AGAROSE GEL ELECTROPHORESIS

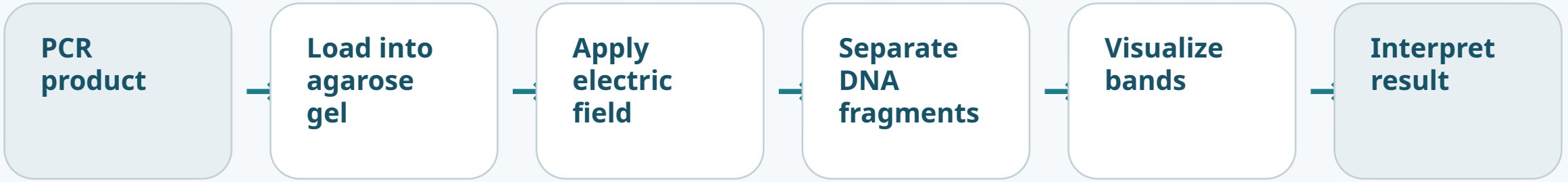
DNA separation, visualization and interpretation



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From PCR to electrophoresis

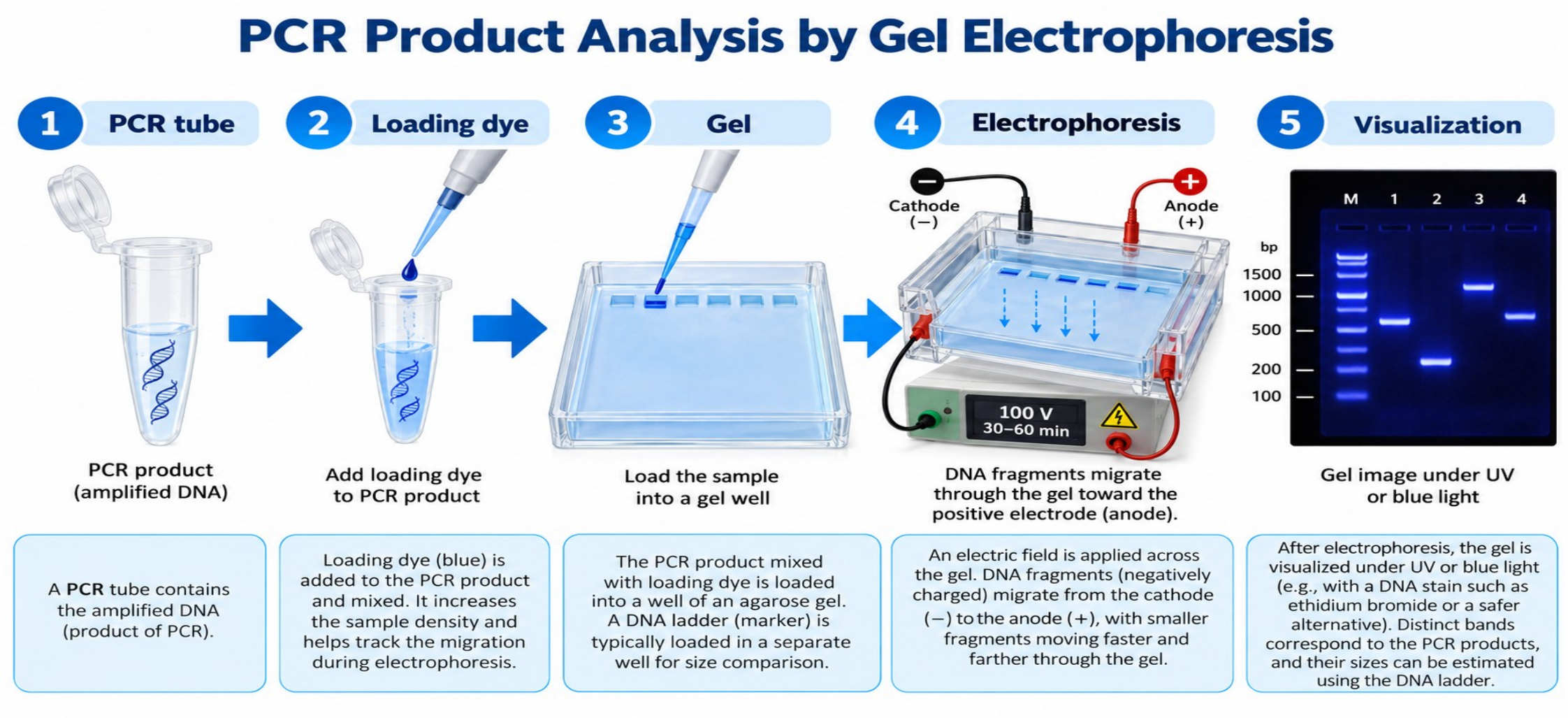
PCR produces DNA. Gel electrophoresis tells us what that product looks like.



Key question: Is the PCR product present, specific and the expected size?

The laboratory workflow

Real laboratory consumables connect PCR and gel analysis.



Accurate pipetting and contamination control remain important after PCR.

What is gel electrophoresis?

An analytical method for separating charged molecules in an electric field.

- DNA fragments are loaded into a porous gel.
- An electric field drives DNA through the gel.
- Fragments separate mainly according to size.
- Separated DNA is visualized as bands.



Why is DNA negatively charged?

The phosphate groups in the sugar–phosphate backbone carry negative charge.

DNA backbone

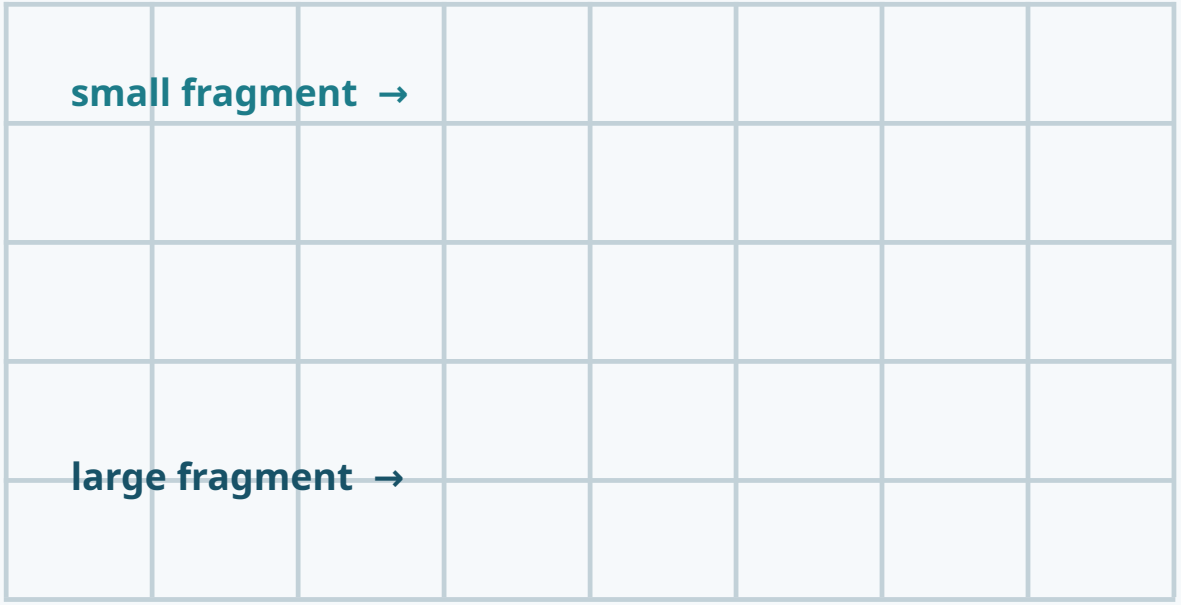


Because DNA is negatively charged, it migrates toward the positive electrode (anode).

Direction of migration: − → +

Agarose is a molecular sieve

Agarose forms a porous matrix that slows DNA fragments as they migrate.



Smaller DNA fragments move more easily through the pores and generally migrate farther in the same time.

Fragment size determines migration

For linear DNA, smaller fragments generally migrate faster than larger fragments.



Agarose concentration affects resolution

Gel percentage is selected according to the size range of DNA fragments.

0.7–0.8% larger fragments	**larger pores**	→ resolution range changes
1.0% general-purpose PCR products	**intermediate pores**	→ resolution range changes
1.5–2.0% smaller fragments	**smaller pores**	→ resolution range changes

Exact useful ranges depend on agarose type, buffer, voltage and fragment size.

Electrophoresis buffer

TAE and TBE provide ions for current flow and help maintain pH.

TAE

Tris–acetate–EDTA
Common for routine DNA electrophoresis
Often preferred when DNA will be recovered from the gel

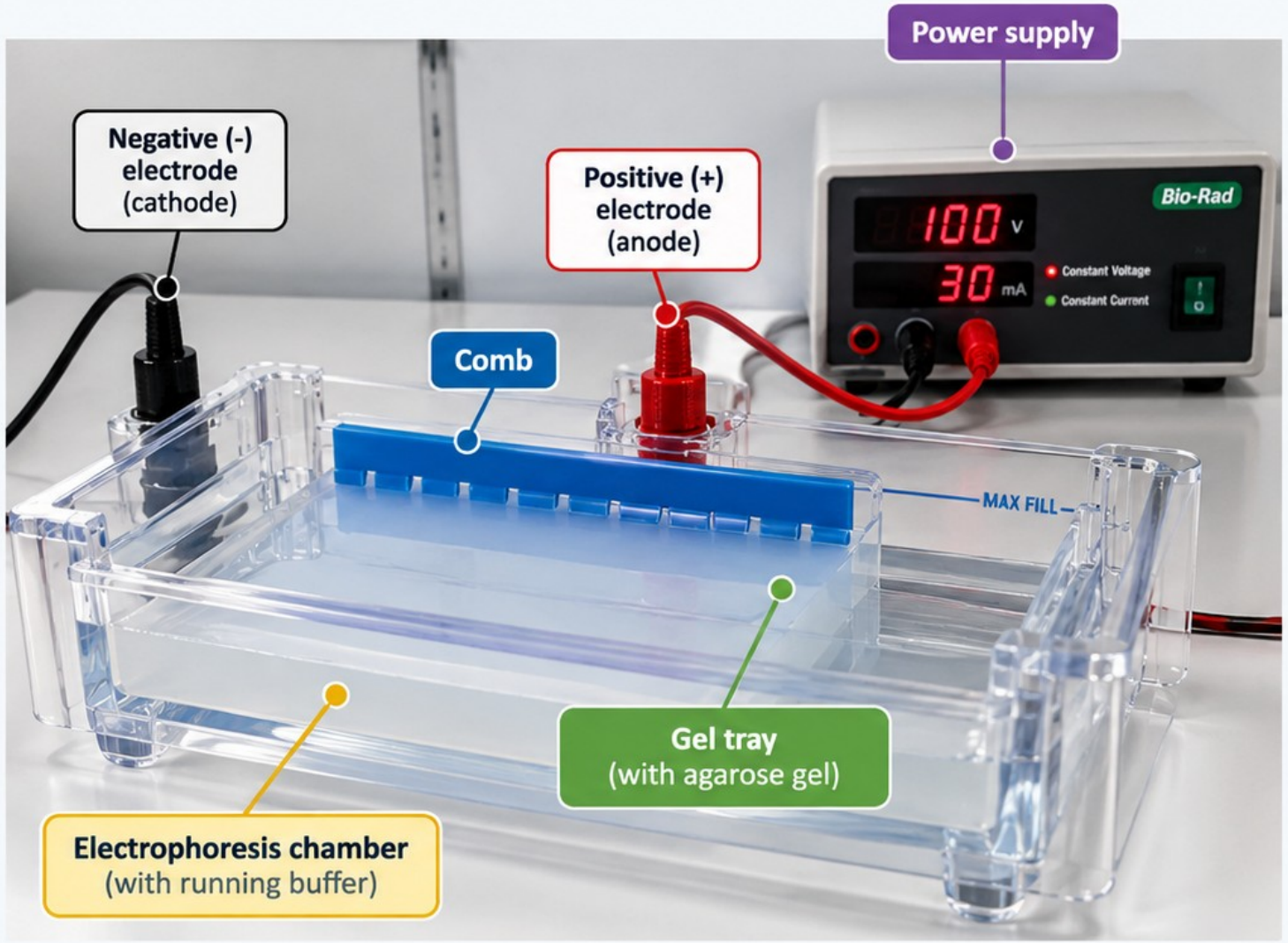
TBE

Tris–borate–EDTA
Higher buffering capacity
Often useful for smaller DNA fragments

The gel and the electrophoresis chamber must contain compatible running buffer.

Main equipment for agarose gel electrophoresis

The system is simple: gel chamber + tray + comb + power supply.



Gel tray

Holds agarose gel.



Comb

Forms sample wells in the agarose gel.



Power supply

Applies constant voltage (e.g. 80–120 V).



Leads/electrodes

Create electric field across the gel.



Negative (–) (cathode)



Positive (+) (anode)

Gel casting: from liquid agarose to a solid gel

Agarose is dissolved in running buffer, cooled, poured and allowed to solidify.

1 Weigh agarose



2 Dissolve in buffer



3 Cool appropriately



4 Pour into tray



5 Insert comb



6 Allow to solidify



Safety: molten agarose can cause burns; staining chemistry may require additional precautions.

The comb forms sample wells

After the gel solidifies, the comb is removed carefully to leave wells.

Comb



After removal

Wells receive DNA samples and ladder.

Loading DNA into the gel

Samples are mixed with loading dye and pipetted carefully into the wells.

1 Mix PCR product with loading dye



Mix PCR product with loading dye (e.g. 6×).

2 Prepare pipette with tip



Use a fresh tip for each sample.

3 Take sample



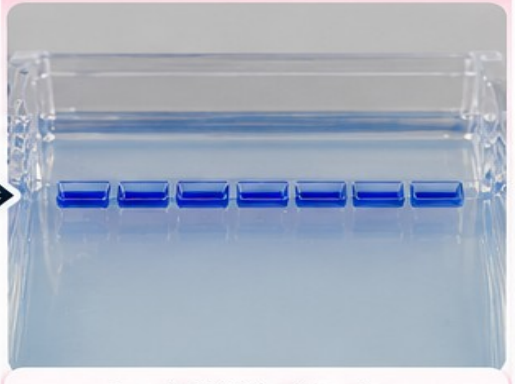
Aspirate the sample (keep the tip inside the liquid).

4 Load into well



Slowly dispense the sample into the well without piercing the gel.

5 Repeat for all samples



Load DNA ladder in a dedicated lane and repeat for all samples.

Micropipettes and tips

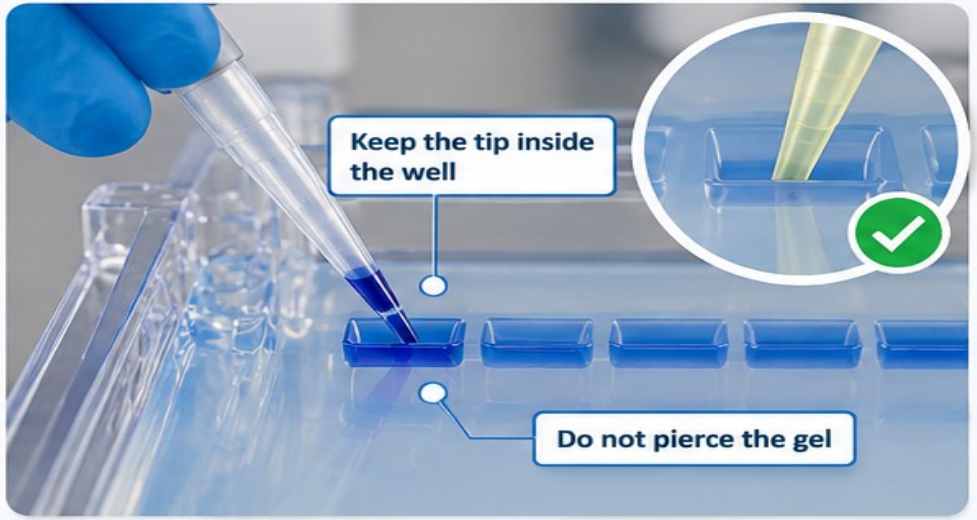


Single-channel micropipettes | Multi-channel pipette

Pipette tips



10 µL | 200 µL | 1000 µL



What does loading dye do?

Loading dye makes sample handling and run tracking easier.

Density agent

Helps the sample sink into the well instead of dispersing into the running buffer.

Tracking dyes

Colored molecules migrate through the gel and indicate progress of the run.

Not the DNA signal

Tracking dyes are not the DNA bands that are visualized after staining.

Do not confuse tracking dye position with DNA fragment size.

DNA ladder: the molecular ruler

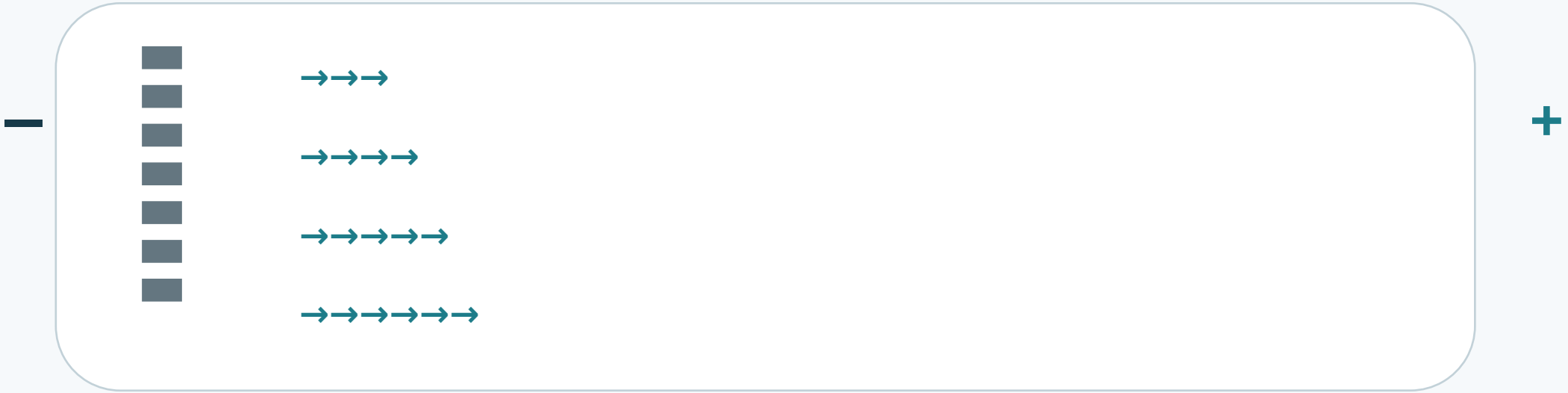
A DNA ladder contains fragments of known sizes and is used to estimate sample fragment length.



- Compare the migration position of the sample band with ladder bands.
- The ladder gives an approximate size estimate.
- Choose a ladder that covers the expected PCR product size.

Running the gel

DNA starts near the negative electrode and migrates toward the positive electrode.



Wells should be positioned near the negative electrode so DNA migrates through the gel.

Voltage influences separation

Higher voltage can shorten run time but may reduce resolution or overheat the gel.

Too low

Slow migration
Long run time
May be acceptable when high resolution is required

Appropriate

Good balance of speed and separation
Follow the validated protocol for the gel system

Too high

Excessive heating
Band distortion
Reduced resolution

Voltage is selected relative to gel size and electrode distance—not by a single universal value.

DNA staining and visualization

DNA is invisible in the gel until a stain or fluorescent dye is used.

Ethidium bromide

Classical intercalating dye; requires appropriate safety procedures.

SYBR-type stains

Fluorescent alternatives widely used for DNA visualization.

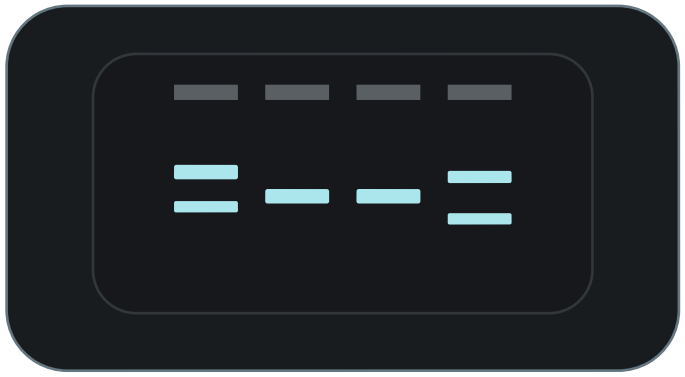
GelRed / GelGreen

Commercial fluorescent nucleic-acid stains.

Visualization may use UV or blue-light illumination depending on the stain.

From gel to image

A gel documentation system captures and stores the fluorescent band pattern.



Gel imaging system

- Capture the gel image.
- Record lane labels and sample identities.
- Estimate fragment sizes using the ladder.
- Store the original, unedited image for documentation.

Successful PCR on an agarose gel

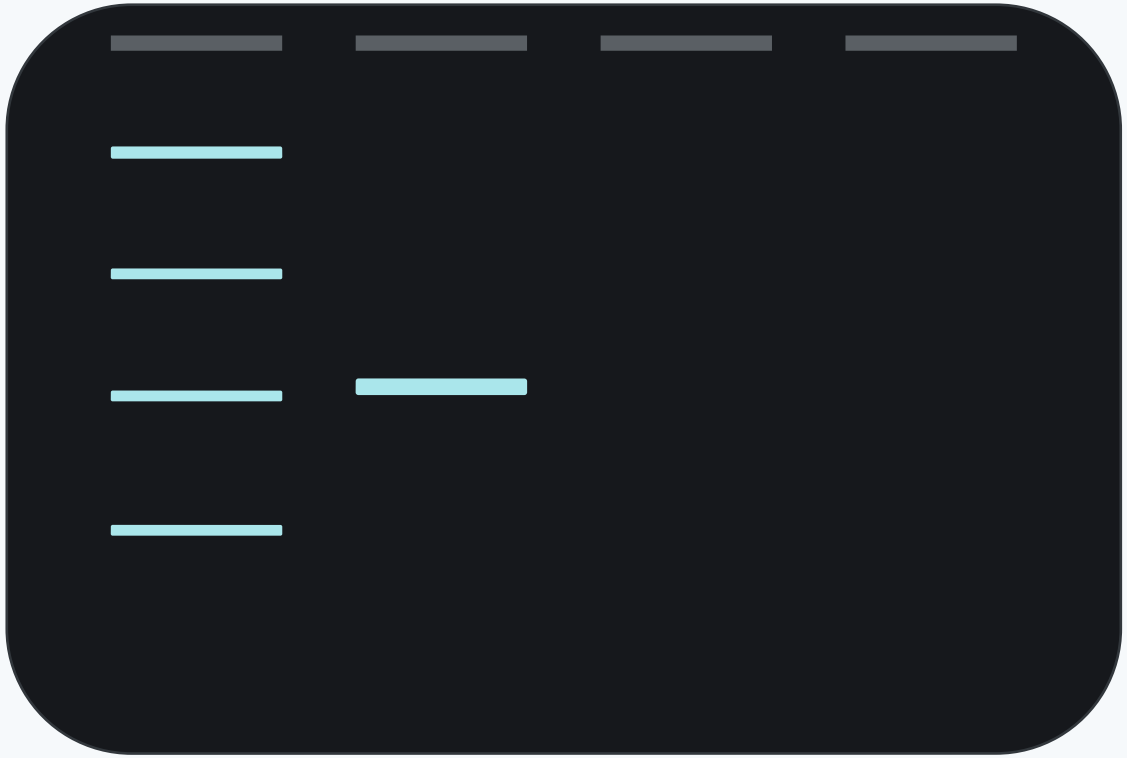
A clear band at the expected size supports successful amplification.



- Positive control: expected band
- Sample: expected band
- NTC: no band
- Band position matches expected fragment size

No band: how should it be interpreted?

A negative lane is meaningful only when the controls are valid.



- If the positive control works and the sample has no band, the target may be absent or below detection.
- If the positive control also fails, interpret the experiment as a PCR failure—not as a biological negative.

Multiple bands: non-specific amplification

More than one discrete band usually indicates amplification of unintended targets.



- Annealing temperature may be too low.
- Primer specificity may be insufficient.
- Mg^{2+} concentration or cycle number may need optimization.
- Confirm expected size before biological interpretation.

Smear: heterogeneous DNA signal

A smear means DNA is distributed across a range of fragment sizes rather than one clear product.



- Degraded template DNA
- Too much DNA loaded
- Non-specific PCR products
- Overheating or poor electrophoresis conditions

Band in the NTC: contamination warning

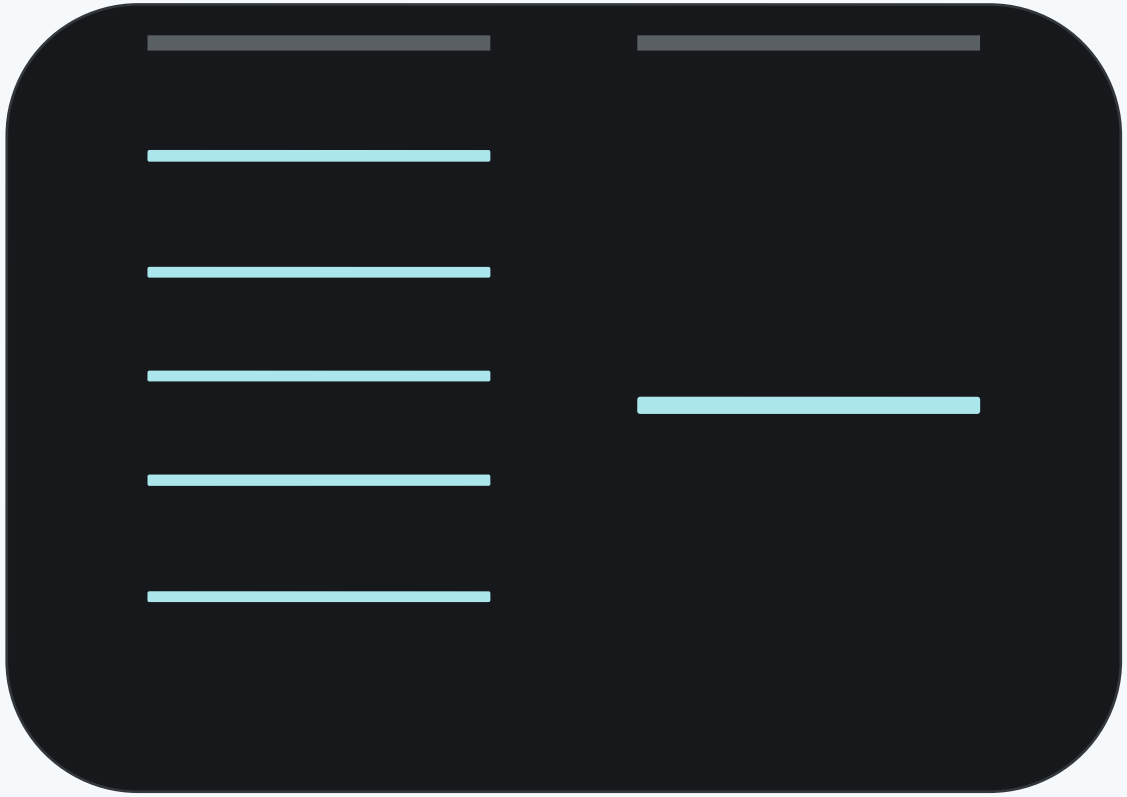
A no-template control should not contain an amplicon.



- Reagent contamination
- Carry-over from previous PCR products
- Aerosol contamination during pipetting
- Review pre-PCR and post-PCR separation

Estimating PCR product size

Compare the unknown band with nearby ladder fragments.



Example

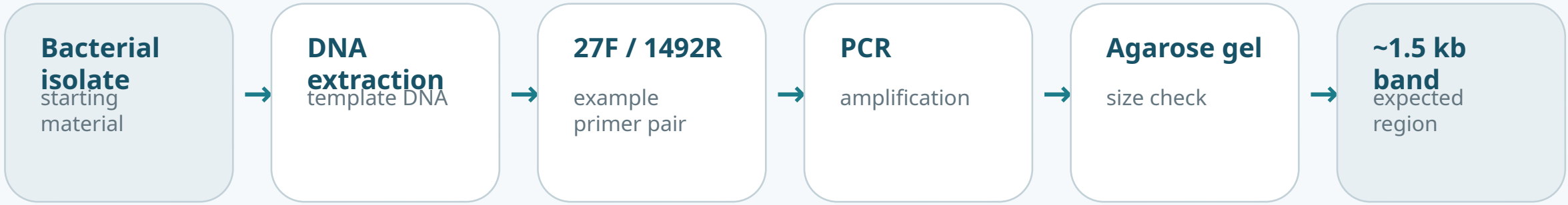
Expected product: ~1500 bp

Observed sample band: between the 1000 bp and 2000 bp ladder bands, close to 1500 bp.

Conclusion: size is consistent with the expected amplicon.

Example: bacterial 16S rRNA gene PCR

A practical workflow from isolate to an approximately 1.5 kb amplicon.



A band of the expected size supports successful amplification; sequence identity is confirmed by sequencing when required.

Systematic gel interpretation

Use the same order every time.

1. Did the ladder separate correctly?

2. Did the positive control work?

3. Is the NTC clean?

4. Is there a sample band?

5. Is its size correct?

6. Is the pattern specific or smeared?

Take-home messages

DNA charge

DNA migrates toward the positive electrode.

Agarose

The porous matrix separates fragments mainly by size.

Ladder

Known-size fragments provide a molecular ruler.

Controls

Positive control and NTC are essential for interpretation.

Band pattern

Single band, multiple bands, smear and no band have different meanings.

Workflow

PCR and gel electrophoresis are interpreted together.

Selected sources and media

- Bio-Rad. Nucleic Acid Electrophoresis — principles, horizontal electrophoresis and imaging systems.
- Thermo Fisher Scientific. Agarose Gel Electrophoresis — DNA separation and ladder guidance.
- Bio-Rad classroom resources: DNA, PCR and agarose gel electrophoresis.
- Real photographs in the course deck: Eppendorf and Bio-Rad product literature.