

1/18

Cyclist® Exo⁻Pfu DNA Sequencing Kit

INSTRUCTION MANUAL

Catalog #200326

Revision #108002

STORAGE CONDITIONS

DMSO: Room Temperature

All Other Components: -20°C

For in Vitro Use Only



Illumina Ex. 1108
Illumina v. Columbia
IPR2018-00291, -00318, -00322,
-00385, and -00797



STRATAGENE®

Instruction Manual

2/18

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Stratagene. Stratagene shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Stratagene Cloning Systems
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (619) 535-5400
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@stratagene.com
World Wide Web www.stratagene.com

Stratagene European Contacts

Location	Telephone	Fax	Technical Services
Austria	660 312 526	660 312 527	017 956 7036
Belgium	0800 96078	0800 96024	027 13 12 11
Germany	0130 840 911	0130 762 088	0699 509 6197
Netherlands	0800 023 0446	0800 023 0447	0800 023 0448
Switzerland	0800 830 250	0800 825 225	01 800 9045
United Kingdom	0800 585 370	0800 783 0889	0171 365 1056

Distributors

All other countries, please contact your local distributor (see *Stratagene Distributors* in this instruction manual for a complete listing).

3/18

Cyclist® Exo⁻ Pfu DNA Sequencing Kit

CONTENTS

Introduction.....	1
Materials Provided.....	2
Additional Materials Required.....	3
Additional Materials Required for Incorporation of Radiolabeled Nucleotide.....	3
Additional Materials Required for End-Labeled Primers	3
Template Preparation.....	3
PCR Products	3
Plasmids	4
Cosmid and Lambda DNA.....	4
M13 DNA	5
Protocol.....	5
Sequencing Reaction.....	5
Sequencing Primers and Primer Sets	8
Troubleshooting	8
Primer End Labeling.....	9
Weights	10
Media and Reagents.....	10
Related Stratagene Products	11
Stratagene Distributors	13
References.....	14
Endnotes	14
Quick-Reference Protocol.....	15

Cyclist® Exo⁻ *Pfu* DNA Sequencing Kit

Catalog #200326

4/18

INTRODUCTION

The Cyclist® Exo⁻ *Pfu* DNA sequencing kit applies the technique of cycle sequencing, also known as linear amplification sequencing, which is a variation of the polymerase chain reaction (PCR). The components of a chain-termination sequencing reaction are cycled through a temperature profile consisting of a heat-denaturation step, an annealing step and an extension step.^{1,2}

There are three basic differences between cycle sequencing and PCR amplification. The first is the presence of only one primer in the cycle sequencing reaction used to prime synthesis of one strand of the DNA. The resultant linear amplification of the sequencing product leads to an increase in the signal generated during the sequencing reaction. The second difference is the presence of dideoxynucleotide triphosphates (ddNTPs) in the sequencing reactions, which create the base-specific terminations required for sequencing. The last difference is the addition of an α -radiolabeled nucleotide triphosphate (α -³⁵S-labeled dATP, [α -³³P]dATP or [α -³²P]dATP), which is incorporated into the growing DNA strand, providing the ability to detect the sequence ladder by autoradiography.

Temperature cycling produces multiple sequencing products labeled with a radioactive nucleotide and terminated at a dideoxynucleotide. Cycling the sequencing reactions results in several advantages.

1. The amount of template necessary for the sequencing reaction is greatly reduced, because multiple sequencing products are formed from each template molecule.
2. The addition of smaller amounts of template minimizes impurities and therefore reduces the amount of template preparation required.
3. Double-stranded DNA (dsDNA) templates, such as PCR products and plasmids, can be sequenced without separate denaturation and annealing steps, because of the high temperature at which the sequencing reactions are run and the multiple heat-denaturation steps.
4. ³²P or ³³P-end labeled primers may be used with the Cyclist Exo⁻ *Pfu* DNA Sequencing kit without any change to the protocol.

5/18

5. Sequencing at elevated temperatures reduces template secondary structure, resulting in fewer strong stops.

Stratagene recommends labeling the cycle sequencing products with α -radiolabeled nucleotides for two reasons: (1) incorporation of α -radiolabeled nucleotides eliminates the extra enzymatic step involved in end labeling the sequencing primers and (2) incorporation of α -radiolabeled nucleotides also generates more highly labeled sequencing products, thereby decreasing the limit of detection. The Cyclist Exo⁻ *Pfu* kit will work with ³²P, ³³P or ³⁵S isotopes.[¶] The Cyclist Exo⁻ *Pfu* kit may be used to sequence M13 DNA, PCR products and purified plasmid DNA, lambda DNA, cosmids or DNA from plaques.

MATERIALS PROVIDED

Materials provided	Quantity	Storage temperature
10× Sequencing buffer (for 100 sequencing reactions) (includes ddNTPs)	500 µl	-20°C
ddATP (1.5 mM)	400 µl	-20°C
ddCTP (1.5 mM)	400 µl	-20°C
ddGTP (1.5 mM)	400 µl	-20°C
ddTTP (1.5 mM)	400 µl	-20°C
Exo ⁻ <i>Pfu</i> DNA polymerase (2.5 U/µl)	100 µl	-20°C
Control sequencing primer [-20 M13/pUC primer (10 ng/µl)]	10 µl	-20°C
pBluescript® II phagemid control template DNA (100 ng/µl)	25 µl	-20°C
Stop solution	2 ml	-20°C
Dimethylsulfoxide (DMSO) ^a	500 µl	Room temperature

^a After initial thawing, store DMSO at room temperature. Do not refreeze.

[¶] See Note on special precautions under the *Protocol* regarding use of ³⁵S nucleotides.

6/18

ADDITIONAL MATERIALS REQUIRED

Additional Materials Required for Incorporation of Radiolabeled Nucleotide

α -³⁵S-Labeled dATP, [α -³³P]dATP or [α -³²P]dATP (1000–5000 Ci/mmol, 10 mCi/ml)
Silicone or mineral oil

Additional Materials Required for End-Labeled Primers

10× Kinase buffer (see *Media and Reagents*)
T4 polynucleotide kinase (see *Related Stratagene Products*)
[γ -³²P]ATP or [γ -³³P]ATP (1000–5000 Ci/mmol, 10 mCi/ml)
Silicone or mineral oil

TEMPLATE PREPARATION

Many different types of DNA preparations may be used as the templates in a cycle sequencing reaction, but as with any DNA sequencing method, higher-purity DNA templates yield cleaner DNA sequence data. Therefore, the quality of DNA sequence required for a project should be considered before deciding which method of DNA template preparation to use.

PCR Products

PCR amplification reactions that result in a single product (no spurious bands or primer dimers) may be prepared with Stratagene's StrataPrep™ PCR purification kit (see *Related Stratagene Products*) or by ammonium acetate–isopropyl alcohol precipitation as described in the following procedure:

1. Add 25 μ l of 5 M ammonium acetate and 50 μ l of isopropyl alcohol to 25 μ l of the PCR amplification reaction. Vortex to mix the reaction.
2. Incubate the reaction at room temperature for 10 minutes.
3. Microcentrifuge the sample for 15 minutes at 14,000 rpm and discard the supernatant.
4. Air dry the sample for 5–10 minutes at room temperature.
5. Resuspend the pellet in 25 μ l of TE buffer (see *Media and Reagents*).

7/18

6. Estimate the DNA concentration and use the appropriate amount in the sequencing reaction.

Notes *Stratagene has obtained consistent in-house results using a 1:10 dilution of a PCR product which gave a single band on an agarose gel, and then sequencing with 1–10 μ l of this dilution. Stratagene also recommends titrating an unfamiliar template initially using two to four concentrations.*

The optimum annealing temperature determined for PCR amplification should be used initially in the cycle sequencing profile.

When sequencing short PCR fragments (<500 bp), Stratagene recommends using 5 μ l of ddNTP in each termination reaction (see step 1 of the Sequencing Reaction). This increased volume results in a higher concentration of ddNTPs in the sequencing reactions. The final volume of the sequencing reaction mixture should be adjusted to 22 μ l (step 2), and 5- μ l aliquots are then added to the termination reactions (step 3).

PCR amplification reactions that result in multiple bands or show evidence of primer dimers must be gel isolated. The band of interest may be recovered using any protocol which leaves the DNA in a low-salt buffer or water.

Plasmids

Plasmids purified by any standard small- or large-scale protocol³ are suitable templates. Stratagene's StrataPrep™ plasmid miniprep kit[‡] or ClearCut™ miniprep kit[‡] provide easy-to-use protocols for plasmid preparation.

Cosmid and Lambda DNA

Cosmid DNA prepared by standard protocols³ is a consistent template for cycle sequencing. Stratagene recommends that small quantities (10–50 pmol) of cosmid DNA be used with a ³²P or ³³P radiolabel. This is true as well for purified lambda DNA. Lambda DNA recovered directly from plaques can be cycle sequenced by using only the top agarose containing the plaque in the manner illustrated on the following page:

Note *It is important to use only the top agarose containing the plaque for template preparation.*

[‡] See Related Stratagene Products.

- 8/18
1. Pick the top agarose containing a plaque and place the plaque into a tube with 25 μ l of TE buffer.
 2. Boil the tube for 5 minutes.
 3. Treat the suspension with RNase A at 1 μ g/25 μ l.
 4. Centrifuge the tube for 1 minute to remove the cellular debris.
 5. Use 10 μ l of the supernatant in the sequencing reaction.

M13 DNA

M13 DNA may be prepared by standard protocols.³ Stratagene recommends using ~20% of the DNA suggested for the dsDNA protocols. Alternatively, M13 DNA may also be prepared from plaques. (As described in *Cosmid and Lambda DNA*, be careful to use only the top agarose containing the M13 DNA.) This procedure also requires the use of [α -³²P]dATP or a ³²P-end-labeled primer.

PROTOCOL

Note See the back page of this instruction manual for a condensed quick-reference protocol that can be detached and used when detailed instructions for this procedure are no longer needed.

Sequencing Reaction

Note Special precautions should be taken when using ³⁵S nucleotides, which may break down into a highly volatile compound when subjected to repeated high-temperature cycles. Accordingly, Stratagene recommends using ³³P instead of ³⁵S. If ³⁵S nucleotides must be used, the following safety guidelines are recommended:

- 1) Use only thick-walled tubes
 - 2) Cover reaction solutions with mineral oil
 - 3) Use oil in the reaction wells
 - 4) Minimize the number of temperature variations and cycles in your thermal cycling program
1. Add 3 μ l of the appropriate ddNTP to each of four termination tubes. Cap the termination tubes and keep the tubes on ice.

9/18

2. For each DNA template, combine the following reaction components on ice and use a pipettor to mix the components *gently* but thoroughly:

Note *It is essential to add DMSO last to avoid precipitation and to thoroughly mix all the components of the reaction prior to aliquoting into the termination tubes.*

Template (200 fmol)
Primer (1 pmol or 5 ng of a 17-mer oligonucleotide)
4 μ l of 10 \times sequencing buffer[§]
10 μ Ci of radioactive label
1 μ l (2.5 U) of Exo⁻ *Pfu* DNA polymerase[‡]
Bring up to 26 μ l with water
4 μ l of DMSO
Final volume equals 30 μ l

For the control reaction, combine the following reaction components:

4 μ l of control template DNA
1 μ l of control primer
4 μ l of 10 \times sequencing buffer[§]
10 μ Ci of radioactive label
1 μ l of Exo⁻ *Pfu* DNA polymerase[‡]
Bring up to 26 μ l with water
4 μ l of DMSO
Final volume equals 30 μ l

3. Aliquot 7 μ l of the reaction mixture from step 2 above into each of the four termination tubes already containing 3 μ l of ddNTP. Mix thoroughly, making sure the reaction mixture and the ddNTP are at the bottom of the termination tube.
4. Overlay the reactions with 15 μ l of silicone oil or mineral oil.

[§] See *Media and Reagents*.

[‡] See *Related Stratagene Products*.

^{*} See *Endnotes*.

10/18

5. Cycle the sequencing reaction through an appropriate temperature profile. Optimum cycling parameters will vary depending on the template and the primer, as well as the type of thermocycler used. Useful starting profiles are given in the following tables:

Standard thermocyclers		
	Temperature	Duration
Initial denaturation	95°C	30 seconds
Link to 30 cycles of		
Denaturation	95°C	30 seconds
Annealing	60°C ^a	30 seconds
Extension	72°C	30 seconds

^a Please note that the annealing temperature must be adjusted to 50°C if using the T3 20-mer and SK 20-mer sequencing primers.

Stratagene's Robocycler [®] 40 and Robocycler [®] Gradient 40 temperature cyclers [‡]		Stratagene's Robocycler [®] 96 and RoboCycler [®] Gradient 96 temperature cyclers [‡]	
Temperature	Duration	Temperature	Duration
95°C	30 seconds	95°C	30 seconds
95°C	30 seconds	95°C	30 seconds
60°C ^a	30 seconds	60°C ^a	30 seconds
72°C	30 seconds	72°C	30 seconds

^a Please note that the annealing temperature must be adjusted to 50°C if using the T3 20-mer and SK 20-mer sequencing primers.

6. Add 5 µl of stop solution (see *Media and Reagents*) below the oil overlay and mix by pipetting.
7. Heat denature the samples at >80°C for 2–5 minutes, then immediately load 2–4 µl of the samples onto a sequencing gel.

[‡] See *Related Stratagene Products*.

11/18

SEQUENCING PRIMERS AND PRIMER SETS

The traditional primers designed for the pBluescript® phagemid vector and its derivatives were used primarily for primer extension reactions at 37°C or less. The advent of PCR and cycle sequencing requires that these primers bind efficiently at higher temperatures. Stratagene has redesigned these primers for exceptional performance in high-temperature primer extension reactions. The new primers maintain nearly the same template positions, but now have higher melting temperatures (see *Related Stratagene Products*).

TROUBLESHOOTING

Observation	Suggestions
Little or no sample after cycling	Sample evaporation possibly due to insufficient oil overlay. Add at least 15 µl of silicone or mineral oil and briefly centrifuge before cycling.
Light or blank film	<p>Primer is not annealing efficiently. Reduce the annealing temperature or redesign the primer.</p> <p>Not enough of the template DNA.</p> <p>Old or insufficiently purified labeled nucleotide may have been used. The specific activity and purity of the labeled nucleotide is essential for optimal labeling. If using ³²P (because the half-life of ³²P is ~14.3 days), check the expiration or reference date on the manufacturer's label to ensure that only fresh labeled nucleotide is used. (The half-life of ³³P is 25.0 days; the half-life of ³⁵S is 87.4 days.)</p> <p>The exposure time of the gel to the X-ray film may have been too short and should be increased.³</p> <p>The reaction components were not thoroughly mixed, or one of the reaction components is missing.</p> <p>Template DNA is contaminated. Make sure excess salt and EDTA have not contaminated the preparation.</p>
Faint band in one of the lanes	<p>The reaction components were not thoroughly mixed, or one of the reaction components is missing.</p> <p>Ensure that ddNTPs are mixed thoroughly before cycling.</p>
High background on the sequencing gel	Too much template DNA. This can be a serious problem with short PCR products. Titrate down the amount of DNA added to the reaction.

(Table continued on the following page)

12/18

(Table continued from the previous page)

Bands in multiple lanes	<p>The primer annealed at multiple sites. Try a higher annealing temperature.</p> <p>Multiple templates are in the sequencing reaction. PCR products may be a particular problem if multiple products or primer dimer artifacts are present and one of the PCR primers is used as a sequencing primer. Gel purification should alleviate the problem.</p> <p>Gel compression artifacts are present. Increase the gel temperature (up to 60°C) or add formamide to the gel.</p>
Blurry or smeared bands	<p>Template DNA is contaminated.</p> <p>Samples are not fully denatured prior to gel electrophoresis.</p> <p>Old or improperly prepared acrylamide solutions were used and/or the DMSO has been frozen and thawed more than once..</p>

PRIMER END LABELING

Primers may be kinased with either [γ -³²P]ATP or [γ -³³P]ATP using the KinAce-It™ kinasing kit (see *Related Stratagene Products*) or by the standard protocol shown below.³

Sequencing End-Labeling Reaction

1. Add the following to a tube:
 - 20 ng of primer
 - 0.5 μ l of kinase buffer
 - 0.5 μ l [γ -³³P]ATP
 - 0.5 (5 U) T4 kinase

Add water to a final volume of 5 μ l
2. Incubate the tube at 37°C for 15 minutes.
3. Heat the entire reaction to 80°C for 10 minutes to deactivate the kinase.

WEIGHTS

Weight of DNA equal to 200 fmol of template DNA		
Template	Size	DNA amount
PCR product	500 bp	60 ng
Plasmid DNA	3,000 bp	400 ng

Weight of DNA equal to 1 pmol of primer DNA		
Template	Size	DNA amount
	17 bp	5.6 ng
	20 bp	6.6 ng
	25 bp	8.2 ng

MEDIA AND REAGENTS**10× Kinase Buffer**

500 mM Tris-HCl[‡] (pH 7.5)
 70 mM MgCl₂
 10 mM dithiothreitol (DTT)[‡]

TE Buffer

5.0 mM Tris-HCl (pH 8.0)
 0.1 mM EDTA[‡]

10× Sequencing Buffer

200 mM Tris-HCl (pH 8.8)
 100 mM KCl
 20 mM MgSO₄
 100 mM (NH₄)₂SO₄
 1% Triton® ×100
 1 mg/ml bovine serum albumin (BSA)[‡]
 20 μM dATP[‡]
 50 μM dCTP[‡]
 50 μM dGTP[‡]
 50 μM dTTP[‡]

Stop Solution

80% formamide
 50 mM Tris-HCl (pH 8.3)
 1 mM EDTA
 0.1% bromophenol blue (BPB)
 0.1% xylene cyanol (XC)

[‡] See *Related Stratagene Products*.

14/18

RELATED STRATAGENE PRODUCTS

Sequencing Primers

Primer	Sequence	Catalog #
T3 20-mer	5' AATTAACCCCTCACTAAAGGG 3'	300301
T7 22-mer	5' GTAATACGACTCACTATAGGGC 3'	300302
M13 (-20)	5' GTAAAACGACGGCCAGT 3'	300303
M13 reverse	5' GGAAACAGCTATGACCATG 3'	300304
SK 20-mer	5' CGCTCTAGAACTAGTGGATC 3'	300305
KS 20-mer	5' TCGAGGTCGACGGTATC 3'	300306

PCR Primer Sets

Primer set	Catalog #
T3/T7 ^{††}	302001
M13 (-20)/Reverse ^{††}	302003
SK/KS	302005

^{††} See *Endnotes*.

Modifying Enzymes

Recombinant Exo⁻ *Pfu* DNA Polymerase^{*} [Catalog #600163 (250 U) (100 reactions)]
 T4 Polynucleotide Kinase (from recombinant *Escherichia coli*) [Catalog #600103 (500 U) and #600104 (2500 U)]

Systems and Kits

Cyclist[®] *Taq* DNA sequencing kit [Catalog #200325 (~100 reactions)]
 KinAce-It[™] kinasing kit (Catalog #200390)
 Deoxynucleotide Mix [25 mM each dNTP (Catalog #200415)]

Nucleic Acid Purification

Lambda DNA Purification Kit [Catalog #200391 (25 1-ml minipreps) and #200392 (50 1-ml minipreps)]
 StrataPrep[™] PCR purification kit [Catalog #400771 (50 purifications) and #400773 (250 purifications)]
 StrataPrep[™] plasmid miniprep kit [Catalog #400761 (50 purifications) and #400763 (250 purifications)]
 ClearCut[™] miniprep kit [Catalog #400732 (50 minipreps) and #400733 (200 minipreps)]

^{*} See *Endnotes*.

^{††} See *Endnotes*.

15/18

Fine Chemicals

Acrylease™ nonstick plate coating [Catalog #300132 (200 ml)]
Tris-HCl [Tris (hydroxymethyl) aminomethane hydrochloride] [Catalog #300181 (500 g) and #300182 (1 kg)]
Dithiothreitol (DTT) (1-threo-1,4-dimercapto-2,3-butanediol) [Catalog #300061 (5 g) and #300062 (25 g)]
EDTA (Ethylenediaminetetraacetic acid-disodium salt) [Catalog #300071 (250 g), #300072 (500 g) and #300073 (1 kg)]
Bovine Serum Albumin [Catalog #300041 (1 ml) and #300042 (10 ml)]
Acrylamide [Catalog #300011 (500 g) and #300012 (2 kg)]
Ammonium Persulfate [Catalog #300022 (25 g)]
Bis-acrylamide [Catalog #300033 (25 g) and #300034 (100 g)]
Acrylamide Denaturing Gel Mix [Catalog #300001 (6% acrylamide), #300003 (8% acrylamide) and #300005 (12% acrylamide)]
6% Polyacrylamide Gel Mix [Catalog #300009 (5 × 100 ml)]
TEMED (*N,N,N',N'*-tetramethylethylenediamine) [Catalog #300161 (50 g)]
Urea [Catalog #300191 (1 kg) and #300192 (5 kg)]

Equipment and Labware

CastAway™ sequencing system [Catalog #401098 (120/100 V) and #401099 (230 V)]
SmartPower™ 4000 power supply [Catalog #400690 (120/100 V), #400691 (230-V CE-marked unit), and #400692 (230-V non-CE-marked unit)]
PicoFuge® microcentrifuge {Catalog #400550 (115/120 V), #400551 [220 V (UK only)], and #400552 [220 V (Continental Europe)]}
ProFuge™ 10k microcentrifuge [Catalog #400554 (120/100 V) and #400556 (230 V)]
RoboCycler® 40 temperature cycler⁺⁺⁺ [Catalog #400830 (100/120 V) and #400832 (230 V)]
RoboCycler® 40 gradient temperature cycler⁺⁺⁺ [Catalog #400860 (100/120 V) and #400862 (230 V)]
RoboCycler® Gradient 96 temperature cycler⁺⁺⁺ [Catalog #400880 (100/120 V) and #400882 (230 V)]
RoboCycler® 96 temperature cycler⁺⁺⁺ [Catalog #400870 (100/120 V) and #400872 (230 V)]
RoboCycler® Infinity temperature cycler⁺⁺⁺ [Catalog #400980 (120/100 V) and #400982 (230 V)]

16/18

STRATAGENE DISTRIBUTORS

Country	Distributor name	Telephone number	Fax number
Argentina	Tecnolab S.A.	541 555 0010	541 553 3331
Australia	Integrated Sciences	(02) 9417 7866	(02) 9417 5066
	Toll free	(800) 252204	
Austria	See inside front cover		
Brazil	Instrucom	11 5561 1771	11 530 0895
Canada	See inside front cover		
Denmark	AH Diagnostics	86 10 10 55	86 16 15 33
Egypt	Clinilab	202 3518763	202 3781507
Finland	Kebo Finland	358 9 804 551	358 9 804 55200
France	Ozyme	1 34 60 24 24	1 34 60 92 12
Germany	See inside front cover		
Hong Kong	Line Analytics Ltd.	2578 5839	2807 2674
India	Wipro Ltd.	91 11 3325677	91 11 3738675
Ireland	B.M. Brownes Ltd.	353 1 295 3401	353 1 295 3818
Israel	Getter Photo Supplies (2000) Ltd.	3 576 1555	3 752 3620
Italy	Eppendorf s.r.l.	02-58.01.34.09	02-58.01.34.38
Japan	Funakoshi Co., Ltd.	3 5684 1622	3 5684 1633
	Toyobo Co., Ltd. (Tokyo)	3 3660 4819	3 3660 4887
	Toyobo Co., Ltd. (Osaka)—Main office	6 348 3785/6 348 3788	6 348 3322
Korea	Koram Biotech Corp.	2 556 0311	2 556 0828
Mexico	Bioselec	355 8928	556 6943
New Zealand	LabSupply Pierce (NZ) Ltd.	9 443 5867	9 444 7314
Norway	MedProbe A.S.	47 22 20 01 37	47 22 20 01 89
Portugal	Biocontec	1 361 3620	1 362 5615
Republic of China	Merck Taiwan Ltd.	886 2 2521 9331	886 2 2536 7734
Singapore	ITS Science & Medical Pte. Ltd.	273 0898	273 0810
South Africa	Whitehead Scientific Supplies	21 981 1560	21 981 5789
Spain	Cultek	91 729 03 33	91 358 17 61
Sweden	AH Diagnostics AB	86 80 08 45	86 80 04 35
	Toll free	800 10299	
Switzerland	See inside front cover		
Thailand	ITS (Thailand) Co., Ltd.	2 308 0611	2 308 0612
United Kingdom	See inside front cover		
United States	See inside front cover		

17/18

REFERENCES

1. Murray, V. (1989) *Nucleic Acids Res.* 17:8889.
2. Craxton, M. (1991) *Methods: A Companion to Methods in Enzymology* 3:20–26.
3. Sambrook, J., Fritsch, E.F., and Maniatis, T., eds. (1989) "Molecular Cloning: A Laboratory Manual," Second Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

ENDNOTES

* Purchase of this product is accompanied by a license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with an Authorized Thermal Cycler. Stratagene's PCR products are sold under licensing arrangements with Roche Molecular Systems, Inc., F. Hoffmann-La Roche Ltd., and The Perkin-Elmer Corporation.

** These products are optimized for use in the Polymerase Chain Reaction (PCR) process covered by patents owned by Hoffmann-La Roche. Purchase of these PCR-related products does not convey any rights under the PCR patents owned by Hoffmann-La Roche. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

*** Practice of the patented Polymerase Chain Reaction (PCR) process requires a license. Stratagene's thermal cycler is an Authorized Thermal Cycler. Its use with Authorized Reagents provides a limited PCR license in accordance with the label rights accompanying such reagents. It may also be used with PCR licenses available from The Perkin-Elmer Corporation.

Cyclist®, PicoFuge®, FeatherVolt®, RoboCycler®, BaseAce®, StrataTherm®, PrimeErase®, and pBluescript® are registered trademarks of Stratagene in the United States.

Acrylease, ClearCut, KinAce-It, ProFige, and StrataPrep are trademarks of Stratagene.
Triton® is a registered trademark of Rohm and Haas Co.

18/18



STRATAGENE®

Cyclist® Exo⁻ Pfu DNA Sequencing Kit

Catalog #200326

QUICK-REFERENCE PROTOCOL

- ♦ Prepare the template DNA
- ♦ Add 3 µl of the appropriate ddNTPs to four termination tubes on ice
- ♦ Prepare the sequencing reaction mixture on ice by adding the following:
 - 200 fmol of template DNA
 - 1 pmol of primer (5 ng of a 17-mer oligonucleotide)
 - 4 µl of 10× sequencing buffer
 - 10 µCi of radioactive label
 - 1 µl of Exo⁻ Pfu DNA polymerase (2.5 U)
 - Bring to 26 µl with water
 - 4 µl of DMSO
 - Final volume equals 30 µl
- ♦ Aliquot 7 µl of the sequencing reaction mixture into each of the 4 termination tubes
- ♦ Cycle the sequencing reaction through an appropriate temperature profile such as the one given in the table below:

	Standard thermocyclers		Stratagene's Robocycler [®] 40 and Robocycler [®] Gradient 40 temperature cyclers		Stratagene's Robocycler [®] 96 and RoboCycler [®] Gradient 96 temperature cyclers	
	Temperature	Duration	Temperature	Duration	Temperature	Duration
Initial denaturation	95°C	30 seconds	95°C	30 seconds	95°C	30 seconds
Link to 30 cycles of						
Denaturation	95°C	30 seconds	95°C	30 seconds	95°C	30 seconds
Annealing	60°C ^a	30 seconds	60°C ^a	30 seconds	60°C ^a	30 seconds
Extension	72°C	30 seconds	72°C	30 seconds	72°C	30 seconds

^a Please note that the annealing temperature must be adjusted to 50°C if using the T3 20-mer and SK 20-mer sequencing primers.

- ♦ Add 5 ml of stop solution
- ♦ Heat denature at 80°C for 2–5 minutes
- ♦ Immediately load 2–4 ml on a sequencing gel