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 μ L final volume, you would use 4 μ L of ready reaction premix and 2 μ L 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	I	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")		
$0.5 \mu l$ (5 x sequence buffer)		
1 μl (1 pmol/μl 溶液 , T7, Sp6)		
100 ng 前後 (double strand DNA の場合)		
Το 5 <i>μ</i> l		
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

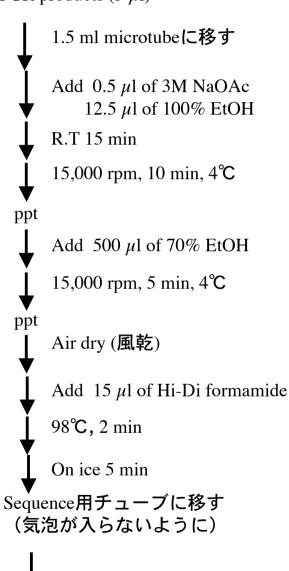
To sequence single- and double-stranded DNA on the GeneAmp® PCR System 9700 (in 9600 emulation mode), 9600, or 2400:

Place the tubes in a thermal cycler and set to the correct volume. Perform an initial denaturation. a. Rapid thermal ramp to 96 °C b. 96 °C for 1 min Repeat the following for 25 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 Rapid thermal ramp to 4 °C and hold until ready to purify. 4. 5. Spin down the contents of the tubes in a microcentrifuge. Proceed to Chapter 4, "Purifying Extension Products." 6.

3. PCR終了後

Sequencer ^

PCR products (5 µl)



^{*}Rapid thermal ramp is 1 °C/second.