Marine meta/genomic libraries: establishing resources for MAMBA project

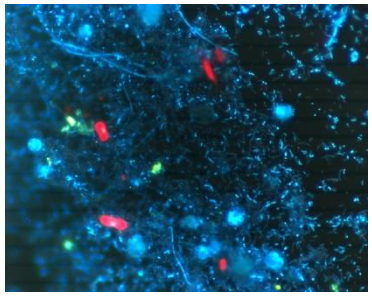

about 70% of the Earth surface marine environments

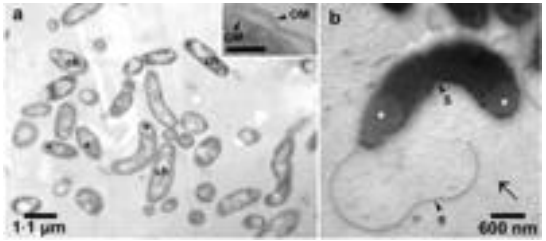
Marine biodiversity

Marine animals

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

Microorganisms





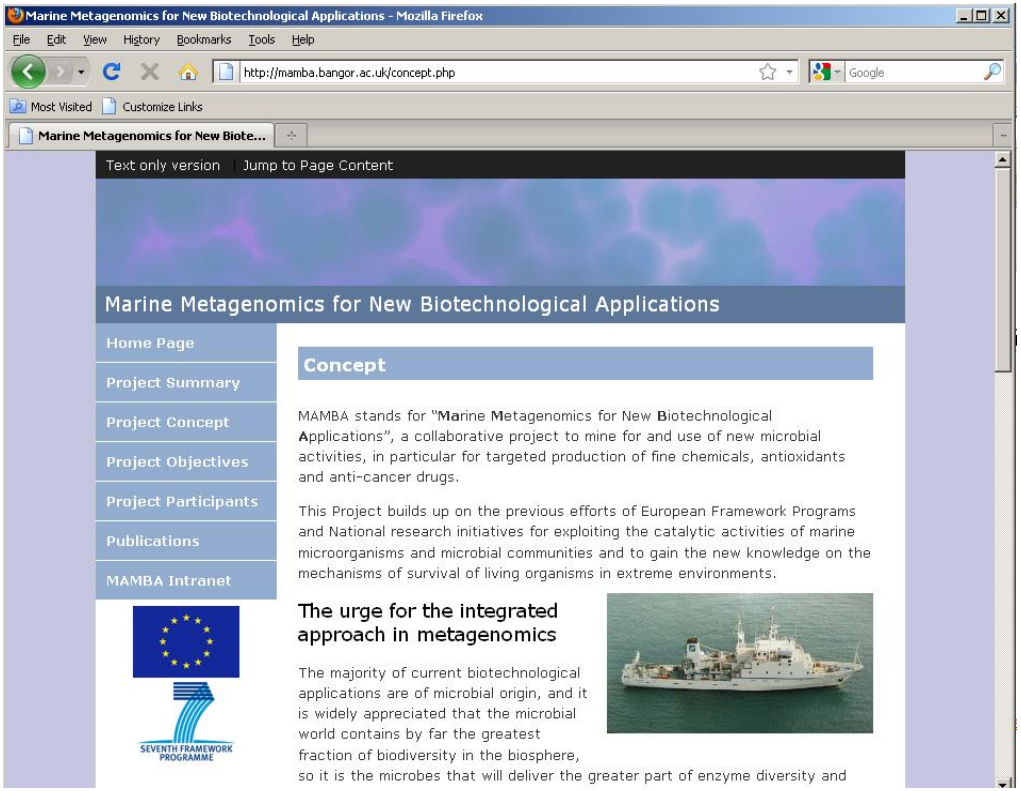
(Yakimov *et al*., 2004)

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

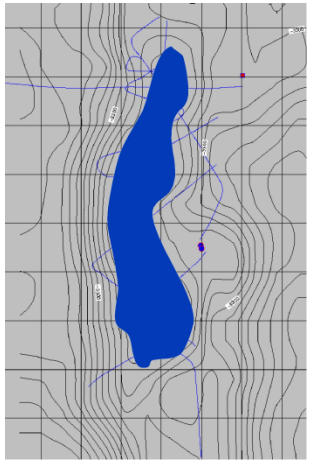


Importance of the topic

MAMBA explores marine microbial life at its limits

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

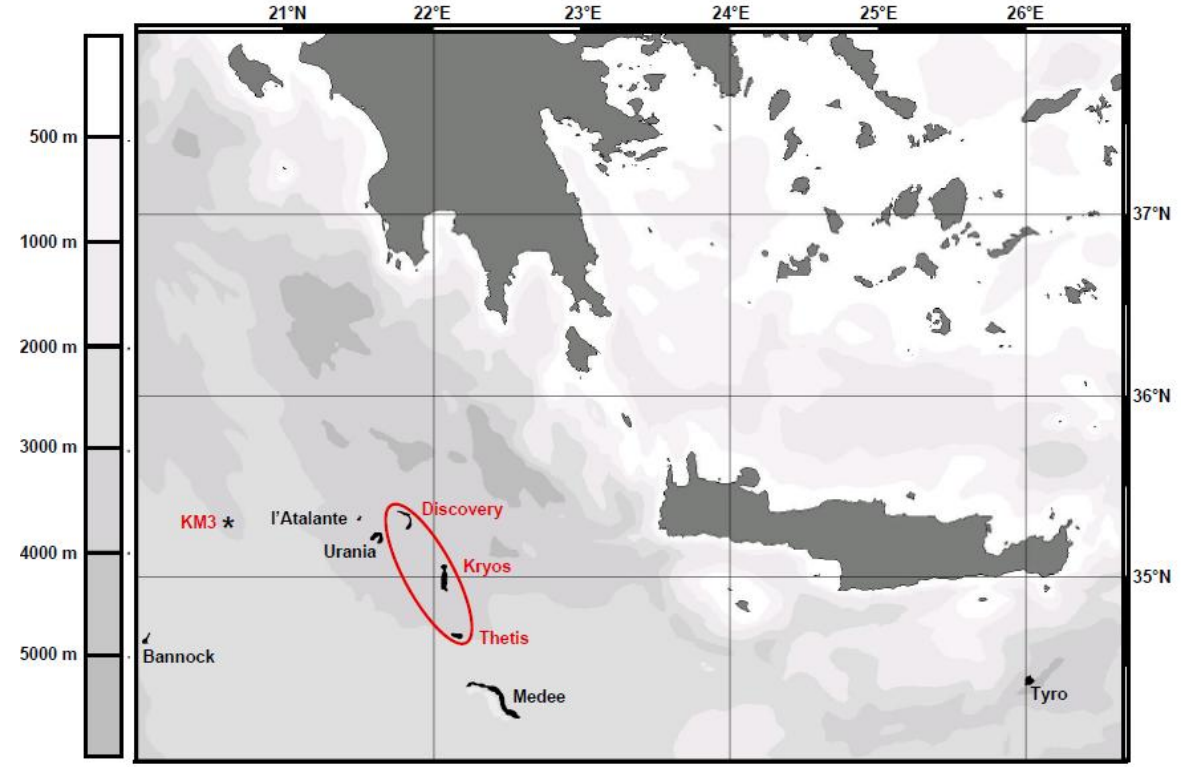
Hypersaline brine lakes at the bottom of the Mediterranean Sea



Kryos basin

Sediment at the bottom of the DHAB

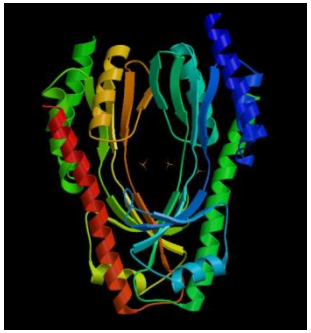
deep hypersaline anoxic basins (DHABs) were formed at most 35,000 years old by Messinian evaporite salt deposits dissolving out of the Mediterranean Ridge

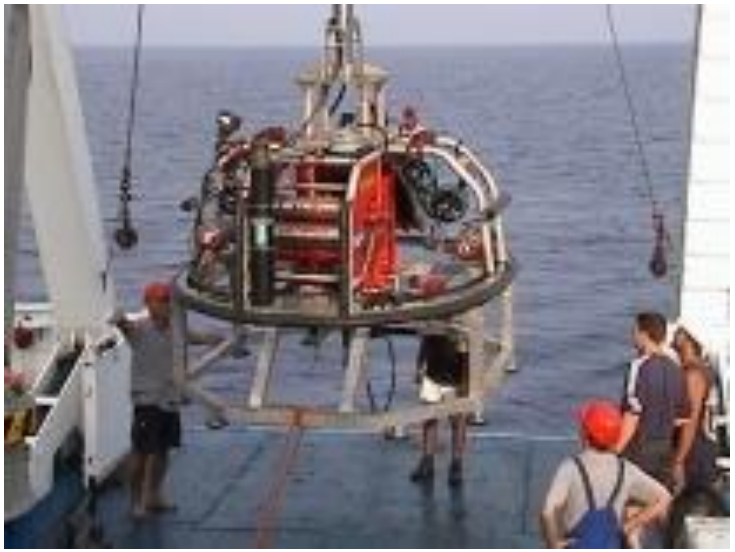


Deep cold water of Atlantic Ocean

Thermal vents in Med Sea

High pressure and low water activity conditions

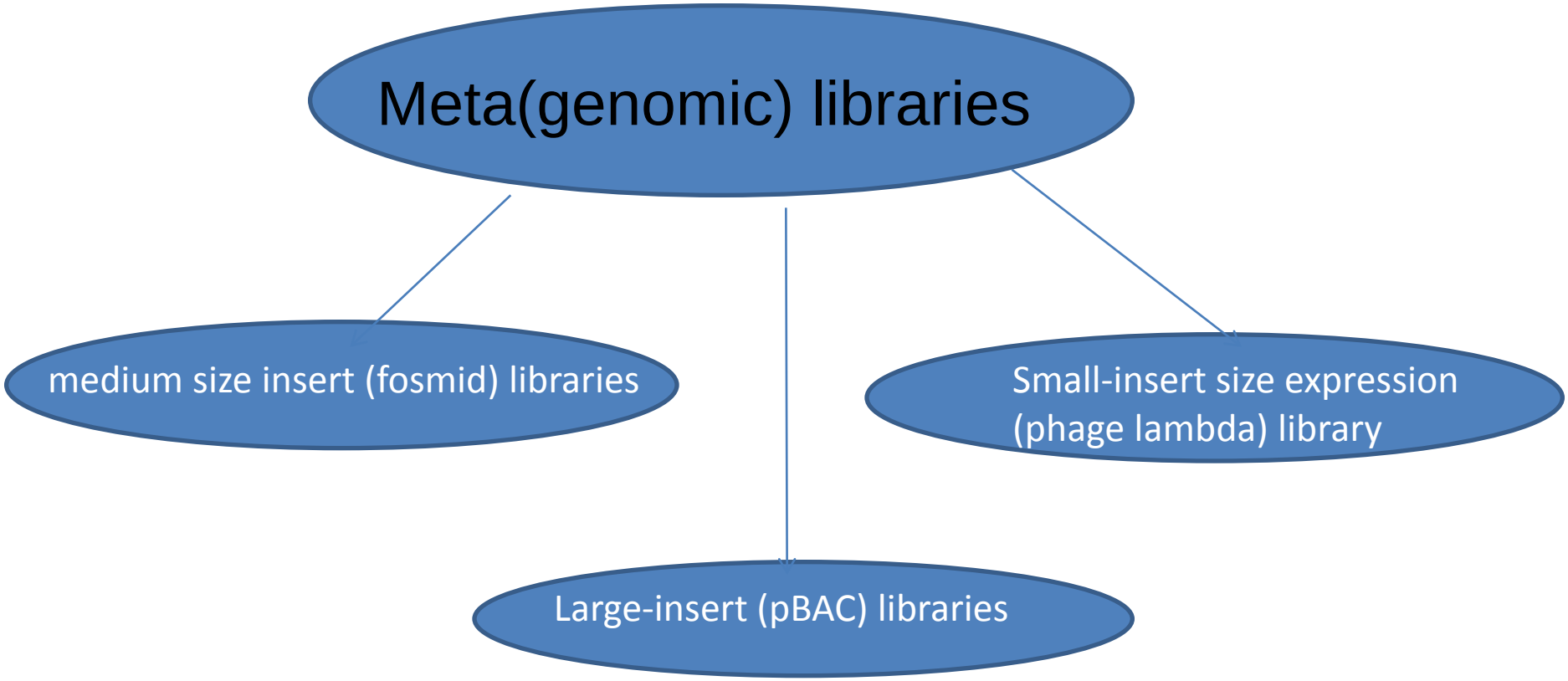




Enzymes isolated from marine systems through metagenomics studies (modified from Kennedy *et al*., 2010)

- *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 sediemnt, Antarctic seawater, Coastal solfataric vent)
- *Alcane hydrolase* (Hydrocarbon seep, Deep sea sediment)
- *Xylanase* (Antarctic seawater)

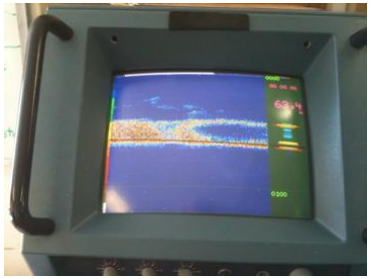
Three types of libraries used in MAMBA project



Genomic material extraction from different marine resources

Samples from:

water, sediments, chimneys, bivalve tissues, etc

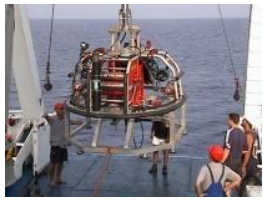


Samples are used as starting material for genomic material production and library production

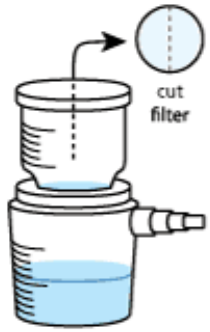
Various protocols used for meta/genomic libraries production:

- ***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***

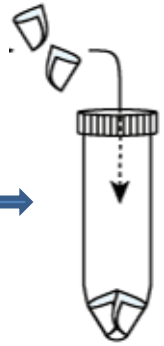
The recovery of environmental nucleic acids from water samples for generation of metagenomic libraries



Water Sample



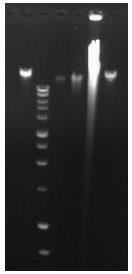
Filtration of water samples



Washing filter pieces

Cell lysis

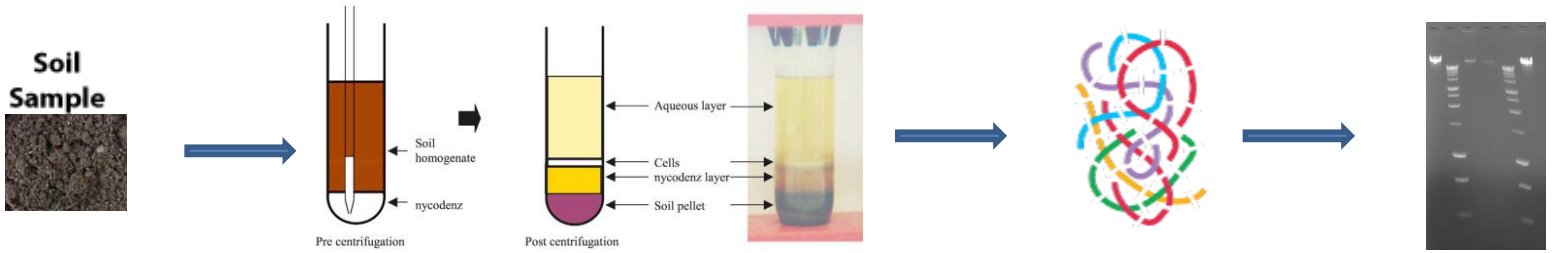
Extraction of genomic DNA



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)

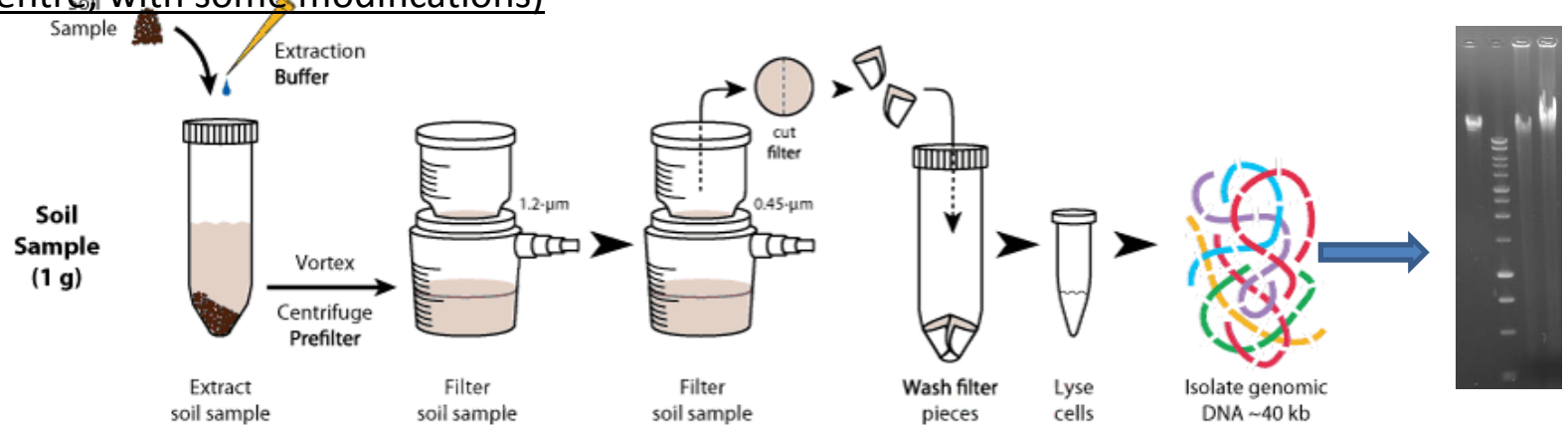


Cell extraction

DNA extraction
~40 kb

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)

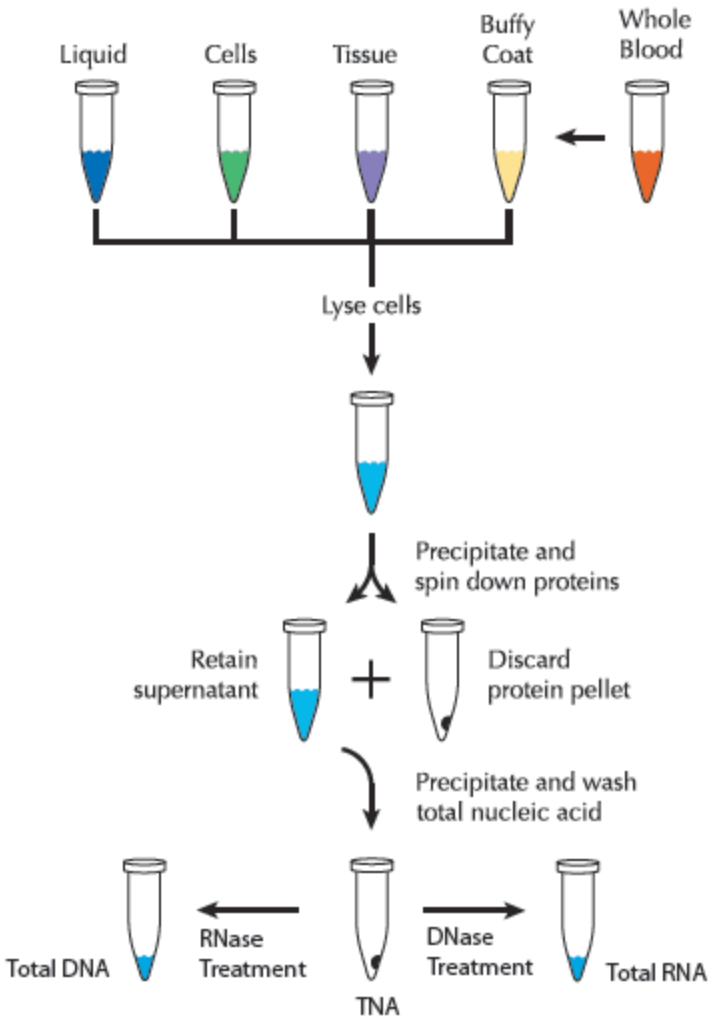


Other methods for genomic material extraction:

BIO 101 Protocol web

G NOME®

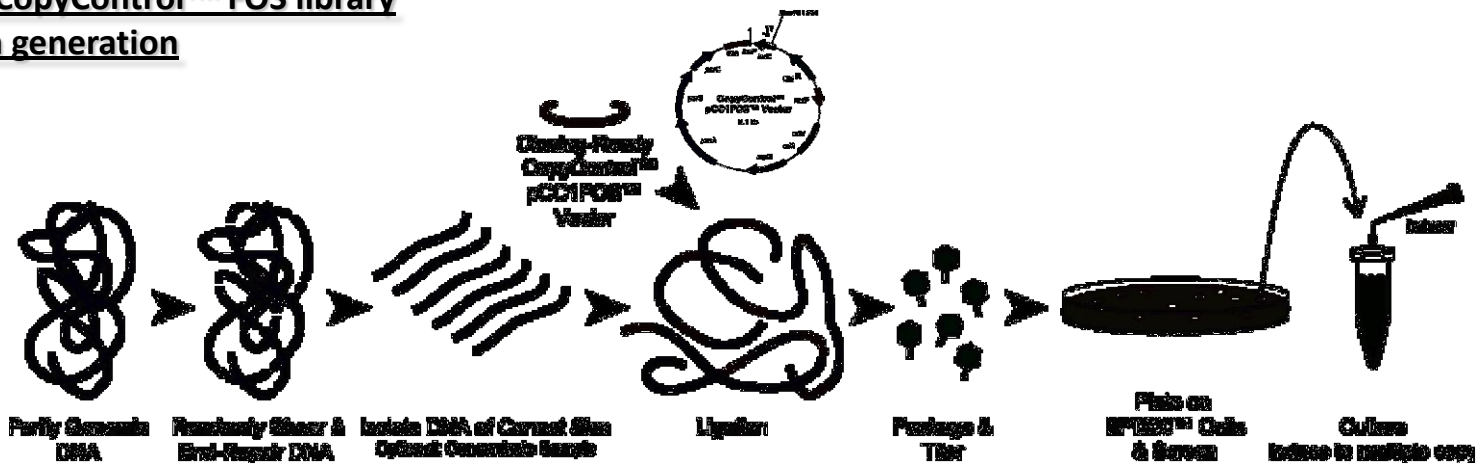
G'NOME (BIO 101/Qbiogene/ ICN)



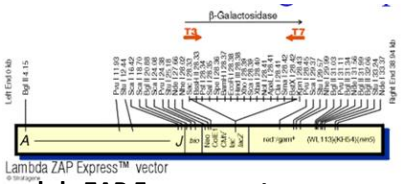
MasterPure Complete DNA and RNA (Epicentre)

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

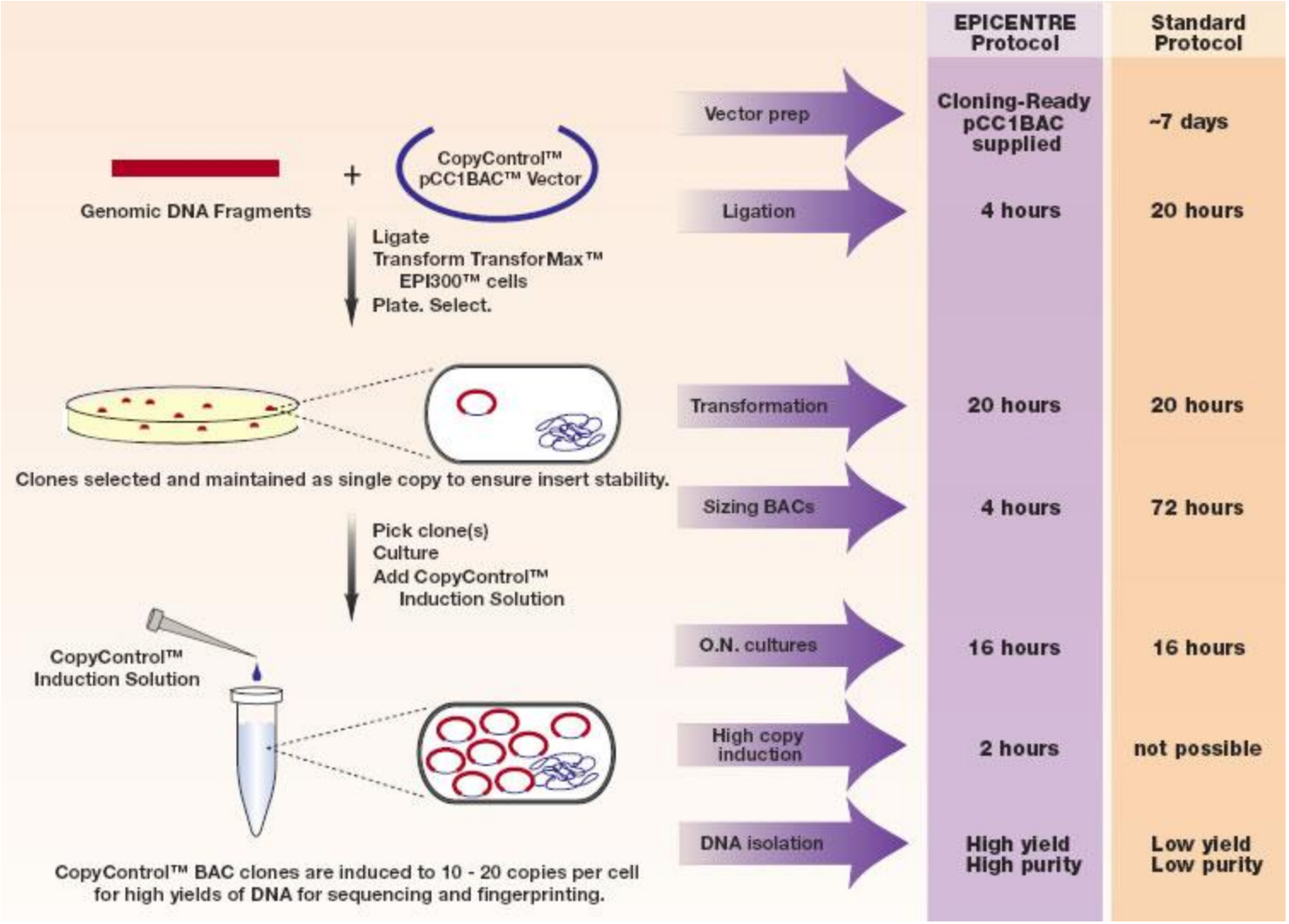


Scheme of ZAP express library production generation



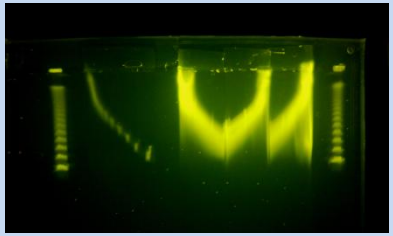
Lambda ZAP Express vector

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

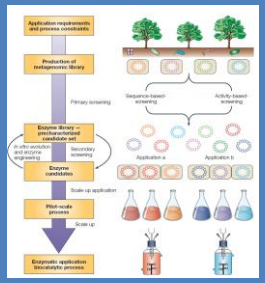


Epicentre™ online resources

Metagenomic library



Activity-based enzymatic screening



Lorenz & Eck 2005
Nat Rev Microbiol

Sequence-based analysis


Chemistry & Biology
Volume 12 Number 8
August 2005
L'Atalante
Discovery
Urania
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Treasures from the Deep