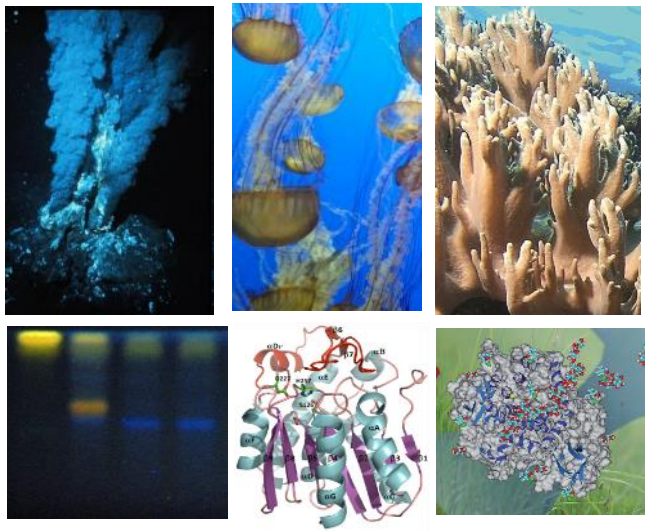
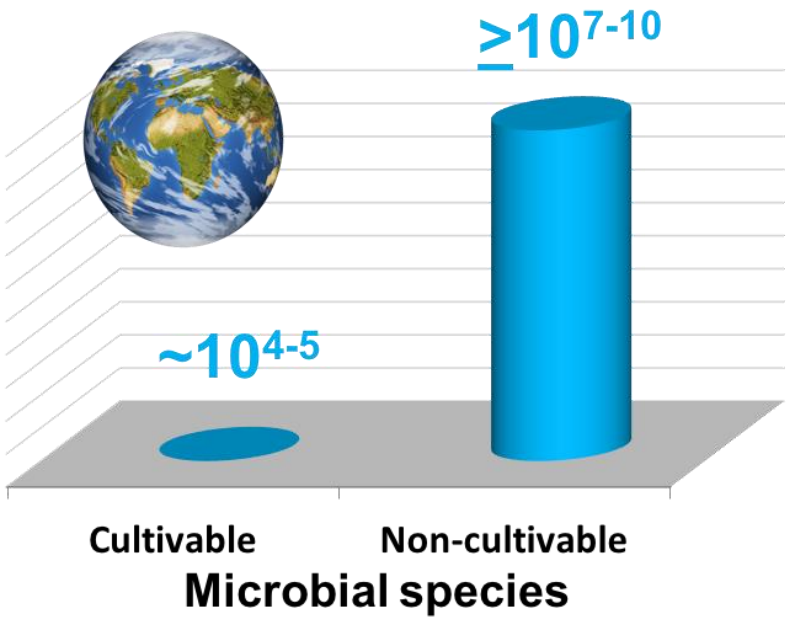
Advanced Marine Biotechnology for accessing the non-cultivated Microorganisms and Biodiversity: MarBioTech





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SINTEF, Trondheim, Norway

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ArcticZymes, Tromsø, Norway

MarBioTech-Mining non-cultivated biodiversity

Milestones and deliverables

- up to 10 libraries produced and delivered for screening and NGS
- RNA extraction of up to 10 multicellular hosts, EST libraries and screening
- Establish marine model hosts and *in vitro* screening systems
- Minimum of 5-10 novel candidate enzymes for each: biofilm inhibiting, PET esterase, fluorescents activity, and 1-2 DNA-/RNA polymerases, single-strange nucleases

WP1: Delivery of unique and highly diverse marine DNAs

WP2: Expanding marine metagenome tool box , for *in vivo* and *in vitro* screening techniques

WP3: Functional screening of marine metagenomes

WP4: Sequencing, conserved motif-based sequence mining & bioinformatic characterization of selected proteins.

WP5: Delivery and characterization of selected high value molecules

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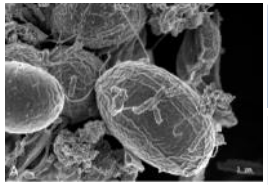
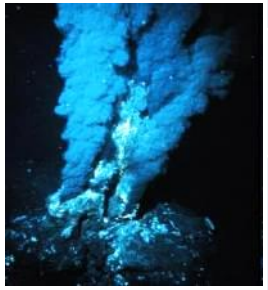
Marine Toolbox

Marine host strains, in vitro systems; anti-biofilm enzymes, PET esterases, DNA/RNA polymerases, biosensors (novel GFP's)

MarBioTech-Samples available





Sites sampled of marine resources available in MarBioTech	Features
Marine macro – and microalgae surfaces.	Diverse communities including symbionts and many non-cultivated bacteria
Marine fish farming	Diverse communities from off-shore fish cages
Seaweed. Mediterranean and Baltic Sea, Portugal, Spain, Germany and Denmark	Plant associated partially halotolerant microbes
Mucus of marine jellyfish (*Aurelia aurita*), Germany	diverse specifically associated bacterial communities
Marine sponges. Baltic and Irish Sea, Germany, Ireland, UK and Portugal	4-18°C, diverse symbionts, highly diverse communities
Marine mussels (*Mytilus edulis*). Baltic and Irish Sea, Ireland, UK and Germany	4-18°C, diverse symbionts
Deep sea hydrothermal vent & chimney (Mid-Atlantic Ridge); vent fields	8-120°C, varying sulfide &hydrogen availability; multiple tall chimneys emitting low pH (~ 4-5) and high temperature fluids (up to 317°C);
Deep sea Atlantik,Pacific, Germany	Diverse communities including many non-cultivated bacteria

Plus uniqe strain collection with > 2,500 marine isolates

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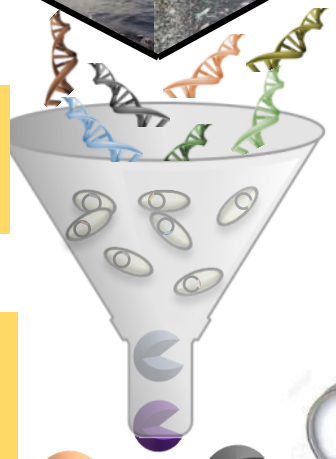
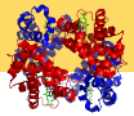
Objectives - Schmitz-Streit, CAU Kiel











→ Extract DNA from 10-20 different existing marine samples and construct fosmid libraries for use in functional screening

→ Prepare DNAs extracted from metagenomes and individual strains for NGS and in silico screening

→ Develop and optimize in vitro screening techniques using sophisticated in vitro transcription/translation systems

→ Establish novel screening systems for detecting marine RNA polymerases and nucleases

Objectives – Uni Hamburg

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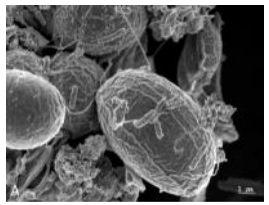
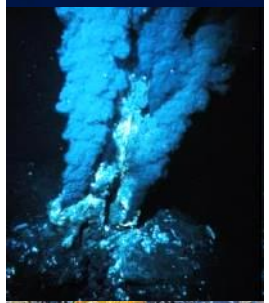
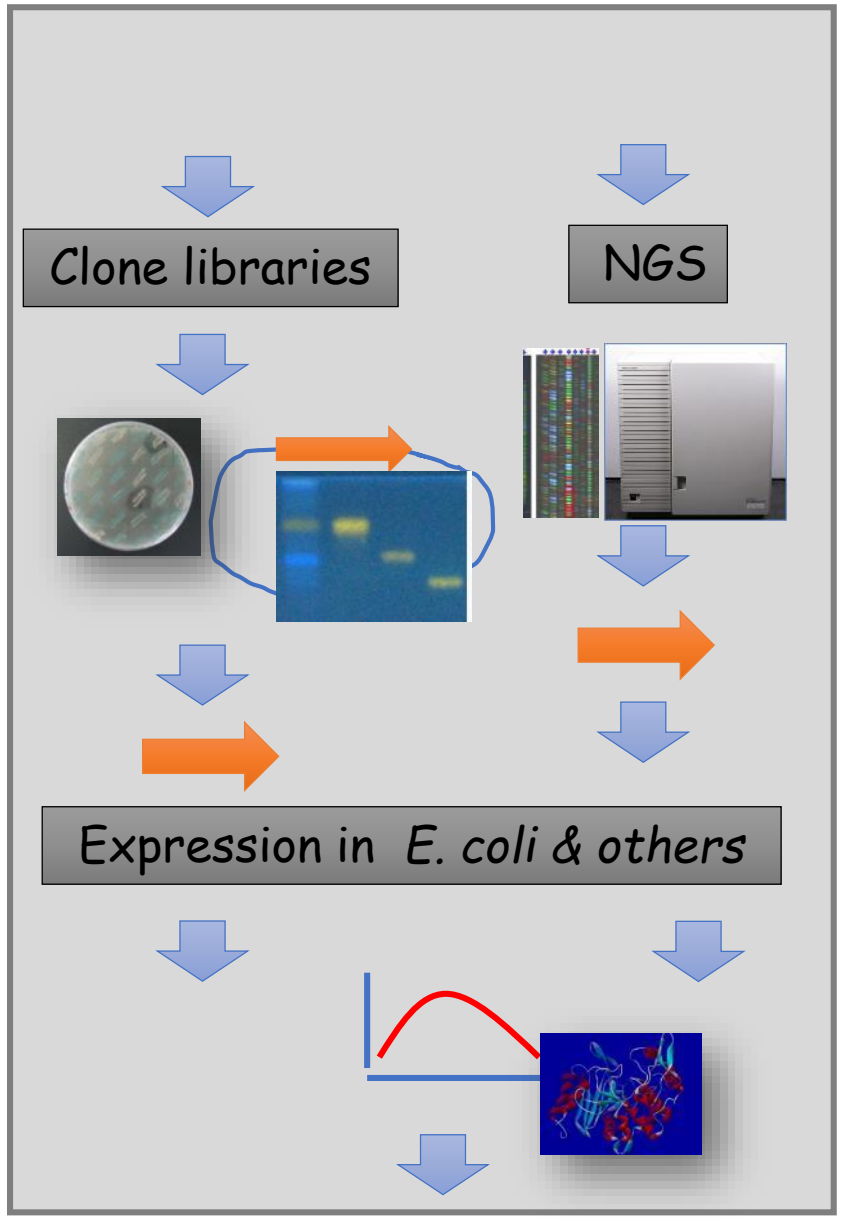
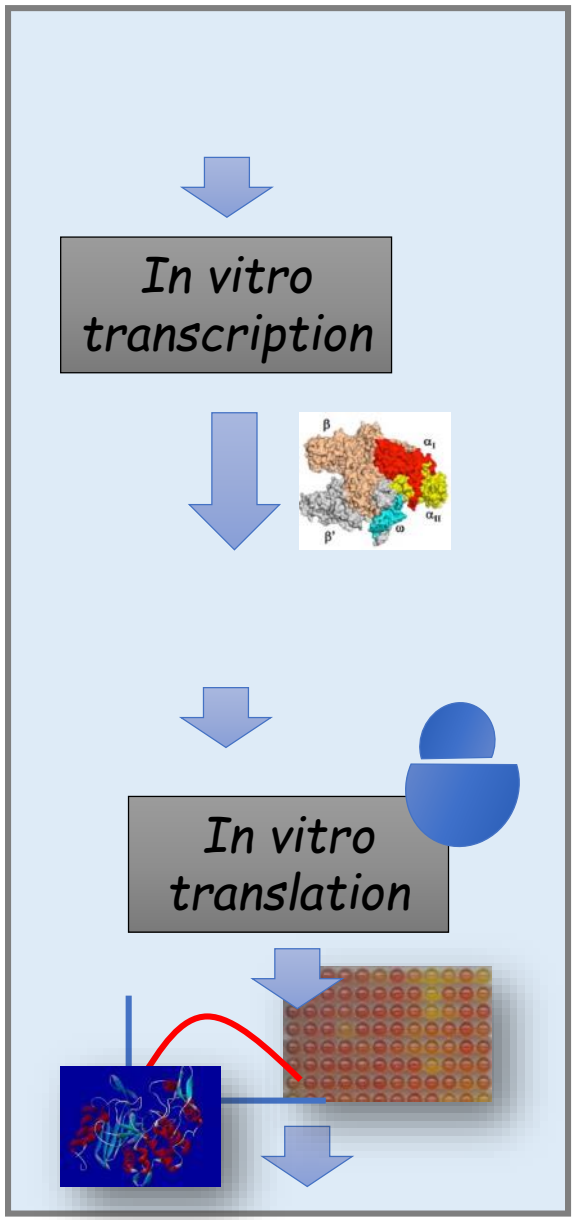
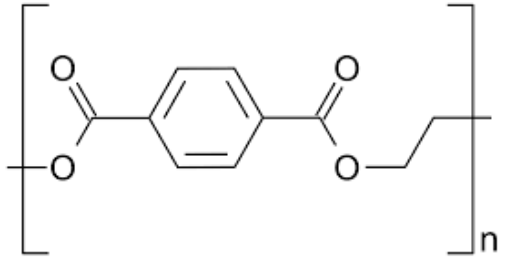




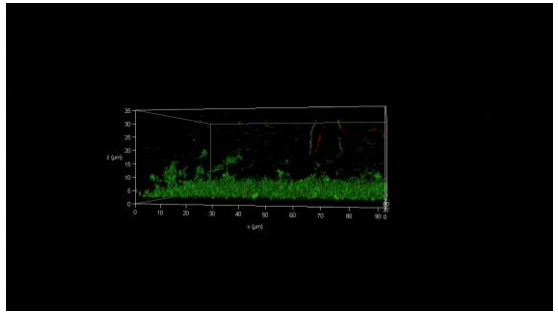




Targets:



PET Hydrolases



Anti-biofilm proteins

Biosensors Variants o, RNA polymerases, etc...

Objectives -SINTEF

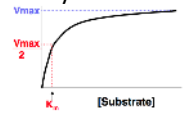
- Devlopment **of new functional assays** for selected enzyme classes (RNA polymerases, DNA nucleases) – WP2 (T2.4)
- Protocol development and robotic and FACS-based (ultra-)high throughput screening of metagenome libraries (fluorescent proteins, **DNA/RNA processing enzymes**) – WP3 (T3.1, T3.2, T3.4, T3.5)
- **Heterologous production** of novel proteins and enzymes for functional characterization – WP5 (T5.1)

Objectives - Nobuhiko Tokuriki, Michael Smith Laboratory, University of British Columbia

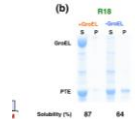
Metagenomic screening → Comprehensive bioinformatics → Enzyme characterizations

Isolated sequences

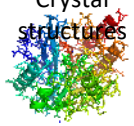
Enzyme kinetics



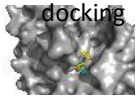
Protein stability



Crystal structures



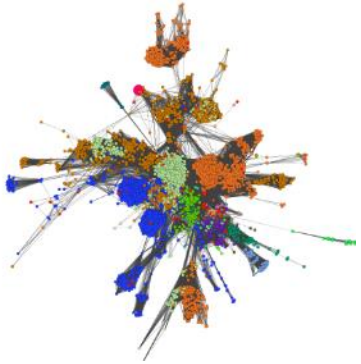
Substrate docking



MD simulations

Diverse biochemical and biophysical characterization of the isolated and it homologous enzymes

Comprehensive bioinformatics

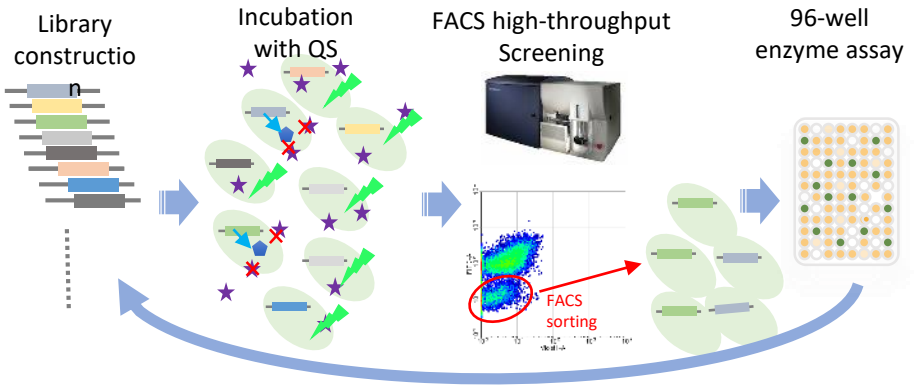


Large scale bioinformatics approaches (sequence similarity networks) to elucidate the function and structure of the isolated sequences.

Bioinfomatics also provide effective enzyme engineering strategies for targeted mutagenesis and rational design to further optimize the function of the target enzyme.

Directed evolution and enzyme engineering

High-throughput enzyme engineering platform



Directed evolution approach to further optimize the target enzymes

Objectives - *ArcticZymes*

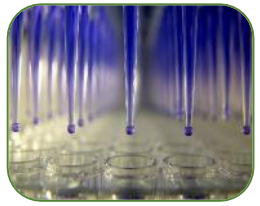
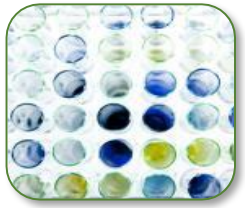
ArcticZymes develops, produces and sells unique enzymes for use in molecular research, diagnostics and biomanufacturing. ArcticZymes is searching for new polymerases, nucleases for molecular applications, and fluorescent proteins, phosphatases and peroxidases for diagnostic purposes.

ArcticZymes main project activities

WP1-2

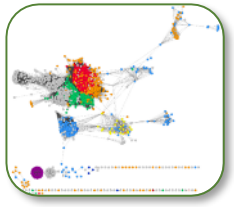
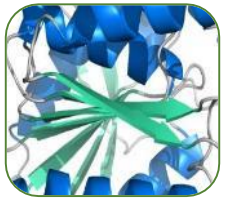

Contribute to selecting starting material and screening parameters.

WP3

Contribute to enzyme assays for functional screening purposes.

WP4

Contribute to enzyme bioinformatics and selection of target sequences

WP5


Purification and biochemical characterization of selected enzymes and proteins.

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