

# Evaluation of the optimal configuration for Pulsed-field gel electrophoresis

Category: Peristaltic pumps

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## Pulsed-field gel electrophoresis for the separation of DNA macromolecules

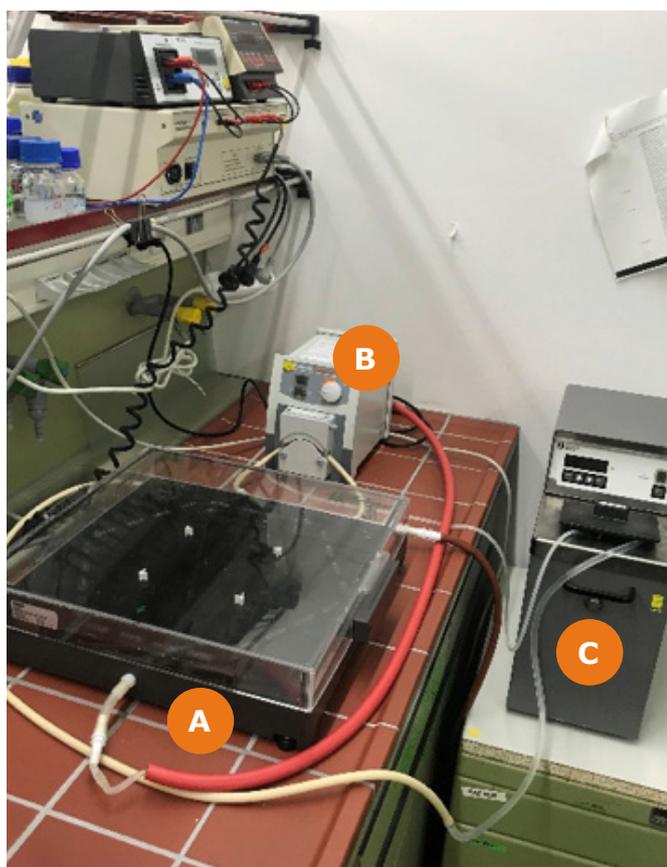
**Pulsed-field gel electrophoresis (PFGE) is also known as clamped homogeneous electrical field electrophoresis (CHEF). It is a special gel electrophoresis type for the separation of DNA macromolecules. This is achieved by alternating anode and cathode at certain intervals, which means that the DNA molecules have to reorient themselves in the electrical field before they can continue to move in the matrix. Smaller molecules orient themselves faster than larger ones, which means that they cover a greater distance in a defined time.**

**The separation of the DNA molecules depends on various factors: voltage, how fast anode and cathode are changing, runtime, buffer concentration and temperature. Due to the long runtimes of PFGE protocols and the sometimes high voltages, a constant and low buffer temperature is crucial for the success of the method. These low temperatures and long runtimes present a special challenge for the tubes used in a PFGE setup for the transport of the buffer solution.**

## Objective: Comparison of three tubes in combination with a peristaltic pump for buffer recirculation in pulsed-field gel electrophoresis (PFGE)

Often standard silicone tubes are used to run a PFGE setup, which is shown in Fig. 1. Connected to the running chamber (A; Fig. 2) is a pump (Heidolph Hei-FLOW Value 01 peristaltic pump with SP quick 1,6 pump head; B) with about 10 m of tube material in a cooling unit (C). Since the apparatus is in continuous use with cold buffer for up to 260 hours, it is important to select the correct tubing to prevent any complications (tearing, formation of air bubbles and loss of pumping power). Silicone tubes wear out very fast, therefore this test should show, if they can be replaced with a more durable one.

Three tubes for peristaltic pumps should be compared regarding their applicability in the field of PFGE. Tested were Heidolph's Tygon® Standard, Tygon® 2001 and PharMed® tubes with an inner diameter of 6,4 mm and a wall thickness of 1,6 mm.



**Fig. 1:** Setup of the PFGE apparatus. (A) shows the gel chamber in which the running buffer is located. This buffer is continuously circulated by a pump (B). About 10 meters of silicone tubing are placed in a cooling unit (C) to keep the buffer constant at low temperature.



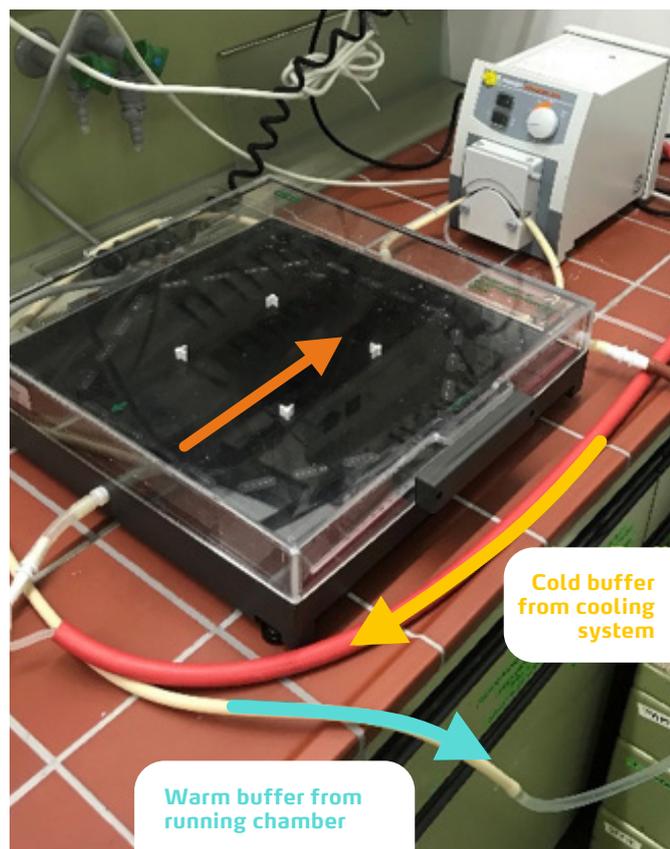
**Fig. 2:** Top view on the gel chamber, the flow direction of the buffer solution is indicated by the red arrow

## Method and results: A/B Protocols of PFGE and evaluation of the three tubes

The following tests have been carried out at the laboratory of Dr. Marlis Dahl, Department of Biology, Division of Biochemistry at the Friedrich-Alexander-University Erlangen-Nuremberg.

The tubing was tested in the setup shown in the figures 3 and 4 according to the following protocols

shown in Table 1: Protocol A was chosen because it is the most frequently used protocol in the laboratory, Protocol B was used because it has the highest requirement regarding the runtime. After the protocols, the tubing was examined and determined if change was needed.



**Fig. 3:** Experimental setup of the PFGE apparatus, showing in which direction the buffer flows (red arrow) inside of the chamber and which tubes transport the buffer to (blue arrow) and from the cooling system (yellow arrow)



**Fig. 4:** The connected pump with the PharMed® tubing. Also shown is the flow or pump direction of the running buffer.

Parameters	Protocol A	Protocol B
Buffer	0,5 × TBE buffer (2 l)	0,75 × TBE buffer (2 l, changed every 48 h)
Buffer temperature	12 °C	8 °C
Set temperature of cooling device	9 °C	4 °C
Voltage in the gel chamber	200 V (6 V/cm)	50 V (1,5 V/cm)
Pole changes (as gradient)	between 40 sec and 80 sec	between 1200 sec and 4800 sec
Set pumping speed	40 rpm	40 rpm
Running time	24 hrs	260 hrs

**Tab. 1:** Test protocols for the examination of the tubes

The Tygon® 2001 tubing turned out to be not usable for the planned purpose, because it was too rigid. Already in the shorter test following protocol A it showed undesired effects: Although controlled for bubble accumulation during the day, the next morning several large bubbles were found as an obstacle to pumping (that is insufficient cooling of the gel). The tube also already looked rather brittle at the area inside the pump head.

The Tygon® Standard tubing allowed good buffer circulation for a little longer period of time, but after some time it cracked. This could be due to the low temperature of the buffer, which makes the tubing less flexible and eventually causes it to break.

The PharMed® tubing performed best. Although the part of the tubing that is inside the pump head stretches after a longer period of use, showing that the material is thinning, it still lasts much longer than a common silicone tubing. The latter can be used in the same way, but the wear is much higher, which means that the part of the tube inside the pump head must be moved regularly, otherwise it will tear.



Tygon® 2001



Tygon® Standard

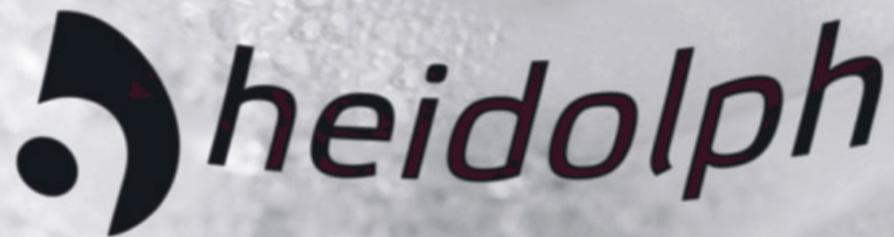


PharMed®

## Conclusion: Heidolph's PharMed® Tubing with Hei-FLOW Value 01 meets the challenge

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These tests showed that conventional silicone tubing can be replaced with the much longer lasting PharMed® tubing for a pulsed-field gel electrophoresis setup using the Hei-FLOW Value 01 peristaltic pump with the SP quick 1.6 pump head. The Tygon® 2001 and Tygon® Standard tubing proved to be not applicable for the PFGE process.



For any technical questions, application support etc. please contact us under:

**EUROPE / AFRICA / MIDDLE EAST /  
ASIA PACIFIC / SOUTH AMERICA**

**Heidolph Instruments GmbH & Co. KG**

Walpersdorfer Str. 12

D-91126 Schwabach

Phone: +49 9122 9920-0

Fax: +49 9122 9920-0

[sales@heidolph.de](mailto:sales@heidolph.de)

[www.heidolph.com](http://www.heidolph.com)

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**NORTH AMERICA**

**Heidolph North America**

1235 N Mittel Blvd. Suite B

Wood Dale IL 60191

Phone: +1 224-265-9600

Fax: +1 224 265-9611

[custserv@heidolph.com](mailto:custserv@heidolph.com)

[www.heidolphna.com](http://www.heidolphna.com)

