

Marine meta/genomic libraries: establishing resources for MAMBA project



A photograph of a vast, deep blue ocean with a small white buoy floating in the center. The water has a textured surface with small waves. The text "about 70% of the Earth surface marine environments" is overlaid in white at the bottom center.

about 70% of the Earth surface marine environments



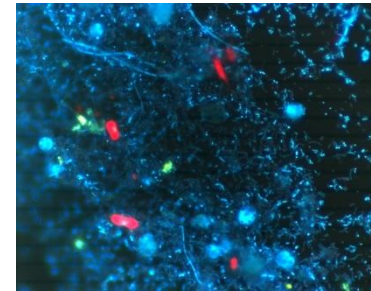
Marine biodiversity



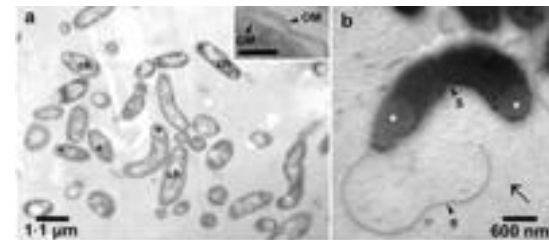
Marine animals



Microorganisms

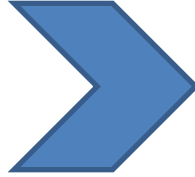


Deep-sea corals *Desmophilum* and *Madrepora* collected at Apulean plateau (MAMBA Cruise 2010)



(Yakimov *et al.*, 2004)

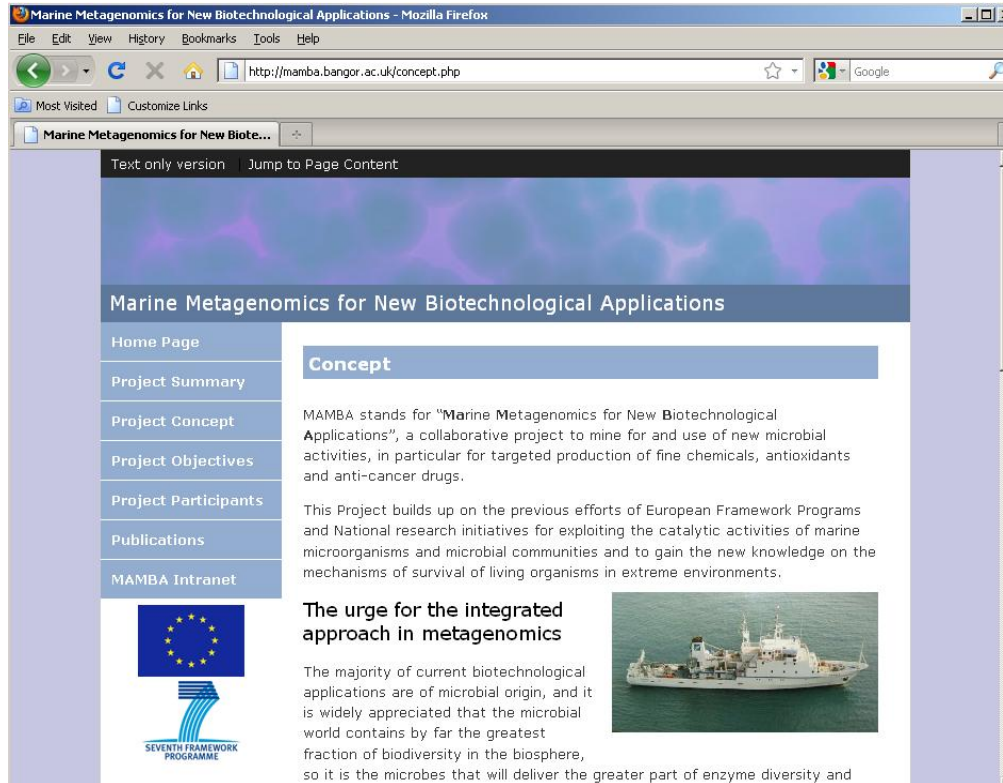
Importance of the topic



Data of US NCI: marine environments contain approx. 100 times more metabolites with potential anti-tumor activities as terrestrial environments

Importance of the topic

Marine microbes: a great resource of products for new biotechnological applications





The screenshot shows a Mozilla Firefox browser window displaying the website "Marine Metagenomics for New Biotechnological Applications". The address bar shows the URL "http://mamba.bangor.ac.uk/concept.php". The website has a blue header with the title "Marine Metagenomics for New Biotechnological Applications". Below the header is a navigation menu with links: Home Page, Project Summary, Project Concept, Project Objectives, Project Participants, Publications, and MAMBA Intranet. The main content area is titled "Concept" and contains the following text:

MAMBA stands for "Marine Metagenomics for New Biotechnological Applications", a collaborative project to mine for and use of new microbial activities, in particular for targeted production of fine chemicals, antioxidants and anti-cancer drugs.

This Project builds up on the previous efforts of European Framework Programs and National research initiatives for exploiting the catalytic activities of marine microorganisms and microbial communities and to gain the new knowledge on the mechanisms of survival of living organisms in extreme environments.

The urge for the integrated approach in metagenomics

The majority of current biotechnological applications are of microbial origin, and it is widely appreciated that the microbial world contains by far the greatest fraction of biodiversity in the biosphere, so it is the microbes that will deliver the greater part of enzyme diversity and

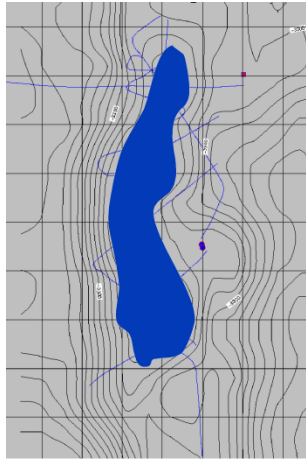


Importance of the topic

MAMBA explores marine microbial life at its limits

(hypersalinity, low and high temperature, high pressure and low water activity conditions, etc.)

Hypersaline brine lakes at the bottom of the Mediterranean Sea

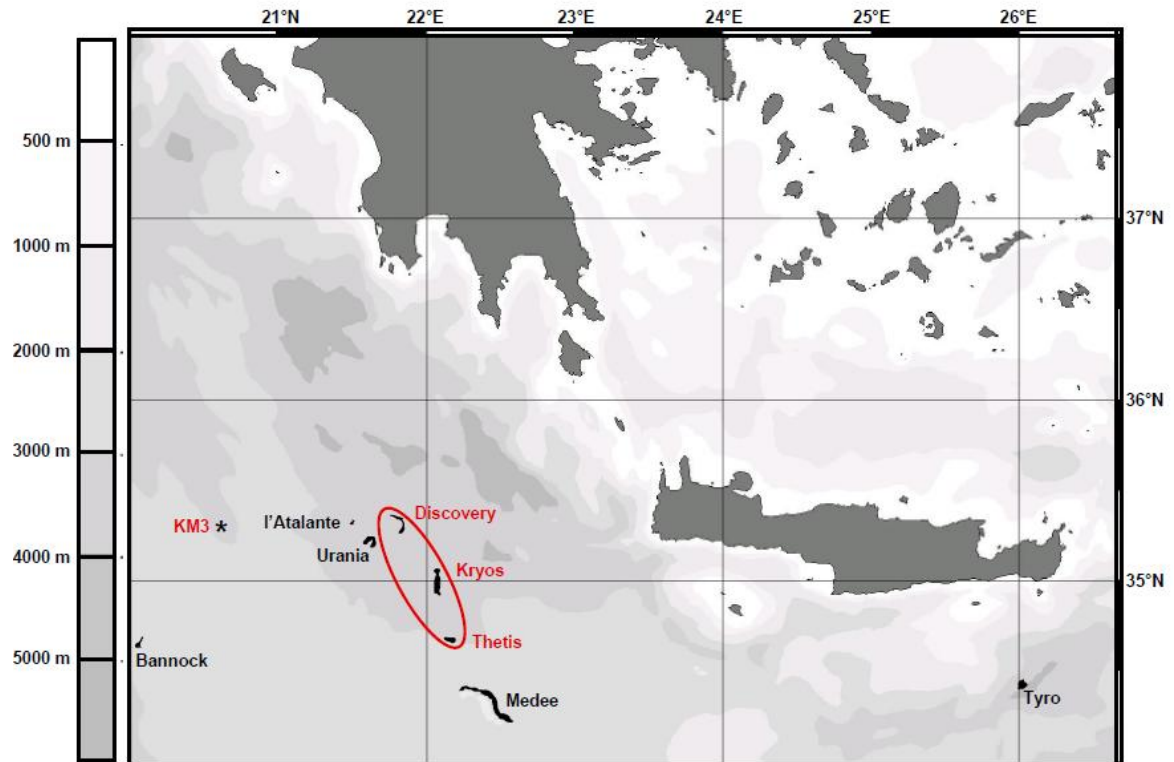


Kryos basin



Sediment at the bottom of the DHAB

deep hypersaline anoxic basins (DHABs) were formed at most 35,000 years old by [Messinian evaporite](#) salt deposits dissolving out of the [Mediterranean Ridge](#)



Deep cold water of Atlantic Ocean

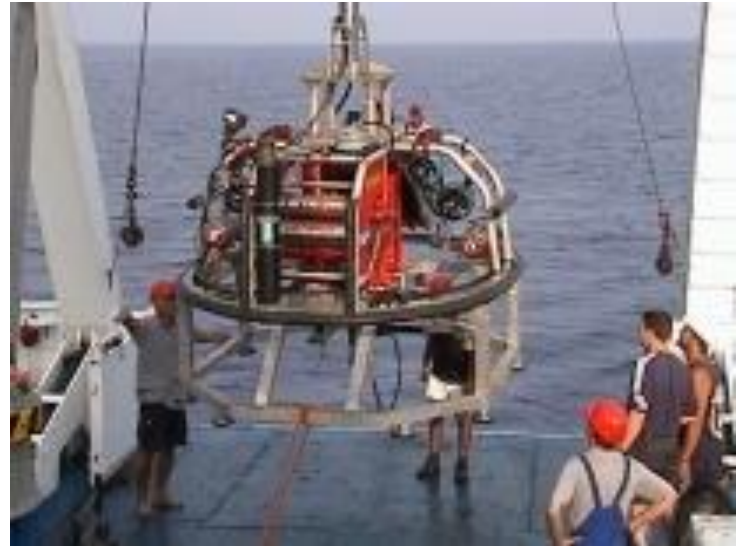
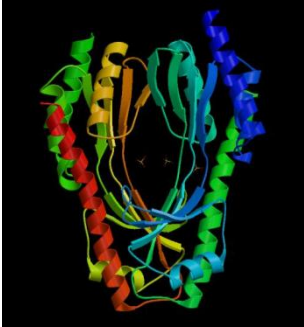


Thermal vents in Med Sea



Milos, Greece 36°40'09"N 24°31'06"E

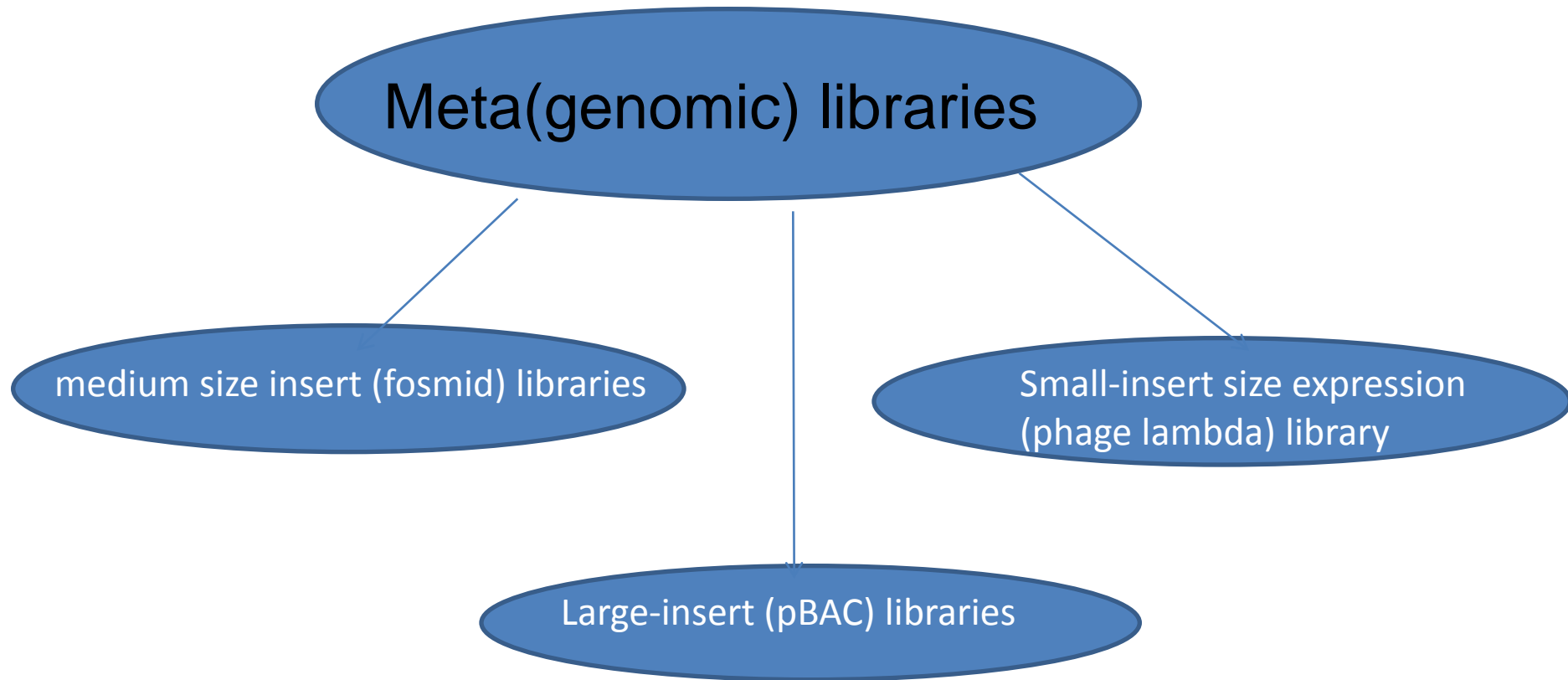
High pressure and low water activity conditions



Enzymes isolated from marine systems through metagenomics studies (modified from Kennedy *et al.*, 2010)

- *Esterase* (Deep sea sediment/basin, surface, Antarctic seawater, Arctic sediment)
- *Lipase* (Tidal flat, Deep sea sediment, Baltic sediment, Antarctic seawater, sea saltern)
- *Cellulase* (Deep sea sediment, Antarctic Seawater, Shipworm, Marine sponge)
- *Chitinase* (Estuary, Antarctic ice, marine hot spring)
- *Amidase* (Marine sediment, sludges)
- *Amylase* (Deep sea sediment, deep sea hydrothermal vent)
- *Phytase* (Fish gut)
- *Protease* (Deep sea sediment, Antarctic seawater, Coastal sulfataric vent)
- *Alcane hydrolase* (Hydrocarbon seep, Deep sea sediment)
- *Xylanase* (Antarctic seawater)

Three types of libraries used in MAMBA project

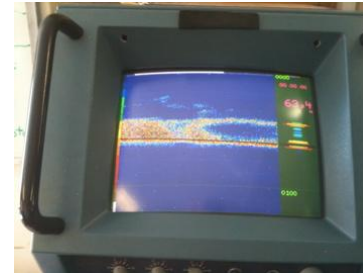
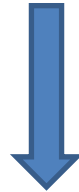


Genomic material extraction from different marine resources

Samples from:



water, sediments, chimneys,
bivalve tissues, etc

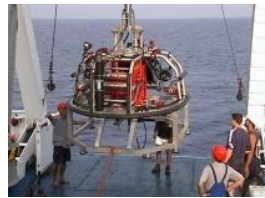


Samples are used as starting material for genomic
material production and library production

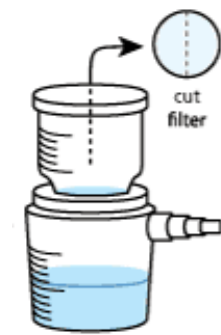
Various protocols used for meta/genomic libraries production:

- ***DNA extraction;***
- ***The medium-size-insert (35-40 kbp) CCFOS fosmid library generation;***
- ***The small-insert (5-10 kbp) lambda phage expression library;***
- ***pBAC library***

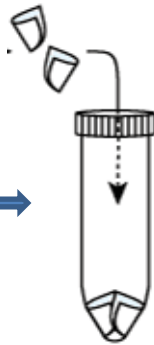
The recovery of environmental nucleic acids from water samples for generation of metagenomic libraries



**Water
Sample**



Filtration of
water samples



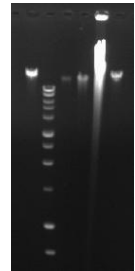
Washing
filter pieces



Cell lysis



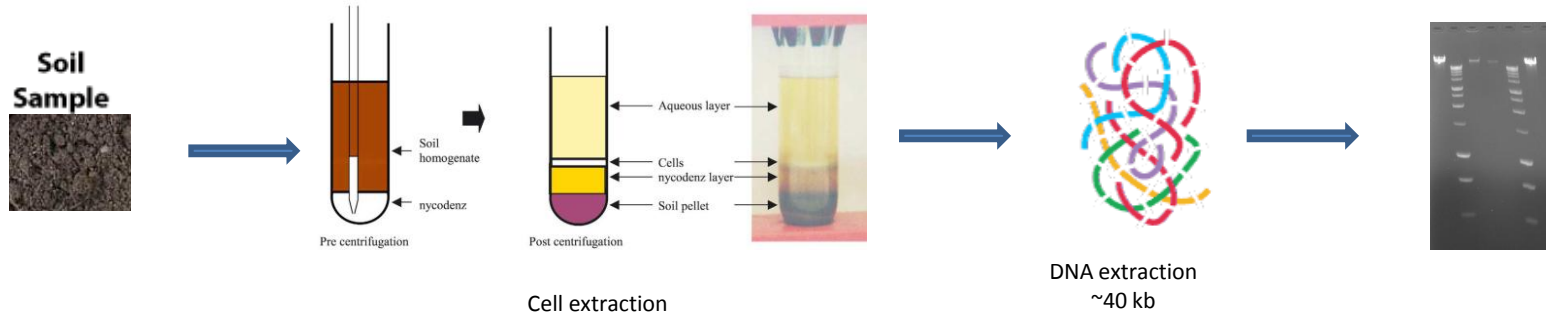
Extraction of
genomic DNA



Meta-G-Nome Kit, Epicentre, modified genomic DNA

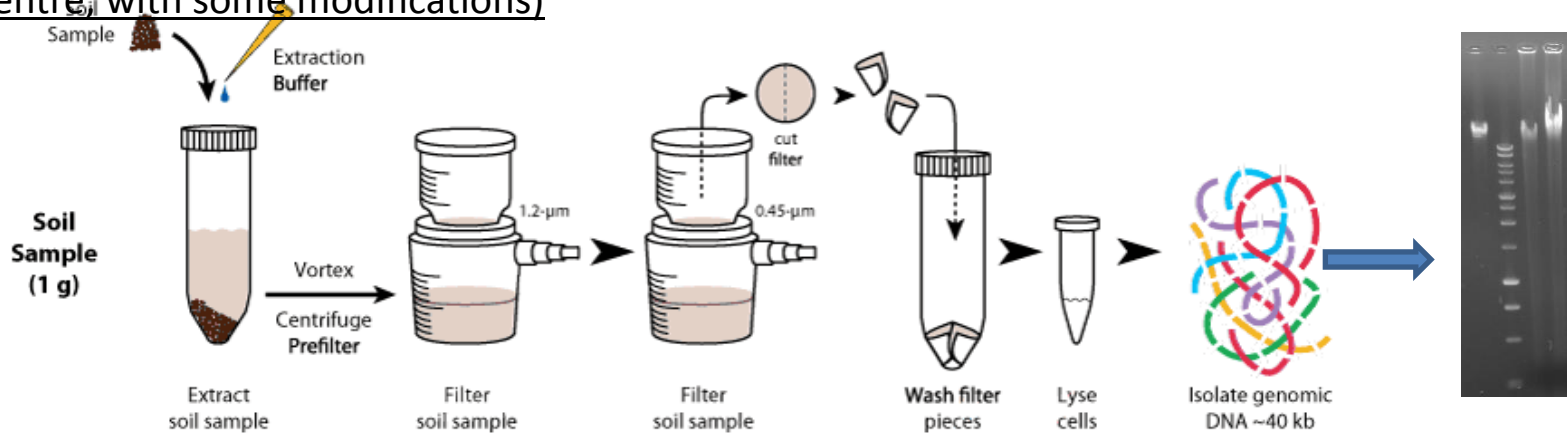
The recovery of environmental nucleic acids from the samples for generation of metagenomic libraries

I. Method -density gradient centrifugation (this method could be used for sediment DNA isolation)



The method of the extraction of bacteria from soil by density gradient centrifugation described by Bakken and Lindahl (1995). This method enables a reasonable cell yield combined with low contamination of the extracted cells with soil particles. The extracted cells can be used to obtain nucleic acids for molecular genetic analysis. The other method - the Meta-G-Nome DNA isolation kit (Epicentre).

II. Method – Meta-G-Nome DNA isolation kit (Epicentre, with some modifications)



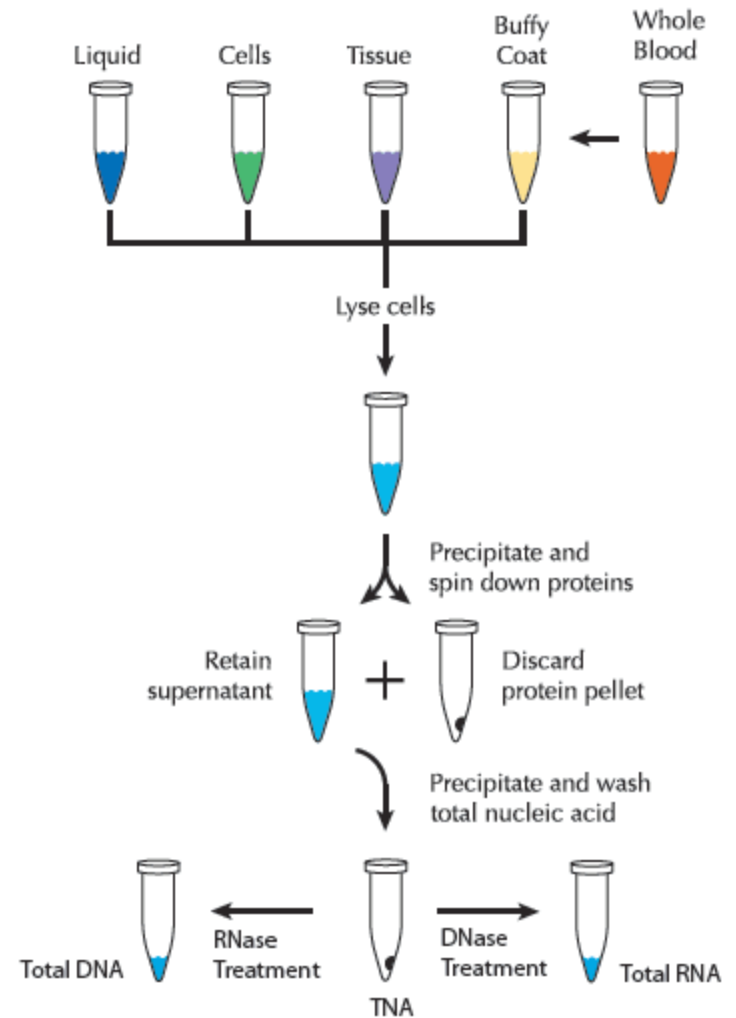
Other methods for genomic material extraction:

BIO 101 Protocol

web

G NOME[®]

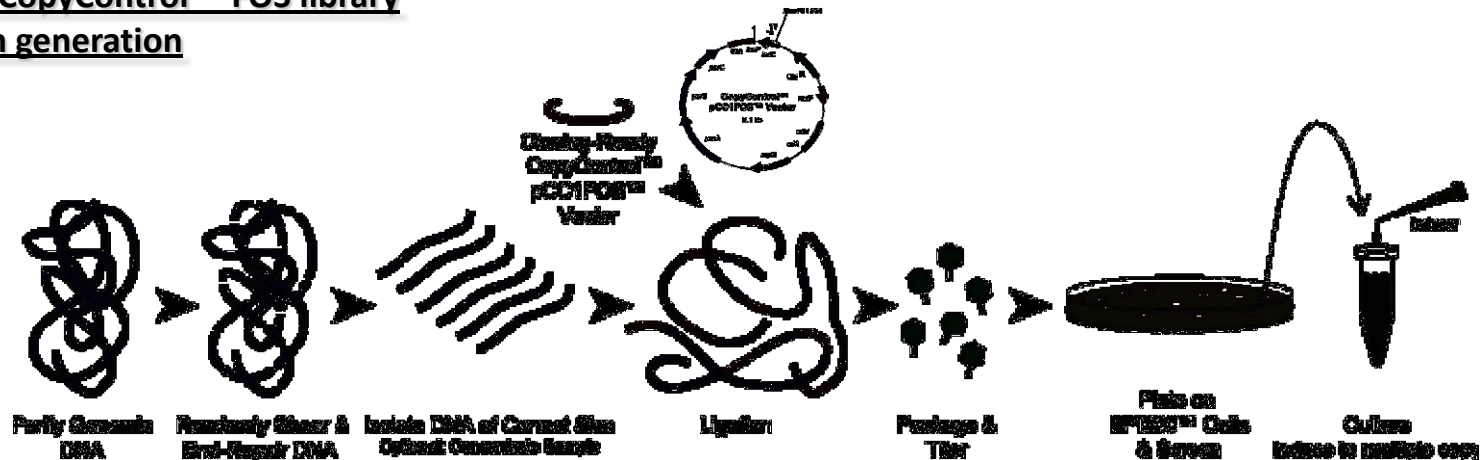
G'NOME (BIO 101/Qbiogene/ ICN)



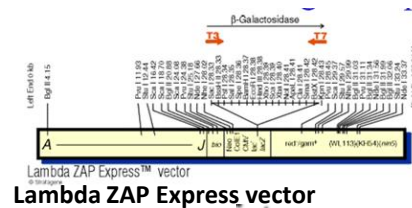
MasterPure Complete DNA and RNA (Epicentre)

The generation of genomic libraries of isolated environmental DNA in CopyControl™ FOS vector and lambda-ZAP cloning/expression system

Scheme of CopyControl™ FOS library production generation



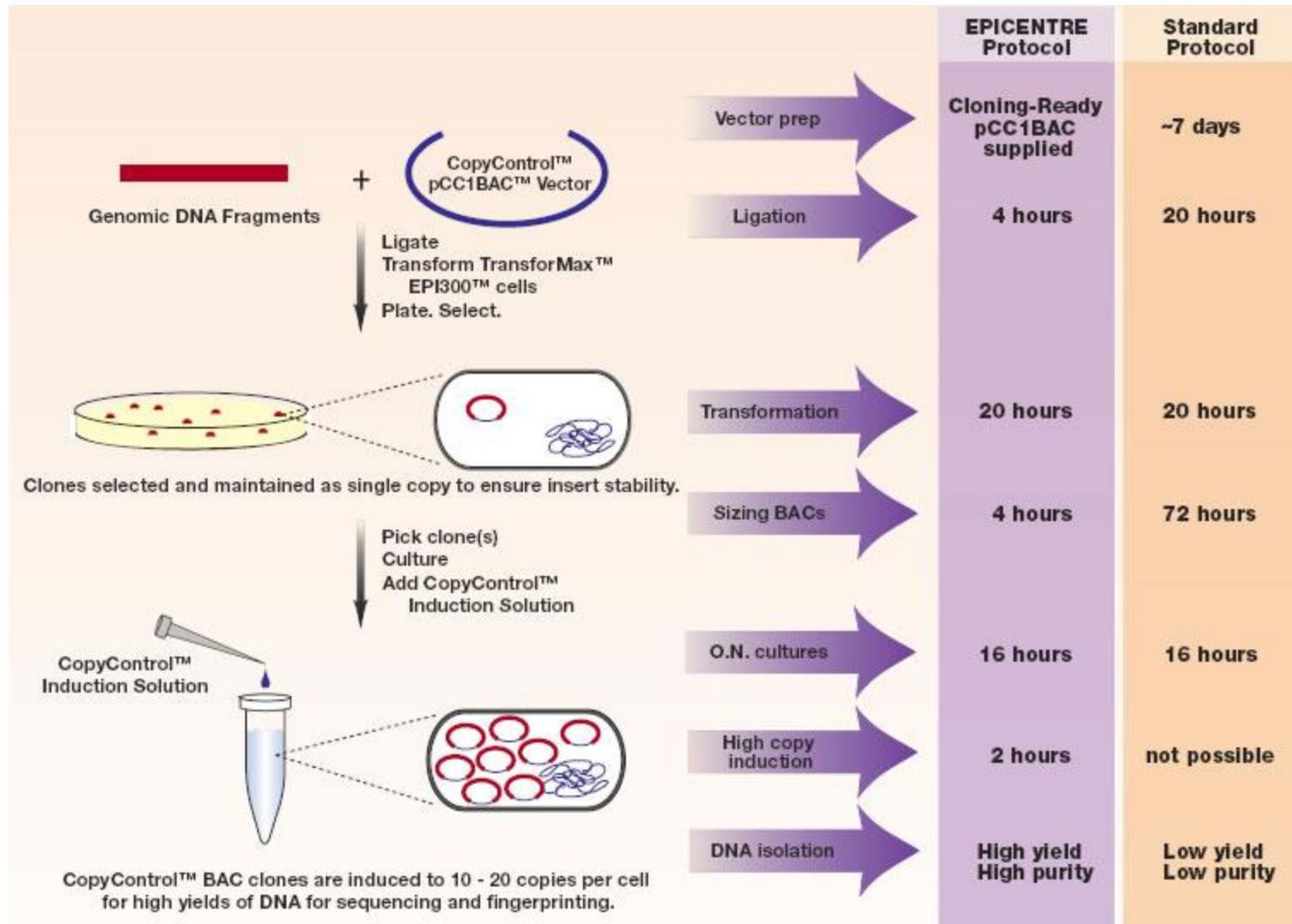
Scheme of ZAP express library production generation



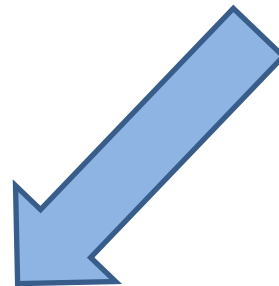
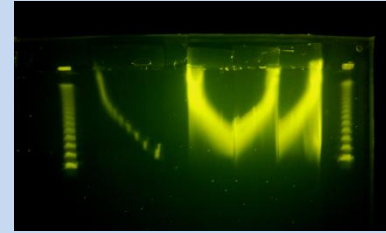
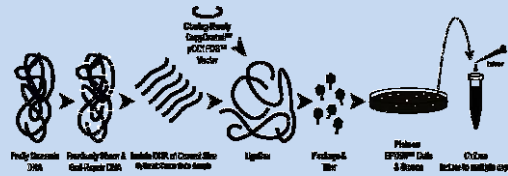
Lambda ZAP Express™ vector



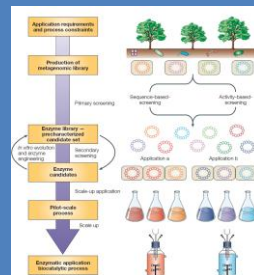
The CopyControl™ BAC Cloning Kit, CopyControl™ BAC Cloning Kits (*Bam*H I, *Eco*R I, or *Hind* III) *Epicentre* protocol



Metagenomic library



Activity-based enzymatic screening



Lorenz & Eck 2005
Nat Rev Microbiol

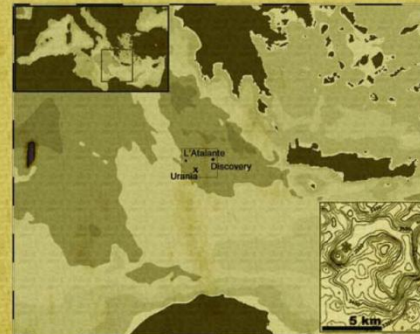
Sequence-based analysis



Chemistry & Biology

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**Metagenomics Reveals
Treasures from the Deep**