

# Cohort I: Change is Good!

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*Cohort I Decompression*  
**Saturday, June 20, 2009**



# The Resource Guide

If you need replacements,  
Resource Guides and  
Laboratory Manuals are  
now spiral-bound



# The Resource Guide

## Recipes and Protocols

- ❖ GPS Coordinates
- ❖ Enrichment
- ❖ Plates
- ❖ Top Agar
- ❖ DNA Prep
- ❖ EM

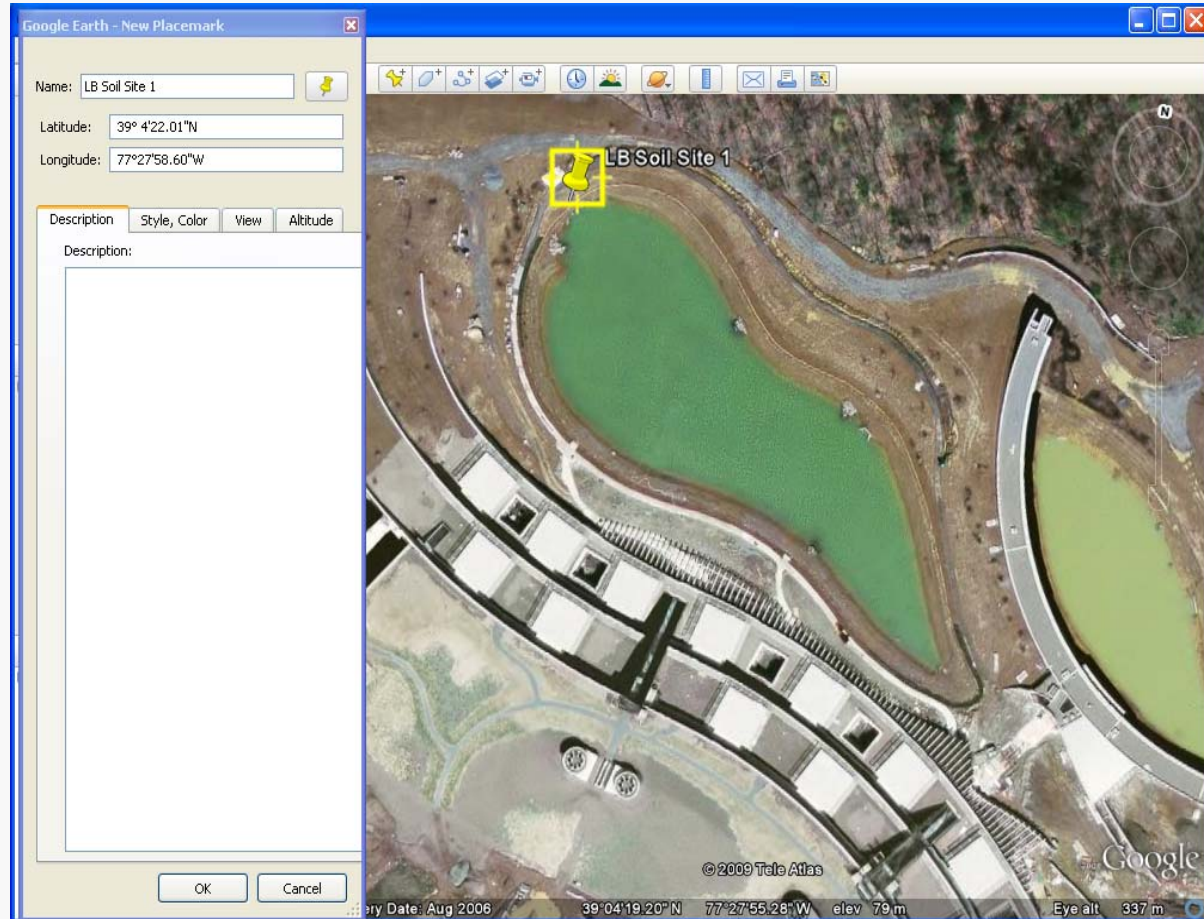


# The Resource Guide

## Recipes and Protocols

### ❖ GPS Coordinates

- You can continue to use your GPS units and/or
- Google Maps
- Instructions on Wiki



# The Resource Guide

## Recipes and Protocols

### ❖ Enrichment

#### ❖ Now the primary means of phage isolation:

- Higher success/more rapid isolation
- Fewer Resources

#### ❖ You will receive:

- Materials for 5 DP samples PLUS
- Disposable flasks (1 per student) for enrichment

#### ❖ Participants can continue to use direct plating but at their own expense



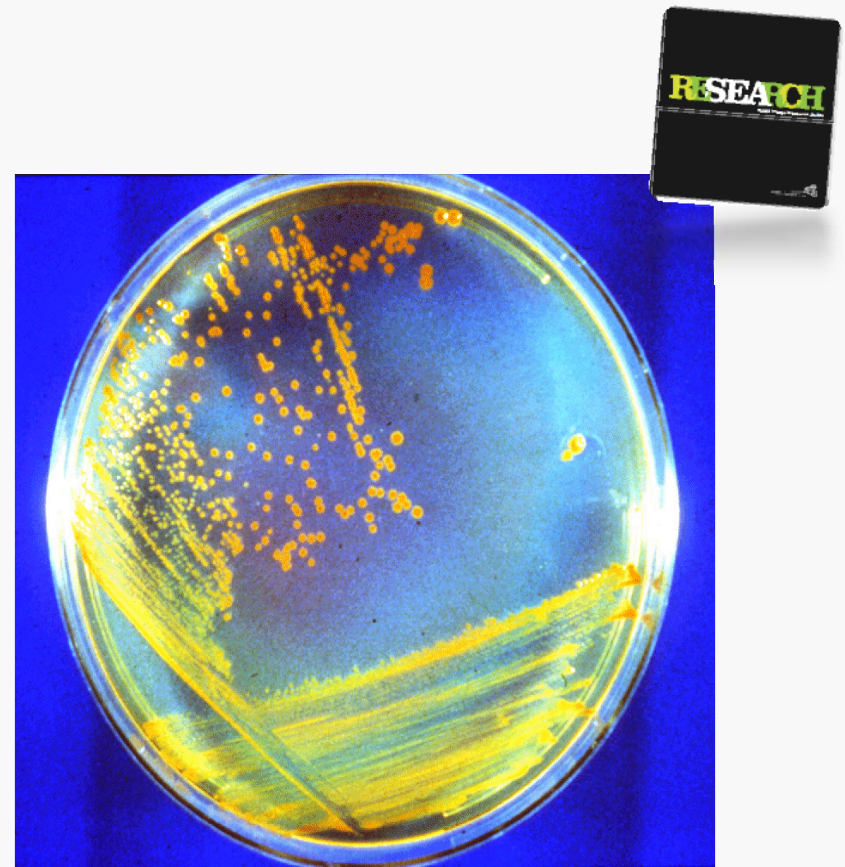
# The Resource Guide

## Recipes and Protocols

### ❖ Plates: Now L-Agar!

- Easier to prepare, just add water/antimicrobials
- More economical
- Titters the same, plaque morphologies comparable
- *M. smegmatis* streak plates take 48-72 (rather than 24-48) hours for single colonies

❖ **NOTE: Liquid media and Top Agar (TA) recipes remain the same!**



# The Resource Guide

## Recipes and Protocols

### ❖ Top Agar (TA)

- From 0.35% final concentration  
(2X = 0.7%)
  
- **To 0.4% final concentration**  
(2X = 0.8%)



# The Resource Guide

## Recipes and Protocols

### ❖ DNA Prep

- Same kit, but the revised protocol defaults to 2 columns!
  - Add 2mL resin to pellet
  - Put 1mL on each of 2 columns
- We're shipping everyone 2 Promega kits (200 columns)
- Other troubleshooting steps on Wiki:
  - Resin wash
  - Lysate dilution



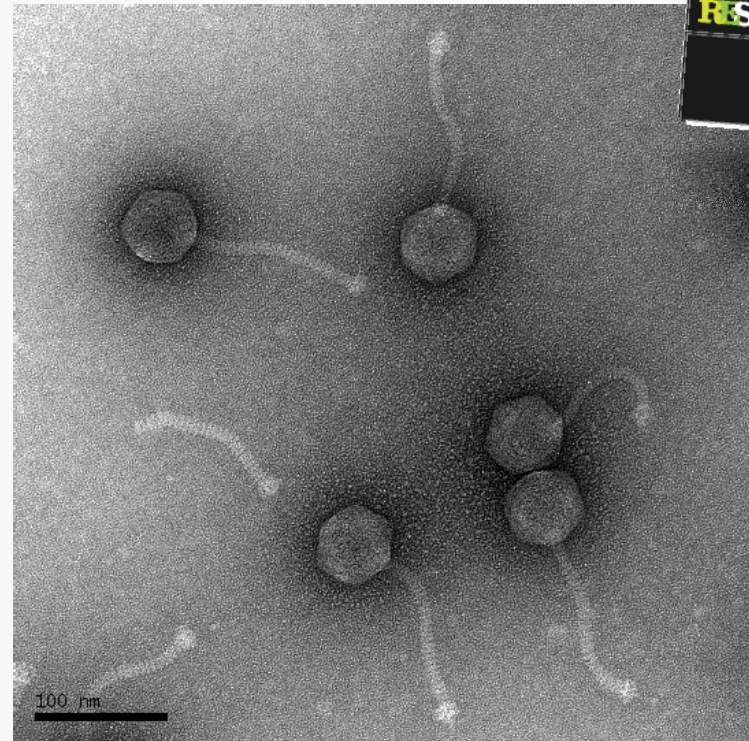


# The Resource Guide

## Recipes and Protocols

### ❖ EM

- The same in the RG, but
- We recommend a spin as per Aaron Best's protocol (on Wiki) if images are difficult to obtain.
- This should get us from 86% to more than 95% with normal lysates



Specimen : 2008 SEA Workshop - Best  
Operator : Fetter  
Voltage : 80 kV  
Microscope Name : Janelia T20  
Name : CCD  
Total Magnification : X345000

Image Name : 43490R1 #3.80k  
Resolution : 4096 x 4096 pixels  
Acquisition Date : 6/27/2008  
Acquisition Time : 9:27:41 AM  
Collection Number :  
Exposure Time : 1.0 s

# The Resource Guide Recipes and Protocols

❖ Newly revised Resource Guide and Laboratory Manual will be posted on the Wiki by mid-July

- Enrichment
- Plates
- Top Agar
- DNA Prep

❖ Other materials on the Wiki

- Lysate washes for EM (A. Best)
- Google Earth instructions



# Shipping Changes '09-'10

## ❖ New for this year:

- Separate shipments from 3 vendors:
  - Fisher
  - New England Biolabs
  - Sigma
- And from the SEAlab



**You have everything you need\***

**No materials requests should be required**

\*Based on reasonable maximum use by first year Cohort schools

## ❖ A few notes:

- These numbers are based on 24 students.
- Standard disclaimer: These are estimates based on reasonable maximum use (not the average!) of *each item* by the first year cohort. The amounts, kits and dates of shipments are subject to change.
- We will do our best to inform you of any changes and to keep the lines of communication open.
- Please inform us of any changes as well!

# Shipping Changes '09-'10

- ❖ Changes are also based on:
  - The switch to Enrichment Protocol
  - The switch to L-Agar plates
  - The switch to a 2-column DNA prep



# Shipping Changes '09-'10



<b>ICE SHIPMENT NUMBER 1</b> Tentative Ship Date:  ASAP post- workshop in order of school start dates	WHAT	AMOUNT	FOR	Store
	AD supplement	2 x 500mL	Culture media	4°C
	Frozen <i>M. smegmatis</i> stock 0.5mL	2 vials	Bacterial cultures	-80°C
	<b>L-Agar Plates</b>	6	Bacterial cultures	4°C
	High-titer phage D29	1 mL	Positive control	4°C

# Shipping Changes '09-'10

STUDENT KIT SHIPMENT	WHAT	AMOUNT	FOR	Store	Notes
Tentative Ship Date: >2 weeks before semester or quarter start	Student Kits	1/student		RT	
	1mL pipettes	Bag 50	Prep		
	Innoculation loops	50-100	Bacterial Cultures		
	NEB buffer charts	1			Per school
	Timers	1 per 2 students			
	Honor in Science	1			Per school
	10X CaCl <sub>2</sub> (1M)	250mL		RT	Dilute before use!!!
	10X Phage buffer	3 x 500mL		RT	



# Shipping Changes '09-'10

FISHER SHIPMENT	WHAT	AMT	FOR/NOTES
Tentative Ship Date: July 20, 2009 Media Prep	L-Agar, powder base 500g	3	Agar plates
	Petri dishes, Cs 500	3	Agar plates
	7H9 powder base 500g	2	Liquid media, TA
	Glycerol 50mL	2	Liquid media, TA
	Bacto Agar 500g	1	TA
Cultures	Glass culture tubes Pk1000	2	Bacterial cultures
	Glass culture tube caps Pk1000	2	"
	250mL Disp. Baffled flasks Cs12	2	"
Laboratory Consumables	15mL conical tubes Cs500	1	General
	50mL conical tubes Cs500	1	"
	Economy microfuge tubes Pk500	4	"
	Syringe filters (0.22um), Bx 50	2	"
	Vinyl gloves Sm Box100	2	"
	Vinyl gloves Med Box100	4	"
	Vinyl gloves Lg Box100	4	"
	Steriflip Bx 25	3	"
	1mL TB syringe Bx100	1	"
	5mL syringe Bx 100	1	"
	DNA Prep and Analysis	Oakridge tubes, Pk10	2
Eppendorf microfuge tubes Pk500		1	"
Isopropanol 500mL		1	"
Wizard DNA prep		2	"
Bench diapers Bx50		1	"
TBE 10X, 1L		3	Gels on RE analysis
Agarose General Purpose 100g		1	"
TAE 10X, 1L		1	QC gels
Agarose, SEAKEM pure 25g		1	"





# Shipping Changes '09-'10

SIGMA SHIPMENT	WHAT	AMT	FOR/NOTES
Tentative Ship Date: July 20	Carbenicillin, 5g	1	Media prep
	Cycloheximide, 1g	1	Media prep
	Tween 80, 25mL	1	Liquid media
	6X Loading dye	1	Electrophoresis
	Ethidium bromide 10mg/mL – 10mL	1	Electrophoresis
<b>STORE AS DIRECTED!</b>			



NEB SHIPMENT	WHAT	AMT	FOR/NOTES
Tentative Ship Date: Sept 15 <sup>th</sup>	All enzymes	2ea	Restriction Analysis
	1kB ladder	1	Electrophoresis
	Lambda/HindIII ladder	1	Electrophoresis
<b>STORE AS DIRECTED!</b>			

# Shipping Changes '09-'10

ICE SHIPMENT #2	WHAT	AMOUNT	FOR	Store	Notes
Tentative Ship Date: 2-3 weeks in	AD supplement	2 x 500mL	Culture media	4°C	
	Nuclease Mix	4 x 1mL		-20°C	
	Phage Precipitant	3 x100mL		4°C	
	JGI Standards and Markers	1set		-20°C	
	TE			RT	



AMBIENT SHIPMENT #2	WHAT	AMOUNT	FOR	Store	Notes	
Tentative Ship Date: October 1st	10X CaCl <sub>2</sub> (1M)	100mL		RT	Dilute before use!!!	
	10X Phage buffer	2 x500mL		RT		
	EM Kit					
	Archiving Kit					

# Shipping Changes '09-'10

STUDENT KITS		
	Lab Notebook	1
	Ruler	1
	Labeling Pen	1
	Trowel	1
	1mL Syringe	5
	15mL Conical Tube	5
	.22µm Syringe Filter	5
	Site Markers	10
	50mL Steriflip	2
	50mL Conical Tube	3
	5mL Syringe	3
	Whatman Filter Paper	2
	EM Tabs	2
	1mL Syringe	1
	.22µm Syringe Filter	1
	Screw-Capped Tube	1
	5x5cm Parafilm	1
	Petri Dish	1
	Screw-Capped Tubes	4
	Other?	



# Shipping Changes '09-'10

FISHER SHIPMENT	WHAT	AMT	FOR/NOTES
Tentative Ship Date: July 20, 2009 Media Prep	L-Agar, powder base 500g	3	Agar plates
	Petri dishes, Cs 500	3	Agar plates
	7H9 powder base 500g	2	Liquid media, TA
	Glycerol 50mL	2	Liquid media, TA
	Bacto Agar 500g	1	TA
Cultures	Glass culture tubes Pk1000	2	Bacterial cultures
	Glass culture tube caps Pk1000	2	"
	250mL Disp. Baffled flasks Cs12	2	"
Laboratory Consumables	15mL conical tubes Cs500	1	General
	50mL conical tubes Cs500	1	"
	Economy microfuge tubes Pk500	4	"
	Syringe filters (0.22um), Bx 50	2	"
	Vinyl gloves Sm Box100	2	"
	Vinyl gloves Med Box100	4	"
	Vinyl gloves Lg Box100	4	"
	Steriflip Bx 25	3	"
	1mL TB syringe Bx100	1	"
	5mL syringe Bx 100	1	"
DNA Prep and Analysis	Oakridge tubes, Pk10	2	DNA prep
	Eppendorf microfuge tubes Pk500	1	"
	Isopropanol 500mL	1	"
	Wizard DNA prep	2	"
	Bench diapers Bx50	1	"
	TBE 10X, 1L	3	Gels on RE analysis
	Agarose General Purpose 100g	1	"
	TAE 10X, 1L	1	QC gels
	Agarose, SEAKEM pure 25g	1	"



## You have everything you need No materials requests should be required\*

❖ \*Last year between July and November, we sent

- Approximately 150 packages in 25 planned shipments plus
- More than 100 special request packages

❖ From this information

- We can send you as much (or more) than you need
- We can consolidate shipments



# Shipping Changes '09-'10

**You have everything you need\***  
**No materials requests should be required**

\*Should something happen...



# Shipping Changes '09-'10

**SEA Materials Request Form**

1. Download and completely fill out this form. **A SEPARATE FORM MUST BE SENT FOR EACH ITEM REQUESTED.** Note that these requests without complete information and justifications will be returned for modification.

2. Email an electronic version with the subject line: **MAMP (Materials Request Form)** to [natexp@hhmi.org](mailto:natexp@hhmi.org). Please allow at least two weeks from the date of form submission to delivery.

Date of Request:

Your Name:

Institution:

4-letter Institution Code:

Shipping Address:

Name of Shipping Contact:

Phone number of Shipping Contact:

Email address of Shipping Contact:

Item	Unit	Amount	Justification

ANY SPECIAL SHIPPING REQUIREMENTS OR REQUESTS:


Materials Request Form Version 18\_2009



1. Download this form from the Wiki for each item
2. Fill out and email to NatExp
3. Allow 2 weeks turn-around

# Shipping Changes '09-'10

- ❖ We plan to do shipping once per week
- ❖ Detailed directions for requesting materials will be posted on the Wiki.
- ❖ And please:
  - ❖ Remember that we have double the institutions this year – plan ahead
  - ❖ Make your justification as detailed as possible
  - ❖ Consolidate any requests



SEA Materials Request Form

1. Download and completely fill out this form. **SEPARATE FORMS MUST BE SENT FOR EACH ITEM REQUESTED.** Note that these requests without complete information and justifications will be returned for modification.

2. Email an electronic version with the subject line: **MMM (Material Request Form) to: [sea@hhmi.org](mailto:sea@hhmi.org)** Please allow at least two weeks from the date of form submission to delivery.

Date of Request:  
Qty/Name:  
Institution:  
4-digit Institution Code:  
Shipping Address:  
Name of Shipping Contact:  
Phone number of Shipping Contact:  
Email address of Shipping Contact:

Item	Unit	Amount	Justification

ANY SPECIAL SHIPPING REQUIREMENTS OR REQUESTS:

Version: Request Form (revision 04/2009)