miDGGE

MOLECULAR BIOLOGICAL TOOL

Profile and identify dominant members within a microbial community

Denaturing Gradient Gel Electrophoresis (DGGE) is a DNA-based technique which generates a genetic profile or "fingerprint" which can be used to identify the dominant members of the microbial community. DGGE has been used to investigate microbial responses in a wide variety of applications including:

- Bioremediation assessment
- Wastewater treatment
- Drinking water treatment
- Biofilm formation
- Microbial induced corrosion
- Identification of microbial contaminants in commercial/industrial products
- And more!

For bioremediation assessment, DGGE profiles and sequence analysis are commonly used for evaluating the similarities/differences in the microbial community composition (dominant bacterial or fungal groups). DGGE highlights differences between samples and changes or "shifts" in microbial community composition over time or following a treatment. For example, DGGE can be used to determine the differences in the dominant bacterial groups in contaminated versus non-contaminated groundwater monitoring wells to evaluate which groups are enriched in impacted zones. Likewise, DGGE can be utilized to determine which bacterial groups are stimulated following a corrective action such as addition of a growth substrate or nutrient.

DGGE fingerprints can be produced and dominant microorganisms can be identified for a variety of target groups:

Target Group	Level	Examples
Bacteria (DGGE-BAC)	Bacterial Community	*Bacteroidetes*, *Clostridia*, *Pseudomonas*, Proteobacteria, among many others
Fungi (DGGE-FGI)	Fungal Community	*Acremonium*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Saccharomyces*, and others
Sulfate Reducing Bacteria (DGGE-SRB)	Specifically targets sulfate reducing bacteria which are functionally important but may represent less than 1% of the total bacterial community	*Desulfobulbus*, *Desulfomonas*, *Desulfuromonas*, *Desulfobacter*, and others
Dehalococcoides (DGGE-DHC)	Specifically targets *Dehalococcoides* spp. which even under conditions favorable for growth may represent less than 1% of the total bacterial population	Allows separation of different strains of *Dehalococcoides*

DGGE
MOLECULAR BIOLOGICAL TOOL

How does DGGE work?

Denaturing Gradient Gel Electrophoresis (DGGE) separates mixtures of amplified 16S rRNA gene segments, which are all the same size, based on nucleotide sequence.

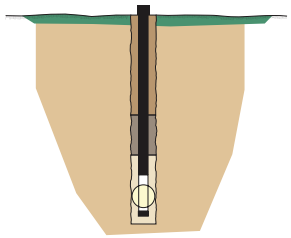
Denaturing—breaking apart the two strands of the DNA molecule.
Gradient Gel —gel with an increasing concentration of a chemical (denaturant) which breaks apart the DNA molecule.

Electrophoresis—application of an electric current across a gel. In response to the current, double-stranded DNA migrates (moves down) the gel. Denaturing the DNA molecule forms Y- and T-shaped structures greatly slowing migration.

DNA contains four nucleotide bases which bond across the two strands of the molecule - "G" forms three hydrogen bonds with "C"; "A" forms 2 hydrogen bonds with "T". Thus, DNA segments with more GC base pairs (high GC content) form stronger bonds between the DNA strands than those with less GC base pairs. Consequently, high GC content DNA segments require a greater concentration of the denaturing chemical before the DNA strands break apart.

The mixture of amplified DNA segments is loaded at the top of the gel. DNA migrates from the top (low denaturant concentration) toward the bottom of the gel (high denaturant concentration). DNA segments with low GC content denature near the top of gel and stop migrating. DNA segments with higher GC content denature further down the gel. DNA with the identical sequences migrate the same distance forming a "band". Individual bands are excised for sequencing and results are compared to a database of 16S rRNA genes to identify the dominant organisms.

Sample Collection

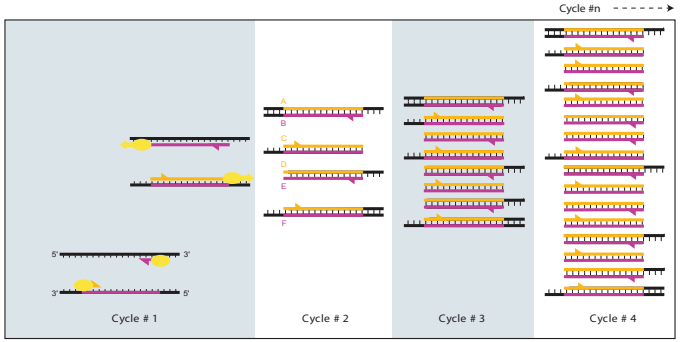


Groundwater, soil, or Bio-Trap ® Sampler collected and shipped overnight on ice (4°c)

DNA Extraction

DNA is extracted from samples upon arrival

Amplification



Variable region of 16S rRNA gene amplified by PCR

Results

DGGE Profile

Dominant bands excised and sequenced

GCCAGCCGTGTTTACAGA…	*Acinetobacter sp.*
GCCTTAAGCAGGCCTTCG…	*Geobacter sp.*
GCCAGCCGTGTTTACAGA…	*Unknown sp X.*
GCCGGCTCTAGCTTCCGT…	*Methylobacterium sp.*

Dominant organisms identified

microbialinsights
www.microbe.com

ERR www.erraus.com.au
Unit 59/148 Chesterville Road
Moorabbin Vic 3189 Australia
Phone: +61 3 9555 3800