Qualitative Analysis of DNA template for Sanger Sequencing

Poor quality DNA templates compromise DNA sequencing results …
Maximize your success with well-prepared DNA templates!

1st BASE
YOUR RESEARCH, OUR BUSINESS
www.base-asia.com

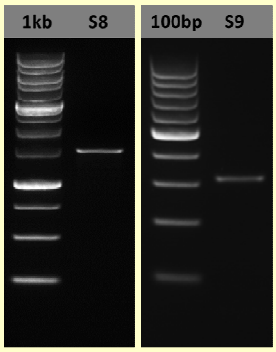
Recommendation: Verify DNA template quality through Agarose gel electrophoresis

1. Recommended Gel % for :
   a. Plasmid DNA & PCR fragments ≥ 400bp , run samples on 1% Agarose gel with 1kb DNA ladder
   b. PCR fragment < 400bp, run samples on 2% Agarose gel with 100bp DNA ladder
2. Load 0.5µg DNA ladder into the first lane, and 1µL of your DNA sample per lane. Run gel electrophoresis, and view gel profile.
3. Capture and print the gel image. Label specifically the name & lane number of the DNA ladder & sample loaded per lane.
4. Attach the gel photo along with your purified DNA samples. Email the gel photo with your Order Form to 1st BASE.

Reading Your Gel Photo

Purified PCR Product

✓ **Good Quality PCR Product**

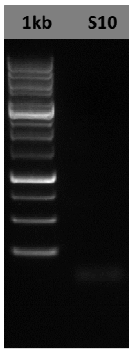


Distinct target PCR fragment, free from degradation and contaminants

DNA Concentration within recommended range

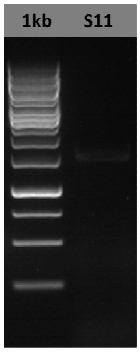
Poorly Optimized PCR reaction / Poorly Purified PCR Product

✗ **Absence of Target DNA**



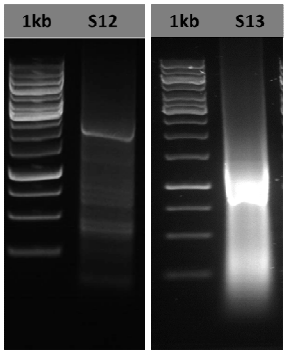
Suggestion:
Amplify and prepare a new batch of target fragment.

✗ **Insufficient Template**



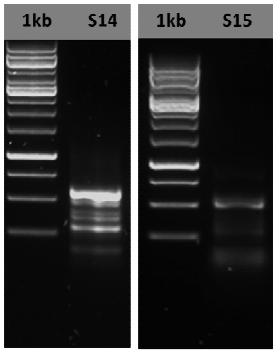
Suggestion:
Amplify and prepare a new batch of target fragment. Pool replicates if necessary.

✗ **Smeared products**

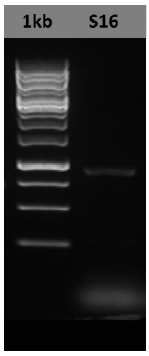


Suggestion:
Amplify and prepare a new batch of target fragment. Optimize PCR condition to obtain distinct target PCR fragment.

✗ **Multiple Products**



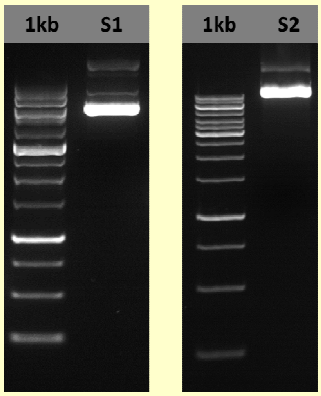
✗ **Primer-dimer Contamination**



Suggestion:
Excise the target fragment for gel-DNA extraction. Check by running purified DNA on gel before sending for DNA sequencing.
For more details, please refer our FAQ website at http://www.base-asia.com/dna_sequencing/support/faqs/

Purified Plasmid DNA

✓ **Good Quality Plasmid DNA**

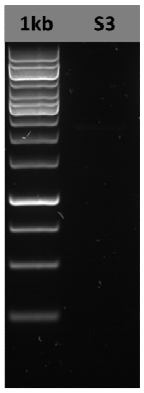


DNA concentration within recommended range (>100ng/uL)

Free from degradation & contaminants

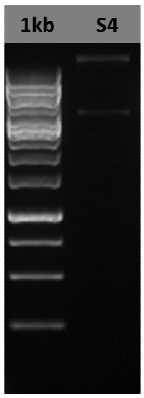
Poorly Purified Plasmid DNA

✗ **Absence of Target DNA**



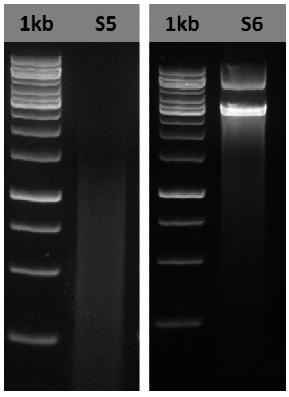
Suggestion:
Extract a new batch of DNA.

✗ **Insufficient Amount of DNA**



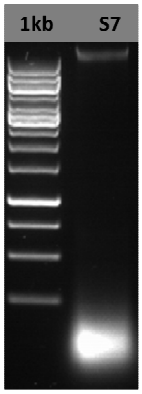
Suggestion:
Extract a new batch of DNA [>100ng/uL].

✗ **Degraded Plasmid DNA**



Suggestion:
Extract a new batch of DNA.

✗ **RNA Contamination**



Suggestion:
Treat with RNase-A.

Note: The DNA ladder is not applicable for sizing comparison of non-linear DNA samples (e.g. plasmid DNA).

For enquiries:

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