A simple equipment for casting denature gradient gel in DGGE analysis

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Abstract

In this study, we develop a simple and convenient equipment for casting denature gradient gel in DGGE analysis. Our findings suggest that a copula-injector as a simple equipment is recommended for DGGE experiments in view of its simplicity and cost-effectiveness, it is suitable for casting denature gradient gel, particularly while handling a little number of fund. With no complicated equipment and technical training, it is very simple and easy to operate for application.

Key words: DGGE, equipment, casting, copula-injector.

Experimental

The intestinal tract of animals harbors a large, active, and complex community of microbes 1. There are at least 400–500 different microbial species, constituting a complex ecosystem 2. Most existing ecological explorations have focused on isolation and characterization of microorganisms obtained from intestinal tract. This technique has acquired valuable information on existing microbial 3. However, the proportion of the microorganisms recovered as colonies on agar plates is typically quite low, too low to only about 0.5% of the microorganism could be detected from the environment 4, therefore, such data provides limited information regarding the actual community structure. Consequently, recent studies focusing on molecular biological techniques to assay microbial ecology, in particular the use of schemes based on characterization of ribosomal RNA sequences, have shown that microbial organism typically differ from those that dominating the intestinal tract 5,6.

Microbial communities living in the animal intestinal tract were characterized using denaturing gradient gel electrophoresis (DGGE), which has been successfully used to analyze various microbial in food, agriculture, and environment 7-9. Banding patterns identified in DGGE provide good insights in understanding the composition change of microbial communities, irrespective of whether or not the bacteria can be cultured. One of the key points with the DGGE fingerprinting patterns obtained from intestinal microflora is the casting denature gradient gel, commercial equipment is high efficiency, but it is few applied on account of the high cost ($1000-2000). In this study, a copula-injector as a simple equipment was compared with commercial equipment. The purpose of this study was to design a cheap, simple and convenient equipment of casting gradient gel for DGGE detection.

Two healthy goslings (10-day-old) provided fresh fecal samples. Their fecal samples from rectum were collected separately. The samples were collected in sterile bags, refrigerated, and immediately taken to the laboratory. We used the Fecal DNA Kit (Tiangen, China) extract DNA of samples as the gold standard for PCR purification.

The bacterial population in faecal samples was fingerprinted by using total fecal DNA as templates for PCR. The PCR amplification of 16S rDNA fragments for DGGE was as described by Nam et al.10.

We cast denature gradient gel by commercial equipment (Fig. 1A) and a copula-injector as a simple equipment (Fig. 1B).

Denature gradient gel was cast according to the manufacturer’s instructions when we used commercial equipment (Bio-Rad) and it was cast as follow steps when we used the simple equipment.

1. Place the copula-injector on a magnetic stir plate and add a stir bar to the “1” glass injector labeled “heavy”. Place the copula-injector above the top of the plate set, but minimize the length of the tubing to a short length of 5-10 cm.

2. Place the copula-injector on a magnetic stir plate and add a stir bar to the “2” glass injector labeled “light”.

3. Stopcock of 2

4. Place the 2 injector labeled “light”

5. Place the 1 injector labeled “heavy”

6. Stir bar

7. Stopcock of the tubing

8. Magnetic stir plate

9. Tubing

Figure 1. Equipment for making denature gradient gel (A is commercial equipment; B is the simple equipment).
Note: The level of the copula-injector stopcock must be placed above the top of the gel sandwich. This creates a hydrostatic head large enough to cast the gels within 10 min from the time the initiators are added to the heavy solution. To create uniform gradients, all of the acrylamide must be in the gel sandwich before polymerization begins.

2. Prepare the heavy and light monomer solutions as same as we cast denature gradient gel by commercial equipment.

3. Combine all reagents except the initiators (APS and TEMED) and degas the solutions for 15 min under a vacuum. Degassing the solutions removes oxygen (oxygen inhibits polymerization.)

4. Immediately prior to pouring, add TEMED and APS in both solutions, mix gently, and pour the appropriate monomer solutions into the copula-injector. The light solution (the one with the lower acrylamide concentration) should be inhaled in the “2” injector labeled “light”, and the heavy solution should be placed in the reservoir “1” injector labeled “heavy”.

5. Turn on the stirring bar in the “1” injector to establish a suitable speed and maintain this same speed throughout casting.

6. Start casting the gels by opening the STOPCOCK of “2” injector. Allow the light monomer solution to enter the “1”injector. Open the STOPCOCK of the tubing once the light monomer solution enter the “1” injector, and begin mixing the solutions and creating the gradient. DO NOT allow any air bubbles to enter the plates.

7. After casting the gels to the top of the plates, carefully insert the combs at an angle; this will help minimize bubble formation in the wells.

Caution: Immediately flush the injector and tubing with water to prevent polymerization of residual acrylamide within the equipment.

The conditions of DGGE detection were performed as described by Nam et al. The gel images were captured as tagged image file format (TIFF) with a Gel Imaging System (Bio-Rad).

To interpret the profiles of the fingerprint of the DGGE, the Quantity One software package (Applied Bio-Rad) was used with pairwise similarity coefficient (Cs). Fingerprints were assigned to a different type when any band differences were observed. Variations in band intensity were not considered to be differences. The fingerprints were similar to Cs more than 90%. Fingerprints were assigned different type when Cs was 0 and identical when Cs was 100%. The results are summarized in Table 1. This study is the first report to estimate the effect of the simple equipment for making denature gradient gel.

The fingerprints of the PCR-DGGE with intestinal bacteria genomic DNA showed that the two equipment making denature gradient gel for DGGE detection have a 100% value of Cs (Fig. 2 and Table 1).

Table 1. Cs matrix values of DGGE fingerprint of making denature gradient gel by simple equipment and commercial equipment (%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>S-equipment (1)</th>
<th>S-equipment (2)</th>
<th>C-equipment (1)</th>
<th>C-equipment (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-equipment (2)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>C-equipment (1)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>C-equipment (2)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

S-equipment: simple equipment. C-equipment: commercial equipment.

The first DGGE measurement in detection of healthy human intestinal bacteria was performed by Millar et al. This method can be used to understand the composition change of microbial communities. Liu et al. compared the sensitivity of DGGE with RFLP method to resolve rRNA fragment successfully, and suggested that DGGE was the most sensitive method based on the three TRE digestions in differentiating rRNA fragment with closely related sequences. They selected tetrameric restriction enzyme (TRE) for digestion. The result showed TRE-RFLP could not differentiate successfully which suggested that DGGE was a rather useful method for screening of clone libraries.

In this pilot study, we demonstrated that the gel cast by the copula-injector was as good as commercial equipment. There is a significant economic beneficial by using the simple equipment. With no complicated equipment and technical training, it is very simple and easy to operate for application.

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