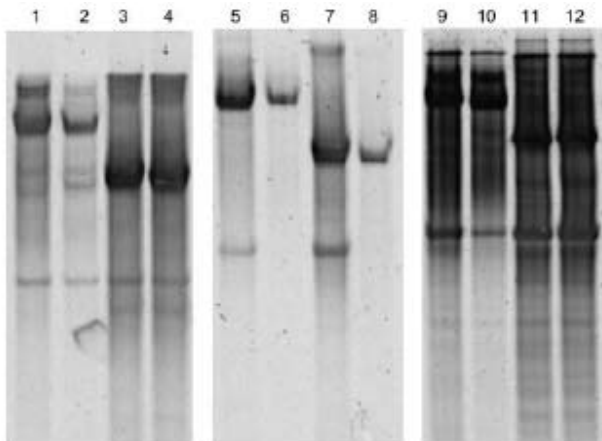


## Development of Temporal Temperature Gradient Electrophoresis for Characterising Methanogen Diversity

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### Abstract

Temporal temperature gradient electrophoretic (TTGE) analysis of 16S rDNA sequences was optimized to monitor the methanogen population present in water and sediments of a small eutrophic lake, Priest Pot, in the English Lake district. The production of nonrepresentative TTGE profiles due to the generation of polymerase chain reaction (PCR) artifacts initially proved problematical. The use of a proofreading polymerase in the PCR was found to be essential and fully optimized protocols were established and tested to ensure confidence that the TTGE profiles truly reflected sequence diversity. TTGE analysis revealed the methanogen population to be less diverse in water than in sediment. The most genetic diversity was observed in TTGE profiles of sediment DNA isolated in winter and the least was in sediment DNA isolated in summer. DNA sequencing analysis of bands recovered from TTGE gels revealed the presence of two methanogen communities. One clustered with *Methanosaeta* species and the other with the *Methanomicrobiales*. Many sequences showed low DNA sequence similarity to known methanogens, suggesting that Priest Pot harbors previously undescribed methanogen species.



**Figure 2.** TTGE analysis of cloned DNA (15Ar13 or 15Ar14) PCR amplified at 10- and 100-fold dilution of template using GC1100 and 1404Ar primers and *Taq* polymerase, *pfx*, or *SuperTaq*. 1: *Taq* 15Ar13 ( $10^{-1}$  dilution); 2: *Taq* 15Ar13 ( $10^{-2}$  dilution); 3: *Taq* 15Ar14 ( $10^{-1}$  dilution); 4: *Taq* 15Ar14 ( $10^{-2}$  dilution); 5: *pfx* 15Ar13 ( $10^{-1}$  dilution); 6: *pfx* 15Ar13 ( $10^{-2}$  dilution); 7: *pfx* 15Ar14 ( $10^{-1}$  dilution); 8: *pfx* 15Ar14 ( $10^{-2}$  dilution); 9: *SuperTaq* 15Ar13 ( $10^{-1}$  dilution); 10: *SuperTaq* 15Ar13 ( $10^{-2}$  dilution); 11: *SuperTaq* 15Ar14 ( $10^{-1}$  dilution); 12: *SuperTaq* 15Ar14 ( $10^{-2}$  dilution).

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