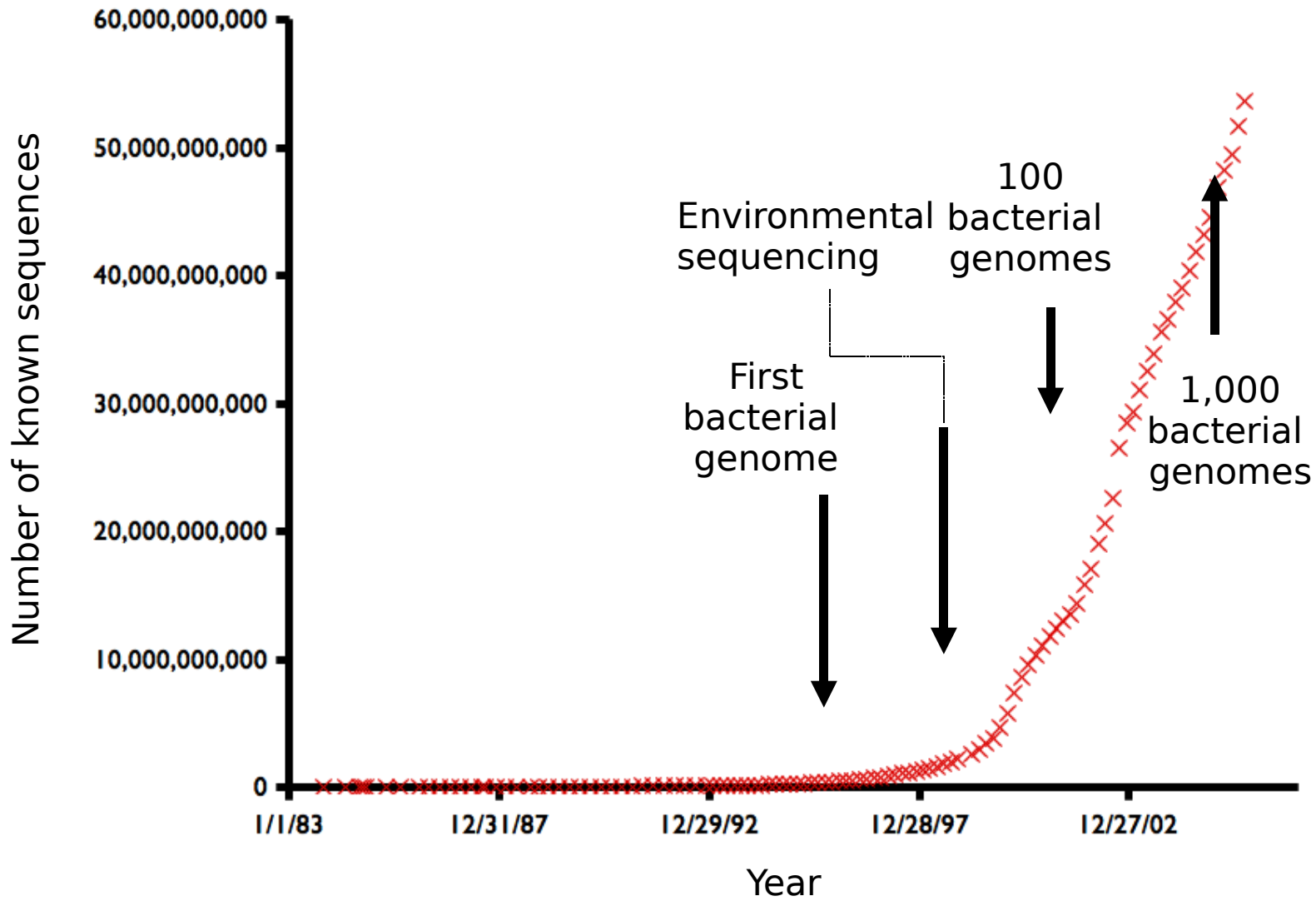
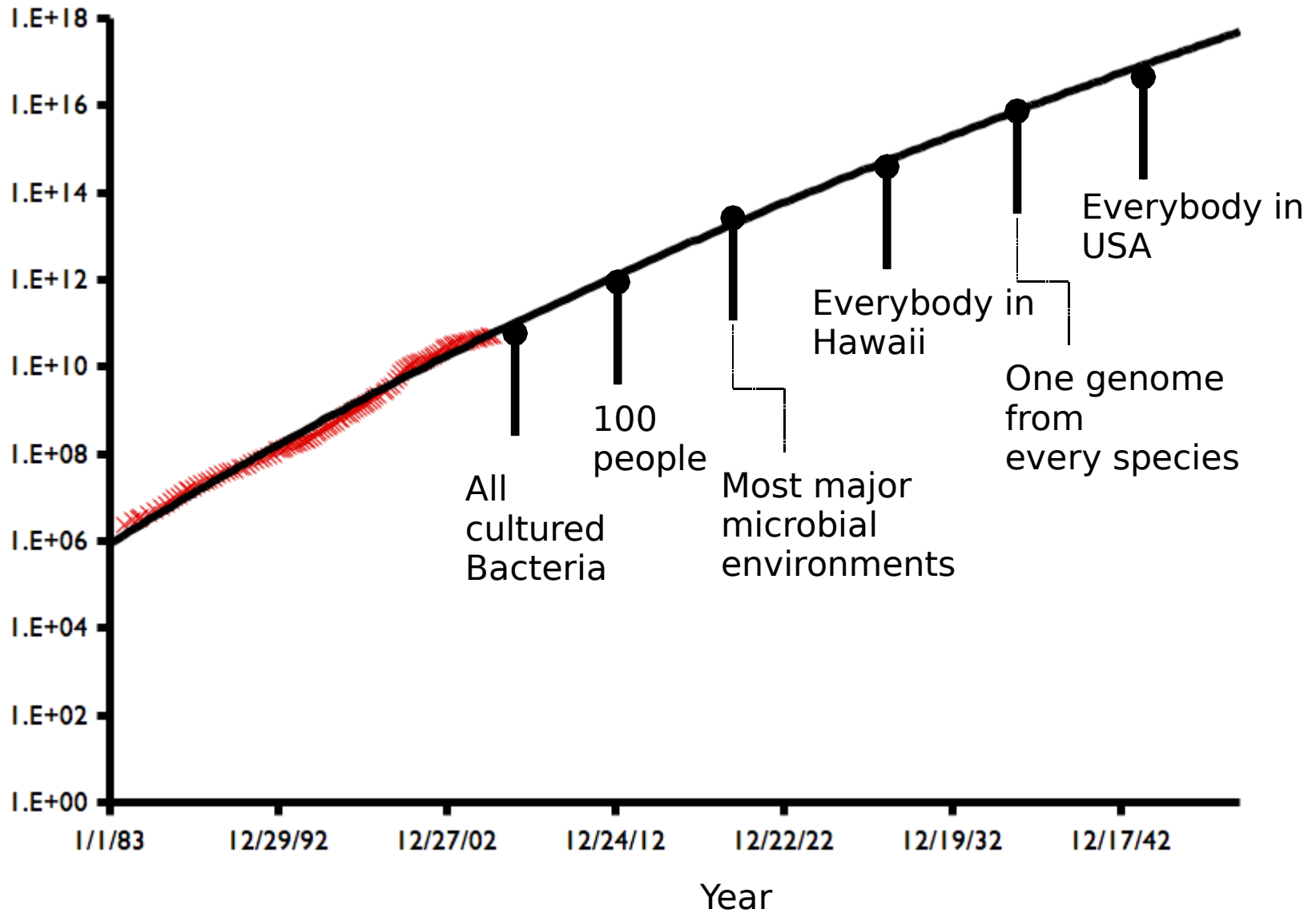


How much has been sequenced?



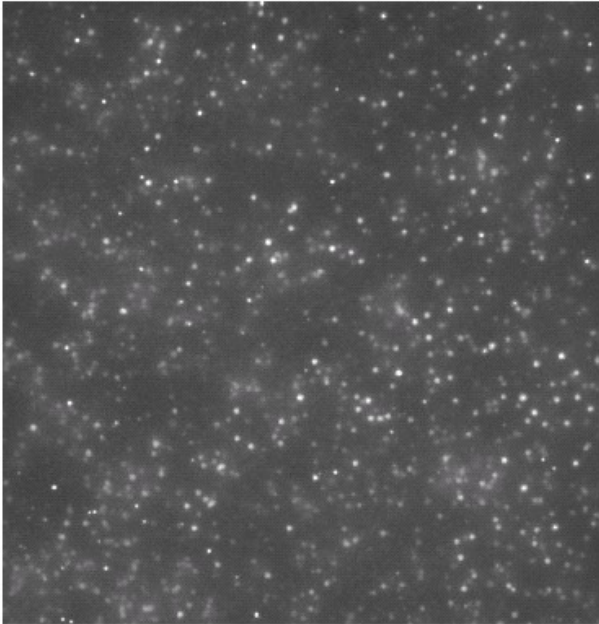
How much will be sequenced?



Why Metagenomics?

- What is there?
- How many are there?
- What are they doing?
- Experimental manipulations?
- Real time metagenomics?

Uncultured Viruses



Epifluorescent
Microscopy

200 liters water
5-500 g fresh fecal matter



Concentrate and purify
viruses or bacteria



Extract nucleic
acids



DNA/RNA LASL

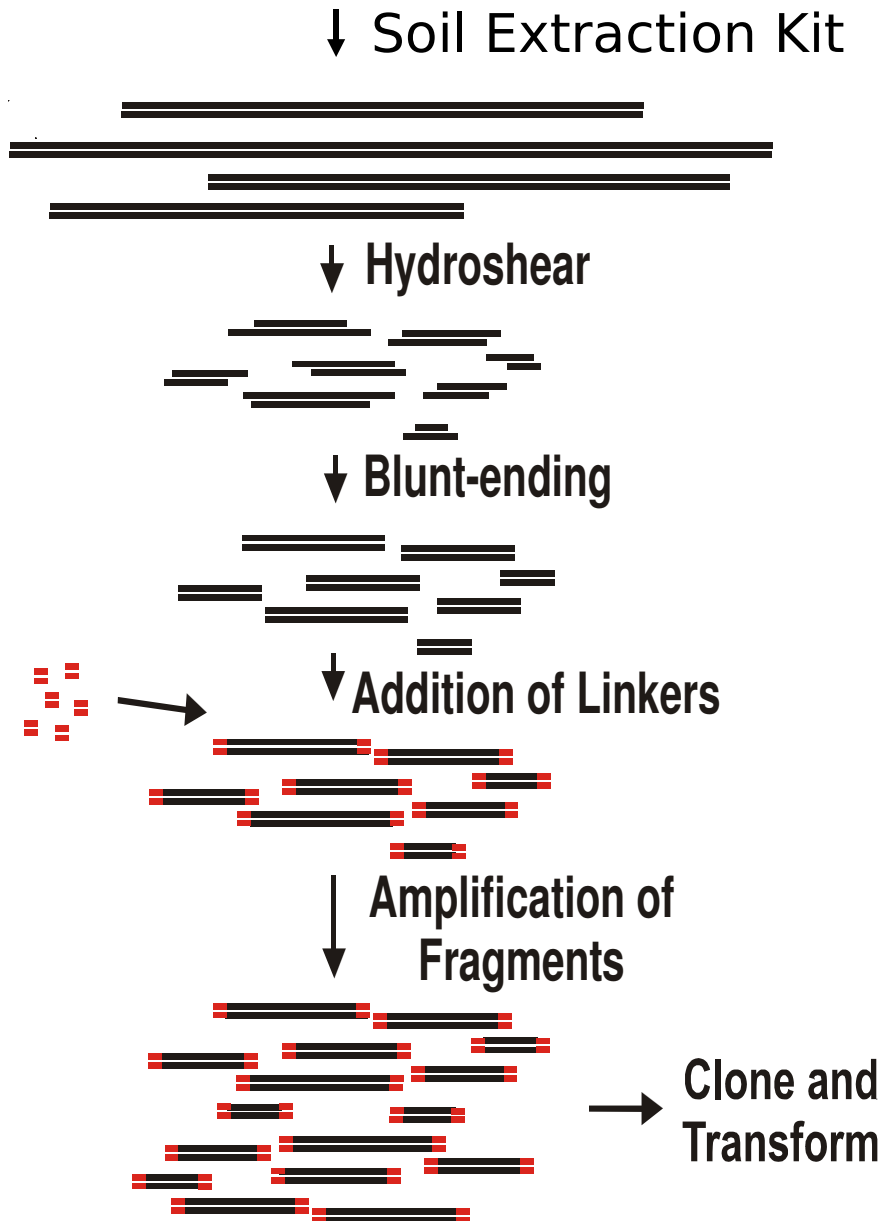


Sequence

How do you sequence the environment?

- Extract DNA
 - Soil extraction kit
 - Water extraction kit
- Create library
 - LASLs
 - fosmids
- Sequence fragments

Linker-Amplified Shotgun Libraries (LASLs)



This method produces high coverage libraries of over 1 million clones from as little as 1 ng DNA

David Mead - Lucigen™

Breitbart (2002) PNAS

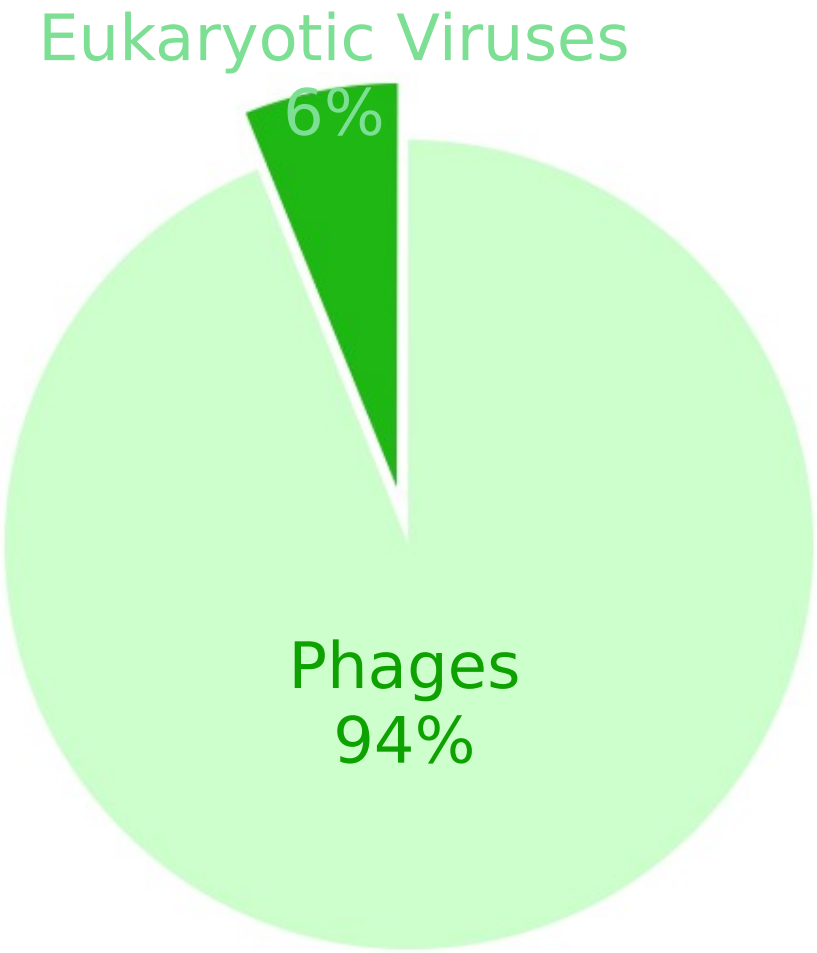
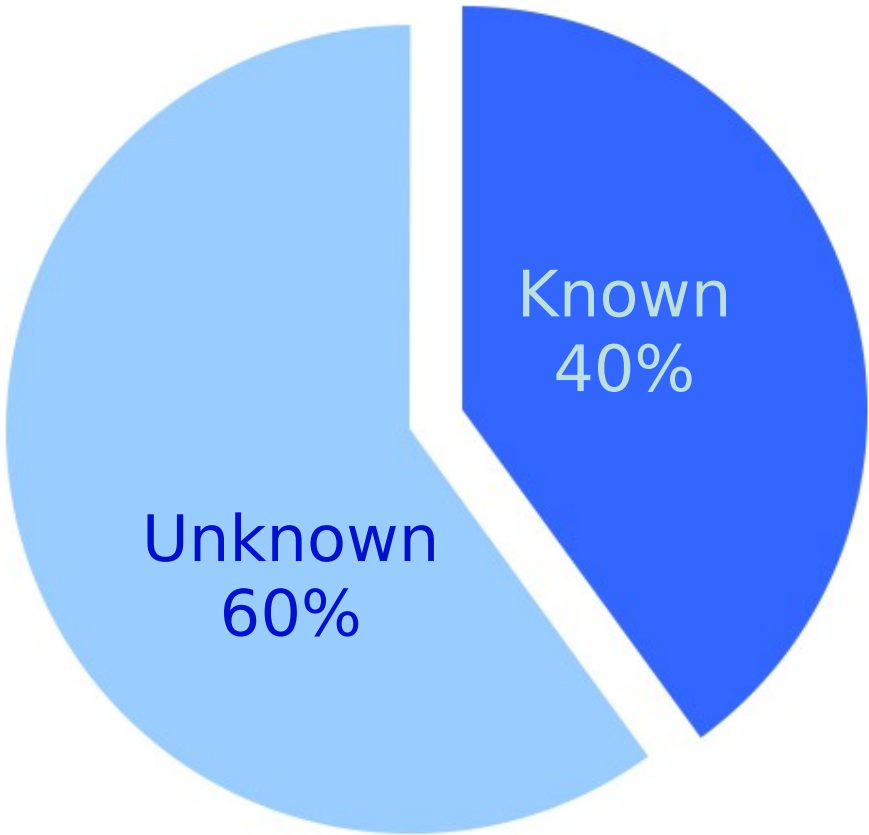
Early Attempts at a Metagenomics Platform

- <http://phage.sdsu.edu/~rob/cgi-bin/remoteblast.cgi>
- Submit BLAST to local and remote databases
 - Local (as fast as possible)
 - NCBI (one search every 3 seconds)
- Many concurrent searches
 - One search versus 1,000 searches
- Parse data into tables for Excel
 - Access to taxonomy etc

Human-associated viruses

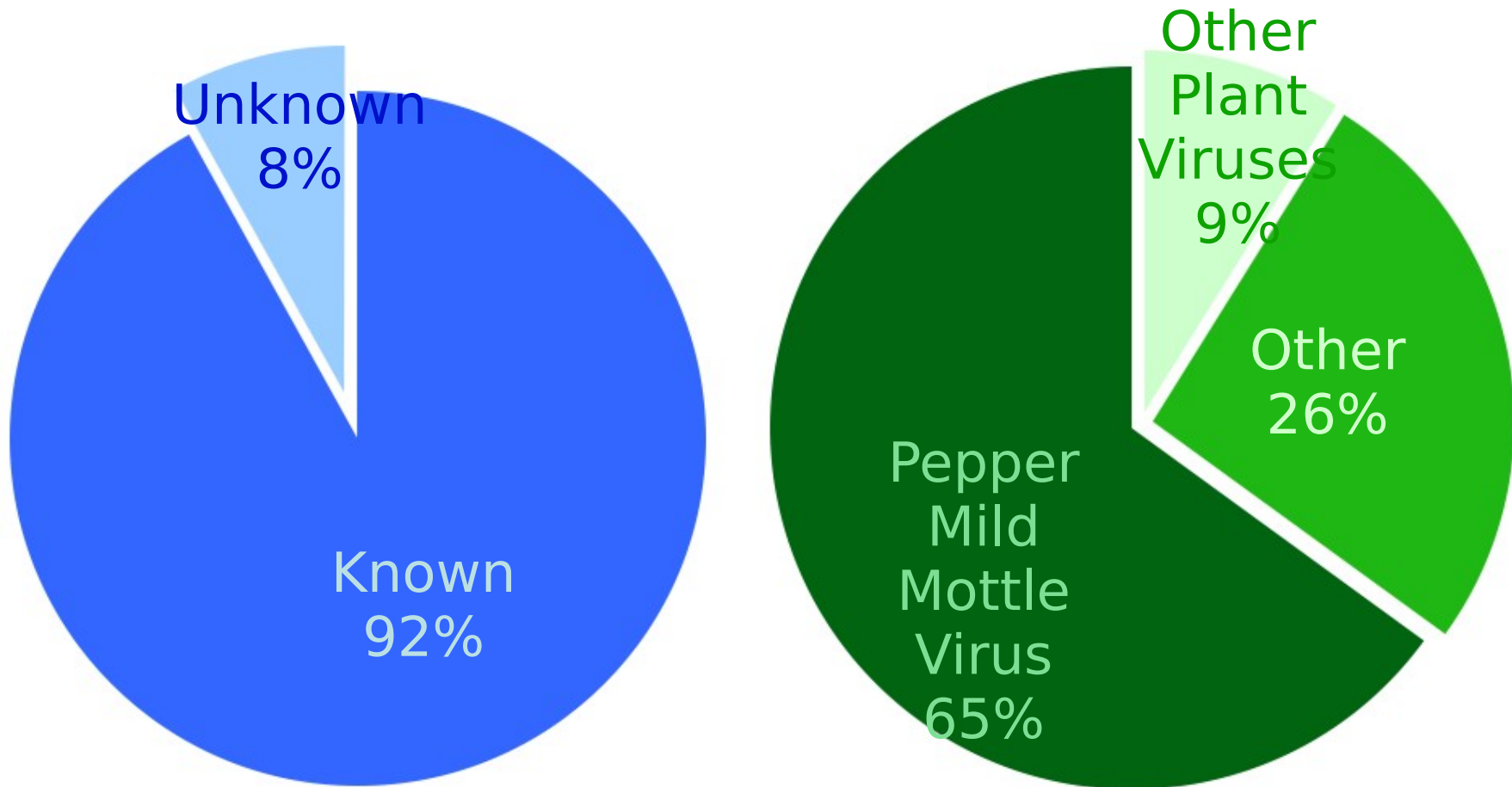
- More bacteria than somatic cells by at least an order of magnitude
- More phages than bacteria by an order of magnitude
- Sample the bacteria in the intestine by sampling their phage

Most Viral DNA Sequences in Adult Human Feces are Unknown Phages



Breitbart (2003) J. Bacteriol.

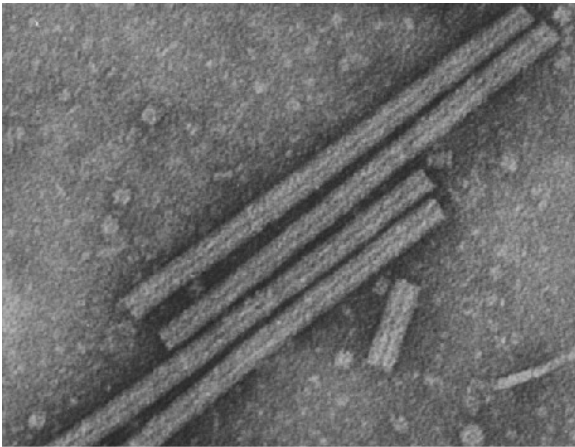
Most Human RNA Viruses are Known



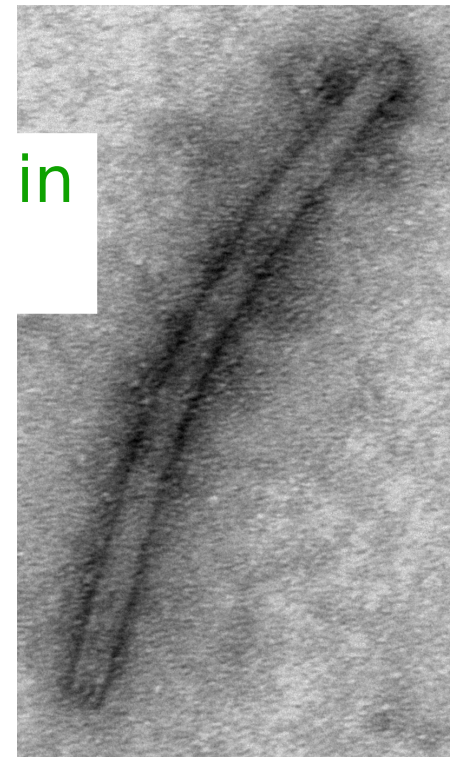
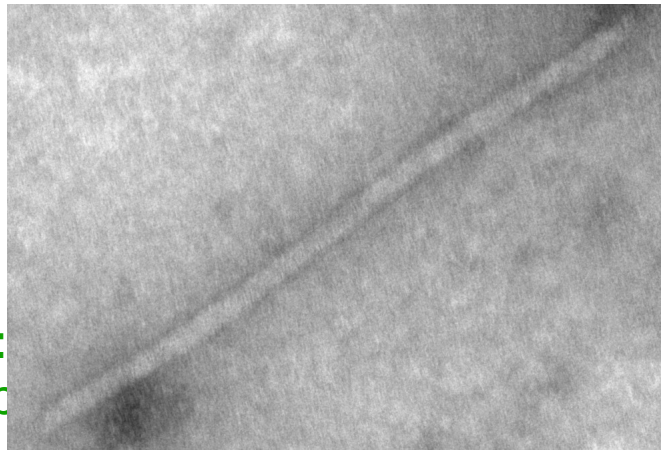
Zhang (2006) PLoS Biology

Pepper Mild Mottle Virus (PMMV)

- ssRNA virus; ≈ 6 kb genome
- Related to Tobacco Mosaic Virus
- Infects members of *Capsicum* family
- Widely distributed – spread through seeds
- Fruits are small, malformed, mottled
- Rod-shaped virions



Viral particles in
fecal sample



TOBACCO MOSAIC VIRUS <http://www.rothamsted.bbsrc.ac.uk/p/i/links/pplinks/virusems/>

PMMV is common in Human Feces

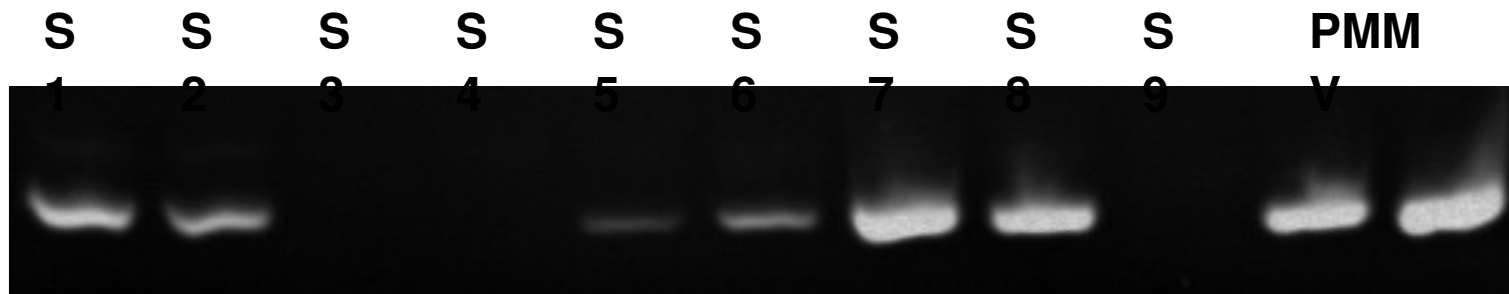
Fecal samples



Extract total RNA



RT-PCR for PMMV



San Diego : 78% people are positive

Singapore : 67% people are positive

10-50 fold increase in feces compared to food
 10^6 - 10^9 PMMV copies per gram dry weight of feces

Which Foods Contain PMMV?

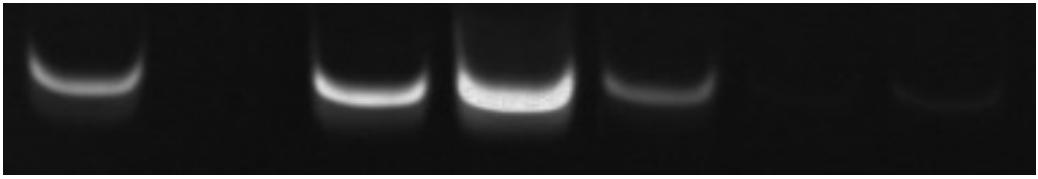


Chili powder

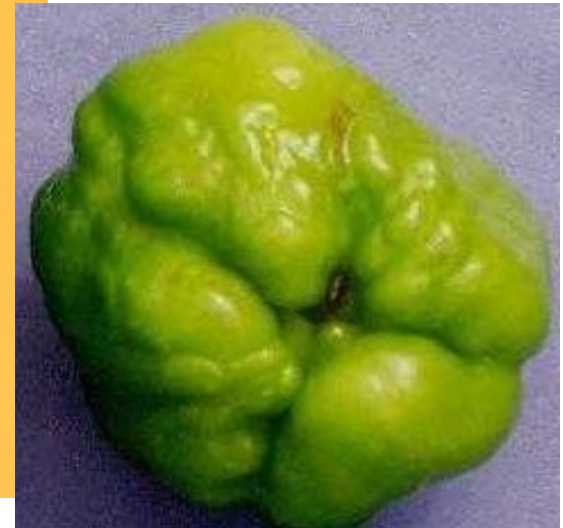
Chili sauces

NOT FOUND IN FRESH PEPPERS

Indian curry
Pork noodle red chili
Chicken rice
Chinese food
Hong Kong chili sauce
Hong Kong green chili
Vegetarian chili



Koch's Postulates



<http://www.sweatnspice.com>

Human microbial metagenome is more important than human genome

NIH started the human microbiome project last year

- Nasal
- Oral
- Skin
- Gastro-intestinal
- Urogenital



Artists impression : not all machines explode!

Uncultured Viruses

200 liters water
5-500 g fresh fecal matter

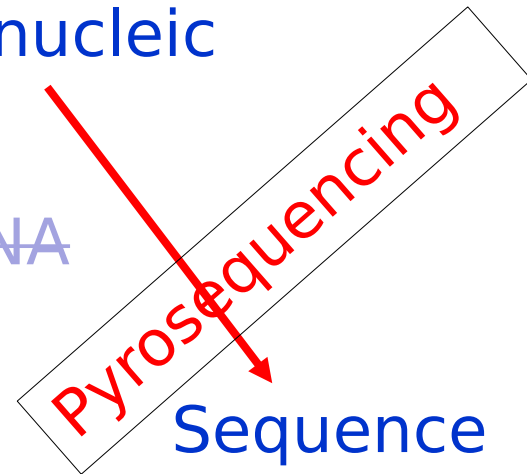


Concentrate and purify
viruses or bacteria

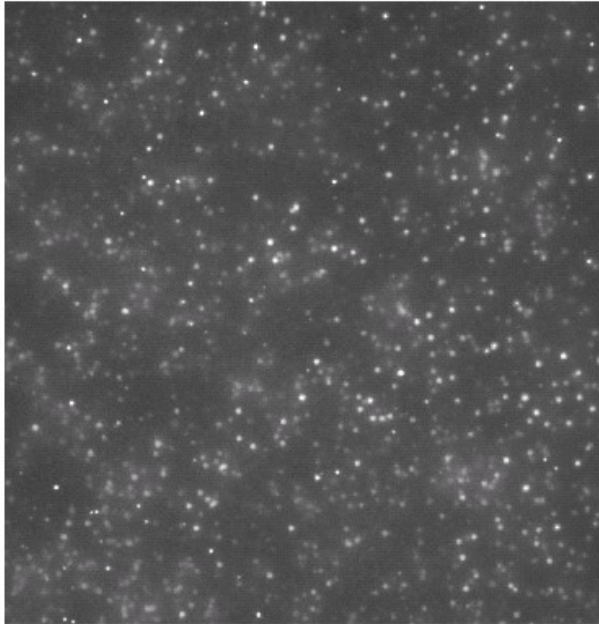


Extract nucleic
acids

~~DNA/RNA~~
~~LAS~~



Sequence



Epifluorescent
Microscopy

BACK!