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SupreDye Cycle Sequencing Kit Quick Guide

Determining DNA Quality and Quantity:

The following methods can be used to examine DNA quality:

- Agarose gel electrophoresis

Purified DNA should run as a single band on an agarose gel.
Note: Uncut plasmid DNA can run as three bands: supercoiled, nicked, and linear.

- Spectrophotometry

The A260/A280 ratio should be 1.7 to 2.0. Smaller ratios usually indicate contamination by protein or organic chemicals.

Agarose gels reveal the presence of contaminating DNAs and RNAs, but not proteins. Spectrophotometry can indicate the presence of protein contamination, but not DNA and RNA contamination.

DNA Quantity Determination:

If possible, quantitate the amount of purified DNA by measuring the absorbance at 260 nm or use agarose gel electrophoresis to estimate.

Recommended Template amount in 20 µL reaction (half the amount for 10 µL reaction):

Template	**Quantity**
PCR product: 100–200 bp 200–500 bp 500–1000 bp 1000–2000 bp >2000 bp	 1–3 ng 3–10 ng 5–20 ng 10–40 ng 20–50 ng
Single-stranded	25–50 ng
Double-stranded	150–300 ng
Cosmid, BAC	0.5–1.0 µg
Bacterial genomic DNA	2–3 µg

Cycle Sequencing Reaction Set-up

Overview:

This section describes how to prepare reactions and perform cycle sequencing for 96- well reaction plates.

Reaction Setup:

If you use 5x Sequencing Buffer for sequencing reactions, be sure the final reaction volume is at a concentration of 1X. Here is the recommended reaction setup:

Reagent	**Concentration**	**20** µL reaction	**10** µL reaction
SupreDye v1.1 or v3.1 Cycle Sequencing Mix	2.5X	2 µL	1 µL
ADS 5x Sequencing Buffer	5X	3 µL	1.5 µL
Primer	—	3.2 pmol	1.6 pmol
Template	—	Use appropriate amount	Use appropriate amount
Water	—	to 20 µL	to 10 µL
Final Volume	**1X**	20 µL	10 µL

***Cycle Sequencing on a Thermal Cycler*:**

Standard cycling conditions

1.	Place the plate in a thermal cycler and set to the correct
2.	Perform an initial denaturation. a. Rapid thermal ramp to 96 °C b. 96 °C for 1 min
3.	Repeat the following for 30 cycles: • Rapid thermal ramp to 96 °C • 96 °C for 10 sec • Rapid thermal ramp to 50 °C • 50 °C for 5 sec • Rapid thermal ramp to 60 °C • 60 °C for 4 min
4.	Rapid thermal ramp to 4 °C and hold until ready to purify.

When the DNA template concentration is low, it is recommended more cycles are performed during reaction. A modified protocol is list here:

1.	Place the plate in a thermal cycler and set to the correct
2.	Perform an initial denaturation. a. Rapid thermal ramp to 96 °C b. 96 °C for 1 min
3.	Repeat the following for 40 cycles: • Rapid thermal ramp* to 96 °C • 96 °C for 10 sec • Rapid thermal ramp to 50 °C • 50 °C for 5 sec • Rapid thermal ramp to 60 °C • 60 °C for 2 min
4.	Rapid thermal ramp to 4 °C and hold until ready to purify.