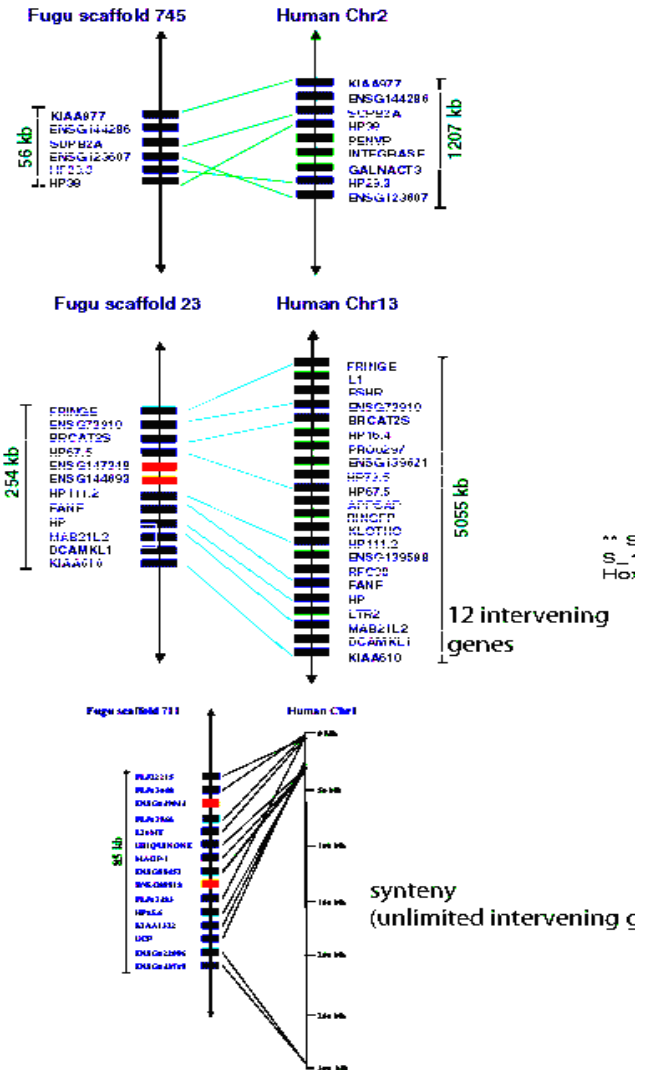
Marine synechococcus beta



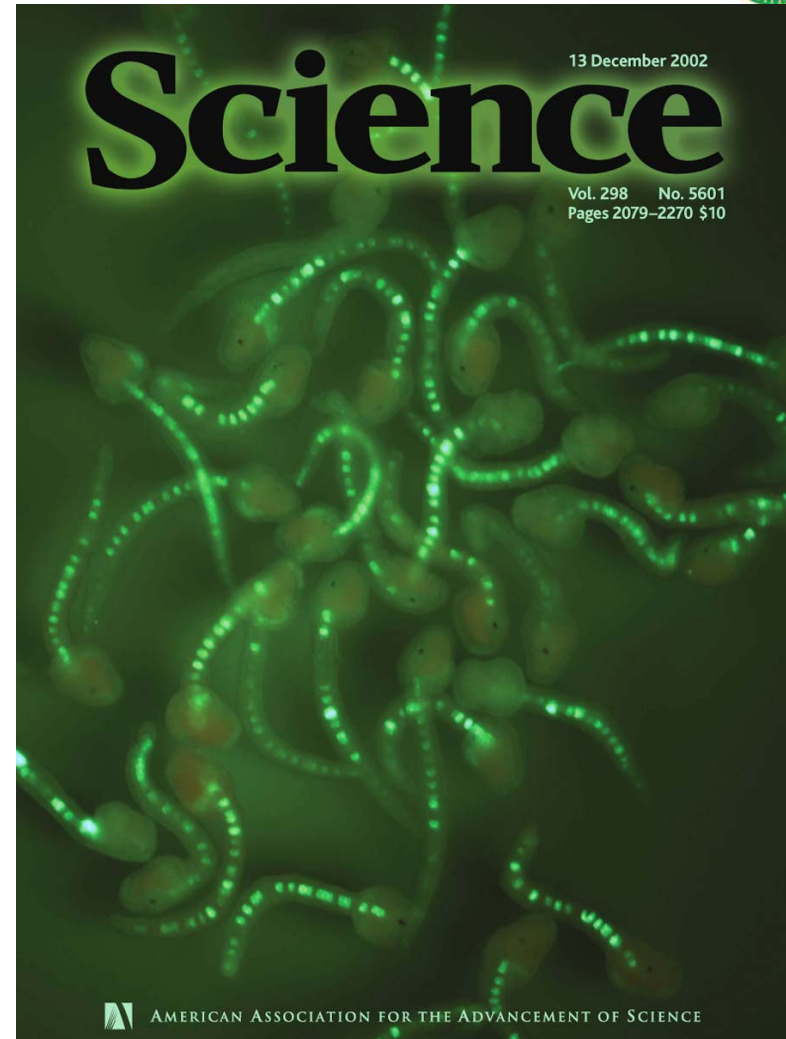
Magnetococcus MC-1

Pufferfish Genome

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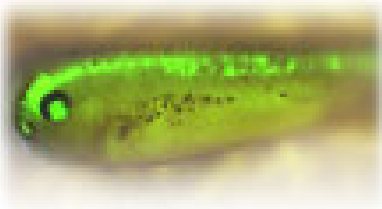
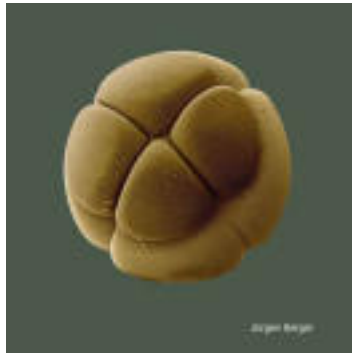


Ciona intestinalis A Primitive Chordate



Xenopus tropicalis

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- Close relative of the well-studied *X. laevis*, a major model organism for developmental biology
- Favorite system for toxicology (EPA)
- Coordinated with
 - WashU BAC map project
 - cDNA projects at NIH, Sanger
 - other projects from international frog research community
- 7x coverage by early '05

Fungi (rots and plant pathogens)

Trichoderma reesei possesses a host of carbohydrate degrading enzymes and is used extensively in industrial processes.



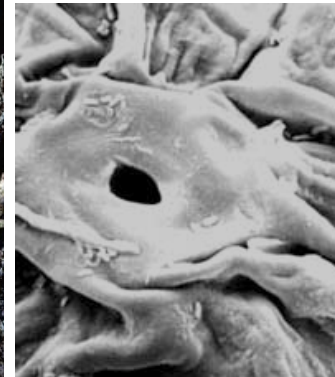
White rot fungi like *P. chrysosporium* are uniquely able to degrade lignin, the second most abundant natural polymer and a major component of biomass



Phakopsora pachyrhizi & *P. meibomia*

Soybean rust was recently found in US.

Highly repetitive sequence

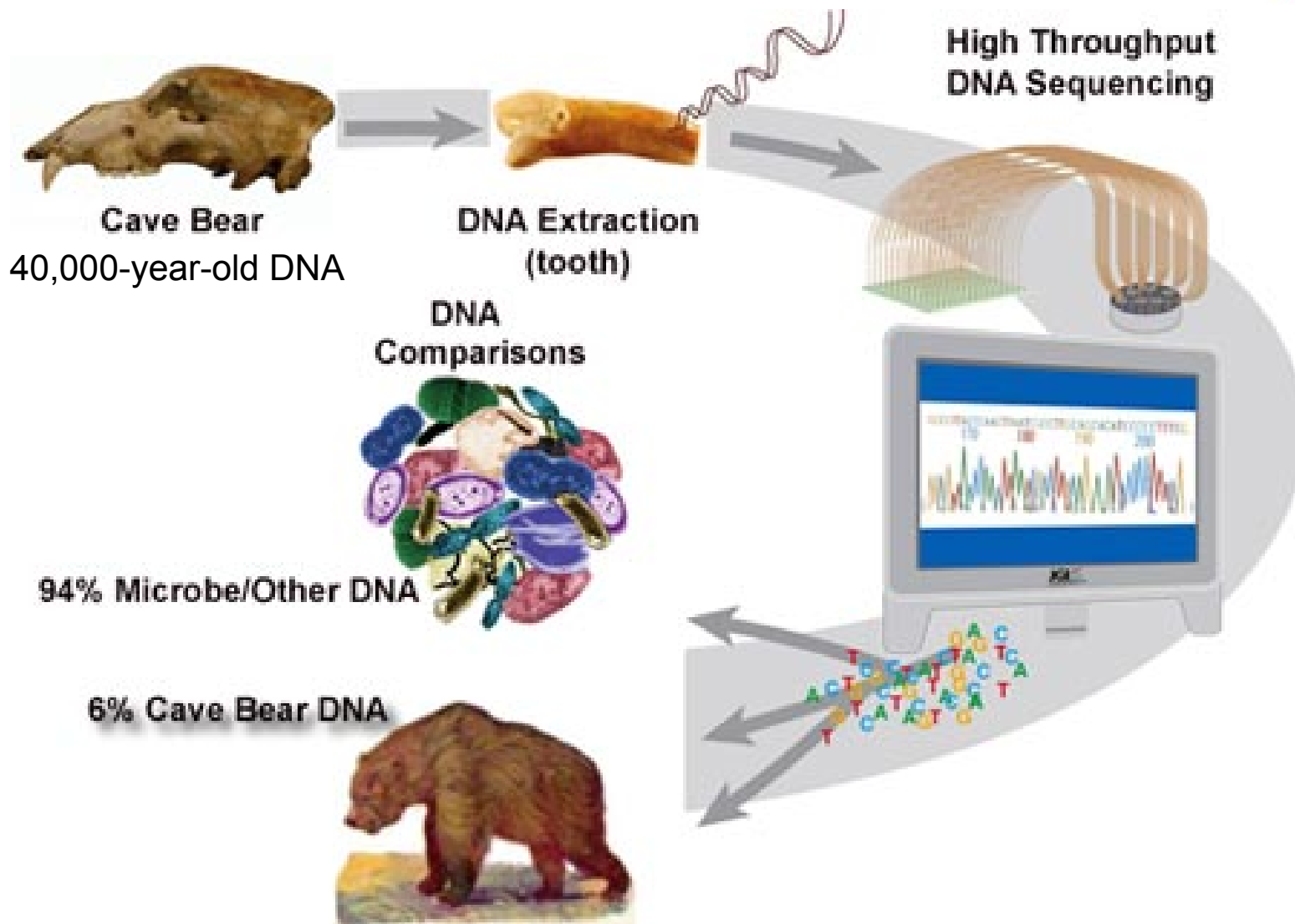


Sudden Oak Death

- In partnership with the USDA, NSF, VBI, California Oak Mortality Taskforce, County Ag Commissioners, City of Walnut Creek, WC Chamber of Commerce.
- 4 TV Stations; various print media



California Agriculture



JGI Community Sequencing Program “Non-traditional User Facility”



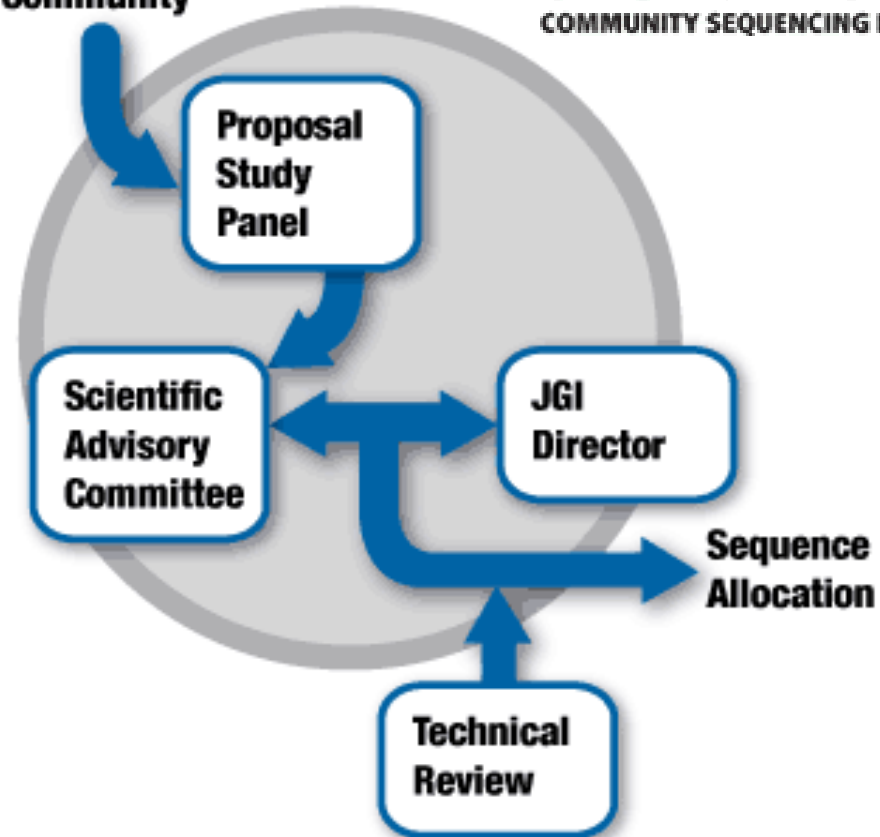
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- Allocation of ~15 gigabases/year for sequencing to advance the frontiers of science supported by DOE
- 56 Proposals received in Feb. '04 totaling 100Gb in requested sequencing (equivalent to the current WW sequencing capacity)
- 150 Proposals received in Feb. '05.
- SAC approved 23 projects beginning Fall '04
- New RFP Spring '06

Proposal from
the Scientific
Community

JGI CSP
COMMUNITY SEQUENCING PROGRAM



Roya de la soja: Una Nueva Amenaza

Martha Lucía Posada-Buitrago Ph.D.
Bióloga Molecular



DOE- Joint Genome Institute
Lawrence Berkeley National Laboratory



Causada por dos especies de hongos:

Phakopsora pachyrhizi

aka aislamiento del “viejo mundo” o
“Asiático”

Patógeno muy agresivo.

Phakopsora meibomiae

aka aislamiento del “Nuevo Mundo” o
“Americano”

No muy agresivo

LEGUMINOSAS (Papilionoideae)

Especies cultivables:

Glycine max

Phaseolus lunatus

Phaseolus vulgaris

Vigna unguiculata

Cajanus cajan

Pachyrhizus erosus

Plantas ornamentales:

Lupinus, poinciana real

Hospederos salvajes:

Kudzu, clavo dulce



Kudzu infectado con la roya de la soja



Situación mundial actual de la roya de la soja



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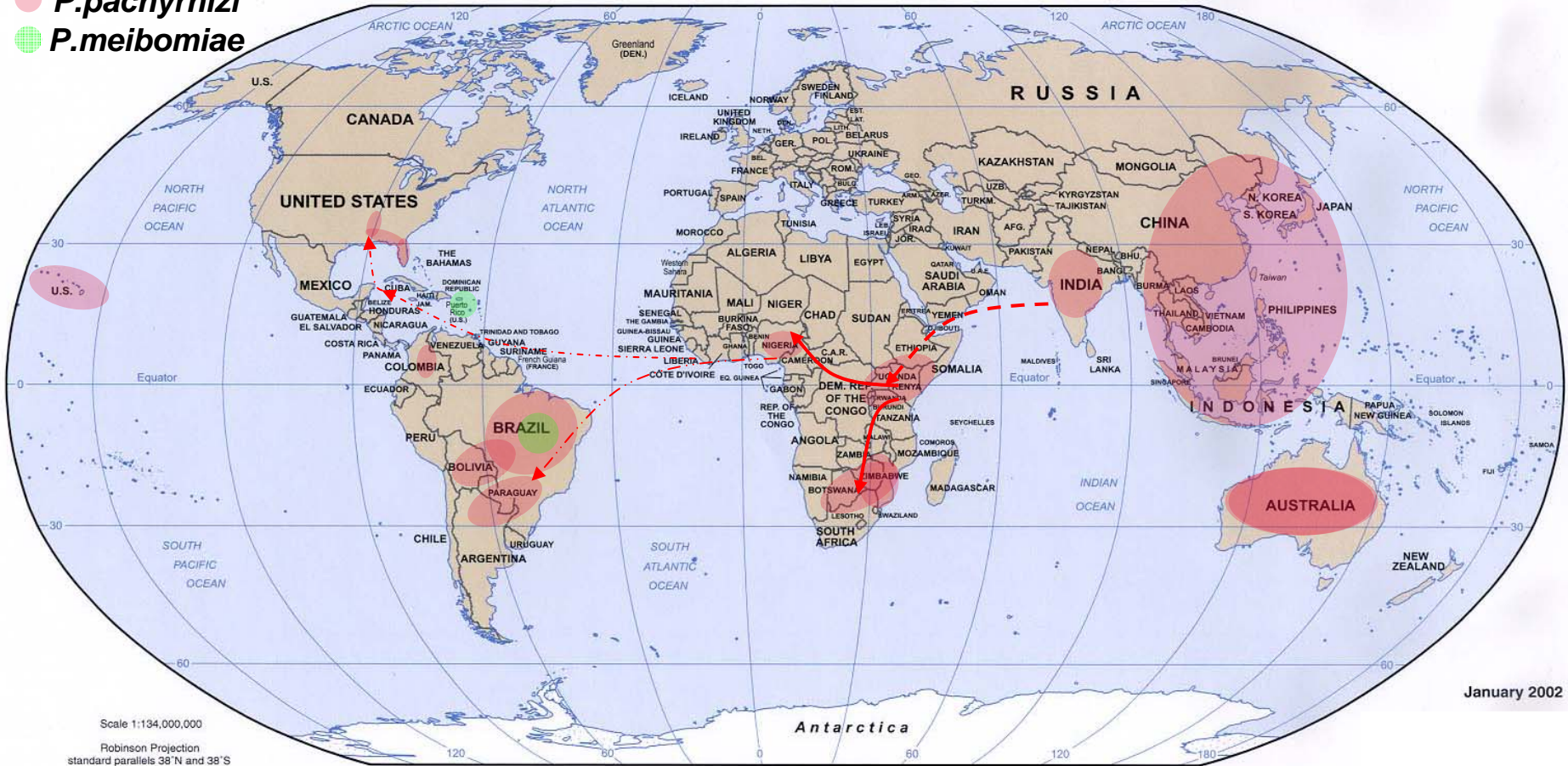


Japon	1904	
Kenya	1997/1998	Se piensa que fue transportada por las corrientes de aire desde Asia
Nigeria	1997/1998	
Rwanda	1997/1998	
Zimbabwe	1997/1998	
South Africa	2001	
Paraguay	2001/2002	Se piensa que fue transportada por las corrientes de aire desde Africa
Brazil	2002	
Argentina	2002	
Bolivia	2003	
Colombia	Junio 2004	
USA	Oct 2004	Huracán Iván

Situación actual de la roya de la soja



- *P.pachyrhizi*
- *P.meibomiaie*



January 2002

Scale 1:134,000,000
Robinson Projection
standard parallels 38° N and 38° S

Defoliación prematura

Disminución de la producción caracteriza por:

- Aumento en el número de vainas vacías por planta
- Disminución en el número de vainas normales por planta
- Disminución en el número de semillas/planta
- Disminución en el peso de semillas/planta
- Disminución en la germinabilidad de las semillas

Campos de soja infectada (Zimbabwe)



Photos by Reid D. Frederick



Síntomas de la roya



Why Containment?



- ❖ Biological activity of organisms
- ❖ Potential benefit to agriculture
- ❖ Need for research
- ❖ Existing regulations



- ❖ Double-pane tempered safety glass
- ❖ Negative air pressure
- ❖ HEPA Air filtration
- ❖ Waste water treatment
- ❖ Steam and ethylene oxide sterilization

- ❖ Authorized personnel only
- ❖ Clean suits
- ❖ Shower out



15-17 days old plants



Inoculation



ca 250.000 spores/pot



Dew chamber. 20°C ,16 - 24 hours



Greenhouse BL- 3



Spore harvesting (14 – 21 dpi)



Cotiledones Infectados



Tallo infectado



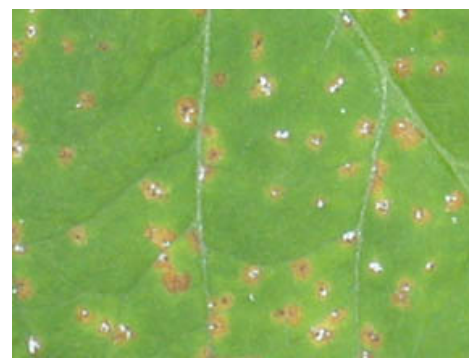
Vainas infectadas



Hojas Infectadas



9 días



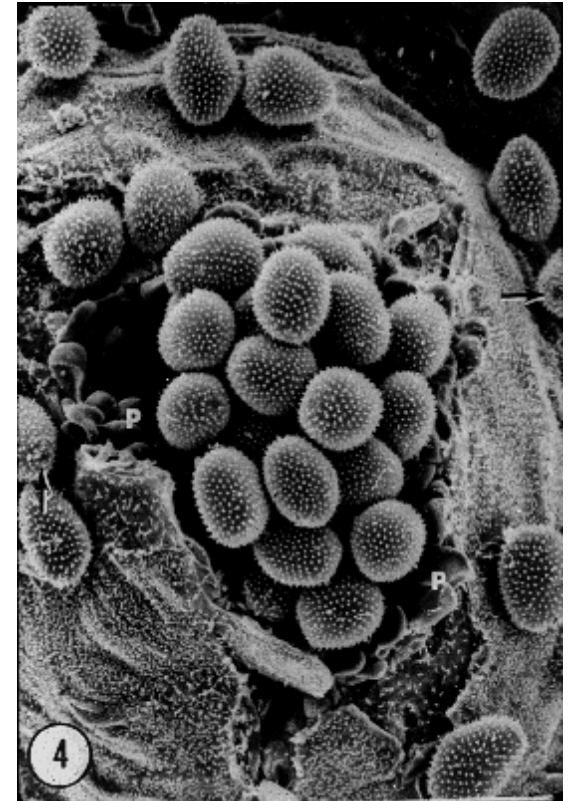
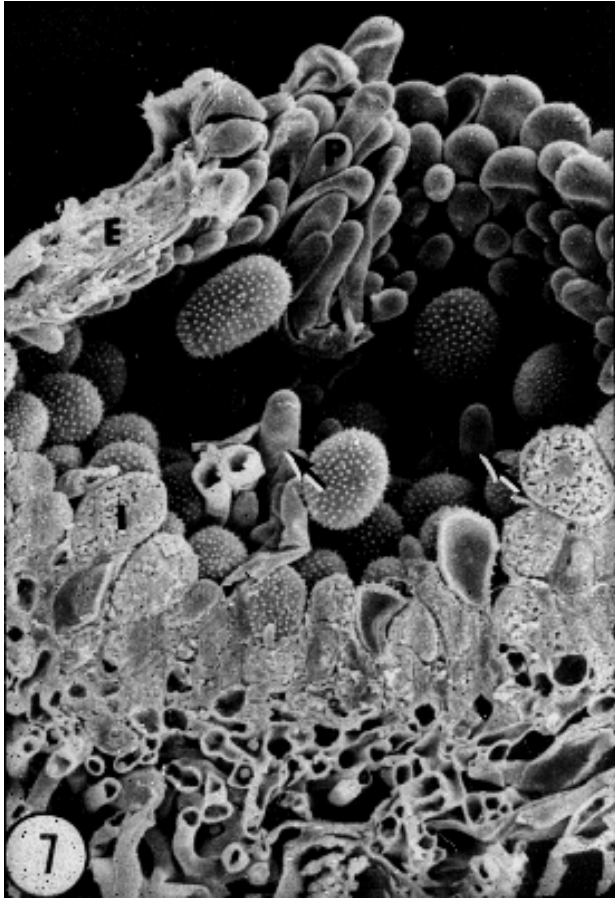
12 días



15 días



18 días



Estrategia nacional de estudio y tratamiento de la Roya de la Soja



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Estudios de expresión génica en planta infectada: Real Time RT PCR, ESTs (genotecas de cDNA en pSPORT1 y genotecas sustractivas –Clontech-). (USDA/ARS/FDWSRU y DOE-JGI).

- Secuenciación del genoma de *Phakopsora pachyrhizi* (USDA/ARS/FDWSRU y DOE-JGI).**
- Proteínas de esporas dormantes, en germinación y de planta infectada (reacciones susceptible y resistente) (USDA/ARS/FDWSRU).**
- Diseño de marcadores genéticos para identificación de aislamientos (USDA/ARS/FDWSRU, DHS).**

Estrategia nacional de estudio y tratamiento de la Roya de la Soja



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- **Búsqueda de resistencia en todas las variedades conocidas de soja (25000) y algunos huéspedes alternativos (Illinois University, USDA/ARS/FDWSRU).**
- **Tratamientos con fungicidas (Illinois University, USDA/ARS/FDWSRU, Cultivadores de Zimbabwe y Suráfrica, EMBRAPA Brasil).**
- **Educación y preparación de personal y trabajadores en campo para identificación del patógeno y acción de emergencia (USDA/ARS/FDWSRU, USDA/APHIS, Universidades estatales de los estados productores de soja). Estudios satelitales climáticos y de movimiento de esporas (Iowa State University - ISU)**
- **Anticuerpos (ISU, US Navy).**

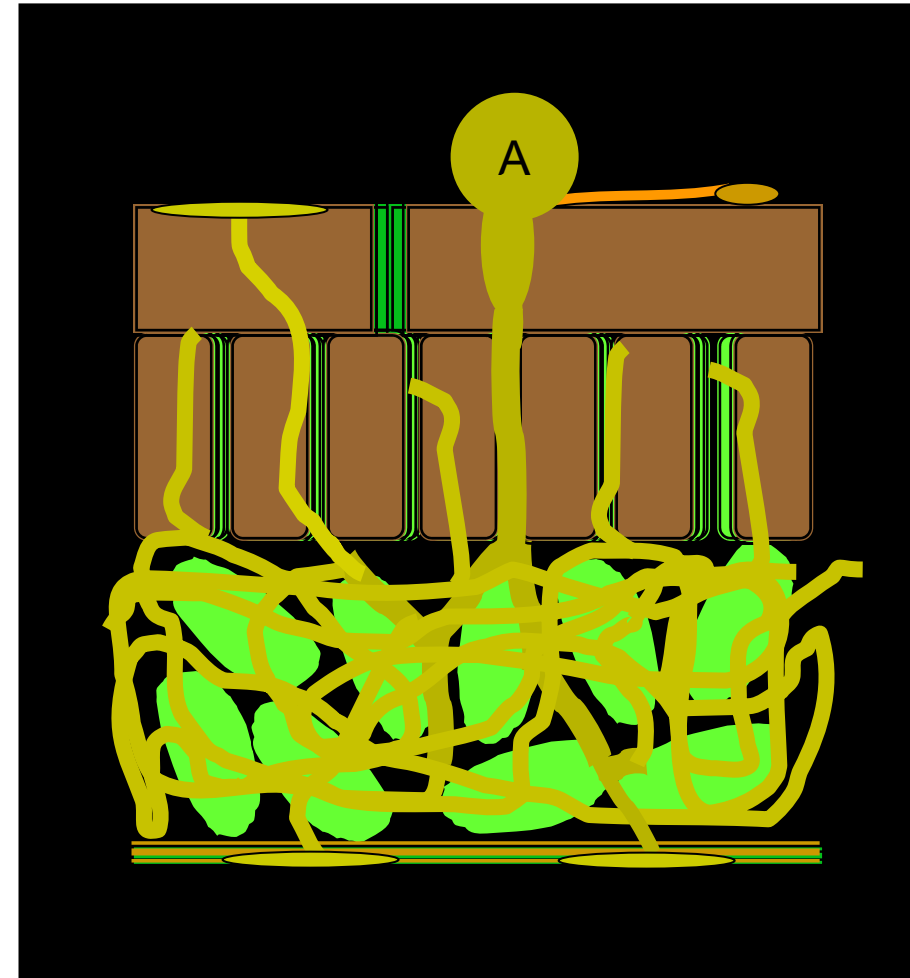
Estudios de Expresión Génica

OBJETIVO



Comprensión de las bases moleculares y bioquímicas del proceso de infección de la roya de la soja que pueda conducir a nuevos métodos de control de la enfermedad.

- 2 h** Desarrollo de los apresorios
- 5 h** Expansión de los apresorios
- 7-12 h** Penetración a través de la cutícula
- 12-16h** Aumento en el diámetro
- 24 h** Hifas primarias emergen desde la tev
- 48 h** Crecimiento Intercelular de las hifas (60µm desde el sitio de penetración)
- 3-8 días** Crecimiento Intercelular de las hifas (75-450 m desde el sitio de penetración)
- 9 días** Esporulación
- 14 días** Máxima esporulación



(Basado en Koch et al. 1983; Keogh et al. 1980)

Germinating
Spores

Resting spores

Hyphal growth*

High sporulation*

16 Hours on
water surface

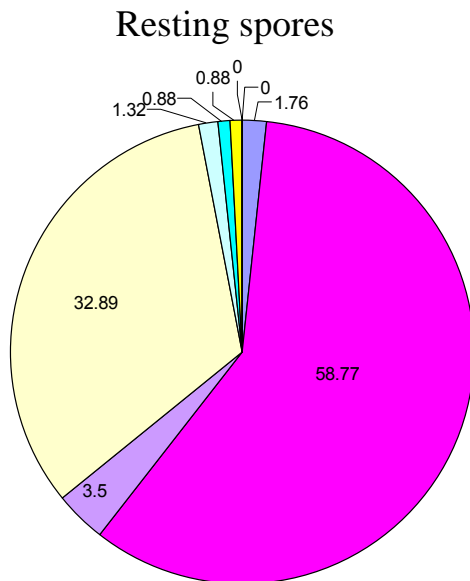
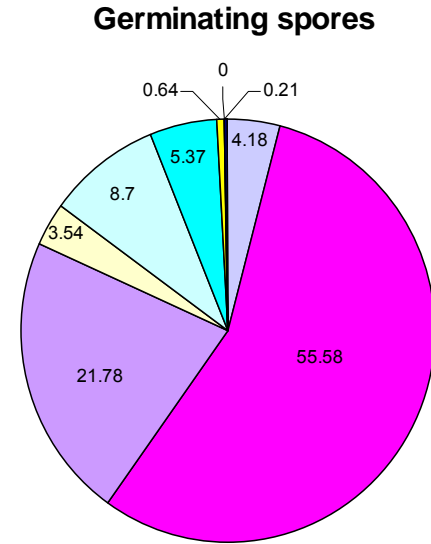
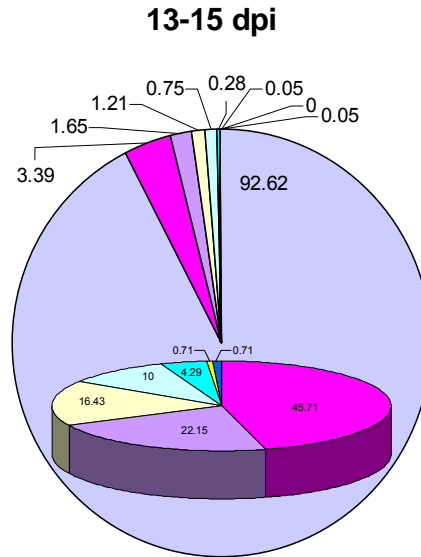
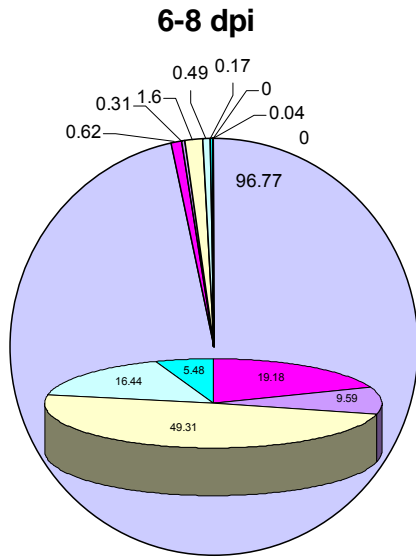
Kept at
– 80°C

6
7
8 Days after
inoculation

13
14
15 Days after
inoculation

mRNA was extracted from infected leaf at each time point and pooled together for the construction of the cDNA libraries. Unidirectional cDNA libraries constructed in plasmid pSPORT1 (Invitrogen).

Description	ESTs	cDNAs	Libraries	Clusters	Consensus	Singlets
6-8 dpi	6100	5374	1	1154	1278	1827
13-15 dpi	6023	4610	1	1291	1387	1356
Resting urediniospores	2295	1762	1	393	455	335
Germinating urediniospores	29601	18638	1	2686	3394	2142
<i>Phakopsora pachyrhizi</i> v2.1	44019	30244	4	5105	6165	4961



- Plant
- Filamentous fungi
- Yeast
- Bacteria
- Vertebrates
- Invertebrates
- Virus
- Oomycete
- Synthetic construct

Percentage of similarity of cDNA clusters from the *Phakopsora pachyrhizi* germinating and resting spores libraries and the infected soybean leaf libraries (6-8 dpi and 13-15 dpi) to proteins from other organisms. Inner pies show the percentage of similarity of cDNA clusters to proteins from other organisms, excluding plant homologs.

cDNA functional categories



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The cDNA clusters were classified into functional categories based on the BlastX hits and the Pfam hits, according to the Expressed Gene Anatomy database (EGAD, TIGR, Rockville, MD).

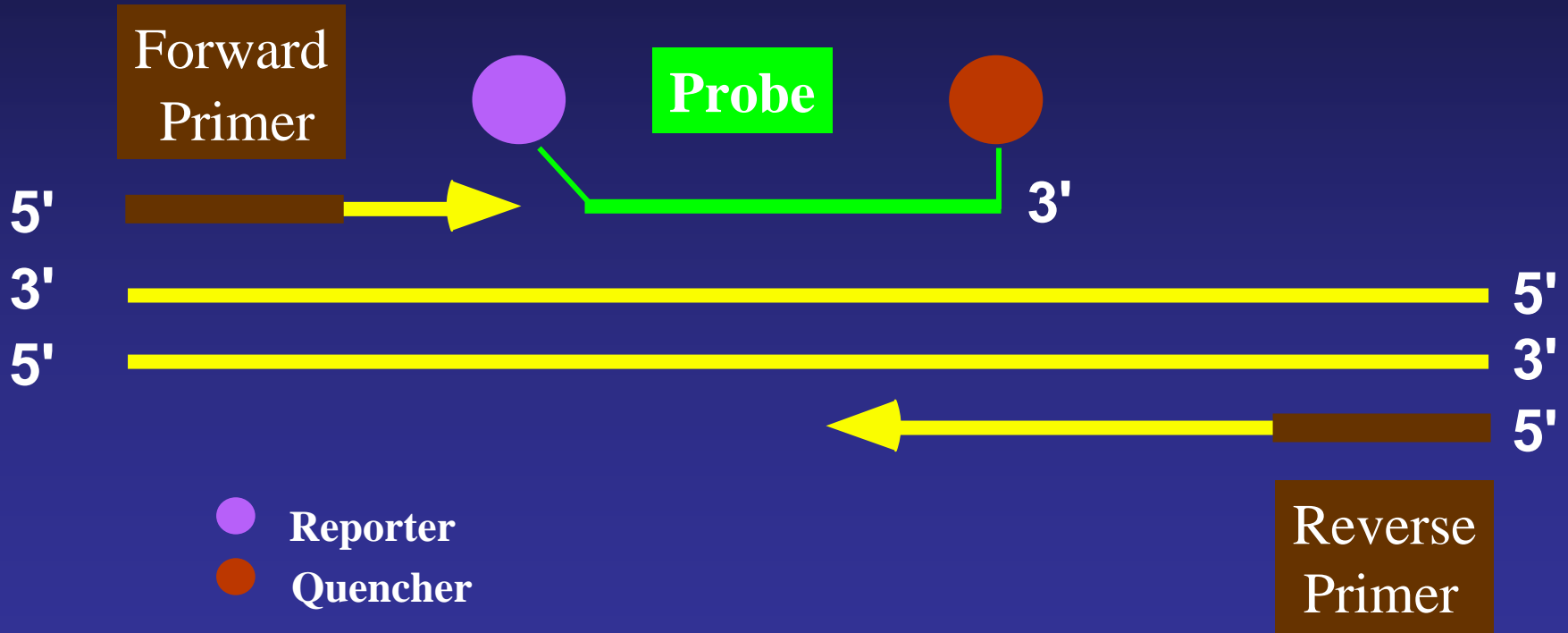
Approximately 23 % of the cDNA clusters from the 6-8 dpi and 13-15 dpi libraries and 40% from the germinating and resting spores libraries show similarity to hypothetical proteins or proteins of unknown function. Several homologs to pathogenesis related proteins (PR proteins) and defense proteins were identified in the infected leaf tissue libraries (Apidaecin, Beta defensin, Thaumatin, etc). In the GS library several homologs to pathogenicity proteins were identified. All the libraries show a high percentage of metabolism related proteins.

Real Time RT-PCR Studies

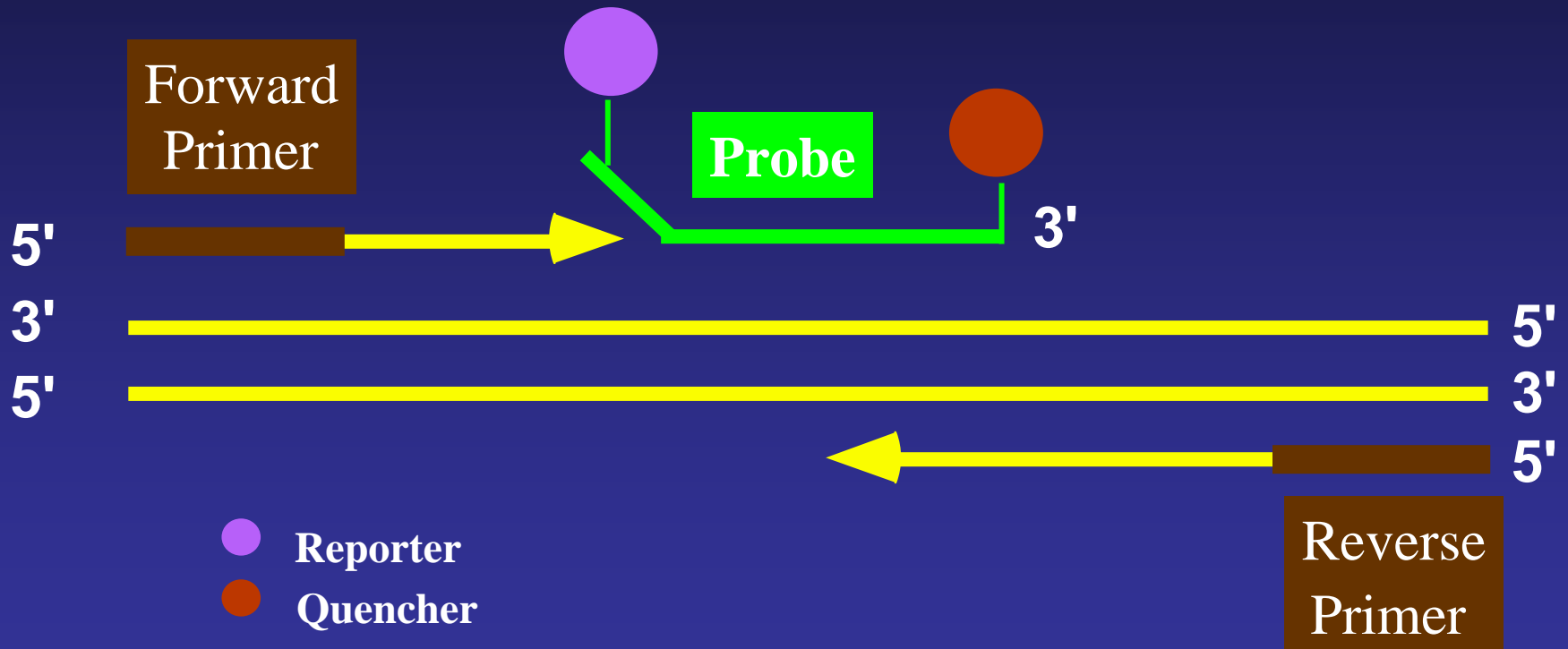


- **Combines the sensitivity of PCR along with hybridization**
- **Post-PCR confirmation steps are eliminated**
 - **No agarose gels & Southern blots**
- **Assays are very rapid**
- **Potential for multiplexing**
 - **Several probes of a particular pathogen**
 - **Specific probes from several pathogens**

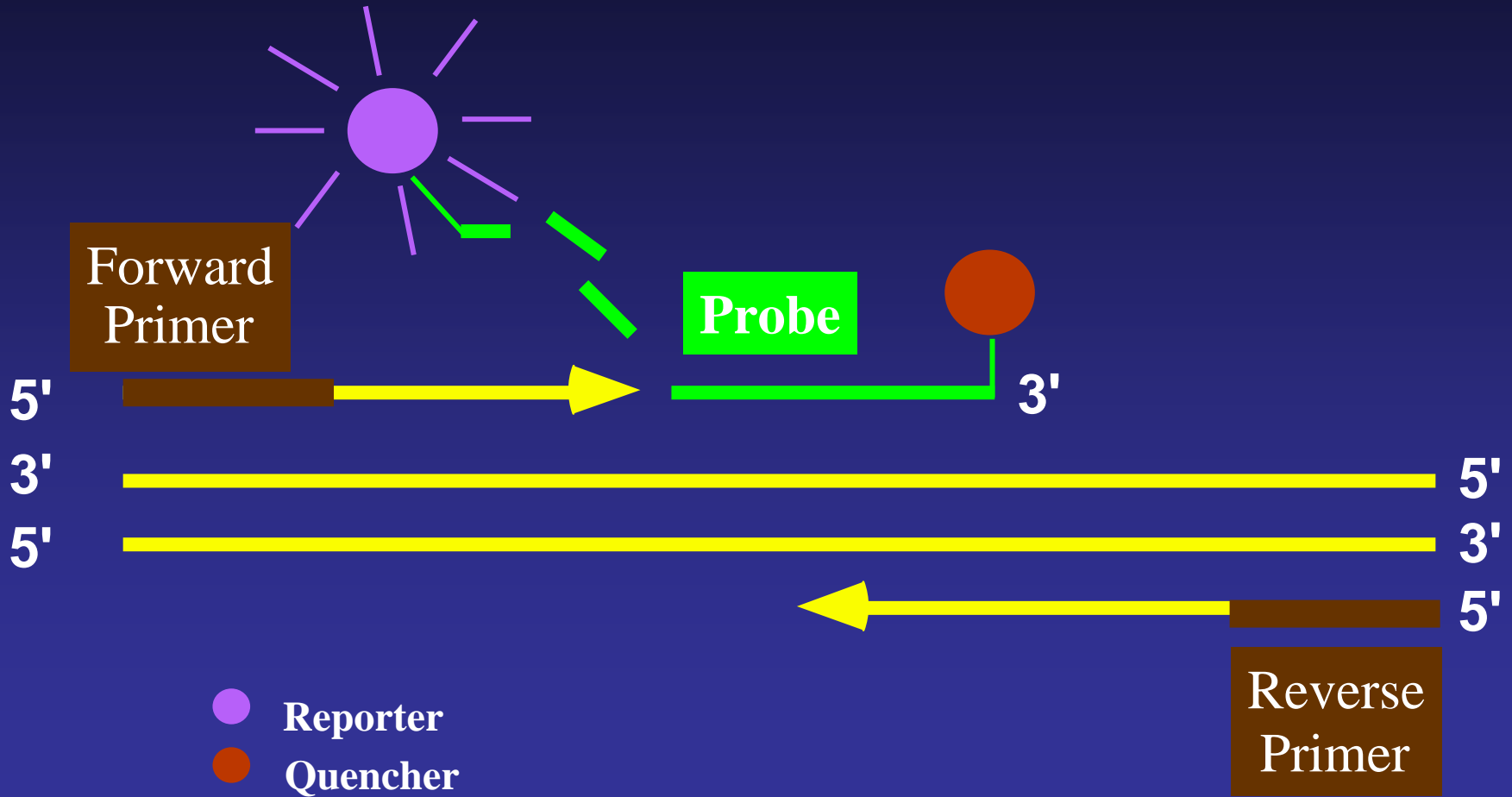
Polymerization



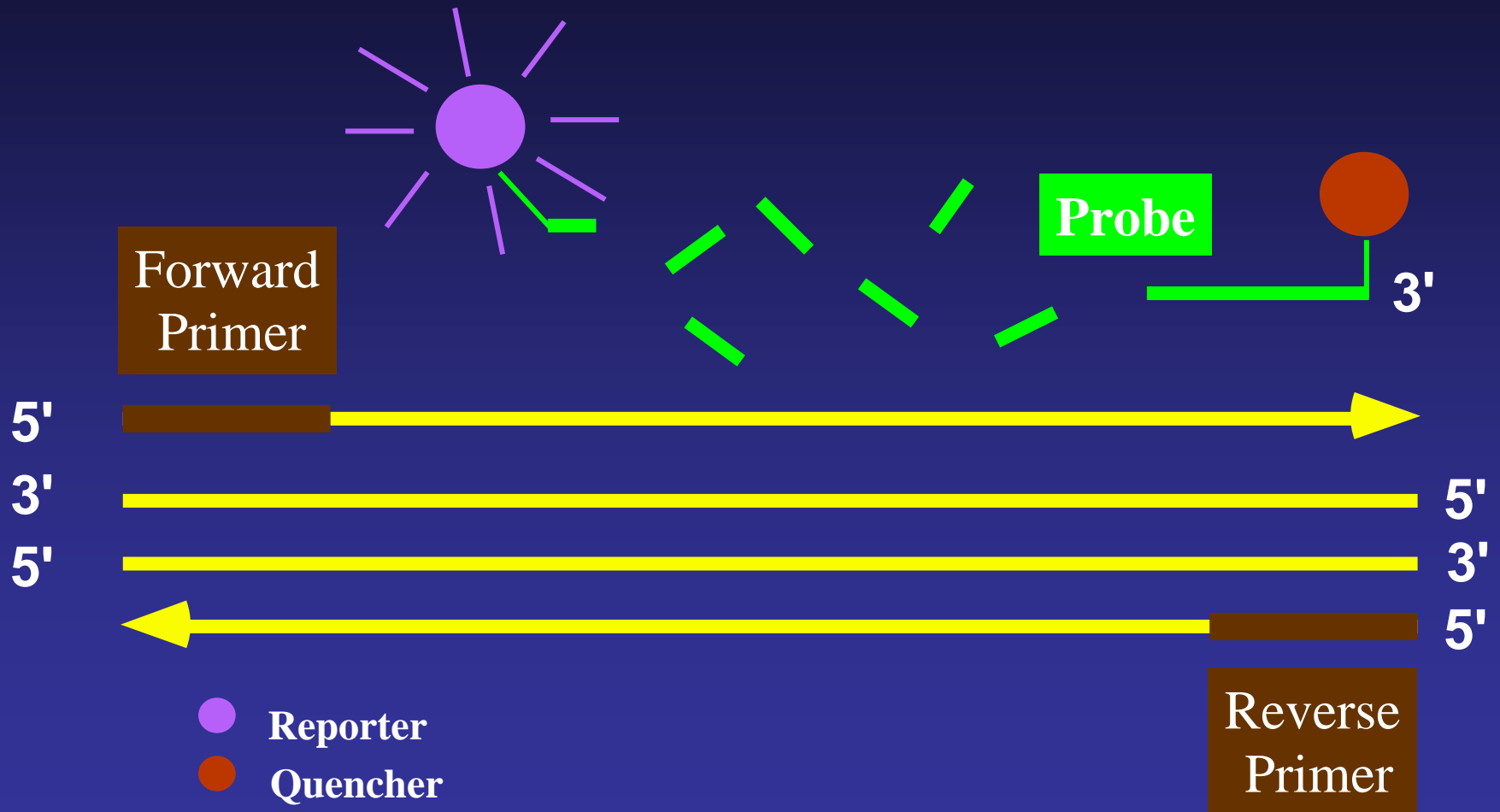
Strand Displacement



Cleavage

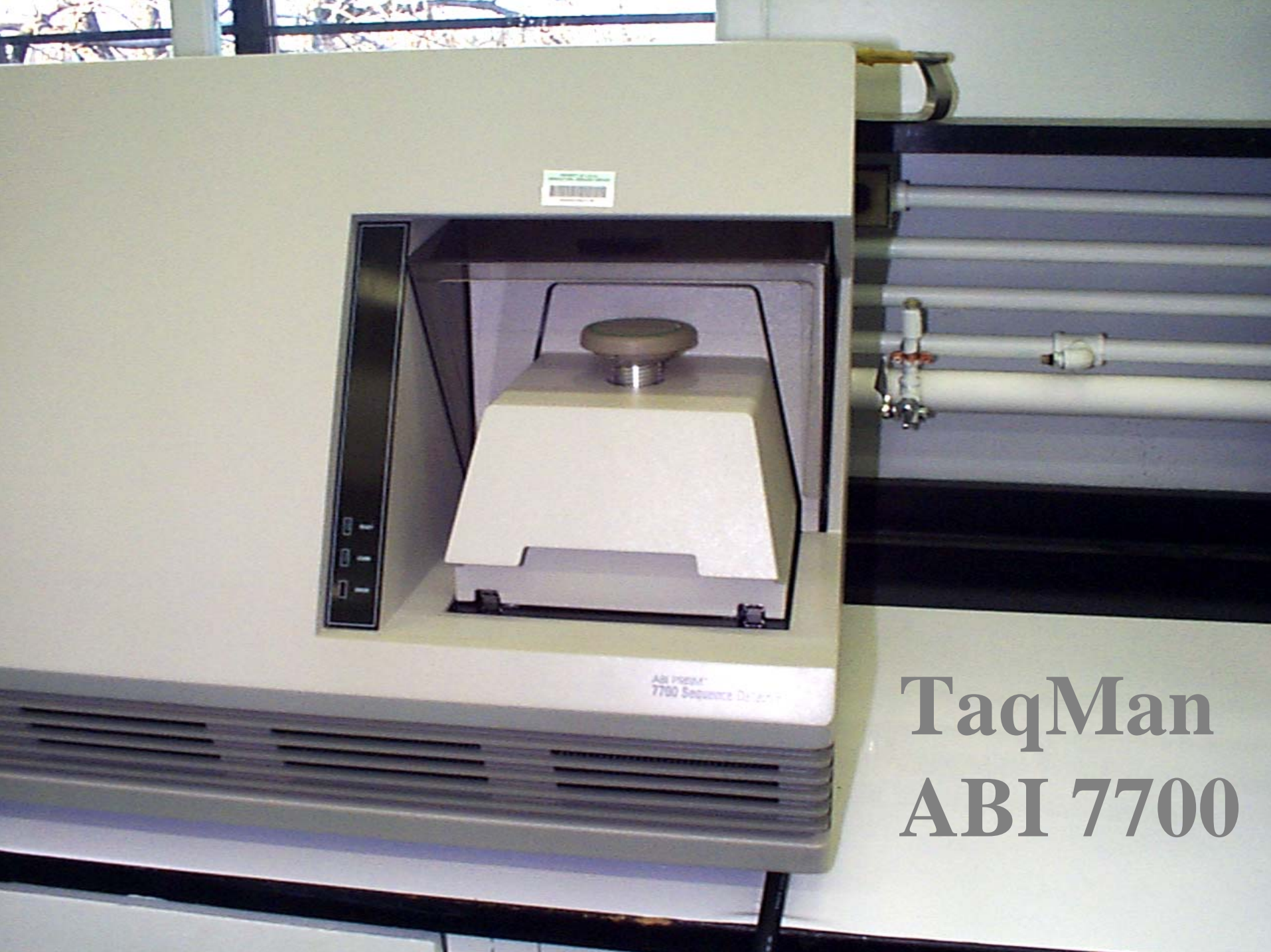


Polymerization Completed





- **Combines the sensitivity of PCR along with hybridization.**
- **Post-PCR confirmation steps are eliminated**
 - **No agarose gels & Southern blots**
- **Assays are very rapid**
- **Potential for multiplexing**
 - **Several probes of a particular pathogen**
 - **Specific probes from several pathogens**



Barcode label

ABI 7700
7700 Sequence Detector

TaqMan
ABI 7700

Smart Cyclor® XC



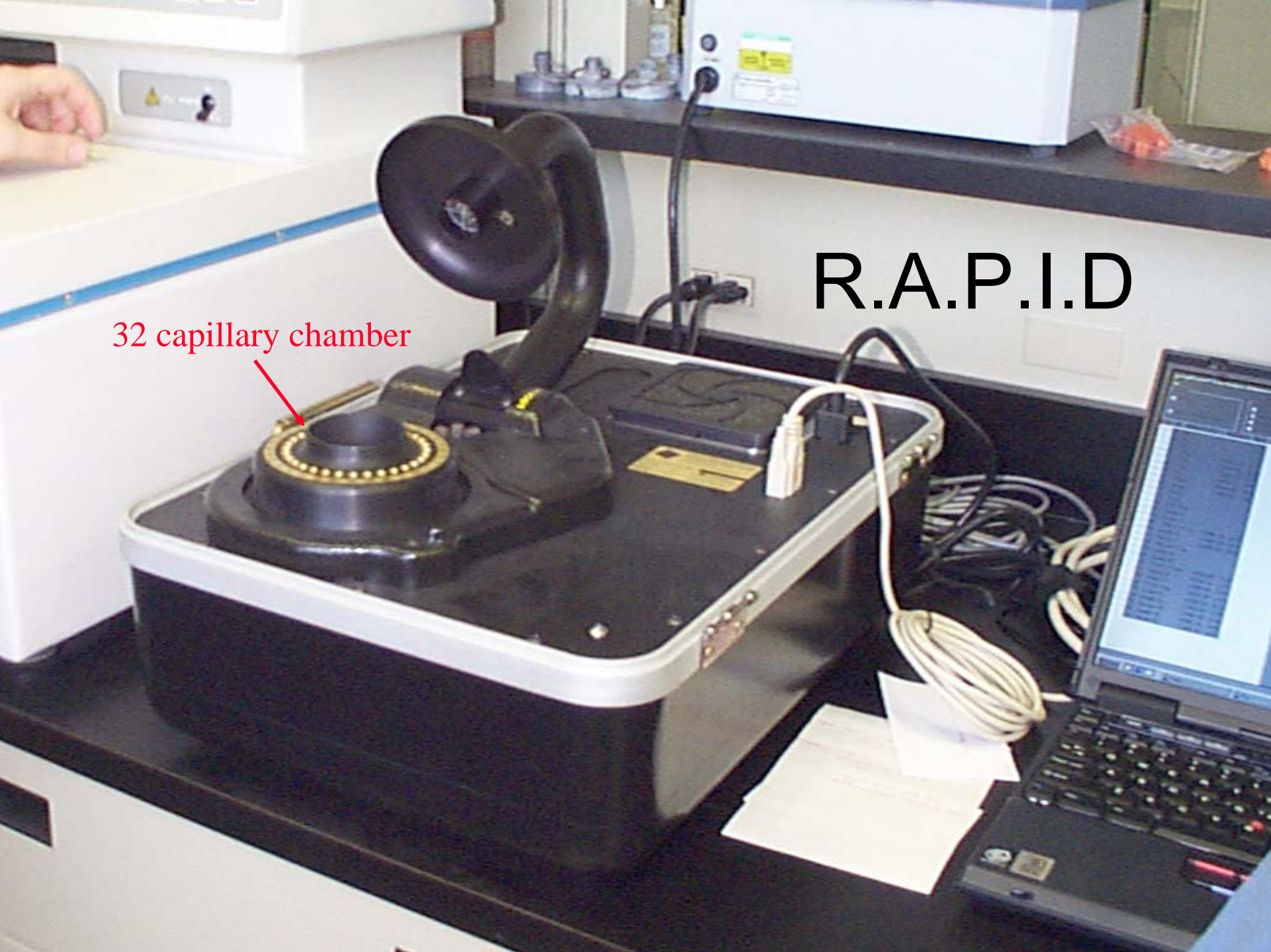
External Case

- Ruggedized
- Air Tight
- Water Proof

Desktop Smart Cyclor
for reference

R.A.P.I.D

32 capillary chamber



Gene Selection

P. pachyrhizi putative Heat-induced catalase, ATP-binding cassette (ABC) transporter, Plasma membrane (H⁺) ATPase and two constitutive genes, putative Alpha and Beta-tubulin, were selected from the ESTs from the germinated spores to study their gene expression during the infection cycle of *P. pachyrhizi* on soybean.

RNA

Total RNA (40ng) from non-infected plant, germinating spores, infected leaf tissue from 1, 2, 4, 6, 8, 10, 12 and 14 dpi were used as template. Positive controls were performed using fungal DNA (25ng), while RNase treated RNA samples and no template were used as negative controls.

Real Time RT-PCR

Real Time RT-PCR was performed in the ABI Prism 7700 (Perkin Elmer) with 40ng of total RNA using the SuperScript One-Step RT-PCR with Platinum Taq Kit (Invitrogen), following the manufacturer's protocol for a 25 μ l reaction.

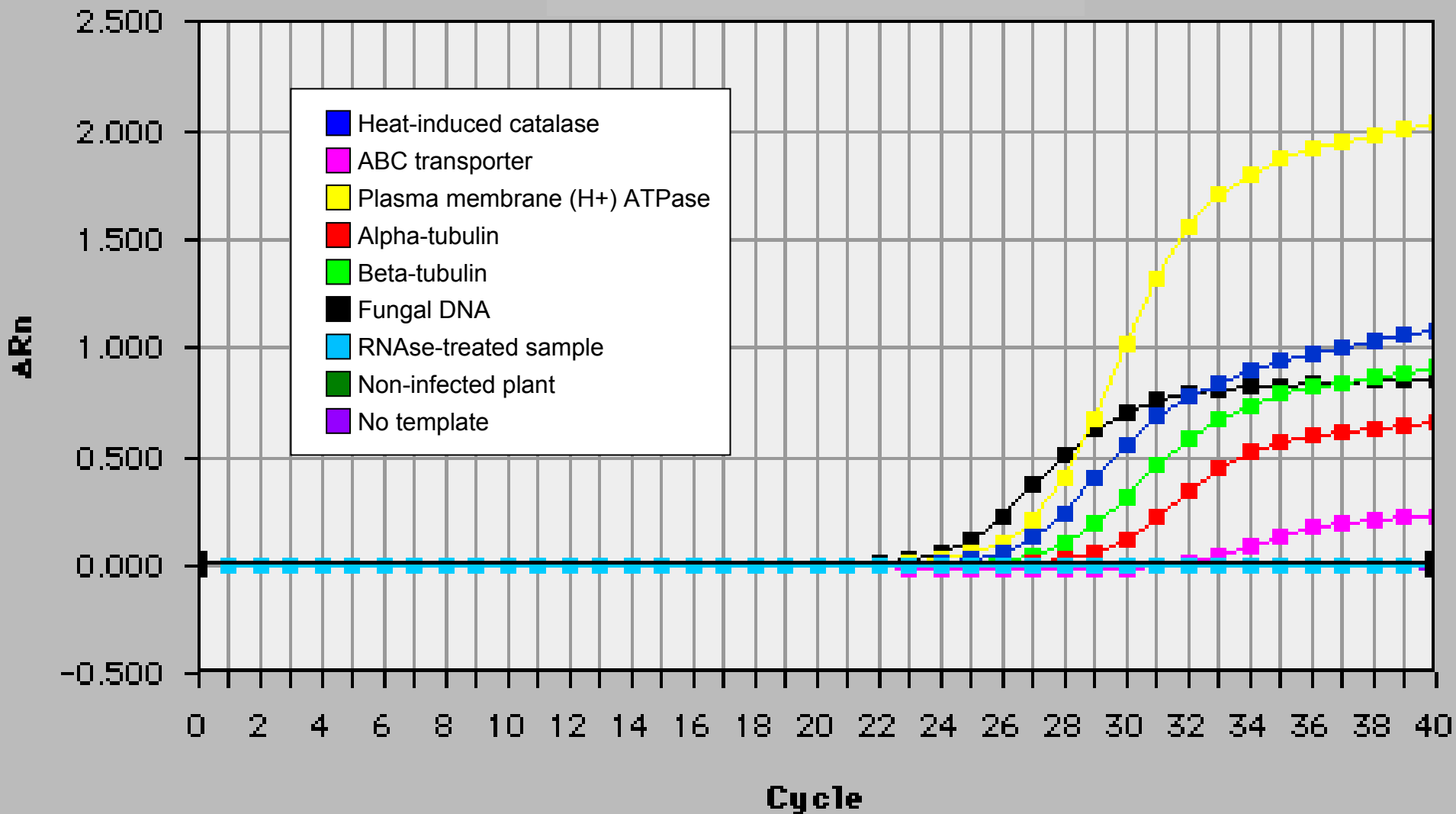
Primers and probes designed for Real Time RT-PCR assays

Putative Gene	Forward Primer	Fluorogenic Probe	Reverse Primer	Amplicon
Heat-induced Catalase	CCTGGTGTAGAGCCTTCTGCA	FAM- ACCCAGTCCTTCAATCGAGGCTATTTTCC-TAMRA	TGACGATGGGTGTCAGGGT	70
ABC Transporter	GAAACATTGGATGTACAACCTGGA	FAM- CCCTATACTCGATTGATTGGTGGACTGCTTG-TAMRA	TCGAGTCGTGCAGCTCATT	76
Plasma membrane (H+) ATPase	TCGTTACACGGCTGGTTT	FAM- TTTATGGAGAGACCATCGGCGGCTT-TAMRA	AGCAATCAGAAAAGCGCCC	68
Alpha-tubulin	CCAAGGCTTCTTCGTGTTTCA	FAM- TCGTTTGGAGGCGGACTGGTTCA-TAMRA	CAAGAGAAGAGCGCCAAACC	65
Beta-tubulin	CCCCGTGCAGTTTTGATTG	FAM- TTGGAACCAGGAACCATGGATTCCG-TAMRA	CCAAAAGTCCCGGATCGA	64

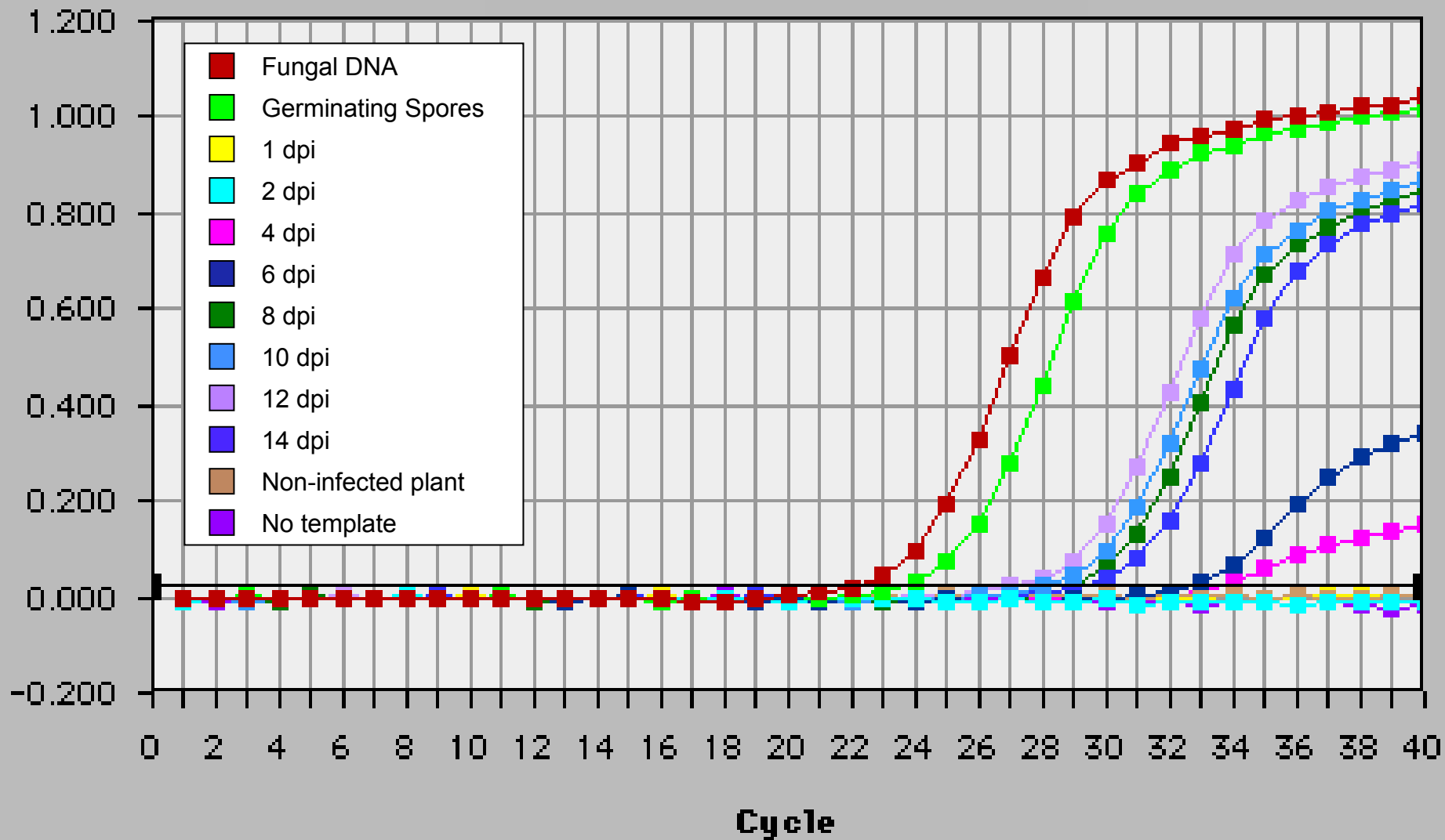
Putative genes of *P. pachyrrhizi* selected from the germinating spores cDNA library. Primers and probes were designed using Primer Express 1.0 (Perkin Elmer). Primers (Operon); Probes (Synthegen).

Thermal cycling conditions (ABI 7700):

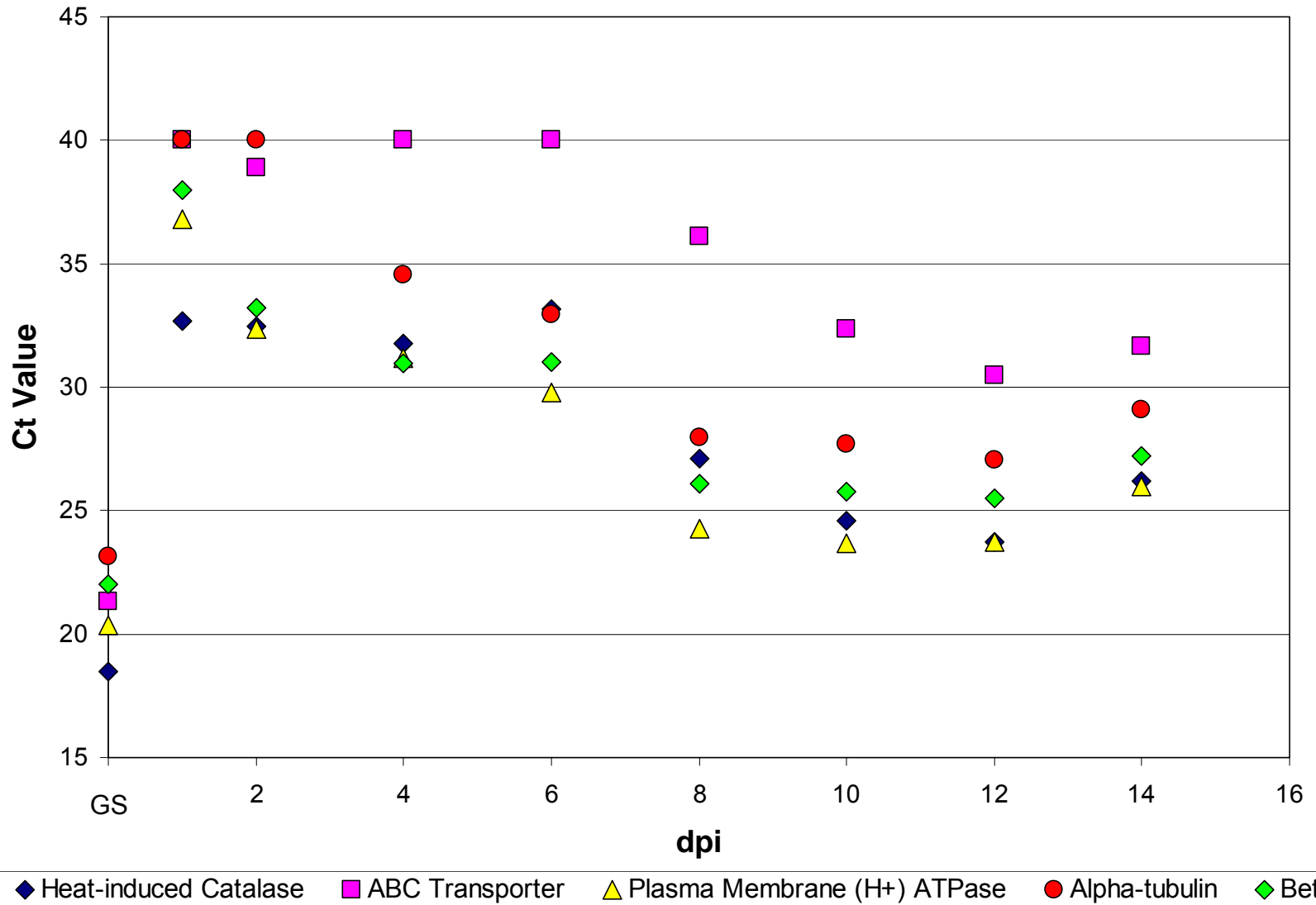
50°C for 15 min (hold)
95°C for 10 min (hold)
40 cycles: 95°C for 15 s
60°C for 1 min



Real Time RT-PCR spectra for 10dpi. Fungal DNA (positive control), RNase treated sample and no template (negative controls).



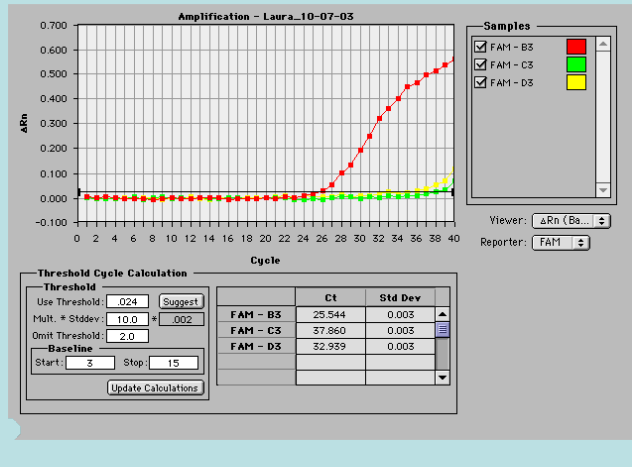
Real Time RT-PCR spectra for Alpha-tubulin



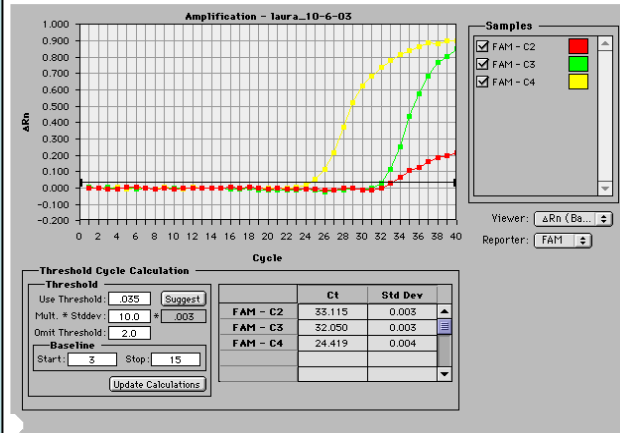
Expression patterns of five putative genes over the infection cycle of *P. pachyrhizi* on *G. max* generated using Real Time RT-PCR. C_T (threshold cycle) is the cycle in which a significant increase in ΔR_n is detected. Germinating spores (GS) were used as a positive control. dpi: days post inoculation

heat-induced catalase - 60C, 1 min

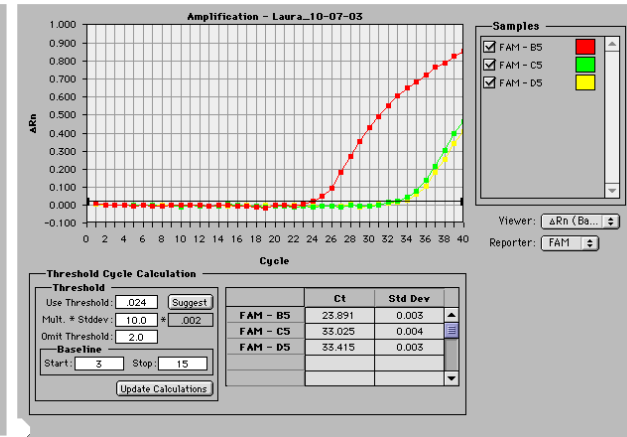
528 2mM MgSO4, 60C, 1 min:
10-7-03; FDNA, NIP, NT



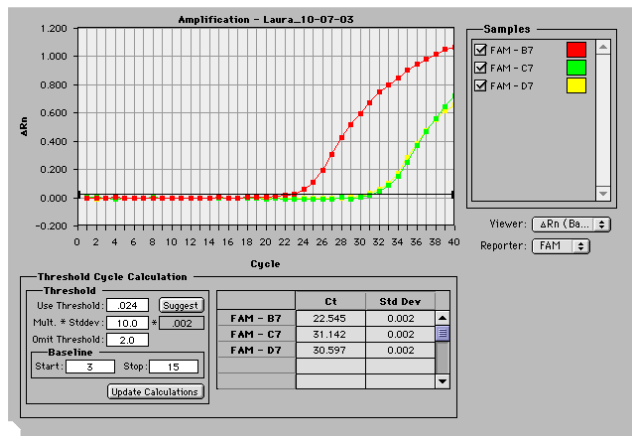
528 3mM MgSO4, 60C, 1 min:
10-6-03; NT, NIP, FDNA



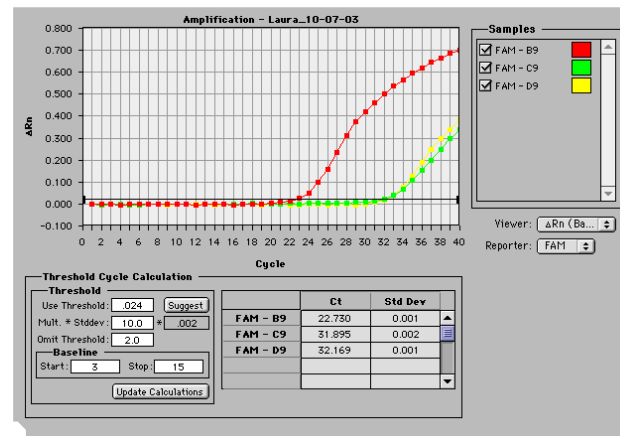
528 3mM MgSO4, 60C, 1 min:
10-7-03; FDNA, NIP, NT



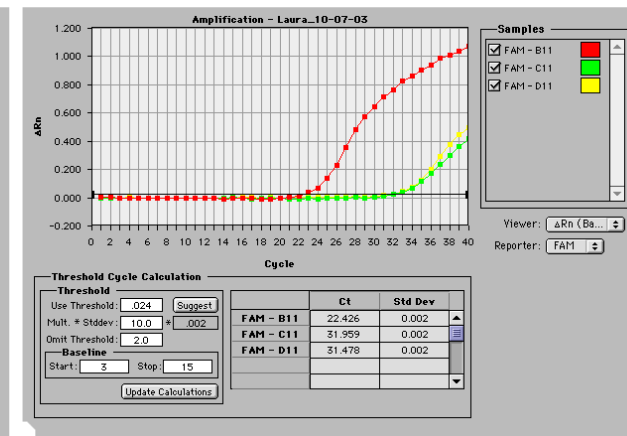
528 4mM MgSO4, 60C, 1 min:
10-7-03; FDNA, NIP, NT



528 5mM MgSO4, 60C, 1 min:
10-7-03; FDNA, NIP, NT

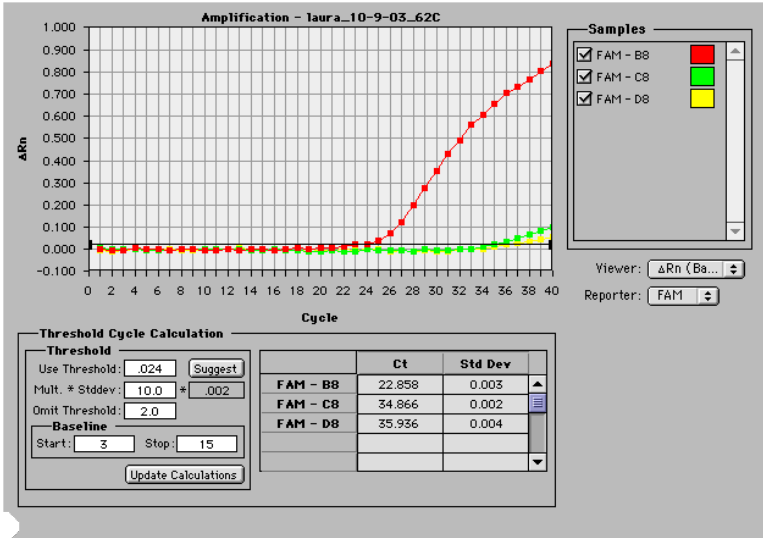


528 5mM MgSO4, 60C, 1 min:
10-7-03; FDNA, NIP, NT

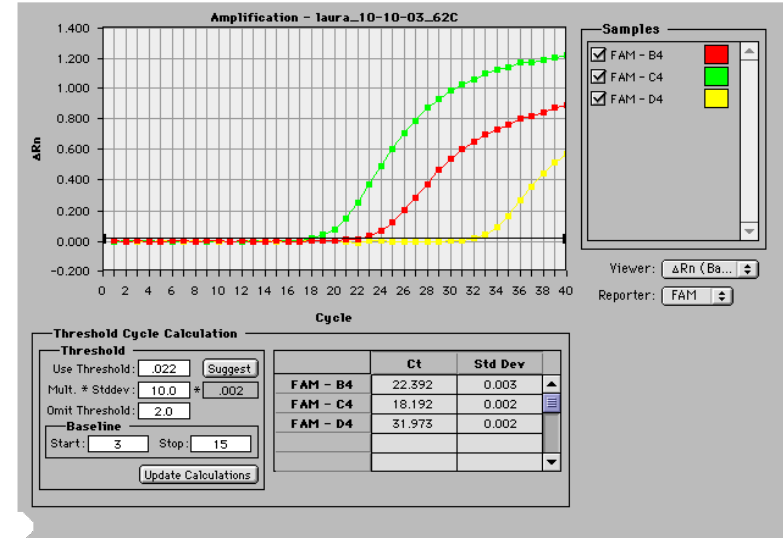


heat-induced catalase – 62 C, 30 sec

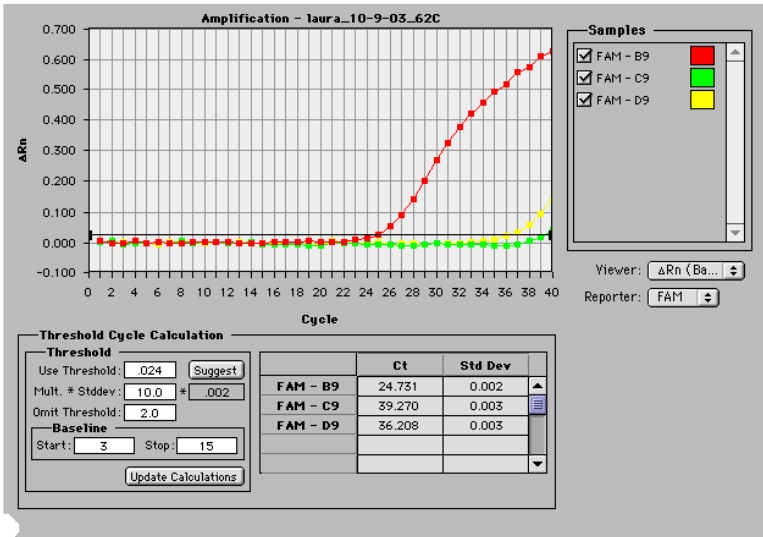
528 4mM MgSO₄, 62C, 30 sec:
10-9-03; FDNA, NIP, NT



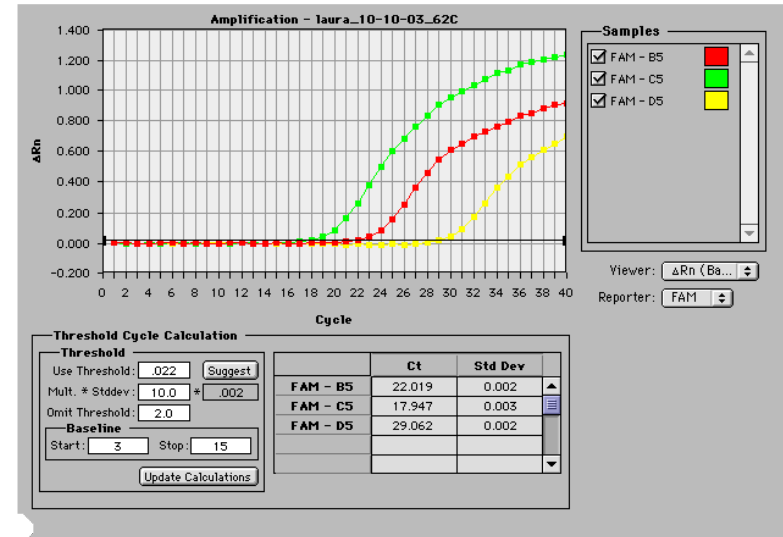
528 5mM MgSO₄, 62C, 30 sec:
10-9-03; FDNA, GS RNA, NT



528 6mM MgSO₄, 62C, 30 sec:
10-9-03; FDNA, NIP, NT



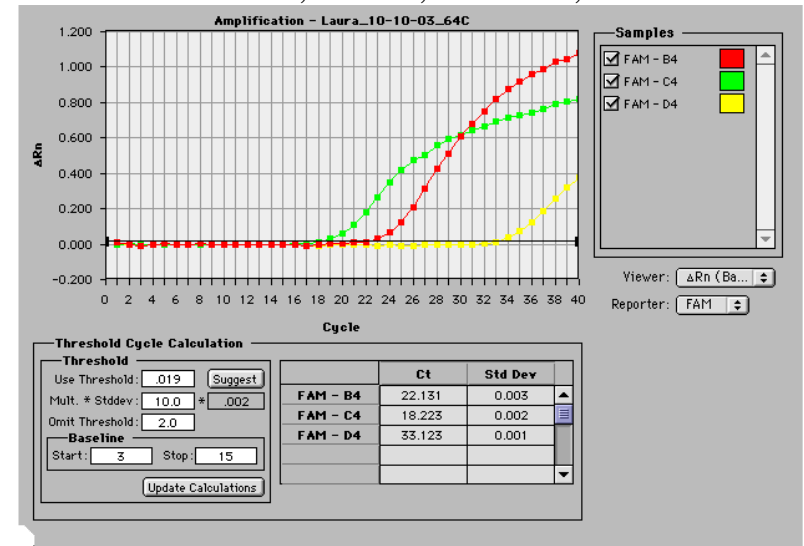
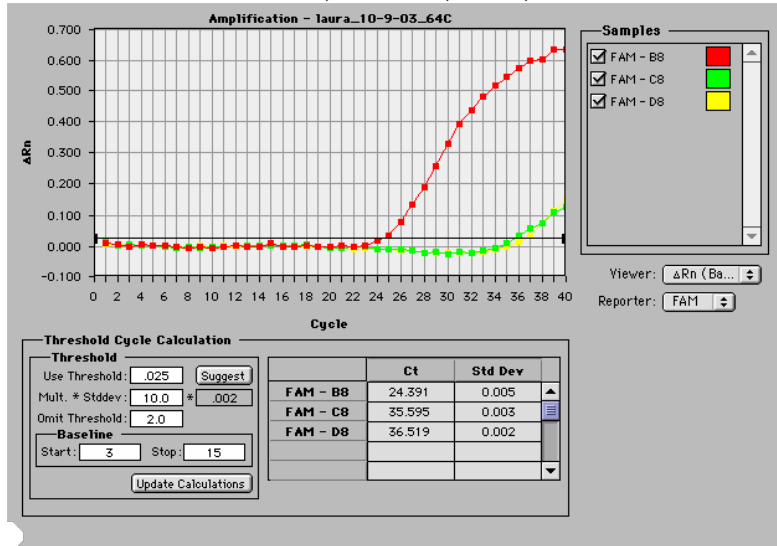
528 5mM MgSO₄/BSA, 62C, 30 sec:
10-9-03; FDNA, GS RNA, NT



heat-induced catalase – 64 C, 30 sec

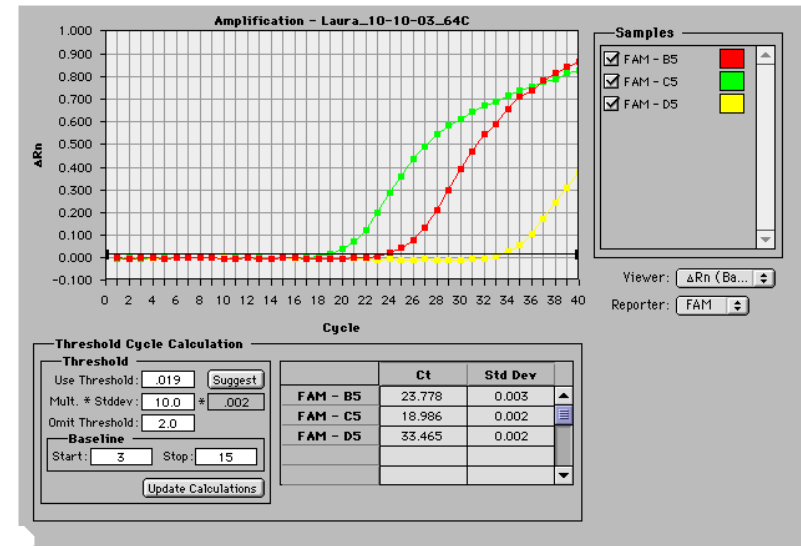
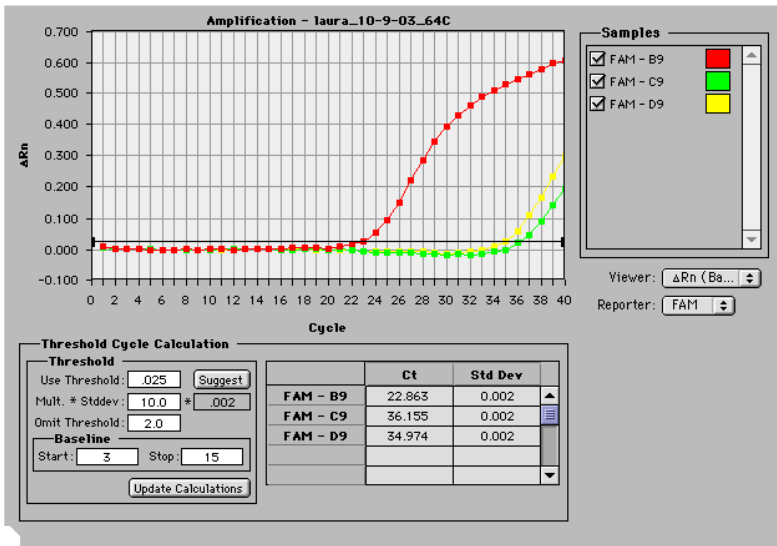
528 4mM MgSO₄, 64C, 30 sec:
10-9-03; FDNA, NIP, NT

528 5mM MgSO₄, 64C, 30 sec:
10-9-03; FDNA, GS RNA, NT



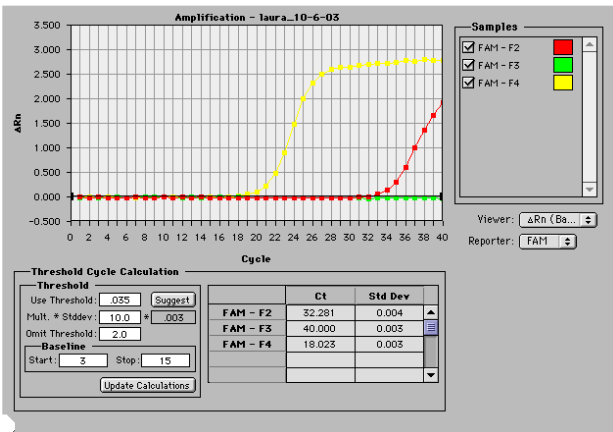
528 6mM MgSO₄, 64C, 30 sec:
10-9-03; FDNA, NIP, NT

528 6mM MgSO₄/BSA, 64C, 30 sec:
10-9-03; FDNA, GS RNA, NT

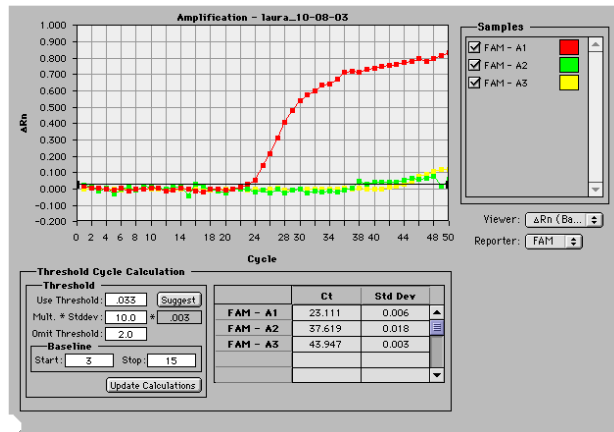


Plasma membrane (H⁺) ATPase – 60C, 1 min

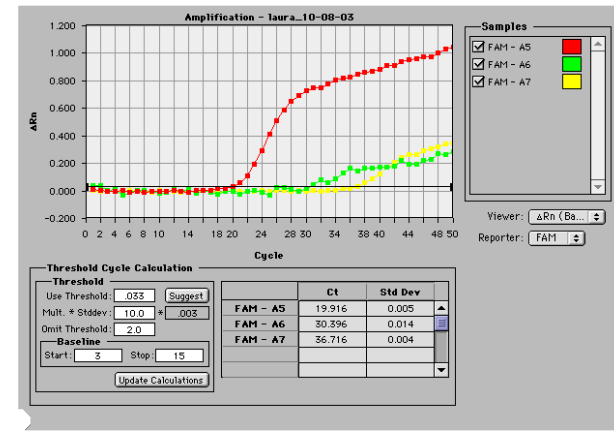
1340 3mM MgSO₄, 60C, 1 min: 10-6-03



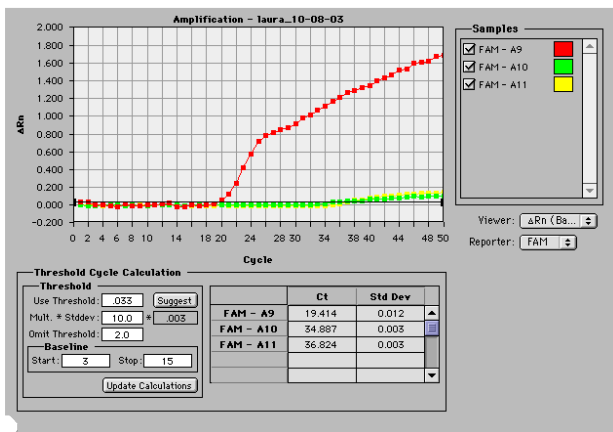
1340 2mM MgSO₄, 60C, 1 min: 10-8-03; FDNA, NIP, NT



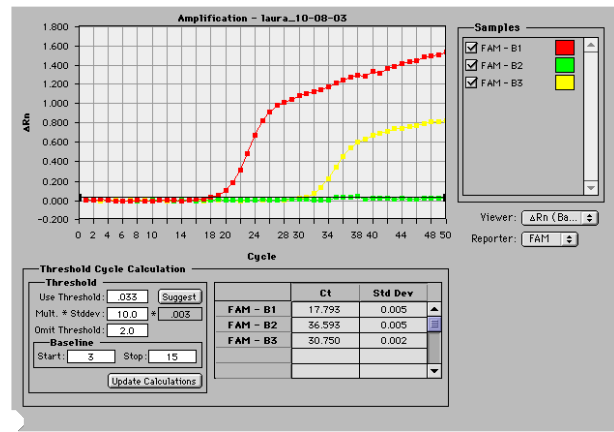
1340 3mM MgSO₄, 60C, 1 min: 10-8-03; FDNA, NIP, NT



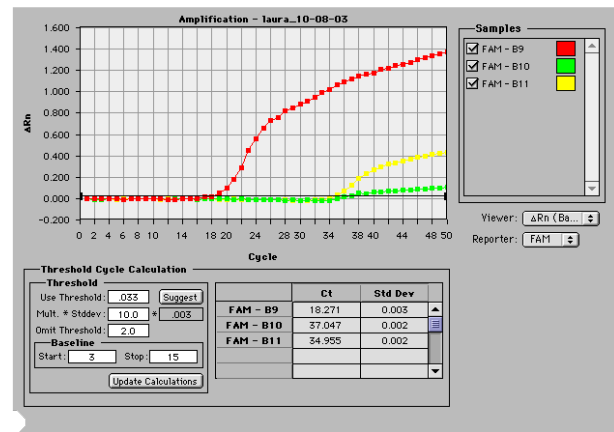
1340 4mM MgSO₄, 60C, 1 min: 10-8-03; FDNA, NIP, NT



1340 5mM MgSO₄, 60 C, 1 min: 10-8-03; FDNA, NIP, NT

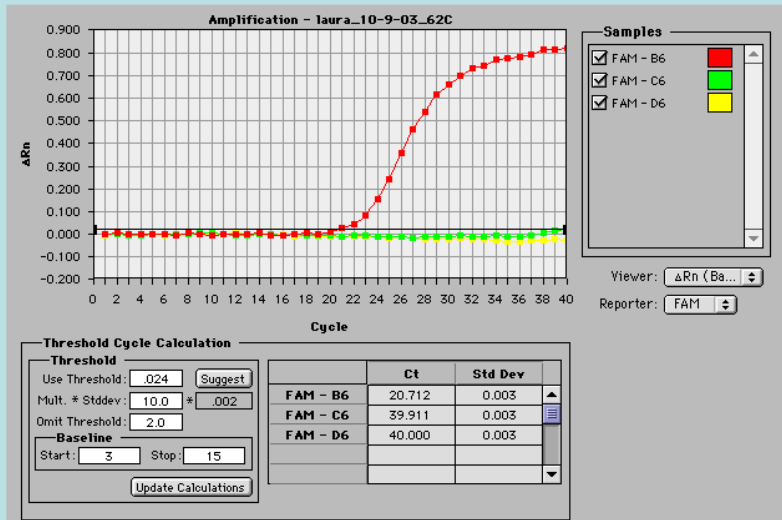


1340 6mM MgSO₄, 60C, 1 min: 10-8-03; FDNA, NIP, NT

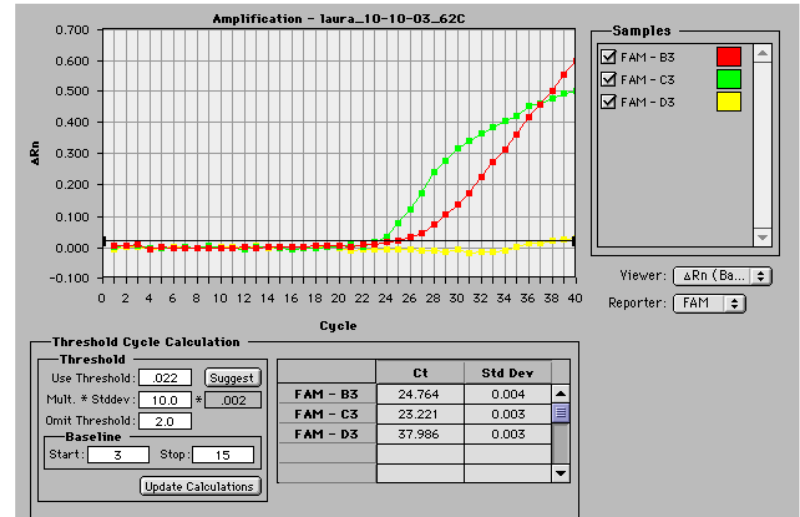


Plasma membrane (H⁺) ATPase – 4mM

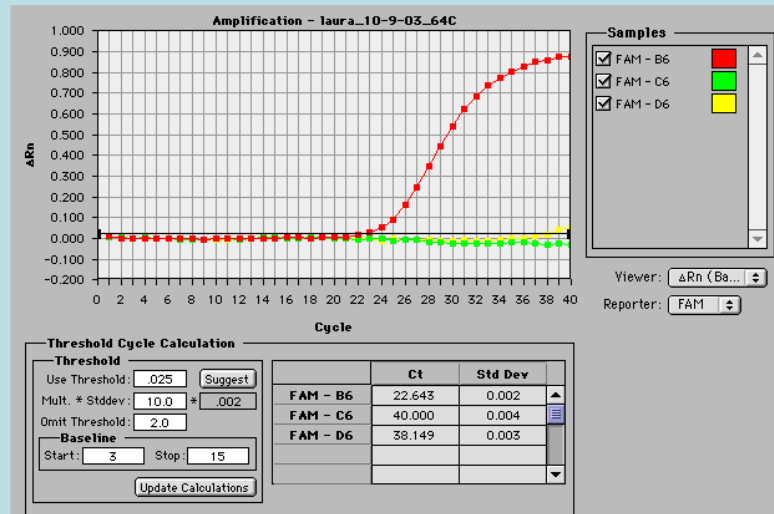
1340 4mM MgSO₄, 62C, 30 sec:
10-9-03; FDNA, NIP, NT



1340 4mM MgSO₄, 62C, 30 sec:
10-9-03; FDNA, GS RNA, NT



1340 4mM MgSO₄, 64C, 30 sec:
10-9-03; FDNA, NIP, NT

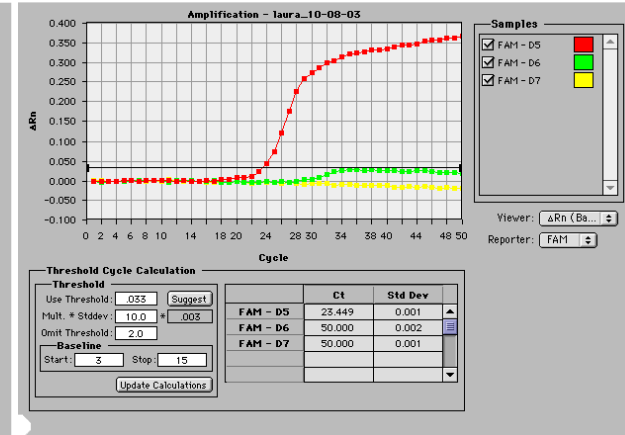
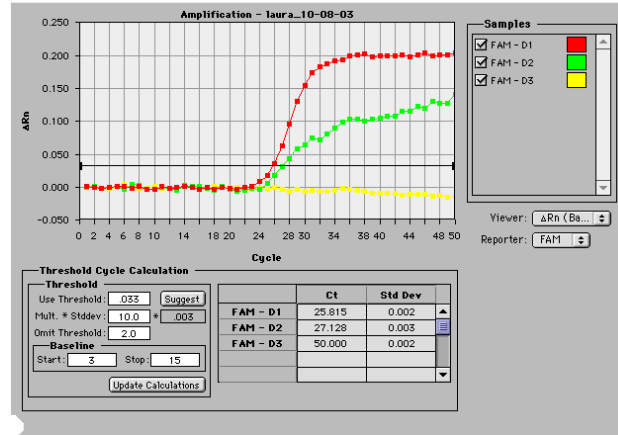
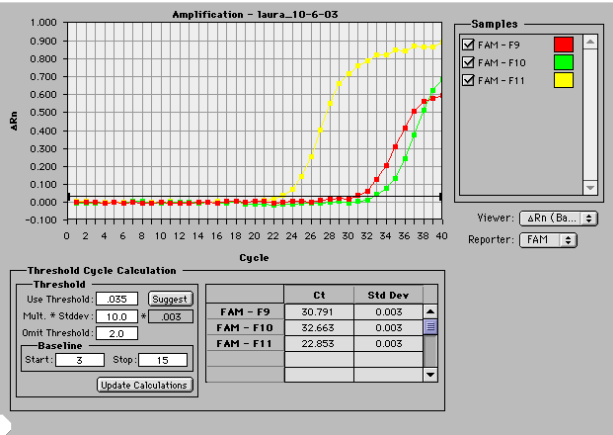


Alpha-tubulin, 60C, 1 min

A-tub 3mM MgSO₄, 60C,
1 min: 10-6-03; FDNA,
NIP, NT

A-tub 2mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT

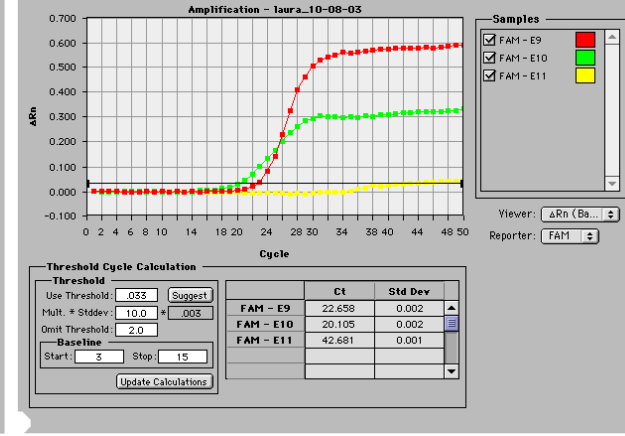
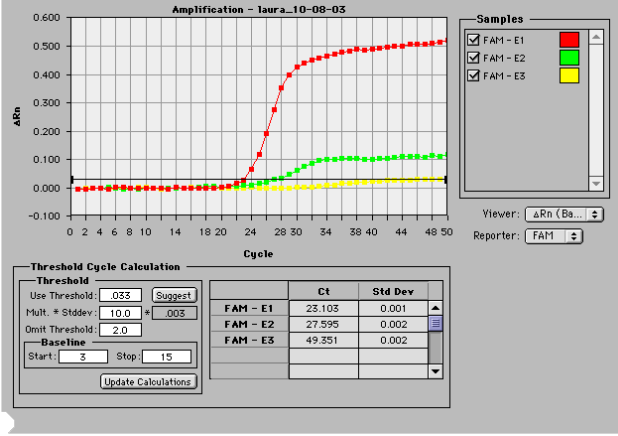
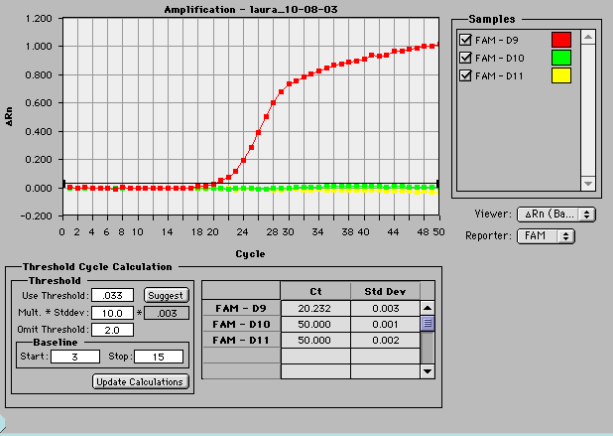
A-tub 3mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT



A-tub 4mM MgSO₄, 60C, 1
min: 10-8-03; FDNA, NIP, NT

A-tub 5mM MgSO₄, 60C, 1
min: 10-8-03; FDNA, NIP, NT

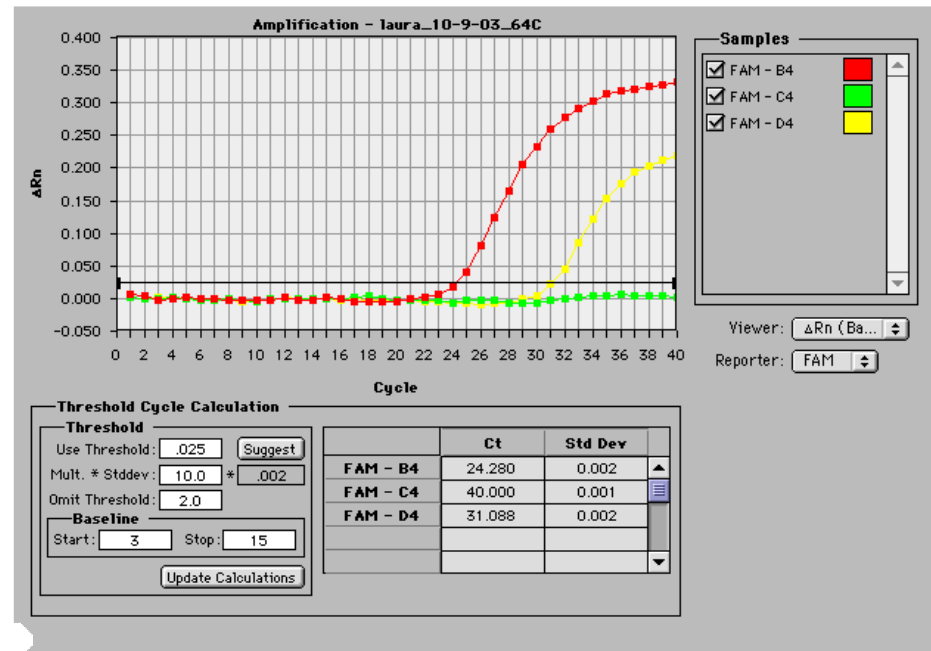
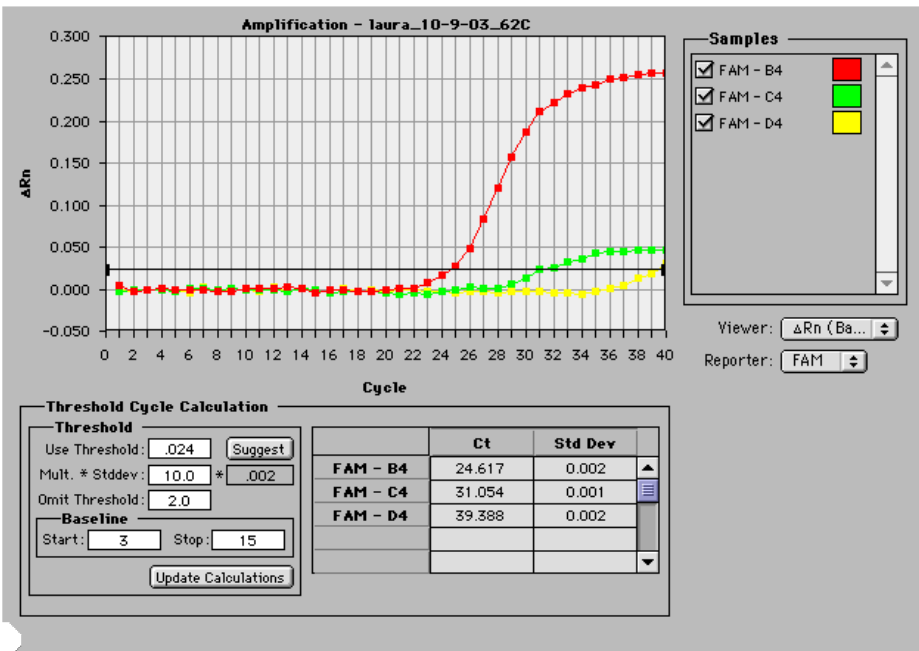
A-tub 6mM MgSO₄, 60C, 1
min: 10-8-03; FDNA, NIP, NT



Alpha-tubulin 4mM MgSO₄, 30 sec

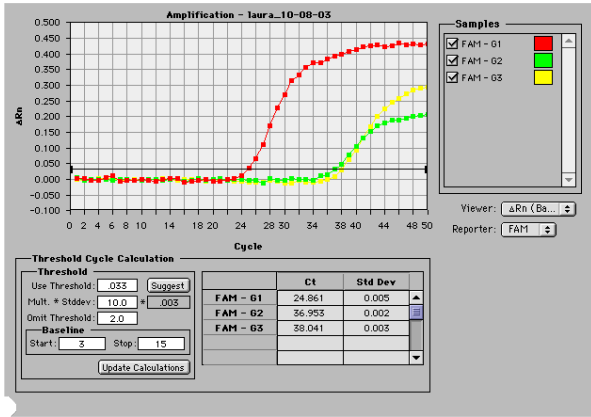
A-tub 4mM MgSO₄, 62C, 30 sec: 10-9-03; FDNA, NIP, NT

A-tub 4mM MgSO₄, 64C, 30 sec: 10-9-03; FDNA, NIP, NT

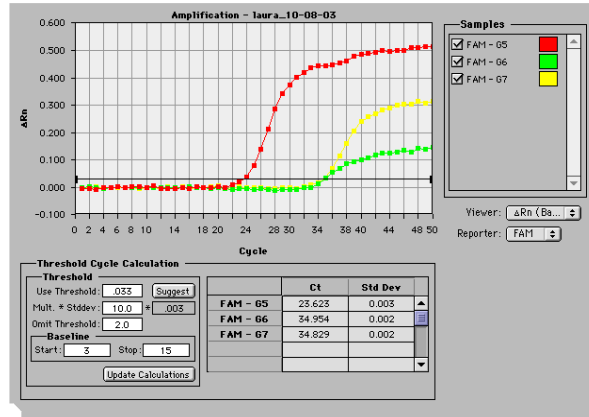


β -tub - 60C, 1 min

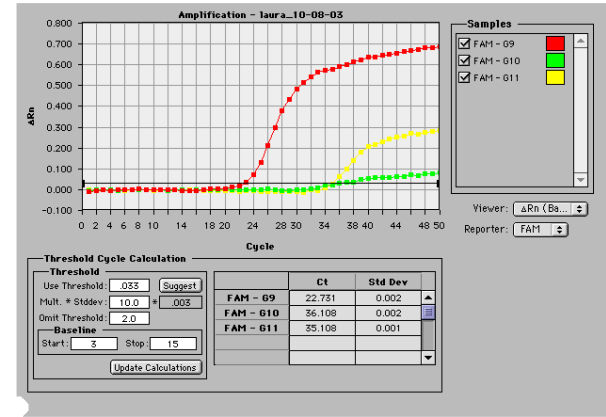
B-tub 2mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT



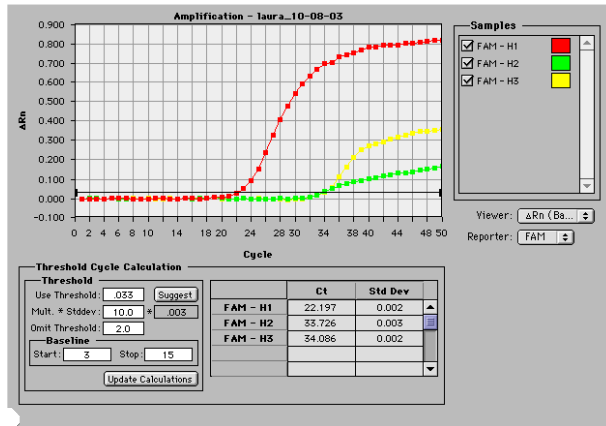
B-tub 3mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT



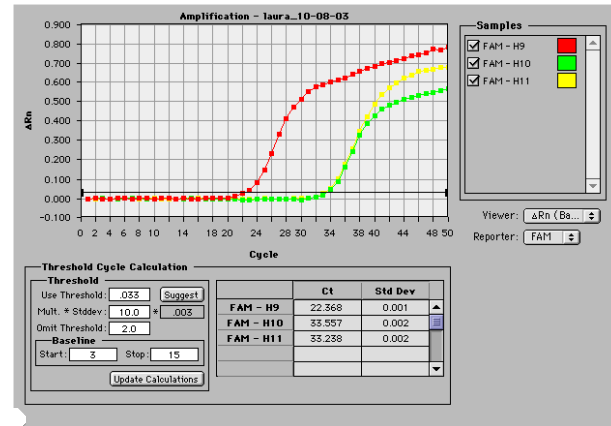
B-tub 4mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT



B-tub 5mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT

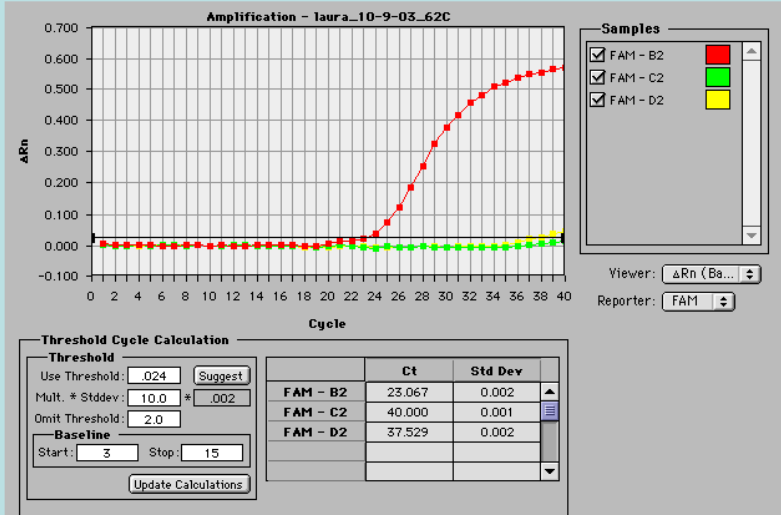


B-tub 6mM MgSO₄, 60C, 1 min:
10-8-03; FDNA, NIP, NT

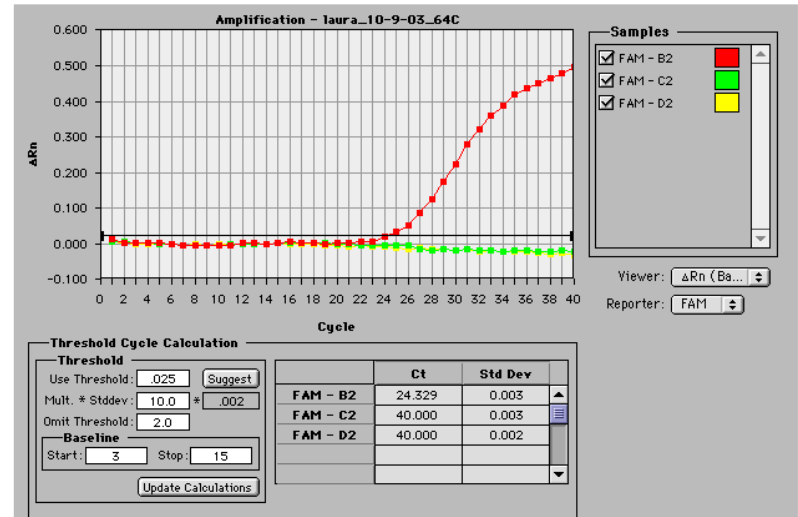


B-tub 4mM MgSO4, 30 sec:

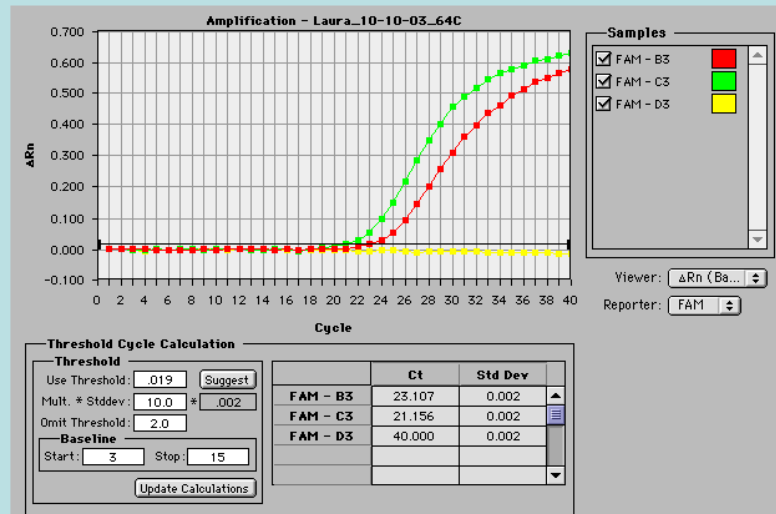
B-tub 4mM MgSO4, 62C, 30 sec:
10-9-03; FDNA, NIP, NT



B-tub 4mM MgSO4, 64C, 30 sec:
10-9-03; FDNA, NIP, NT

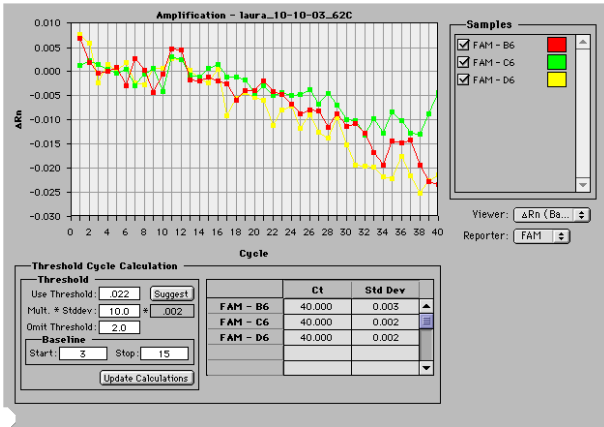


B-tub 4mM MgSO4, 64C, 30 sec:
10-9-03; FDNA, GS RNA, NT

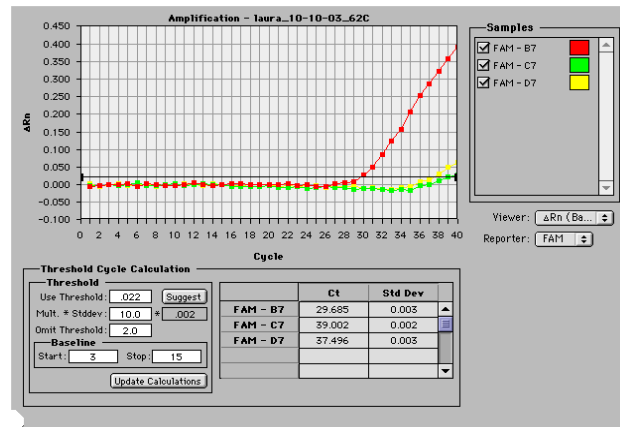


ABC transporter (ATP-binding cassette transporter) - 62C, 30 sec

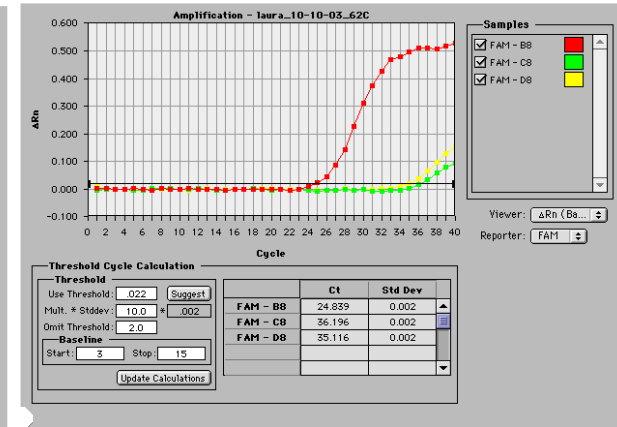
917 2mM MgSO₄, 62C, 30 sec: 10-10-03; FDNA, NIP, NT



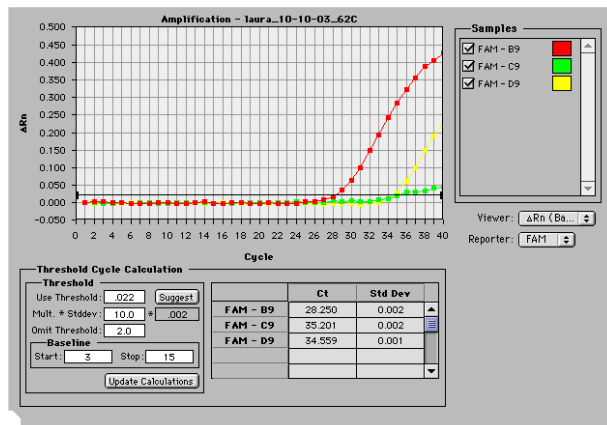
917 3mM MgSO₄, 62C, 30 sec: 10-10-03; FDNA, NIP, NT



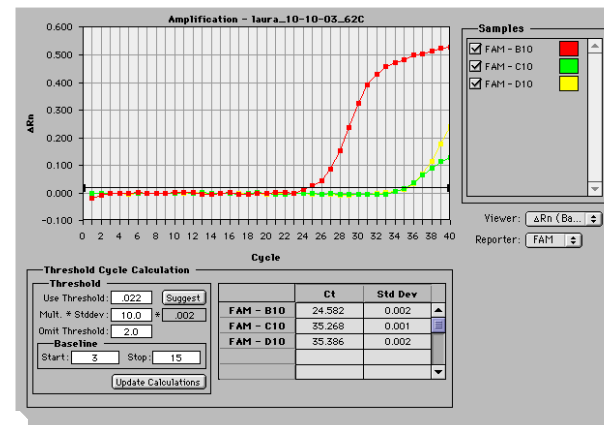
917 4mM MgSO₄, 62C, 30 sec: 10-10-03; FDNA, NIP, NT



917 5mM MgSO₄, 62C, 30 sec: 10-10-03; FDNA, NIP, NT

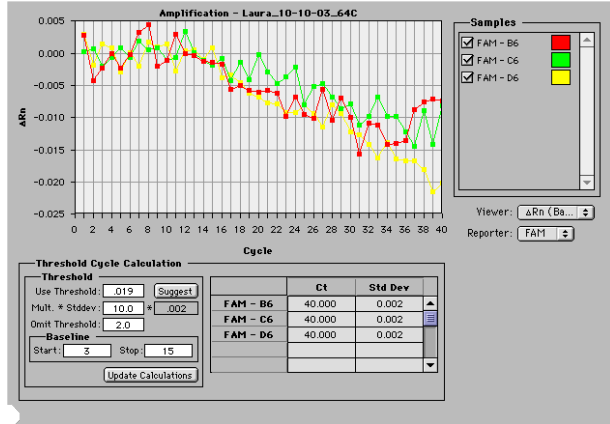


917 6mM MgSO₄, 62C, 30 sec: 10-10-03; FDNA, NIP, NT

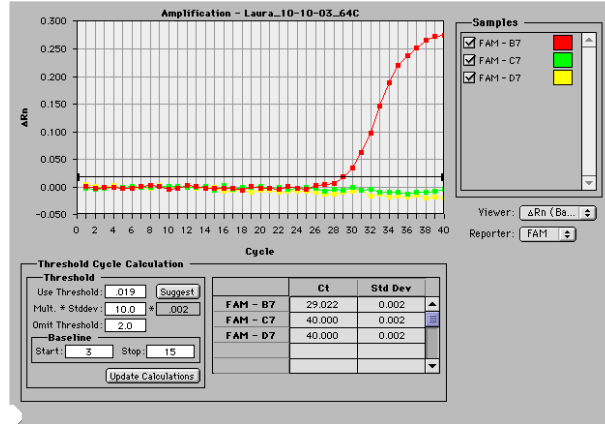


ABC transporter (ATP-binding cassette transporter) - 64C, 30 sec

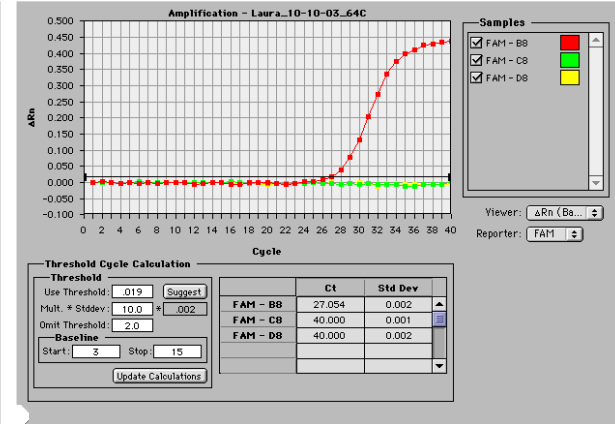
917 2mM MgSO₄, 64C, 30 sec: 10-10-03; FDNA, NIP, NT



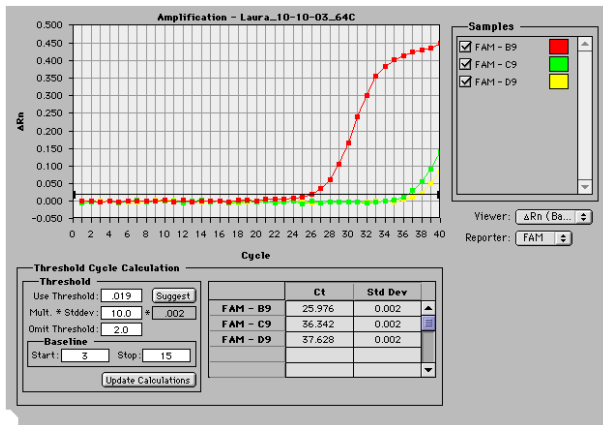
917 3mM MgSO₄, 64C, 30 sec: 10-10-03; FDNA, NIP, NT



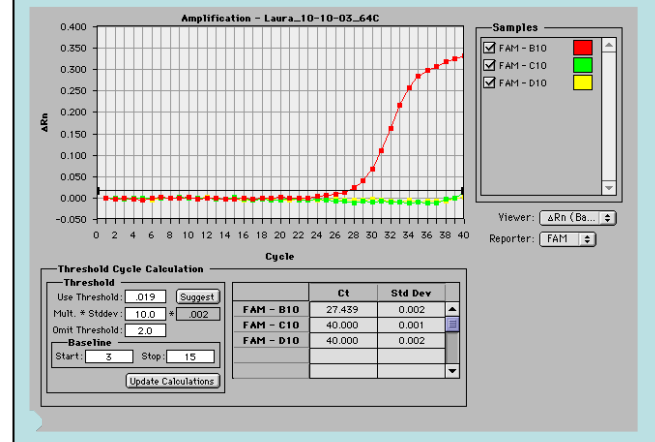
917 4mM MgSO₄, 64C, 30 sec: 10-10-03; FDNA, NIP, NT



917 5mM MgSO₄, 64C, 30 sec: 10-10-03; FDNA, NIP, NT



917 6mM MgSO₄, 64C, 30 sec: 10-10-03; FDNA, NIP, NT



Specific objectives

- Develop a suppression subtractive hybridization (SSH) library of the resistant interaction and identify transcripts/ESTs (Expressed Sequence Tags)

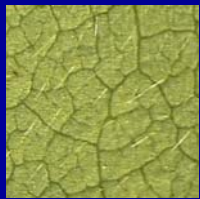
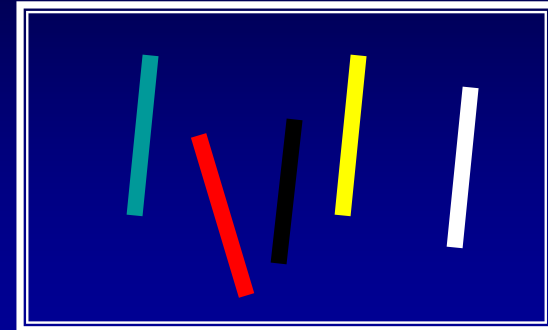
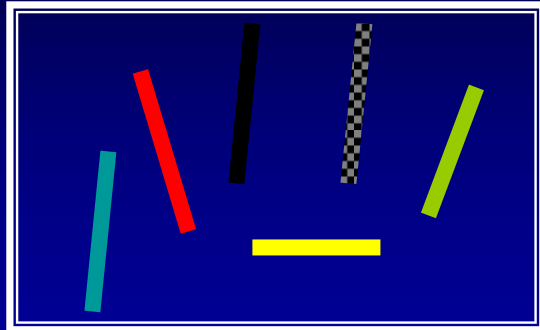
Suppression subtractive hybridization (SSH) cDNA library

Two libraries:

pooled RNA from $t = 1, 6, 12, 24, 48$ hpi
(each from first trifoliolate, from 4 plants)

- Forward Subtraction:
 - Tester = Komata/HW94 [Resistant/immune]
 - Driver = Komata/TW72 [Susceptible]
- Reverse Subtraction:
 - Tester = Komata/TW72 [Susceptible]
 - Driver = Komata/HW94 [Resistant/immune]

Suppression subtractive hybridization (SSH) cDNA library

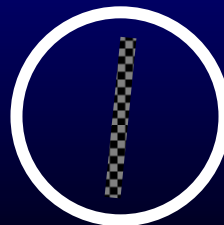
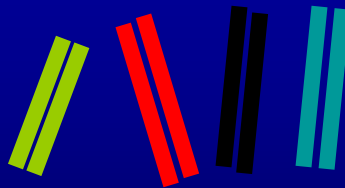


Resistant rxn
"Tester"



Susceptible rxn
"Driver"
(overabundance)

Suppress "common"
transcripts & pcr-select
"uncommon" from
tester



Activator or up-
regulated gene in a
resistant reaction

Suppression subtractive hybridization (SSH) cDNA library

- Our unique approach:
“driver”= susceptible interaction
- This should identify not just the general “defense-related” genes of typical pathogen invasion, also genes that are differentially turned on that prevents the disease from progressing
- Suppression should allow for the identification of unique, rare gene expression

Suppression subtractive hybridization (SSH) cDNA library

Results:

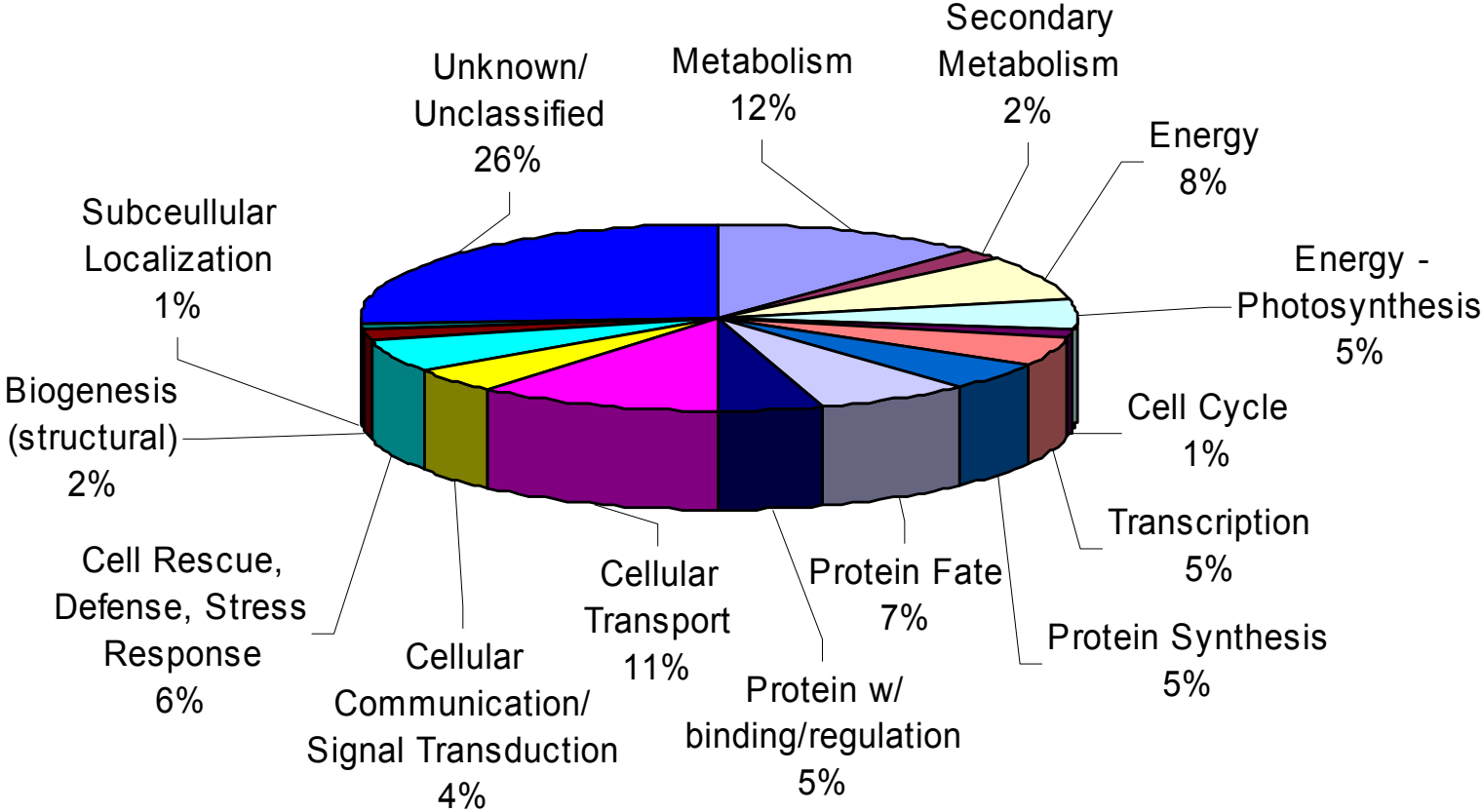
Forward Subtraction Library:

- 1056 clones sent for single-pass sequencing
[Nucleic Acid Facility (NAF) at USDA-ARS-ERRC in Wyndmoor, PA]
- 45 clones did not sequence
- Due to method (blunt-digest) clones with multi-inserts (~15%), 1182 ESTs
- Insert sizes of EST ranged from 52nt to >600nt, no full-length transcripts were identified
- A low-redundant subset of 979 EST

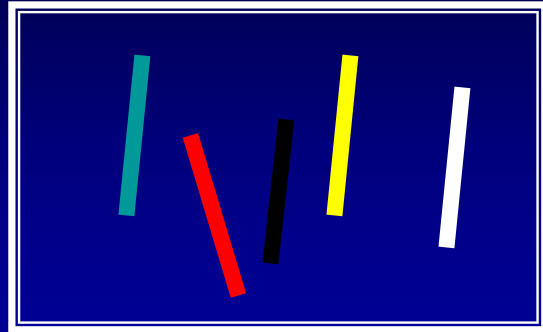
Subtractive suppressive cDNA library Data Analysis

- Comparative genomics using sequence-similarity tool BLAST (Basic local alignment search tool)
 - BLASTx = protein database
 - EST = dbEST
 - Unigene = compiled cluster of sequences from ESTs/mRNA/genomics projects
- Further analysis into Functional Categories (MIPS- Munich Information Center for Protein Sequences)

Results: Forward Subtraction Functional Categories



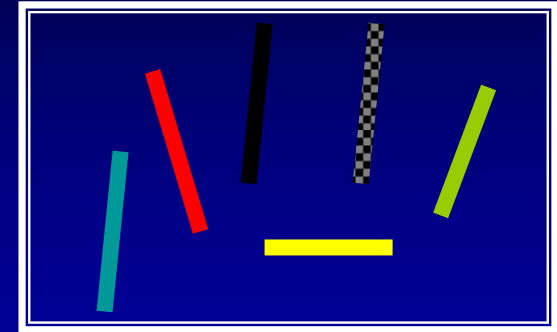
Suppression subtractive hybridization (SSH) cDNA library



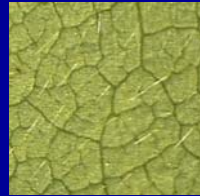
Susceptible rxn
"Tester"



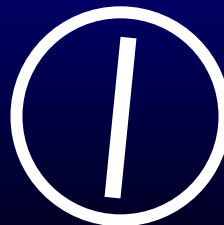
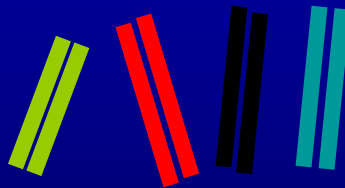
Reverse
subtraction



Resistant rxn
"Driver"
(overabundance)



Suppress "common"
transcripts & pcr-select
"uncommon" from
tester



Repressor/other
regulated gene in a
susceptible reaction

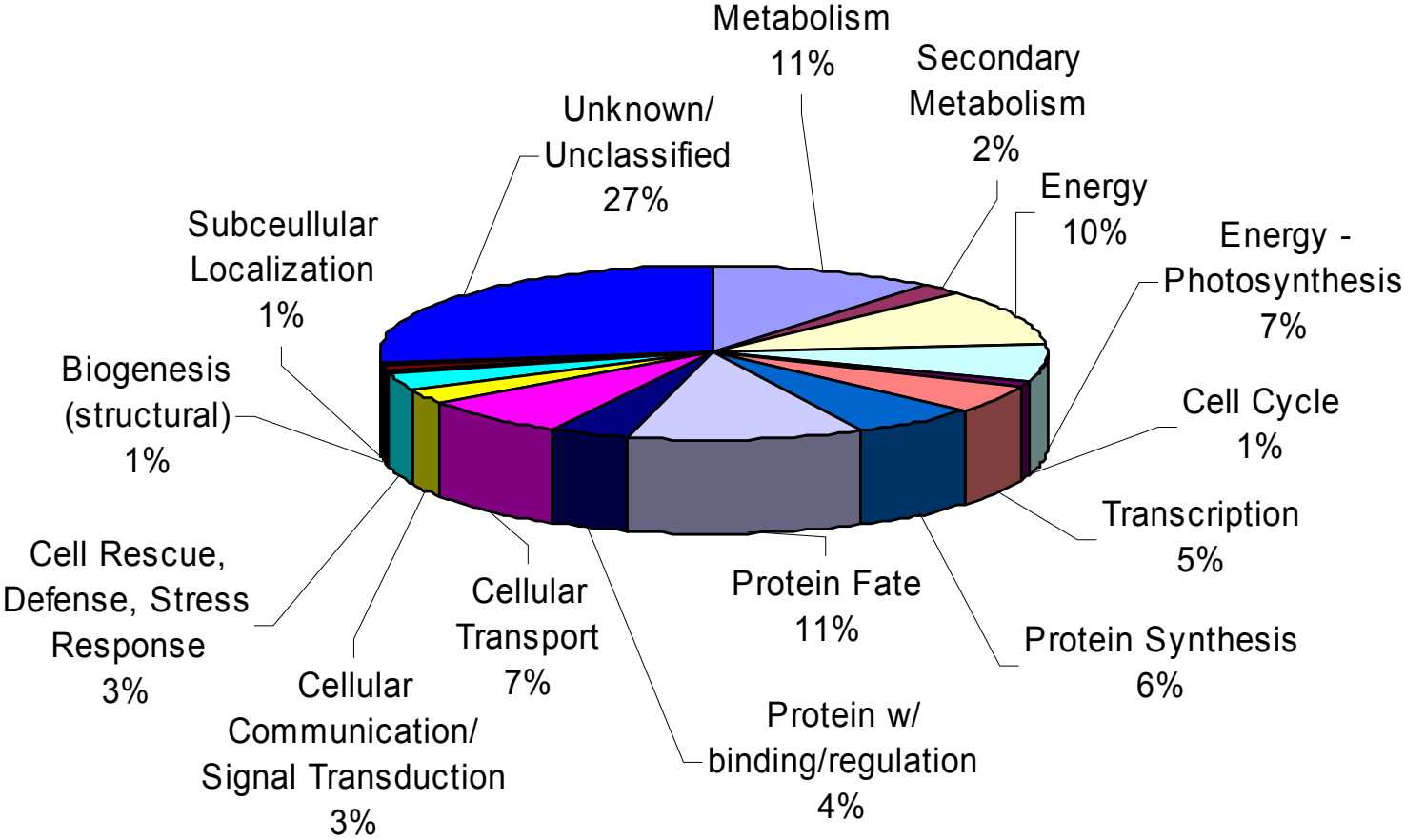
Suppression subtractive hybridization (SSH) cDNA library

Results:

Reverse Subtraction Library:

- 672 clones sequenced (NAF)
- 12% with multi-inserts, 590 ESTs sequence edited & computer analyzed
- 555 low redundant ESTs further analyzed and grouped into functional categories

Results: Reverse Subtraction Functional Categories



Specific objectives

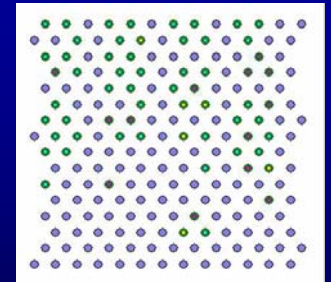
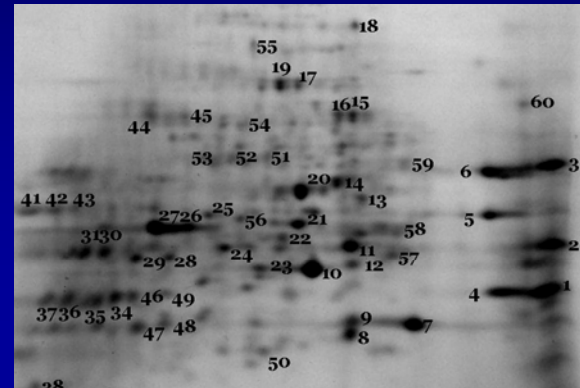
- Develop a suppression subtractive hybridization library of the resistant interaction and identify transcripts/ESTs (Expressed Sequence Tags)
- Protein profiling of germinating and resting urediniospores from *P. pachyrhizi*

Enriched extracellular proteins from germinating and resting urediniospores

- Vacuum infiltrate leaflets
- Low spin, collect infiltrate - 45 μ m filter
- Concentrate, dialysis, acetone-precipitation

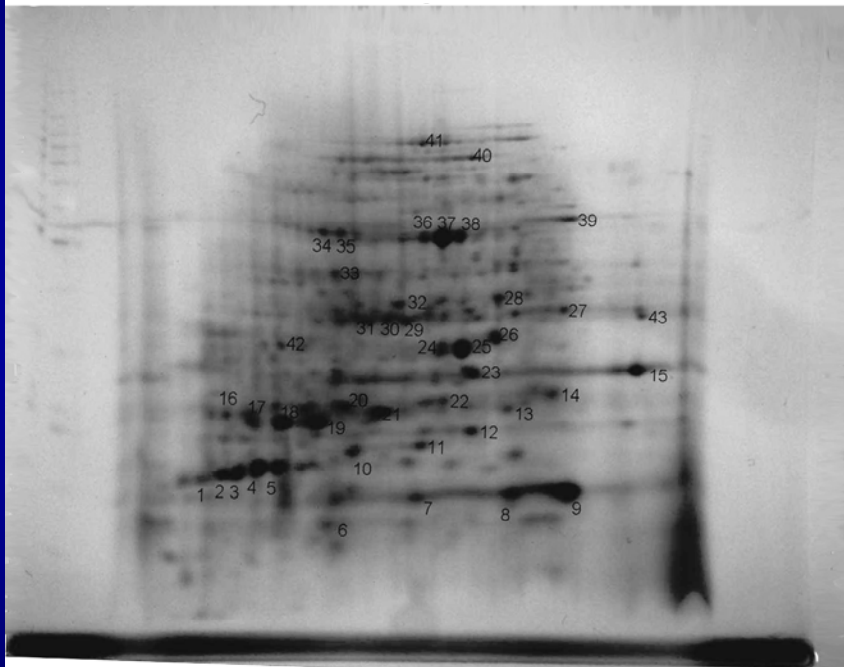
Enriched extracellular proteins from germinating and resting urediniospores

- 2-D protein gel
- Pick spots for MALDI
- In-gel trypsin-digestion
- MALDI/TOF-TOF mass spectrometry
ABI4700

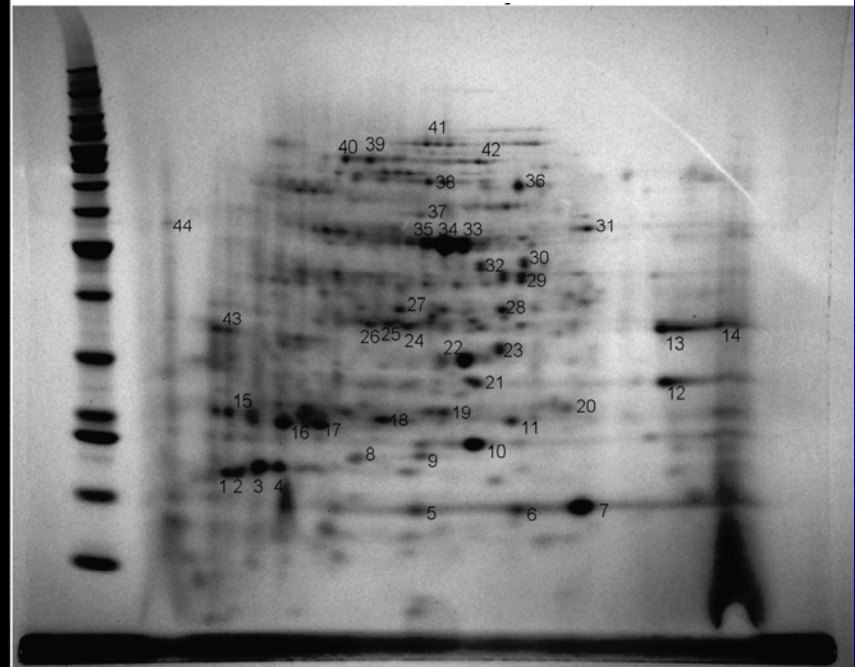


Protein profiling with 2D-gel and MALDI/TOF-TOF mass spec 12hpi

Germinating spores



Resting spores



Selected spots were blasted against the “nr” database and the EST database (six reading frames)

Enriched extracellular proteins from soybean leaves from **resistant** and **susceptible** interaction

Time points: 0h, 12h, 24h, 48h, 72h, 6dpi

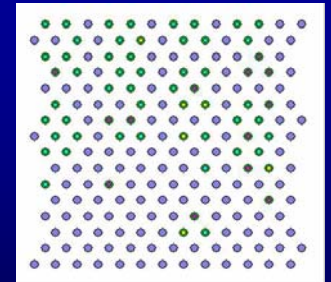
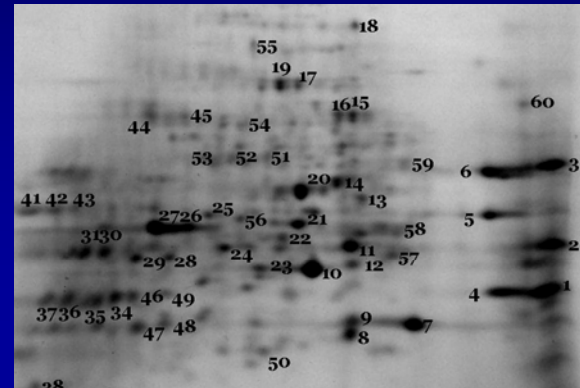
Treatments: Mock, **HW94-1**, **TW72-1**

Soybean cv.: *G. max* cv. Komata

- Vacuum infiltrate leaflets
- Low spin, collect infiltrate - 45µm filter
- Concentrate, dialysis, acetone-precipitation

Enriched extracellular proteins from soybean leaves from resistant and susceptible interaction

- 2-D protein gel
- Pick spots for MALDI
- In-gel trypsin-digestion
- MALDI/TOF-TOF mass spectrometry
ABI4700



Enriched extracellular proteins from soybean leaves from **resistant** and **susceptible** interaction

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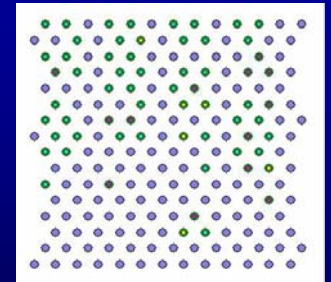
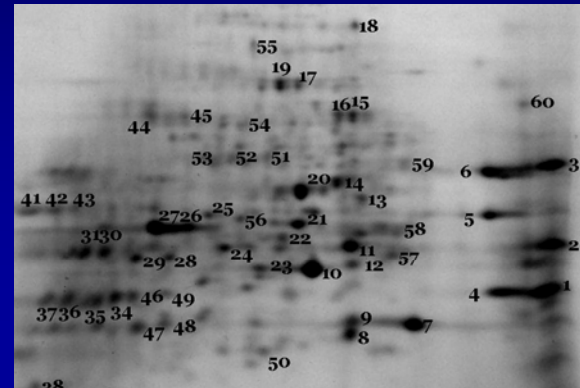
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ABI4700



GENOME SEQUENCING PROJECT

Phakopsora pachyrhizi
Phakopsora meibomiaae

Initial Genome Project Strategy

Random shotgun libraries:

General 3kb insert size in vector pUC18,

Mid-size 8-10kb insert in vector p21

Fosmid (40kb insert size) in pCC1FOS

**cDNA libraries from different stages
of *P.pachyrhizi* (in pSPORT1)**

Sequencers:

ABI3730

MegaBACE 4000

Informatics:

Reads processing by Phred

Reads assembly by Phrap

Verification

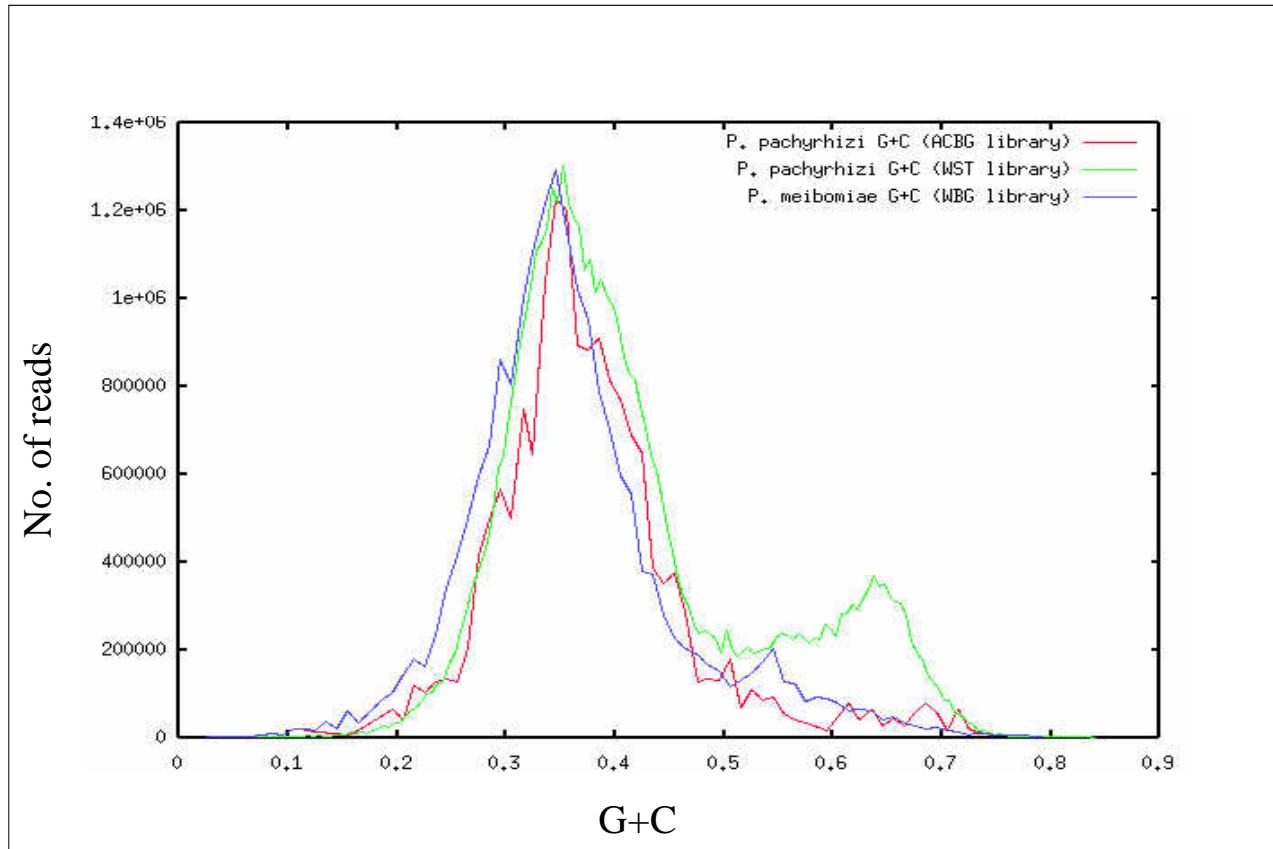
Genome annotation

	Library (Insert size)	Bases sequenced
<i>P. pachyrhizi</i>	3 Kb	146.60 Mb
	8 Kb	264.28 Mb
	40 Kb	5.75 Mb
Total		416.63 MB
<i>P. Meibomiaae</i>	3 Kb	125.20 Mb
	8 Kb	5.97 Mb
Total		131.17

Several independent methods were used to estimate the genome size. Although there were considerable uncertainties associated with most of the methods, they consistently yielded a genome size above 500 MB.

Estimation Method	Genome Size
cDNA Coverage	720 Mb
All-Pairs Read Alignment	500-800 Mb
Gene Density	300-700 Mb
Shotgun Fosmid Coverage	600-950 Mb

G+C content in *P. pachyrhizi* and *P. meibomia*



Phakopsora pachyrhizi and *Phakopsora meibomia* G + C content estimation

The mean G+C content in *P. pachyrhizi* and *P. meibomia* is 34-35%, estimated with the “G+C content program” (Chapman) on sequences from three different genomic libraries.

Fosmid sequencing



Random fosmids

Stanford University:

Finished	87
Incomplete	28

Selected fosmids

Lawrence Livermore
National Laboratory:

Probes designed for	120
Selected	50
To go	70
Sequencing	24
Finished	0

Probes designed based on ESTs selected by high similarity to “interesting” genes from other fungi, unknown genes highly expressed in germinating spores from *P. pachyrrhizi* and represented in the “sequenced” proteins from germinating and resting spores.

Known mitochondrial genome sequences were blasted against the entire set of reads. Potential mitochondrial sequences were assembled with the Phred Phrap Package. This resulted in single contig assemblies for both fungal mitochondrial genomes.

Genome analysis and annotation:

DOGMA Dual Organellar GenoMe Annotator ([http:// bugmaster.jgi-psf.org/dogma](http://bugmaster.jgi-psf.org/dogma)).

tRNAscan-SE 1.21 ([http:// www.genetics.wustl.edu/eddy/tRNAscan-SE/](http://www.genetics.wustl.edu/eddy/tRNAscan-SE/))

MacVector 7.1 (Accelrys)

Blast algorithm

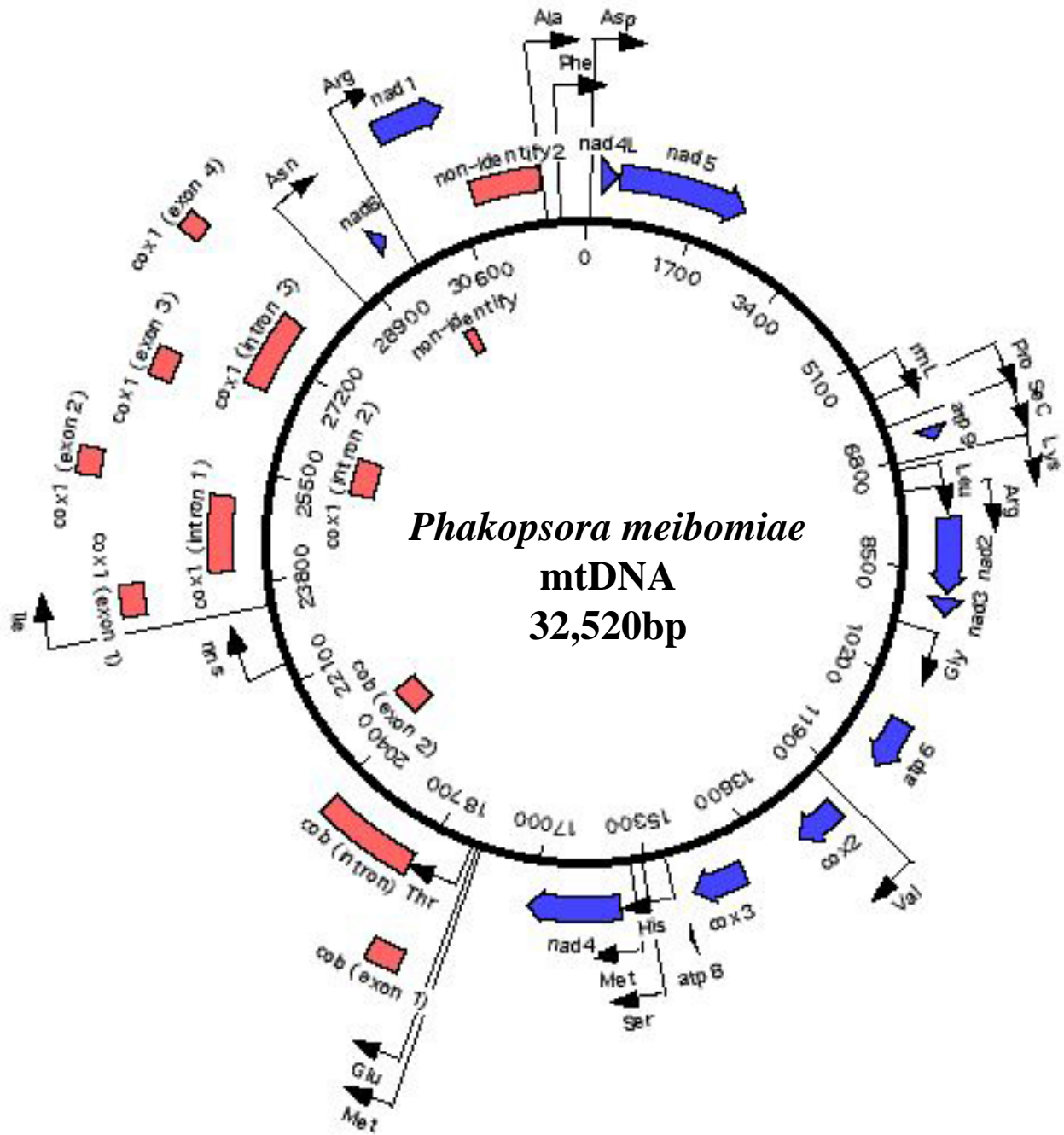
Mitochondrial Genomes

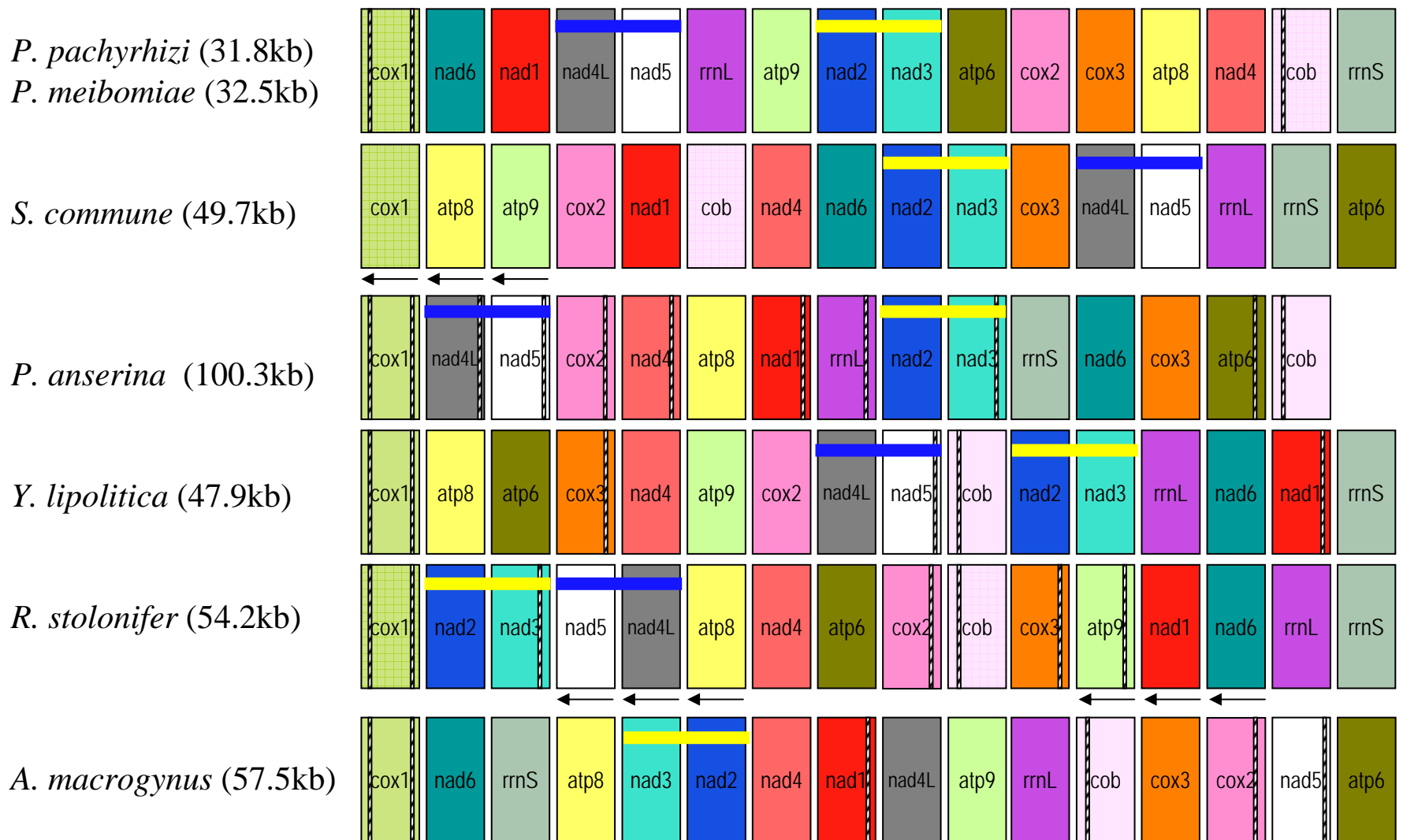


The complete nucleotide sequence of the mitochondrial (mt) genome was determined for *Phakopsora pachyrhizi* and *P. meibomia*.

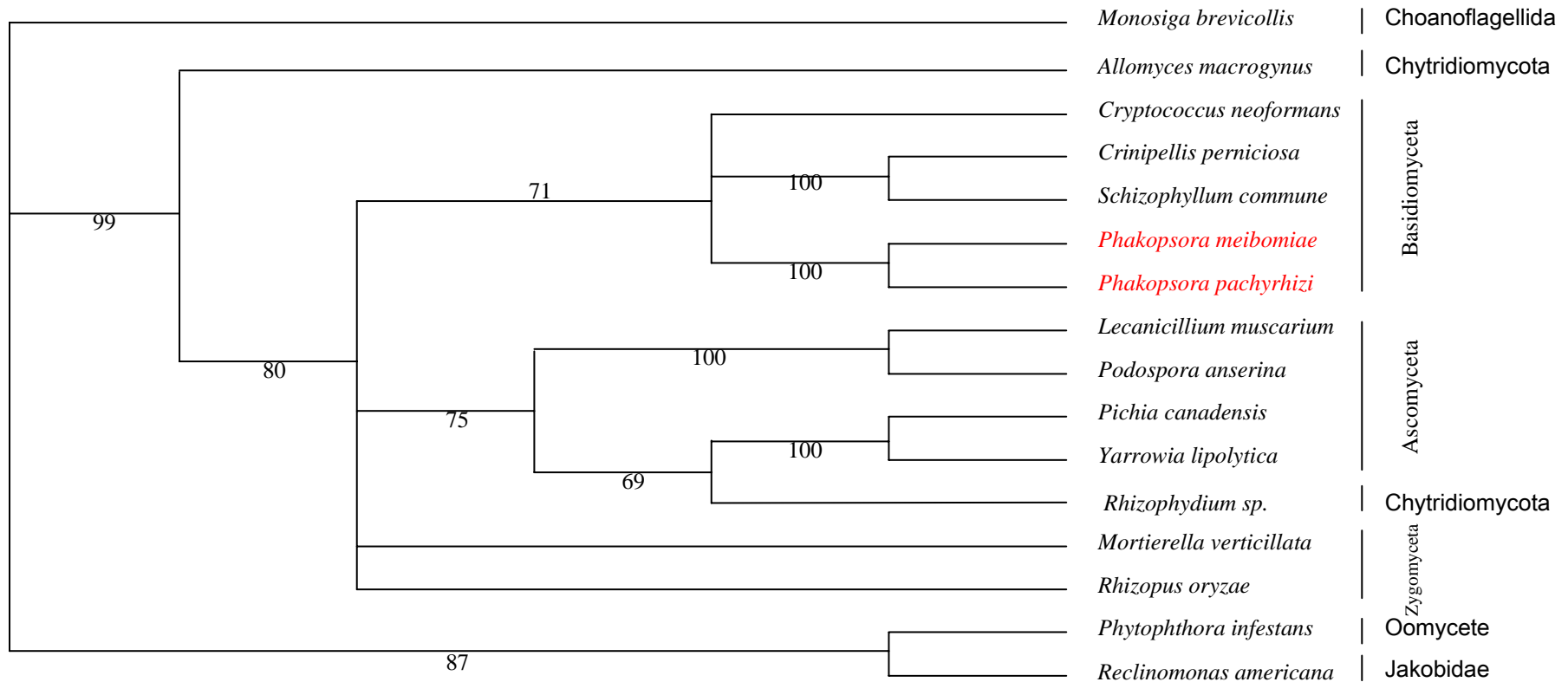
These 32 kb genomes contain the genes encoding ATP synthase subunits 6, 8, and 9 (*atp6*, *atp8*, and *atp9*), cytochrome oxidase subunits I, II, and III (*cox1*, *cox2*, and *cox3*), apocytochrome b (*cob*), reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), the large and small mitochondrial ribosomal RNAs (*rrns* and *rrnl*) and *tRNAs* for all amino acids.

	<i>P. Pachyrhizi</i>	<i>P. meibomia</i>
Size	31.82 Kb	32.52 Kb
G+C	34.6 %	34.9 %

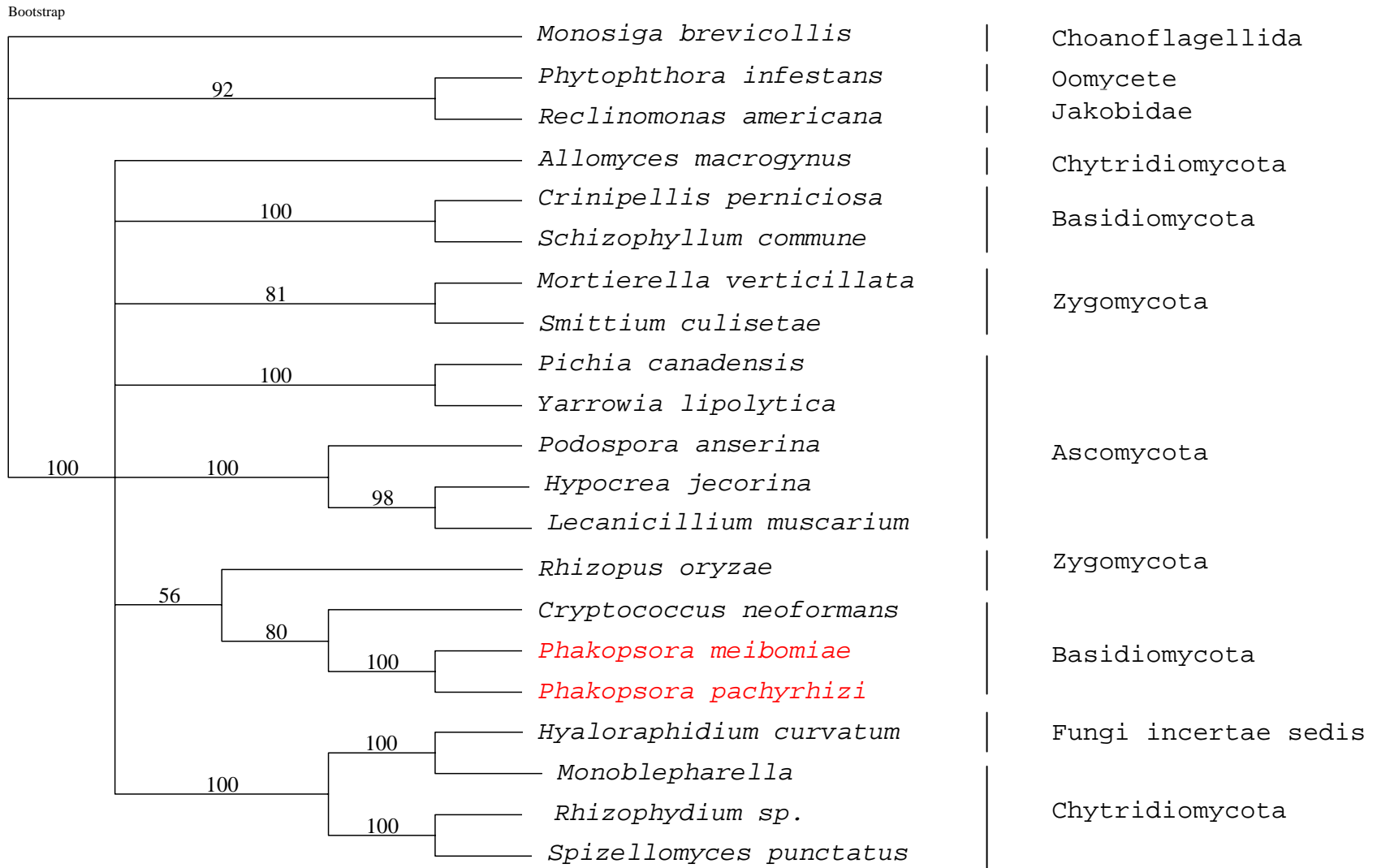




Comparison of mitochondrial genomes from the four phyla of fungi. Protein-coding and rRNA genes are represented by boxes; arrows indicate the direction of transcription. Lines within genes represent presence of intron(s).



Phylogenetic tree of 1582 amino acid position from four mitochondrial-encoded proteins from 16 taxa. The genes encoding *cob*, *cox1*, *nad1* and *nad5* are present in all organisms compared. Parsimony-bootstrap support was calculated from 100 replicates using Paup 4.0b10. *Monosiga brevicollis*, *Phytophthora infestans* and *Reclinomonas americana* were included as outgroups.



Phylogenetic tree of 1296 amino acid position from seven mitochondrial-encoded proteins from 21 taxa, including 18 species from all fungal phyla and *Monosiga brevicollis*, *Phytophthora infestans* and *Reclinomonas americana* as outgroups. The genes encoding cob, cox1, cox2, cox3, nad1, nad4 and nad5 are present in all organisms compared. Parsimony-bootstrap support was calculated from 100 replicates using Paup 4.0b10.

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National Laboratory

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Jarrod Chapman

Laurie Gordon

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