Supplementary Information

Hydrogel Beads for Enhanced Nucleic Acid Analysis in Complex Fluid Matrices

The aim of this study is the efficient capture of DNA from complex biological matrices, followed by its detection through PCR amplification.

To determine the PCR conditions, a gradient PCR was performed. For this purpose, the MCF-7 system, previously established by Marc Zinggeler, was employed.

Reference DNA was obtained from DSMZ and stored at –80 °C until use. The MCF-7 cell lysate was previously prepared and stored by Marc Zinggeler. PCR amplification was conducted using this lysate to generate a detectable amplicon.

Materials and Equipment Reagents:

Material / Reagent	Supplier
Nuclease-free water	Qiagen
Forward Primer: Mcf7-FW (10 µM)	Tib-Molbiol
Reverse Primer: Mcf7-RV (10 µM)	Tib-Molbiol
2× Hot Start PCR Master Mix	Biotechrabbit
MCF-7 cell lysate	Provided by Marc Zinggeler
Agarose	Merck
TAE Buffer (1×)	Carl Roth
GelRed (10,000×)	Biotium
6× Loading Dye	Genaxxon
DNA Ladder (100–1,000 bp)	Genaxxon

Equipment:

Equipment	Manufacturer
Thermal Cycler (PTC-255)	MJ Research
Power Supply (PowerPac Basic)	Bio-Rad
Gel Imaging System (EZGelImager)	Bio-Rad

PCR Protocol

A master mix was prepared for 9 reactions, with a final volume of 50 µL per reaction.

PCR Reaction Mix

Component	Stock Concentration	Volume per Reaction	Volume for 9 Reactions	Final Concentration

Nuclease-free water	–	23.00 µL	198.00 µL	–
2× Hot Start Master Mix	2×	25.00 µL	225.00 µL	1×
Forward Primer (FW)	100 µM	0.50 µL	9.00 µL	1 µM
Reverse Primer (RV)	100 µM	0.50 µL	9.00 µL	1 µM
Template DNA (lysate)	–	1.00 µL	9.00 µL	–
Total Volume		50.00 µL	450.00 µL	

All components except the template DNA were combined to prepare a master mix. A volume of 49 µL from this mix was used as a negative control, to which 1 µL of nucleasefree water was added in place of template DNA.

The remaining master mix received 8 µL of MCF-7 lysate and was distributed into 7 tubes (50 µL each):

- Tubes 1–7: PCR with MCF-7 lysate as template

- Tube 8: Negative control

The residual volume was reserved to compensate for pipetting losses.

PCR Thermal Cycling Conditions

As the primers have melting temperatures (Tm) of approximately 55 °C, a gradient PCR was employed in this initial experiment.

Step	Temperature	Time
Initial Denaturation	95 °C	2 min
Denaturation (×25–35)	95 °C	30 s
Annealing (gradient)	50–70 °C	30 s
Extension	72 °C	60 s
Final Extension	72 °C	5 min

Agarose Gel Electrophoresis

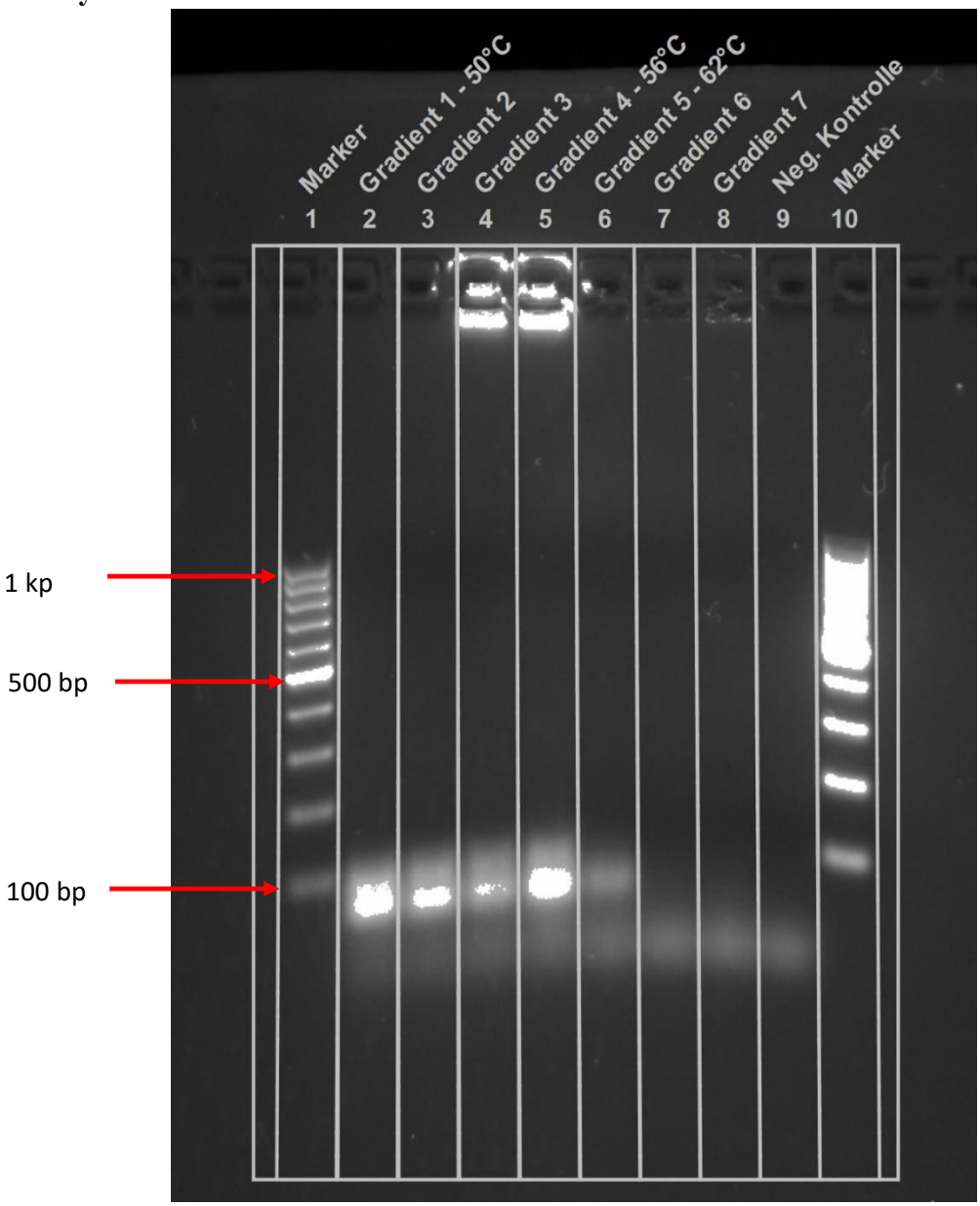
A 2% agarose gel was prepared by dissolving 0.8 g of agarose in 40 mL of 1× TAE buffer and heating until fully dissolved. After cooling, the evaporated volume was compensated with deionized water, and 4 µL of GelRed (10,000×) was added. The gel was then cast using a comb to form wells.

For each PCR product:

- 5 µL of product was mixed with 1 µL of 6× loading dye.
- 5 µL of this mixture was loaded onto the gel.
- Electrophoresis was conducted at 70 V for 1 hour.
- Images were acquired using the EZGelImager system (below).

Summary

PCR amplification was successful. The optimal annealing temperature was determined to be **approximately 57 °C under the tested conditions.**



Gel image