

1. Sequence PCR

Using BigDye Terminator v1.1/3.1 Sequencing Buffer

The BigDye® Terminator v1.1/3.1 Sequencing Buffer (5X)* is supplied at a 5X concentration. If you use it for sequencing reactions, be sure the final reaction volume is at a concentration of 1X. For example, for a half reaction in 20 µL final volume, you would use 4 µL of ready reaction premix and 2 µL of BigDye sequencing buffer as shown below.

標準法

Reagent	Concentration	Volume
Ready Reaction Premix	2.5X	4 µL
BigDye Sequencing Buffer	5X	2 µL
Primer	—	3.2 pmol
Template	—	See “Template Quantity” on page 2-6.
Water	—	to 20 µL
Final Volume	1X	20 µL

(1/8 scaleでも可能)

PCR tubeにとる

0.5 µl (tube name “BD”)

0.5 µl (5 x sequence buffer)

1 µl (1 pmol/µl溶液, T7, Sp6...)

100 ng前後
(double strand DNAの場合)

To 5 µl

5 µl

Note: The use of this buffer without optimization may result in deterioration of sequence quality. Applied Biosystems does not support diluted reactions or guarantee the performance of BigDye® chemistry when it is diluted.

2. PCR条件

To sequence single- and double-stranded DNA on the GeneAmp® PCR System 9700 (in 9600 emulation mode), 9600, or 2400:

1.	Place the tubes in a thermal cycler and set to the correct volume.
2.	Perform an initial denaturation. a. Rapid thermal ramp to 96 °C b. 96 °C for 1 min
3.	Repeat the following for 25 cycles: <ul style="list-style-type: none">• 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.
5.	Spin down the contents of the tubes in a microcentrifuge.
6.	Proceed to Chapter 4, “Purifying Extension Products.”

*Rapid thermal ramp is 1 °C/second.

3. PCR終了後

PCR products (5 μ l)

