# Molecular markers for detection and phylogenetic analysis of methanogenic consortia

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Methanogenic archaea are phylogenetically diverse group of strictly anaerobic microorganisms that occure in anoxic environments, such as peatlands, freshwater sediments, land fills, anaerobic digesters and the intestinal tracts of ruminants. The common feature of this physiological group of archaea is the ability to formation of methane from different substrate: $CO_2$ and $H_2$, formate, methanol, methylamines and/or acetate. Methanogenesis mediated by Archaea is a crucial element of global carbon cycle, taking into account the fact that ~1 billion tons of methane per year is produced in this way. In the light of this information it is important to study the biology and diversity of methanogenic archaea.

As majority of methanogens are uncultivable, thus the main method used for their diversity studies is based on (meta)genomic approach. Two marker genes: 16S rRNA and *mcrA* (encoding the α subunit of the methyl coenzyme M reductase) are commonly used for the detection and phylogenetic analysis of methanogenic consortia.

In this study, we propose novel molecular markers for methanogenic consortia analyses. We created and validated a set of degenerated PCR primers for the amplification of genes encoding key enzymes involved in the methanogenesis, including: *mcrB* and *mcrG* (β and γ subunits of the methyl coenzyme M reductase), *mtaB* (methanol-5-hydroxybenzimidazolylcobamide Co-methyltransferase), *mtbA* (methylated [methylamine-specific corrinoid protein]:coenzyme M methyltransferase), *mtbB1* (dimethylamine--corrinoid protein Co-methyltransferase), *mer* ($F_{420}$-dependent methylene-$H_4$MPT reductase) and *cdhD1* (δ subunit of acetyl-CoA decarbonylase/synthase complex).

Specificity of the developed primers was verified by a sequencing of the PCR products obtained from different environmental samples (e.g. anaerobic digesters, mine waters and peatlands). Phylogenetic analysis of the amplicons' sequences confirmed that selected markers and developed PCR primers can be used as a specific tool for an in depth diversity analyses of methanogenic consortia.