

Outlines

Used to:

1. Organize material
2. Discover connections between pieces of information
3. Create a “blueprint” for the finished manuscript

Two types:

1. Working outline

Revised as new material is discovered and added

May include material that will not be included in the final manuscript

2. Final outline

Reflects the structure and flow of the final manuscript version

Two Principles of Outline Construction:

1. Attempt to bring related material together under general headings
2. Arrange sections so that they relate logically to each other

I. Introduction

a. denaturing gradient gel

electrophoresis (DGGE)

i. **popularity** of the method (Muyzer and Smalla, 1998).

ii. Describe the **mechanics** of the method.

1. As the DNA encounters an appropriate denaturant concentration, a sequence-dependent partial separation of the double strands occurs.

2. conformational change in the DNA tertiary structure causes a reduced migration rate and results in a DNA band pattern

a. representative of the sampled microbial community.

b. methods for the **analysis of DGGE band patterns**.

i. **pairwise matching** of DGGE bands in separate lanes has been used to describe relationships between

communities (van der Gucht et al., 2001; Sigler et al., 2002).

ii. common **diversity indices** can incorporate band number and intensity

1. phylotype number (Casamayor et al., 2000)

2. phylotype abundance (Øvreås et al., 1997; Konopka et al., 1999; Nübel et al., 1999; McCaig et al., 2001; Sigler and Zeyer, 2002a)

3. **limitations** of this approach

a. band information is often limited to less complex systems

i. PCR amplification biases

1. preferential- and nonspecific amplification (reviewed by van Wintzingerode et al., 1997; Suzuki and Giovannoni, 1996)

2. heterogeneity in rrn copy number (Farrelly et al., 1995).

c. Problem: **No standardization** among laboratories, but in general..

- i. PCR product length analyzed is between 200 and 600 base pairs (bp).
- ii. The acrylamide percentage of the gel is commonly either 6 or 8 percent
- iii. runs are performed at a temperature of 60° C
- iv. denaturant concentrations are from 20% to as high as 70% or more
- v. much inconsistency exists in the choice of electrophoresis volt-hours (V·h)
 1. ranges from a minimum of 130 V for 3.5 h (455 V·h; Cocolin et al., 2001) to 2100 V·h (100 V for 21 h; Gejman et al., 1998).

d. preliminary experiments showed that **extended electrophoresis times**

resulted in sub-optimal band
separation and resolution.

- i. Research question: can extended electrophoresis times can impact community analyses performed by assessing DGGE band patterns?