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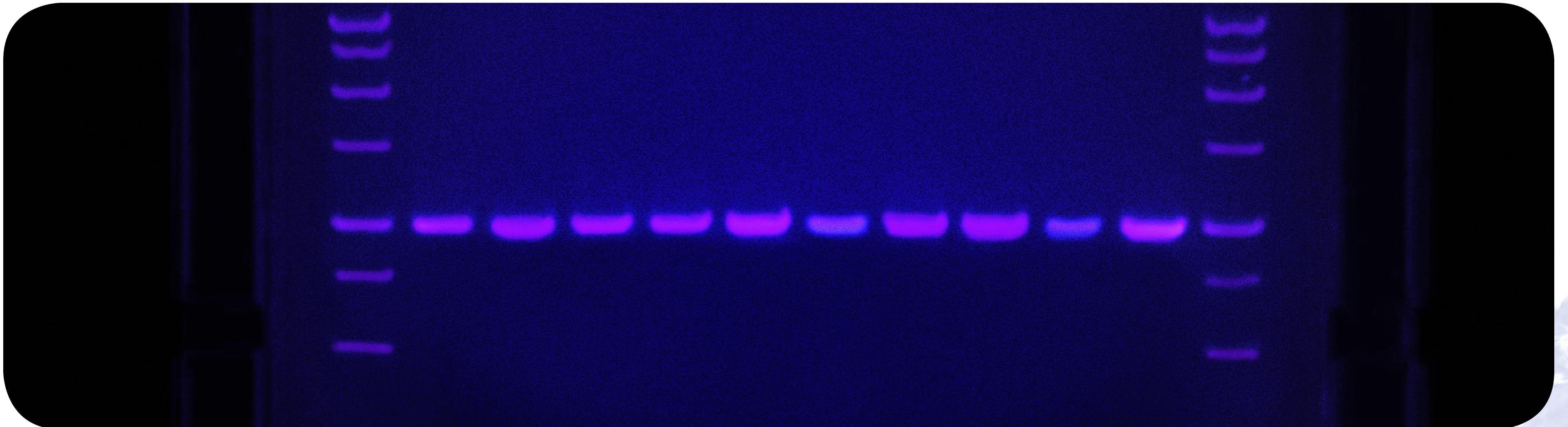
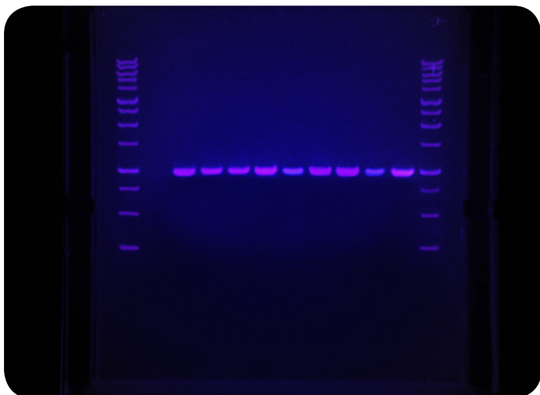
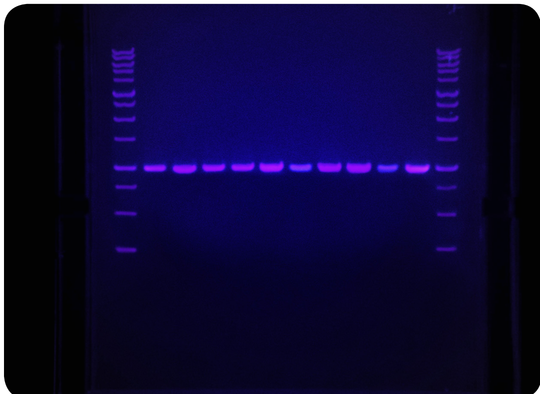
PCR TROUBLESHOOTING





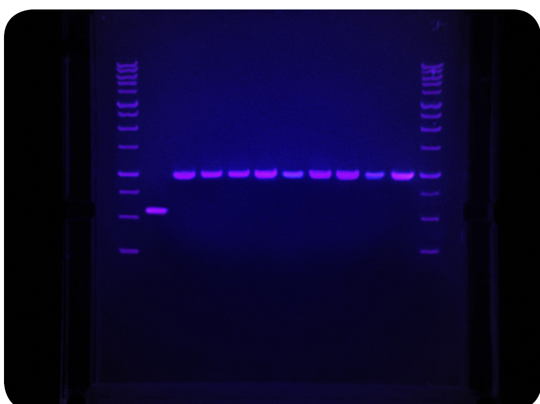
No Product

1. Verify annealing temperature (Ta) matches the calculated primer melting temperature (Tm) value.
2. Repeat the PCR with a checklist to confirm all components are added.
3. Ensure A260/280 ratio falls between 1.6 and 2.0.
4. Prepare new template.
5. Decrease annealing temperature.
6. Assume 1 minute per kb of target sequence for extension.



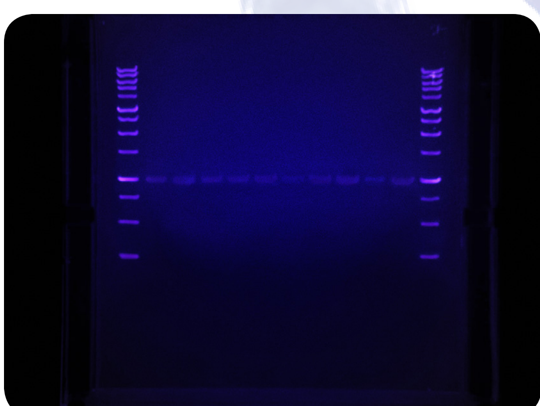
Misleading Product

1. Errors in primer or template sequence. Sequence the primers or template. Use new primers or template.
2. Use high fidelity DNA polymerase.
3. Multiple copies of the target sequences in the template.
4. Use appropriate concentration of Mg2+.
5. Unequal concentrations of dNTPs. Use equal concentrations.



Incorrect Product

1. Verify correct primer was used.
2. Increase annealing temperature.
3. Verify correct template DNA was used. Sequence if necesary.
4. Analyze template for alternate primer binding sequences.
5. Low molecular weight artifacts may indicate primer-dimer formation.



Weak Amplification (Faint Bands)

1. Check primer and template concentrations.
2. Increase cycle number by 2-5 cycles.
3. Check DNTP expiration date and ensure concentrations are appropriate for target sequence: For most PCRs, use 50μM per nucleotide (200μM total), but for longer targets, you may need up to 200μM per nucleotide (800μM total).

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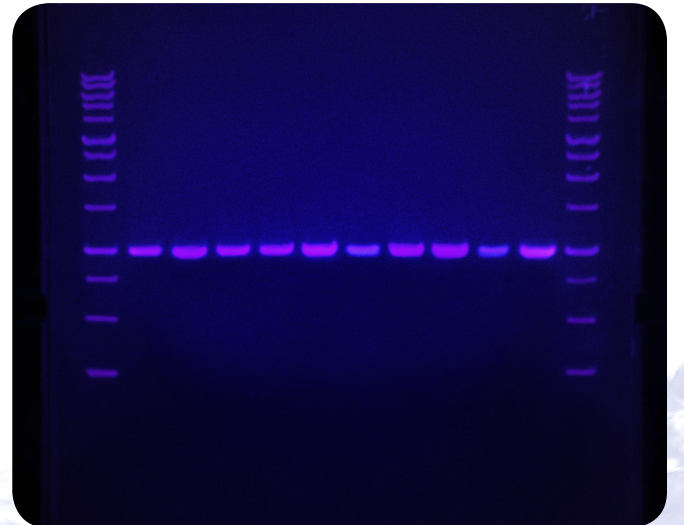
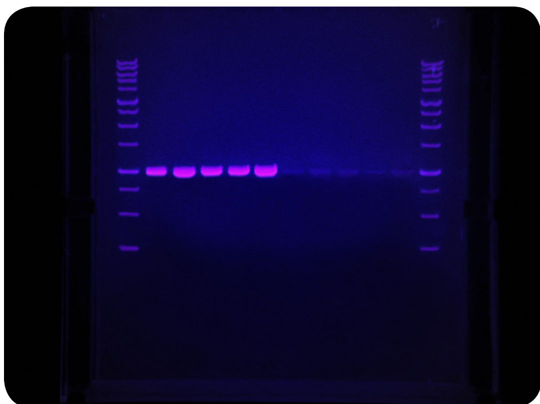
AGAROSE GEL TROUBLESHOOTING



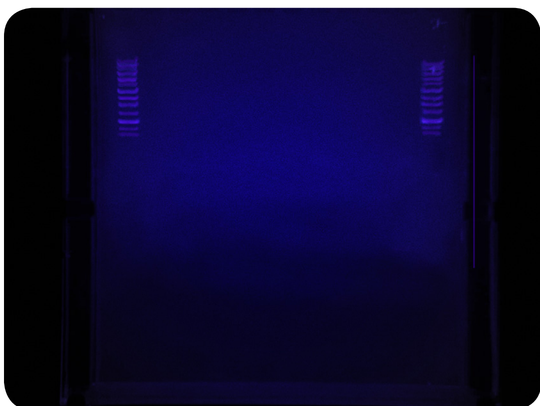
No Bands

1. Increase sample loading volume. Optimal range = 2 - 100 ng of DNA per lane.
2. Ensure ethidium bromide (EtBr) is added to gel before pouring.
3. Verify correct orientation of electrodes.
4. Ensure gels are properly stored, prepare fresh whenever possible.
5. Ensure ethanol has been completely eliminated (mini-preps).
6. Verify marker dyes are present to ensure sample did not run.



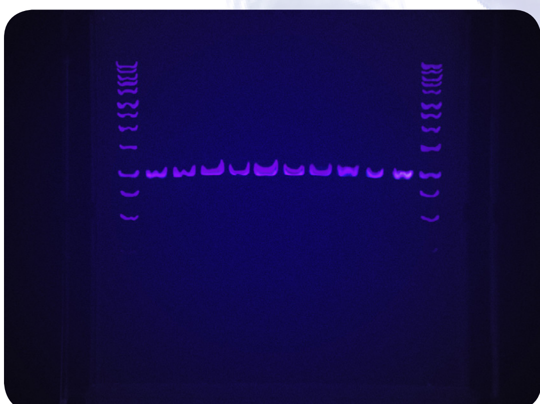
Too Bright/Too Dim

1. Check concentration of sample being loaded.
2. Bright: Decrease sample loading volume or EtBr concentration.
3. Dim: Increase sample loading volume or EtBr concentration.
4. Decrease sample concentration to ensure multiple bands of similar molecular weight are not combining into a single band.



Gel Percent

1. Poor resolution: Gel percentage is too low.
2. Bands stacked near top of gel: Gel percentage is too high.
3. For targets smaller than 500 bp, prepare 1% - 1.5% gel.
4. For targets larger than 10 kb, prepare 0.7% – 1% gel.



Smiles & Birds

1. Birds (individual wells): Remove comb slowly from wells after gel has solidified.
2. Birds (individual wells): Ensure wells are thoroughly rinsed of agarose after removing comb.
3. Whole gel smile: Gel did not solidify evenly resulting in uneven density. Prepare new gel.
4. Whole gel smile: Gel or running buffer may be too old. Prepare fresh gel and running buffer.

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