



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L703

PCR Amplification and Gel Electrophoresis

Standard Operating Procedure

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1. Purpose

This SOP describes standard polymerase chain reaction (PCR) amplification of target DNA fragments and visualization of PCR products by agarose gel electrophoresis. PCR is used in the EA Program for confirming DNA extraction success, screening for target genes before ddPCR or sequencing, and verifying amplicon size and specificity prior to library preparation (Lorenz, 2012; Mullis, 1990). Gel electrophoresis separates DNA fragments by size, providing a visual confirmation that amplification produced the expected product.

2. Scope

- **Applies to:** Standard endpoint PCR amplification of environmental DNA extracts (soil, water, tissue) and visualization of products on agarose gels; applicable to gene-specific screening, amplicon verification, and colony PCR
- **Does not apply to:** Quantitative PCR (qPCR) or digital PCR (ddPCR; see [SOP-L701](#) and [SOP-L702](#)); RT-PCR for RNA targets; polyacrylamide gel electrophoresis (PAGE)
- **Location:** Molecular biology bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve primer selections and cycling protocols
Trained Personnel	Set up PCR reactions and run gels per this SOP
New Students	Complete training before independent use

4. Definitions

- **Agarose gel:** A porous polymer matrix cast from dissolved agarose in TAE or TBE buffer; DNA fragments migrate through the gel under an electric field and are separated by size; typical concentrations are 1–2% (w/v).
- **Amplicon:** The specific DNA fragment produced by PCR between a defined pair of primers; its length (in base pairs) is determined by the primer binding positions and is used to verify PCR specificity on a gel.
- **Annealing temperature (T_a):** The temperature during the PCR extension cycle at which primers bind to the template; typically 3–5 °C below the calculated primer melting temperature (T_m); incorrect T_a causes non-specific amplification or failed reactions.
- **DNA ladder (size standard):** A mixture of DNA fragments of known sizes run alongside samples on a gel; used to estimate the size of amplified products.
- **Ethidium bromide (EtBr) / SYBR Safe:** Fluorescent intercalating dyes added to agarose gels or post-staining baths to visualize DNA under UV light; EtBr is a potent mutagen requiring dedicated waste disposal; SYBR Safe is a lower-hazard alternative.

- **Master mix:** A pre-mixed reagent solution containing DNA polymerase, dNTPs, buffer, and MgCl_2 in optimized concentrations; reduces pipetting error and variability between replicate reactions.
- **Melting temperature (T_m):** The temperature at which 50% of a primer-template duplex is dissociated; calculated from primer sequence and salt concentration; the basis for selecting annealing temperature.
- **Non-specific amplification:** PCR products of unexpected size caused by primer mis-binding; detected as extra bands on a gel; remedied by increasing annealing temperature or redesigning primers.
- **Primer:** A short (18–25 bp) single-stranded oligonucleotide that binds to a complementary sequence on the template DNA and defines the start point for DNA polymerase extension.
- **TAE / TBE buffer:** Running buffers for agarose gel electrophoresis; TAE (Tris-acetate-EDTA) gives sharper bands for larger fragments; TBE (Tris-borate-EDTA) provides better resolution for smaller fragments and is more resistant to overheating.

5. Safety and Hazards

Hazards

- **Chemical:** Ethidium bromide (EtBr) and SYBR Safe are nucleic acid stains. EtBr is a suspected mutagen; handle with gloves at all times and dispose of gels and buffers as hazardous waste. SYBR Safe is a safer alternative but should still be handled with gloves.
- **Electrical:** Gel electrophoresis uses high voltage (80–120 V). Do not touch the buffer or gel while the power supply is on. Always disconnect the leads before opening the gel box.
- **UV radiation:** Transilluminators emit UV light that can cause skin burns and eye damage. Use a UV shield, wear UV-blocking safety glasses, and minimize exposure time. Blue-light transilluminators are preferred when used with SYBR Safe.
- **Biological:** Environmental DNA extracts may originate from samples containing pathogens. Handle with standard precautions.

5.1. Required PPE

- ☐ Safety glasses (UV-blocking if using a UV transilluminator)
- ☐ Lab coat
- ☐ Nitrile gloves (change frequently; required at all times when handling gels, stains, or PCR products)

5.2. Emergency Procedures

For EtBr contact with skin, wash immediately with soap and water. For UV exposure, flush eyes with water and seek medical attention. Refer to [SOP-L003 Hazardous Materials Handling](#).

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Thermal cycler (Bio-Rad C1000 Touch or equivalent)	Mol bio bench	Verify block type matches tube format
Gel electrophoresis system (tank, comb, casting tray)	Mol bio bench	Mini-gel (7 x 10 cm) or midi-gel
Power supply	Mol bio bench	Capable of 10–300 V
Gel imaging system (transilluminator + camera)	Mol bio bench	UV or blue-light; with gel documentation software
Micropipettes (2, 10, 100, 1000 uL)	Mol bio bench	Calibrated annually
Microwave	Lab	For melting agarose
Microcentrifuge	Mol bio bench	For brief spin-downs

6.2. Reagents and Consumables

Item	Notes
Taq DNA polymerase or high-fidelity polymerase	e.g., GoTaq (Promega), Q5 (NEB), or OneTaq (NEB)
5x or 10x PCR buffer (with MgCl ₂)	Supplied with polymerase
dNTP mix (10 mM each)	Store at –20 C
Forward and reverse primers (10 uM working stocks)	Store at –20 C
Nuclease-free water	For master mix and negative controls
PCR tubes or plates (0.2 mL, thin-wall)	Matched to thermal cycler block
Agarose (molecular biology grade)	For gel preparation
1x TAE or TBE buffer	Running buffer; prepare from 50x stock
DNA stain (SYBR Safe or ethidium bromide)	SYBR Safe preferred
DNA ladder (100 bp and/or 1 kb)	e.g., NEB 100 bp or 1 kb Plus
6x gel loading dye	Often included with ladder or polymerase
Filter pipette tips (10, 100, 1000 uL)	Barrier tips mandatory to prevent cross-contamination

7. Procedure

7.1. PCR Workspace Preparation

! Contamination Prevention

PCR is extremely sensitive to contamination. Follow these rules:

- Set up reactions in a designated pre-PCR area, physically separated from post-PCR work (gel loading, product analysis).
- Never bring PCR products, gels, or gel imaging equipment into the pre-PCR area.
- Use dedicated pipettes for pre-PCR work. Use filter tips exclusively.
- Wipe bench surfaces with 10% bleach followed by 70% ethanol before setting up reactions.
- Change gloves frequently, especially after touching any surface outside the pre-PCR area.

7.2. Master Mix Preparation

Prepare all components on ice. Calculate volumes for the number of reactions plus 10% overage to account for pipetting losses.

Standard 25 uL reaction (GoTaq example):

Component	Volume per reaction	Final concentration
5x GoTaq Reaction Buffer	5.0 uL	1x
dNTP mix (10 mM each)	0.5 uL	200 uM each
Forward primer (10 uM)	1.25 uL	500 nM
Reverse primer (10 uM)	1.25 uL	500 nM
GoTaq DNA Polymerase (5 U/uL)	0.125 uL	0.625 U
Nuclease-free water	14.875 uL	–
Subtotal (master mix)	23.0 uL	
Template DNA	2.0 uL	Variable (1–10 ng)
Total	25.0 uL	

1. Thaw buffer, dNTPs, and primers on ice. Vortex briefly and spin down.
2. Prepare the master mix in a sterile 1.5 mL tube, adding polymerase last. Mix by pipetting; do not vortex.
3. Aliquot 23 uL of master mix into each labeled PCR tube.
4. Add 2 uL of template DNA to each tube. Include controls:
 - **Positive control:** DNA known to amplify with these primers
 - **Negative control (NTC):** 2 uL of nuclease-free water
 - **Extraction blank:** DNA extract from [SOP-L700](#) extraction blank
5. Cap tubes, vortex briefly, and spin down.

7.3. Thermal Cycling

Program the thermal cycler with the following general protocol. Adjust the annealing temperature for your specific primer set.

Step	Temperature	Time	Cycles
Initial denaturation	95 C	2 min	1
Denaturation	95 C	30 s	30–35
Annealing	T_a C	30 s	30–35
Extension	72 C	1 min/kb	30–35
Final extension	72 C	5 min	1
Hold	4 C	Hold	–

where T_a is the annealing temperature, typically 3–5 C below the lower primer T_m . For primers with T_m of 55–60 C, start at 55 C. Optimize by running a gradient if needed (Lorenz, 2012).

Record the cycling parameters, primer names, and annealing temperature in the lab notebook.

7.4. Agarose Gel Preparation

1. Weigh agarose for a 1–2% gel (w/v), depending on the expected amplicon size:

Agarose %	Effective separation range
0.8%	500–10,000 bp
1.0%	250–7,000 bp
1.5%	100–3,000 bp
2.0%	50–1,500 bp

2. Add agarose to 1x TAE (or TBE) buffer in an Erlenmeyer flask.
3. Microwave in 30-second intervals, swirling between intervals, until the agarose is fully dissolved and the solution is clear.
4. Cool to approximately 55 C (comfortable to hold).
5. If using SYBR Safe, add 5 uL per 50 mL of gel. Swirl gently.
6. Pour into the casting tray with the comb in place. Allow to solidify (approximately 20–30 minutes at room temperature).

7.5. Loading and Running the Gel

1. Remove the comb carefully. Place the gel in the electrophoresis tank and fill with 1x TAE (or TBE) buffer until the gel is submerged by 2–3 mm.
2. Load 5 uL of DNA ladder into the first well.
3. Add loading dye to each PCR product (if not already included in the PCR buffer). Load 5–10 uL per well.
4. Record the loading order in the lab notebook.

5. Connect the leads: DNA migrates toward the positive (red) electrode. Confirm the orientation.
6. Run at 80–120 V for 30–45 minutes, or until the leading dye front has migrated approximately two-thirds of the gel length.
7. Turn off the power supply and disconnect the leads before removing the gel.

7.6. Gel Imaging

1. Place the gel on the transilluminator (blue light for SYBR Safe; UV for EtBr).
2. Capture an image using the gel documentation system.
3. Save the image with the naming convention: YYYY-MM-DD_primers_project_gel.tif
4. Evaluate the image:
 - Positive control: visible band at the expected size
 - NTC and extraction blank: no visible band
 - Samples: band at the expected size indicates successful amplification
5. Upload the image to the project GitHub repository under **results/gels/**.

8. Troubleshooting

Symptom	Probable Cause	Corrective Action
No bands in any lane	Failed reaction; wrong cycling parameters	Check primer and dNTP stocks; verify cycling program
Bands in NTC	Contamination	Discard reagents; clean workspace with bleach; use fresh aliquots
Multiple bands	Non-specific amplification	Increase annealing temperature; reduce primer concentration; redesign primers
Smear instead of band	DNA degradation or overloaded gel	Reduce template amount; check DNA quality on NanoDrop
Bands too faint	Insufficient template or cycles	Increase template to 5–10 ng; increase to 35 cycles
Bands in samples but not positive control	Positive control degraded	Prepare fresh positive control DNA

9. Quality Control

- Every PCR run must include: positive control, NTC, and extraction blank
- No bands should appear in the NTC or extraction blank. Any amplification in controls invalidates the run (Salter et al., 2014).
- Band size must match the expected amplicon size (compare to the DNA ladder)
- For quantitative downstream applications (library prep, sequencing), confirm band intensity is roughly uniform across samples

10. Waste Disposal

Waste Type	Container	Disposal Method
EtBr-stained gels and buffer	Designated EtBr waste container	EH&S hazardous waste pickup
SYBR Safe-stained gels	Standard trash (check local EH&S policy)	Non-hazardous per manufacturer
Used PCR tubes and tips	Standard trash	Non-hazardous after thermal cycling
Unused EtBr stock solution	Chemical waste	EH&S hazardous waste pickup

11. References

- Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments*, (63), e3998. <https://doi.org/10.3791/3998>
- Mullis, K. B. (1990). The unusual origin of the polymerase chain reaction. *Scientific American*, 262(4), 56–65. <https://doi.org/10.1038/scientificamerican0490-56>
- Salter, S. J., Cox, M. J., Sherrill-Mix, S., Turner, R. M., Sherrill-Mix, S., Sherrill-Mix, S., & Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, 12, 87. <https://doi.org/10.1186/s12915-014-0087-z>

12. Related SOPs

SOP	Title	Relationship
L003	Hazardous Materials	EtBr and UV safety
L700	DNA Extraction	Upstream: provides template DNA
L701	ddPCR Primers	Primer design and validation
L704	Amplicon Library Prep	Downstream: library preparation
L106	Freezers	Reagent and DNA storage

13. Training Record

Before performing PCR and gel electrophoresis independently, personnel must:

1. Read this SOP and [SOP-L700](#) in full
2. Demonstrate master mix preparation with correct volumes and controls
3. Run a supervised PCR and gel, producing a clean positive control band with no NTC contamination
4. Demonstrate safe handling of DNA stains and proper gel disposal
5. Be signed off by the PI or lab manager