# Marine metagenomic large insert libraries as rich source for biofilm inhibiting molecules

Marine microbial consortia are highly diverse and have evolved during extended evolutionary processes of physiological adaptations affected by a variety of ecological conditions and selection pressures. They harbor an enormous diversity of metabolically complex microbes with still unknown and probably new physiological characteristics, and are thus rich sources for isolating novel biologically active and pharmacologically valuable natural products. However, to date, the biodiversity of marine microbes and the versatility of their bioactive compounds and metabolites have not been fully explored. In our recent publication, we in detail describe (*i*) sampling in the marine environment, (*ii*) construction of metagenomic large insert libraries and (*iii*) exemplarily the function-based screen of metagenomic clones for identification of quorum quenching (QQ) activities.

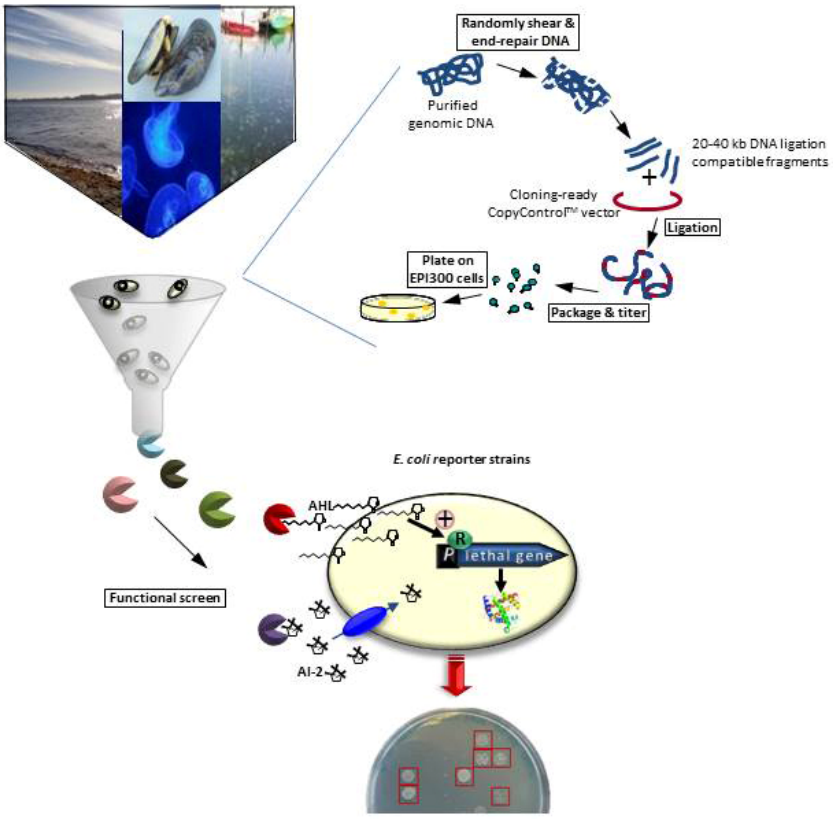


Fig. 1. Identification of quorum quenching activities in environmental samples. Total DNA is isolated from marine environmental samples and the genetic information is stored in metagenomic libraries. Cell-free culture supernatants and cell extracts of these metagenomic clones are functionally screened for quorum quenching (QQ) compounds using E. coli-based reporter strains. Degradation or modification of the signaling molecules by QQ biomolecules allows growth of the reporter on agar plate.

Marine surface or deep water can be sampled using membrane pumps, Conductivity-, Temperature-, Depth sensor (CTD) equipped with a 24 Niskin 10 L bottle rosette or *in situ* pumps (volumes up to 5,000 L). After collecting, pre-filtration with filters of 10 µm pore size is performed directly followed by a consecutive filtration with polycarbonate or polyvinylidenfluoride membrane filters of 0.22 µm pore size. DNA from those filters is commonly extracted by a direct lysis of the microorganisms. Subsequently, a fosmid vector kit (e.g. Copy Control$^{TM}$ Fosmid Library Production Kit; Epicentre, Madison/USA) is used to construct a large insert library. The fosmid vector is carrying the single-copy and an additional inducible high-copy number origin of replication enabling stable and successful cloning of up to 40 kb DNA fragments as well as expression of toxic proteins and unstable DNA sequences. Additionally, the fosmid allows increased DNA yields in vector preparations and functional screens of clone libraries by induction to high copy numbers. Further, beside sequence-based analyses, functional screens for novel genes can explore the genetic potential of the habitat in metagenomic libraries by directly monitoring products or enzymatic activities of metagenomic clones.

In our publication, we exemplarily describe the functional screening of metagenomic libraries for natural quorum sensing interfering compounds. Quorum sensing (QS), the bacterial cell-cell communication, is based on the accumulation and perception of small signaling molecules (autoinducers) and enables the bacteria to detect an increasing cell density by sensing the signaling molecule concentration. This allows the bacteria to change their gene expression to coordinate behaviors that require high cell densities, e.g. pathogenicity and biofilm formation. Most bacteria are able to grow in biofilms, in which they effectively resist antimicrobial agents. This leads to a general problem, since traditional treatments of bacteria and prevention strategies become mostly ineffective. Novel strategies for biofilm inhibition are thus urgently required, and compounds interfering with QS are promising targets for alternative strategies. Recently, reporter strains AI1-QQ.1 and AI2-QQ.1 were established to identify such QQ compounds interfering with acyl-homoserine lactone (AHL) and autoinducer-2 (AI-2) based cell-cell communication. Both, cell extracts and culture supernatants of fosmid single clones or clone pools (up to 96 single clone equivalent to one microtiter plate) can be rapidly and easily screened for QQ activities. In order to identify the respective open reading frame (ORF) of a confirmed fosmid conferring QQ activity, two alternative methods can be used (*i*) subcloning or (*ii*) *in vitro* transposon mutagenesis. Such naturally occurring QQ compounds are attractive candidates for future use in biotechnology and medicine, in particular in those cases where therapy with antibiotics fails or their administration is strictly regulated.

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## Publication

Construction and Screening of Marine Metagenomic Large Insert Libraries.
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