FOCUS

Published as a service to the molecular biologist by Bethesda Research Laboratories, Inc.
P.O. Box 577, Gaithersburg, Maryland 20877 (301) 840-8000
Toll Free (800) 638-8992 • Telex: 908750 BRL GARG
BRL GmbH Offenbacher Strasse 113, 6078 Neu Isenburg, West Germany
Telephone: 06102-3206 • Telex: 417899 BRL NID

Vol. 3 No. 3 July 1981

Replacement Synthesis Method of Labeling DNA Fragments

Dr. Patrick O'Farrell
Dept. of Biochemistry and Biophysics
School of Medicine, University of California, San Francisco

Facile methods for the labeling and manipulation of DNA fragments can greatly simplify procedures associated with recombinant DNA research. Here I describe a convenient and efficient method for the synthesis of labeled hybridization probes using the T4 DNA polymerase[1]. This polymerase, the product of bacteriophage T4 gene 43, contains two enzymatic activities, a DNA polymerase and a 3' exonuclease[2]. As shown in Figure 1, in the absence of deoxynucleotide triphosphates[3], the 3' exonuclease acts on double-stranded DNA fragments; determination of the degradation rate permits controlled excision of nucleotides to produce DNA fragments with recessed 3' termini of defined length[1]. On addition of labeled deoxynucleotide triphosphates these molecules act as primed templates[1,4,5], and the polymerase activity accurately regenerates the intact double helix now labeled in a defined region that has the specific activity of the labeled substrates. Hybridization probes prepared by this method lack artificial double-stranded hairpin structures that can be produced during nick translation[5-8]; probes specific for only a single strand can be easily prepared for analysis of the direction of transcription; and the isolation of labeled subfragments is greatly simplified. As a guide to application of the method, some example protocols are given below, and here, the relevant parameters of the reactions are described. Before discussing the reactions, it should be pointed out that a major factor contributing to the convenience of these protocols is that we have found that we can use a single buffer for restriction enzyme cleavage and the labeling reactions[1]. At this point more than twenty different restriction enzymes have been shown to function efficiently and accurately in TA buffer. Additionally, lambda exonuclease, exonuclease III, T4 DNA ligase and terminal transferase also work in this buffer. Thus several sequences of enzymatic steps can be carried out by a simple series of enzyme additions to the same reaction vessel.

The Exonuclease Reaction

The 3' exonuclease activity, or proof-reading activity[9], of the T4 DNA polymerase acts on all 3' hydroxyl termini whether the ends are blunt or have either 3' or 5' overhangs. Although the exonuclease may show some sequence dependence[3], experiments examining the rate of excision of nucleotides at the

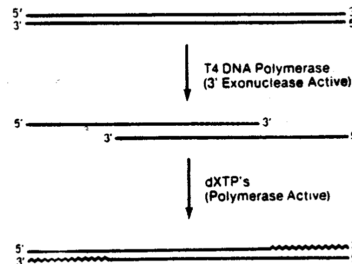


Figure 1

end of numerous restriction fragments have shown negligible sequence-specific differences. The enzyme attacks all ends indiscriminately and non-processively so that the size decrease of all fragments is equal and linear with time. The size distribution of fragments remains very narrow at all stages of the exonuclease reaction. The enzyme appears to have a relatively low affinity for the 3' ends and binds substantially and non-productively to internal regions of DNA. Thus, it is very difficult to saturate the 3' ends with enzyme; in most practical ranges of enzyme and DNA concentrations the rate of the exonuclease reaction is dependent on the enzyme:DNA ratio. The dependence is roughly linear at enzyme:DNA ratios below 2.5 units/μg DNA (a level which gives an exonuclease rate of about 40 nucleotides/min/3' end. At higher enzyme: DNA ratios, the dependence of the exonuclease rate is distinctly non-linear and reaches an apparent minimum rate of approximately 120 bases/min/3' end at a ratio of 30 units of enzyme/μg DNA[10].

It should be noted that, since the 3' exonuclease degrades single-stranded DNA much faster than double-stranded DNA[11], upon degradation to the halfway point, a restriction fragment will dissociate into two half-length single strands which will then be rapidly degraded to completion. As a result, the very center of a restriction fragment cannot be labeled by the replacement synthesis method, and the size of the smallest restriction fragment in a mixture of fragments dictates the maximum extent to which all the fragments can be labeled. This factor can occasionally be used as an enzymatic method for the selective purification of the larger restriction fragments in a mixture. That is, the smaller fragments can be degraded to completion while the partially degraded larger fragments can be repaired by polymerization.

continued on page 2

In the presence of deoxynucleotide triphosphate, the exonuclease reaction is masked by the polymerization reaction; however, an idling reaction comprised of alternating steps of exonuclease and polymerization continues[12]. The presence of a single species of nucleoside triphosphate is adequate to arrest the exonuclease reaction, ... the addition of all four triphosphates leads to the r... polymerization of the degraded DNA.

The Polymerization Step

Although the polymerization rate can exceed 15,000 nucleotides per minute[13], in the absence of accessory proteins the T4 DNA polymerase replicates base-paired regions extremely inefficiently. The secondary structure which forms in the 5′ region following its conversion to a single-stranded form by the exonuclease reaction will impede the subsequent polymerization step[4]. Stable hairpins will transiently arrest polymerization at distinct points, and electrophoretic analysis will show distinct bands representing incomplete replacement synthesis[14]. We have avoided this problem by simply allowing the polymerization reaction to proceed for an excessively long time. To obtain a product in which the proportion of incomplete molecules is negligible, we have followed the general guideline that the time of replacement synthesis should be twice as long as the exonuclease reaction step. In those cases where the product is to be used as a hybridization probe without prior separation by electrophoresis, the presence of some incomplete molecules is irrelevant and in these cases the polymerization time is generally reduced.

Because of competition between the polymerization reaction and the exonuclease reaction, a fraction of the substrate nucleoside triphosphates is hydrolyzed rather than incorporated[5]. It appears that the exonuclease can remove a 3′ terminal adenosine residue almost ten times faster than it removes a 3′ terminal cytosine residue[3]. Consequently the proportion of dCTP (maximally about 80%) which is incorporated rather than hydrolyzed is higher than the proportion of dATP (maximally about 50%) which is incorporated. For this reason we prefer to use labeled dCTP as precursor, but efficient labeling can be achieved with any nucleotide.

Generally, to achieve efficient use of isotope, the concentration of the labeled nucleotide is kept low and is often limiting. Since the K_m of the T4 DNA polymerase is about 20 μM, the low nucleotide concentration is presumed to reduce the rate and efficiency of the reaction. If a completely repaired product is required, unlabeled nucleotide is added to the reaction after an initial labeling period (see protocol 2). This "chase" reaction does not significantly reduce the specific activity since the majority of the replacement synthesis is completed in the early parts of the polymerization reaction.

Parameters and Properties to be Considered in Applying the Method

Although this discussion will consider only the replacement synthesis method of labeling DNA, it should be emphasized that the controlled excision at the 3′ terminal nucleotides by the T4 DNA polymerase has numerous other applications. Whether the particular application requires a highly accurate product (markers for gel electrophoresis, substrates for enzyme reactions) or high specific activity (hybridization probes for detection of homologous immobilized DNA) or both (probes for solution hybridization), the T4 DNA polymerase reaction provides a simple and suitable method.

The importance of high specific activity is a frequently emphasized feature of labeling reactions (especially those designed to produce hybridization probes); here I would like to emphasize that the specific activities are approaching a practical limit. In the replacement synthesis method, those sequences initially degraded and subsequently replaced in the polymerization step will be resynthesized at the specific activity of the available substrates. For a single deoxytriphosphate a specific activity of 3000 Ci/mmol (commercially available), the specific activity of the replaced sequences will be 5 × 10^9 cpm/μg DNA. (With present highly sensitive methods for film detection of ^{32}P, it would be possible to detect about 2 × 10^{-16} grams of DNA or about 4 molecules of bacteriophage lambda DNA, if it were labeled to this specific activity.) Obviously if only a fraction of the sequences are replaced, the average specific activity will be reduced by a corresponding fraction. At these specific activities radioisotopic damage leads to substantial fragmentation of the labeled DNA and if higher specific activities were obtained the quality and stability of the product would be compromised. At a specific activity of 5 × 10^9 cpm/μg, roughly one single-stranded break per 200 bases is expected in one day. The severity of the effects of isotopic damage depends on the specific activity to which the DNA is labeled and the starting fragment size (target size). In our experience hybridization probes labeled to a specific activity of about 10^8 cpm/μg continue to be useful for about two weeks; however, the decline in the hybridization signal is much greater than the twofold decline anticipated to be due to radioactive decay.

The described labeling method does not produce a uniform distribution of label throughout the restriction fragment. In some cases this can be used to advantage to produce 3′ (end) specific labeling or to label a limited region around a particular restriction enzyme site. The non-uniformity of the labeling can influence intensities of hybridization on Southern analysis. If the probe consists of a single *intact* restriction fragment with a small labeled region, the position of the label within the molecule will not affect the signal, but with increasing time after preparation of the probe, isotopic damage will fragment the probe and result in a diminution of the signal obtained from sequences indirectly linked to the labeled regions.

Because of the asymmetry of the labeling, the replacement synthesis method can be used to specifically label one strand or another of a cloned DNA fragment. Figure 2 illustrates the selective labeling of individual strands of a 1 kb insert within the *Hind* III site of pBR322. Such probes can be of great utility in defining the direction of transcription.

Thus, the methods presented here for the use of T4 DNA polymerase exemplify the utility of this enzyme in the synthesis of defined DNA probes.

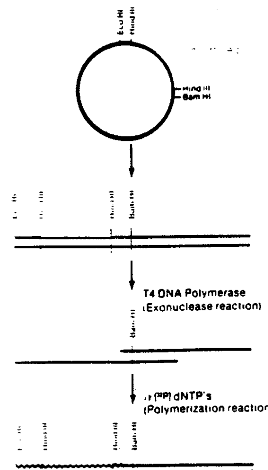


Figure 2

continued on page 3

Protocols

The Reaction Buffer (Tris Acetate Buffer: TA)

Final Composition

33 mM Tris acetate pH 7.9

66 mM potassium acetate

10 mM magnesium acetate

100 μg/ml nuclease-free BSA (or human serum albumin, Worthington)

0.5 mM DTT

Stored as frozen aliquots of a 10X buffer

Small variations in the buffer composition have no detectable effect on the reactions. The following experimental protocols take advantage of this to simplify the reaction mixtures.

Example 1: Preparation of a Hybridization Probe

For this example consider a 5 kb hybrid plasmid with a single *Hind* III site adjacent to a 1 kb inserted fragment. The object of this protocol is to prepare a hybridization probe for detection of the sequences present on the insert.

Reaction Mix

2 μl of 10X TA buffer stock

16 μl H_2O

2 μl of plasmid DNA at 100 μg/ml in TE (10 mM Tris pH 7.5, 1 mM EDTA)

Restriction Cleavage

Add 2 units of *Hind* III and incubate at 37°C for 30 minutes (this is a 5-fold excess level of digestion and is in the range that we usually use to ensure complete cleavage).

Exonuclease Step

Add directly to the above reaction 0.25 units of the T4 DNA polymerase. (There is no need to inactivate the restriction enzyme.) Note that the ratio of polymerase to DNA determines the rate of the exonuclease reaction.

In general it is convenient to dilute the enzyme to about 0.25 units per μl using the dilution buffer: 0.2 $KHPO_4$, pH 6.5, 500 μg/ml BSA, 50% glycerol, 10 mM 2-mercaptoethanol.

At an enzyme to DNA ratio of 1.25 units/μg DNA we have found that 20 nucleotides/min will be excised from each 3′ end and the ratio is standardly used.

Incubate this reaction 70 mins.

Calculation of the Moles of Nucleotide Excised

Total DNA in moles of nucleotide is 6×10^{-10} moles

$$\text{Fraction of DNA degraded} = \frac{1400 \text{ nct/end} \times 2 \text{ ends}}{5000 \text{ bp} \times 2 \text{ nct per bp}} = 0.28$$

Abbreviation: nct = nucleotides

Moles of nct excised = $6 \times 10^{-10} \times 0.28 = 1.68 \times 10^{-10}$

Assuming random nucleotide composition and distribution $1/4 \times 1.68 \times 10^{-10} = 4.2 \times 10^{-11}$ mole of each of the triphosphates would be required to resynthesize the degraded region.

Resynthesis Step

Add directly to the above reaction

1 μl 2 mM dATP, 2 mM dGTP and 2 mM dTTP

4×10^{-11} moles of [α-^{32}P]-dCTP

Incubate at 37°C for 35 minutes. This short polymerization is adequate for the synthesis of hybridization probes, but some truncated molecules may persist.

The reaction is stopped by the addition of 5 μl of 100 mM EDTA.

If the labeled isotope was produced recently and if it is of high quality, 60 to 80% of the input isotope should be incorporated.

To ensure low backgrounds in filter hybridization, we remove the free isotopes by spin dialysis.[15] Additionally, to remove other contaminants which appear to contribute to the background, we use the following procedure.[16]

To 1 volume of the labeled DNA after spin dialysis, add 3 volumes of 8 M $NaClO_4$

Under these conditions, the DNA will stick to glass.

Wet a GFC filter with 6 M $NaClO_4$, 50 mM $NaPO_4$, pH 7, 10 mM EDTA (PPE) and place on a vacuum filtration apparatus. Apply the DNA across the surface of the filter (1 cm^2 of GFC will retain about 10 μg of DNA). Wash the filter with PPE and with 100% ethanol. Place the filter in a microfuge tube and wet with TE. After 15 min at room temperature, pierce the bottom of the tube with a needle, insert the microfuge tube "piggyback style" into an intact tube and centrifuge to recover the liquid and the labeled DNA. The recovery is about 50%.

Example 2: Purification of Labeled Subfragments as Hybridization Probes

In this example consider a bacteriophage lambda clone isolated from a bank of clones. The clone contains a 15 kb insert bracketed by *Eco* RI sites and with an additional four *Eco* RI sites within the insert. The object of this protocol is to prepare hybridization probes from each of the five resolvable *Eco* RI fragments comprising the inserted sequences.

Reaction Mix

2 μg of the recombinant phage DNA in TE buffer.

Add 2 μl 10X TA buffer and H_2O to give a final volume of 20 μl.

Restriction Cleavage

Add 20 units *Eco* RI and incubate for 30 minutes.

Exonuclease Step

Add 2.5 units of T4 polymerase directly to above.

Assuming that the smallest *Eco* RI fragment is larger than 1 kb, 500 nct can be excised from each 3′ terminus without losing any of the fragments. Incubate for 25 min.

Calculation of the Moles of Nucleotide Excised

Total DNA as moles of nct = 6×10^{-9} moles.

Total number of ends = 12 due to 6 sites of *Eco* RI cleavage + 2 ends of lambda = 14.

Fraction of total DNA degraded

$$= \frac{500 \text{ nct/end} \times 14 \text{ ends}}{50{,}000 \text{ bp (lambda DNA)}} \times 2 \text{ nct/bp} = 0.07.$$

Moles of nct excised = $6 \times 10^{-9} \times 0.07 = 4.2 \times 10^{-10}$ and for individual nct ~ 1×10^{-10} moles.

Resynthesis Step

Add 1 μl of 2 mM dATP, 2 mM dGTP and 2 mM dTTP.

Add 2.5×10^{-10} moles of α[^{32}P]-dCTP (a slight excess).

Incubate for 12 minutes at 37°C.

To ensure complete regeneration of the intact duplex, 1 μl of 2 mM dCTP is added and the reaction is continued for 40 minutes.

Unincorporated isotope is removed by spin dialysis.

Gel Separation of DNA

Because of accumulating radioisotopic damage of the DNA the best results are obtained if the gel is run immediately after labeling.

Bands visualized by ethidium bromide straining are cut out, weighed, and 3 volumes of 8 M $NaClO_4$ are added.

After the agarose has dissolved, the DNA is collected on GFC filters as outlined above.

Example 3: Preparation of Labeled Size Standards

We have found frequent use for labeled standards for gel separations and Southern transfers. The T4 DNA polymerase reaction provides an extremely convenient method for the preparation of such standards.

Reaction

20 μl of *Eco* RI-cut lambda DNA at 100 μg/ml (The restriction of this DNA is done in TA buffer and a stock of the cut DNA is kept frozen.)

1 μl of T4 DNA polymerase at 2.5 units/μl.

Incubate for 5 min.

Add 1 μl of 2 mM dGTP, 2 mM dATP, 2 mM dTTP.

Add approximately 1 μCi of α[^{32}P] dCTP.

Incubate approximately 1 min.

Add 1 μl of 1 mM dCTP.

Incubate for about 10 minutes.

Stop the reaction by heating to 70°C for 5 minutes.

References

1. O'Farrell, P.H., Kutter, E. and Nakanishi, M. (1980) *Molec. Gen. Genet.* *179*, 421-435.
2. Goulian, M., Lucas, Z.J. and Kornberg, A. (1968) *J. Biol. Chem.* *243*, 627-638.
3. Hershfield, M.S. and Nossal, N.G. (1972) *J. Biol. Chem.* *247*, 3393-3404.
4. Huberman, J.A., Kornberg, A. and Alberts, B.M. (1971) *J. Mol. Biol.* *62*, 39-52.
5. Richardson, C.C., Inman, R.B. and Kornberg, A. (1964) *J. Mol. Biol.* *9*, 46-69.
6. Schildkraut, C.L., Richardson, C.C. and Kornberg, A. (1964) *J. Mol. Biol.* *9*, 24-45.
7. Kelly, R.B., Cozzarelli, N.R., Deutscher, M.P., Lehman, I.R. and Kornberg, A. (1970) *J. Biol. Chem.* *245*, 39-45.
8. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* *113*, 237-251.
9. Brutlag, D. and Kornberg, A. (1972) *J. Biol. Chem.* *247*, 241-248.
10. Nakanishi, M. and O'Farrell, P.H., Unpublished observations.
11. Huang, W.H. and Lehman, I.R. (1972) *J. Biol. Chem.* *247*, 3139-3146.
12. Englund, P.T. (1971) *J. Biol. Chem.* *246*, 3269-3276.
13. Mace, D.M. and Alberts, B.M. Manuscript in preparation.
14. Challberg, M.D. and Englund, P.T. (1979) *J. Biol. Chem.* *254*, 7820.
15. Neal, M.W. and Florini, J.R. (1973) *Anal. Biochem.* *55*, 328.
16. Chen, C.W. and Thomas Jr., C.A. (1980) *Anal. Biochem.* *101*, 339.

BRL is pleased to make available a wide variety of enzymes for DNA synthesis. Please contact BRL for the pamphlet on our DNA polymerases.

Enzymes for Nucleic Acids Research

T4 DNA Polymerase

T4 DNA polymerase is purified from *E. coli* D110 infected with T4 bacteriophage that is rec A–, *am* N 55–, *am* H39– (gene 42–, 30–) according to the procedure of Morris, *et al.*[1] The enzyme is functionally assayed by first allowing 3'→5' exonucleolytic degradation of *Bam* HI-cleaved pBR322 (40%) followed by addition of deoxynucleoside triphosphates and 5'→3' polymerization (fill in) to yield full length, linear pBR322[2].

This DNA is then cut with *Hae* III to demonstrate the faithful regeneration of the *Hae* III sites in pBR322. In addition, BRL incubates T4 DNA polymerase with ϕX174 RF DNA and ϕX174 viral strand DNA to insure the absence of double and single strand endonucleases.

Unit Definition: One unit is defined as the amount of T4 DNA polymerase catalyzing the incorporation of 1 nmol of total nucleotide into an acid precipitable product in 30 min. at 37°C using denatured or nicked calf thymus DNA as template-primer[1].

References

1. C. F. Morris, H. Hama-Inaba, D. Mace, N. K. Sinha nd B. Alberts, *J. Biol. Chem.* (1979) *254*, 6787-6796
2. O'Farrell, P. (1981) *Focus* 3 (3), 1-3. (This issue)

Product	BRL No.	Size	Quantity	Price
T4 DNA Polymerase	8005SA	50 U	each	$ 60.00
	8005SB	250 U	each	220.00

Applications of DNA Polymerases

Product	Preparation of Hybridization Probes	Modification of DNA Termini	Labeling of Specific DNA Strands	Second Strand Synthesis	Sequencing
T4 DNA Polymerase BRL #8005	+	+	+		
DNA Polymerase I (*E. coli.*) BRL #8010	+			+	
Large Fragment DNA Polymerase I (Klenow Fragment) BRL #8012	+	+	+	+	+
Terminal Transferase BRL #8008		+			

RNA Polymerase II (Wheat Germ)

DNA-dependent RNA polymerase II synthesizes RNAs which ultimately function as messengers for protein synthesis. Wheat germ RNA polymerase II is isolated by the method of Jendrisak and Burgess[1]. The enzyme is essentially homogeneous and is inhibited by low levels of α-amanitin. The BRL enzyme is assayed to insure the absence of RNAse, DNAse, exonuclease, and phosphatase.

Unit definition: One unit incorporates 1 nmol of ATP into TCA precipitable material in 15 min at 25°C using calf thymus DNA as template.

Reference:

1. Jendrisak, J.J. and Burgess, R.R. (1975) *Biochemistry 14*, 4639.

Product	BRL No.	Size	Quantity	Price
RNA Polymerase II	8036SA	25 units	each	$ 35.00
(Wheat Germ)	8036SB	100 units	each	120.00

Enzymes for Nucleic Acids Research

Polynucleotide Phosphorylase

Polynucleotide phosphorylase (MW 260,000) is isolated from *Micrococcus luteus* by a modification of the procedure of Klee[1]. It polymerizes ribonucleotide diphosphates (NDPs) without requiring any primer or template. In the presence of inorganic phosphate it can catalyze the breakdown of RNA into nucleotide diphosphates. It is used to synthesize homopolymers (such as poly A) or random copolymers. The BRL enzyme is assayed to insure the absence of RNAse, DNAse, exonucleases, phosphatase, and RNA polymerase.

Unit Definition: One unit of the enzyme polymerases one μmol of ADP into poly A in 15 min at 37°C.

References

1. Klee, C.B. (1971) *Procedures in Nucleic Acids Research* (G.L. Cantoni and D.R. Davies, eds.), Vol. 2, p. 896, Harper and Row, New York.

Product	BRL No.	Size	Quantity	Price
Polynucleotide	8034SA	40 units	each	$ 33.00
Phosphorylase	8034SB	160 units	each	120.00

Polynucleotide Phosphorylase-T (Primer Dependent)

Polynucleotide phosphorylase is isolated from *Micrococcus luteus* by a modification of the procedure of Klee[1]. It is made primer dependent by trypsin treatment and has a molecular weight of 220,000. This enzyme polymerizes nucleoside diphosphates in the presence of an oligonucleotide (ApApA) and the primer is incorporated into the product. The usefulness of this enzyme is in its ability to extend defined oligonucleotides. The BRL enzyme is assayed to insure the absence of RNAse, DNAse, exonucleases, phosphatase, and RNA polymerase.

Unit definition: One unit of the enzyme polymerizes one μmol of ADP into poly A in 15 min at 37°C in the presence of primer.

Reference:

1. Klee, C.B. (1971) *Procedures in Nucleic Acids Research* (G.L. Cantoni and D.R. Davies, eds.) Vol. 2, p. 896, Harper and Row, New York.

Product	BRL No.	Size	Quantity	Price
Polynucleotide	8035SA	7 units	each	$ 60.00
Phosphorylase	8035SB	28 units	each	200.00
(Primer dependent)				

Vaccinia Capping Enzyme (Guanylyltransferase)

Vaccinia capping enzyme has three major applications in molecular biology. Firstly, capping enzyme has been used to map transcription initiation sites of *Xenopus laevis* preribosomal RNA[1], fibroin mRNA of *Bombyx mori*[2], early vaccinia virus transcripts[3,4] and early herpes virus transcripts[5]. Secondly, RNAs of brome mosaic[6], satellite necrosis, influenza virus[7], and yeast mitochondria[8] have been labeled by capping enzyme prior to sequence determination. Thirdly, capping enzyme has been used to study the role of the cap structure in mRNA binding to ribosomes and in translation[9].

BRL is the sole commercial source for vaccinia capping enzyme. It has been purified to a level such that it is free from interfering levels of RNAse and phosphatase.

Unit Definition: The amount of enzyme that incorporates 1 pmol GMP into the 5′ termini of triphosphate-terminated poly A in 30 minutes at 37°C

References

1. Reeder, R.H., Sollner-Webb, B., and Wahn, H.C. (1977) *Proc. Nat. Acad. Sci. 74*, 5402.
2. Tsujimoto, Y., and Suzuki, Y. (1979) *Cell 16*, 425.
3. Wittek, R., Cooper, J.A. Barbosa, E., and Moss, B. (1980) *Cell 21*, 487.
4. Venkatsasn, S., and Moss, B. (1981) *J. Virol. 37*, 738.
5. Mackem, S., and Rolzman, B. (1981) *Proc. Nat. Acad. Sci.* (In Press)
6. Alquist, P., Dasguota, R. Shih, O.S. Zimmerman, D., and Kaesberg, P. (1979) *Nature 281*, 277.
7. Moss, B. Keith, J.M., Gershowitz, A., Ritchey, M.B., and Palese, P. (1978) *J. Virol 25*, 312.
8. Levens, D., Ticho, R., Ackerman, E., Rabinowitz, M. (1981) *J. Biol. Chem. 256*, 5226.
9. Muthukrishnan, S., Moss, B., Cooper, J.A., and Maxwell, E.S. (1978) *J. Biol. Chem. 253*, 1710.

Product	BRL No.	Size	Quantity	Price
Capping Enzyme	8024SA	25 units	each	$ 80.00
(Guanylyltransferase)	8024SB	100 units	each	256.00

Fragments

Fragments is published as a regular feature of *Focus* in an effort to provide information concerning new procedures and products of interest to the molecular biologist.

BRL welcomes and encourages all articles or notes for publication in *Focus* on procedures helpful to molecular biologists. All material should be sent to:

Dr. Howard Young
Director of Technical Services
Bethesda Research Laboratories
8705 Grovemont Circle
Gaithersburg, Maryland 20877

Recent Improvements to BRL Lyphozyme™

Reconstitution Procedure

Recent studies at BRL have developed an improved method for enzyme reconstitution. This procedure routinely yields greater than 70% of the enzyme activity within one hour of reconstitution (Table 1). Full activity is routinely achieved within 2-4 hours of reconstitution. Once reconstituted, the stability of Lyphozyme™ is comparable to the soluble form.

Table 1

Recovery of Unit Activity After Reconstitution of Lyphozyme™

Enzyme	% Unit Activity After Reconstitution[1]			
	1 hr	2 hr	4 hr	Overnight
Bam HI	80	80	80	100
Eco RI	70	90	100	100
Hae III	100	100	100	100
Hinc II	100	100	100	100
Hind III[2]	88	88	100	100
Hinf I	100	100	100	100
Kpn I	86	100	100	100
Sal I	88	100	100	100
Sma I	100	100	100	100
Taq I	100	100	100	100
Xba I	100	100	100	100

[1]Lyphozymes were reconstituted with 37°C glycerol-water (50%), gently vortexed and left on ice; unit activity was determined at 1, 2, and 4 hours after reconstitution. An overnight reconstitution (−20°C) with cold glycerol was used as control.

[2]*Hind* III was reconstituted with cold glycerol.

Polyethylene Glycol Inhibition of "Sanger 'Dideoxy' Elongation Reactions"

With the development of an M13 cloning vehicle, M13 mp7[1], DNA sequence analysis of inserts cloned at any of the six cloning sites (*Bam* HI, *Eco* RI, *Acc* I, *Sal* I, *Hinc* II, and *Pst* I) can be rapidly carried out using the 26 bp universal primer fragment of Anderson *et al.*[2] and the "dideoxy" chain terminating protocols of Sanger *et al.*[3] With the exception of single-stranded DNA which is isolated from the budding bacteriophage, all of the reagents required for these analyses are available commercially and practical experience has shown their quality to be at a high level. Recently, it has been noticed, both at BRL and elsewhere, that the extraction of the single-stranded DNA from the M13 virions can be a source of variability in the "dideoxy" sequencing reaction. In certain cases, this variability can be explained by contamination of the polymerase elongation reaction with residual polyethelene glycol carried forward from the bacteriophage purification step. Therefore, to eliminate this potential problem, BRL recommends that following deproteinization by phenol extraction, the aqueous phase be extracted several times with $CHCl_3$ (i.e., add 0.1 ml chromatograhic grade $CHCl_3$ to 0.05 ml aqueous phase in a 1.5-ml sterile microfuge tube) and one time with diethyl ether (0.1 ml). The DNA is then concentrated by ethanol precipitation overnight at −70°C and resuspended in 50 µl, 20 mM Tris-HCl (pH 7.5), 0.1 mM Na_2 EDTA, and 10 mM NaCl.

References

1. Messing, J.R., Crea, R., and Seeburg, D.H. (1981) *Nucleic Acids Res.* 9, 309.
2. Anderson, S., Gait, M.J., Mayol, L. and Young, I.G. (1980) *Nucleic Acids Res.* 8, 1731.
3. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463.

Important Notice

Please note that there is a change in the Unit Assay Conditions for Nuclease BAL-31, printed on page 35 of the 1981/82 BRL Catalog. For the most effective use of BRL's Nuclease BAL-31, the proper Unit Assay Conditions are as follows:

20 mM Tris-HCl (pH 8.1)
600 mM NaCl
12 mM $CaCl_2$
12 mM $MgCl_2$
1 mM Na_2 EDTA
1.0 mg/ml [^{3}H] λ DNA
0.05 - 0.5 units enzyme
Assay at 30°C

Also, please note that there is an error in the price of Plasmid pBR322 listed in the 1981/82 BRL Catalog. The price for 130 µg is $275.00, not $75.00, as listed on page 185.

Batch Preparation of Plasmid DNA Using RPC-5 ANALOG -

Developed by the DNA Sequence Analysis Group, BRL

After further development and testing, BRL has determined that the chromatography* matrix, RPC-5 ANALOG, can be employed in a "batch" method for the low resolution separation of certain nucleic acid species. This procedure eliminates the requirement for high pressure liquid chromatography equipment, heretofore necessary for employing this matrix. The batch method is both rapid and convenient, and proven to be superior when used for the purification of up to milligram quantities of plasmid DNAs. Outlined below is the rapid method developed by BRL for isolating plasmid pBR322 DNA from one liter of an amplified bacterial growth.

Nucleic Acid Enrichment Step Prior to Binding to RPC-5 ANALOG

1. Prepare a bacterial lysate using standard high salt method[1]. Include a RNAse digestion step. Remove protein from the crude lysate by organic solvent extraction; first, with an equal volume of freshly distilled phenol that has been saturated with 0.1M Tris-HCl (pH 8.0), then with an equal volume of chloroform. Nucleic acids are precipitated from the aqueous phase of the extract with two to three volumes of ethanol.
2. Resuspend the precipitated nucleic acids in 1.0 ml of DNA Buffer 0.2M NaCl, 10mM Tris-HCl (pH 7.2), 10mM EDTA.
3. Load the dissolved nucleic acids on a Sepharose-4B column (1 × 50 cm) equilibrated in DNA buffer. Elute the nucleic acids using this buffer.
4. Pool the fractions of the void volume (typically 10-20 ml) which contain the high molecular weight nucleic acids. Determine the concentration of nucleic acid by reading the absorbance at 260nm.

Step 1: Preparation of BRL RPC-5 ANALOG

Dry RPC-5 ANALOG is pre-treated as described in the BRL Product Profile. Briefly, the chromatography matrix is pelleted by centrifugation for 10 minutes at 3000 RPM in a clinical centrifuge (room temperature). the pelleted matrix is resuspended in 10 ml DNA buffer per gram dry weight and gently mixed for 5 minutes at room temperature. This procedure is repeated twice.

Step 2: Absorption of Nucleic Acids

Up to 1 mg of nucleic acid extract can be bound to 5 grams of RPC-5 ANALOG. The enriched nucleic acid extract in a volume of 10 to 20 ml is added to the pelleted, washed RPC-5 ANALOG, for 15 minutes at room temperature, with gentle mixing. The matrix, containing bound nucleic acids, is pelleted by centrifugation for 10 minutes at 3000 RPM at room temperature in a clinical centrifuge. The nucleic acids, bound to the pelleted matrix, can then be eluted in a step-wise manner using DNA buffer of increasing salt concentration; scale-up is possible as long as the initial ratio of nucleic acid to RPC-5 ANALOG (1 mg/5 g) remains constant. The matrix-bound nucleic acids are eluted in a step-wise fashion with increasing concentrations of NaCl. With bacterial lysates containing pBR322 we have found that 0.5M NaCl removes Form I plasmid DNA and 0.65M NaCl removes high molecular weight DNA. However, the preuse salt concentration needed to remove Form I of the particular plasmid DNA will vary according to the size and base composition of the DNA.

Step 3: Elution of Nucleic Acids

For step-wise elution, at each salt concentration a washed pellet of ANALOG matrix, containing bound nucleic acids, is resuspended in 2 ml elution buffer per gram dry weight RPC-5 ANALOG. After the addition of elution buffer, the solution is mixed gently for 15 minutes at room temperature. This is followed by centrifugation at room temperature for 10 minutes at 3000 RPM in a clinical centrifuge. The A_{260} of the supernatant fluid is determined, then the pellet is washed two more times with elution buffer to insure that all the nucleic acid will be eluted. These steps are continued up to 1 M NaCl. The matrix is then washed with 2 M NaCl; *the solution in which the RPC-5 ANALOG is stored until further use.

Using the conditions described above, milligram quantities of purified plasmid DNA have been obtained with greater than 95% recovery. Similar results are obtained using RPC-5 ANALOG under high pressure liquid chromatography conditions[2]. Both methods utilizing this chromatography matrix require one-third the time necessary to purify plasmid DNA by the conventional density centrifugation procedure in cesium chloride and ethidium bromide. The plasmid DNA recovered from RPC-5 ANALOG was determined to be 99% free from contaminating cellular DNA and RNA as compared to 90% purity obtained using cesium chloride density centrifugation. The DNA eluted from RPC-5 ANALOG was found to maintain all of its biological activity and had the added feature of never being exposed to potential carcinogens (ethidium bromide).

Furthermore, the "batch" method has been conveniently used to isolate and purified other species of DNA, including pBR322, pBR325, M13 mp7 RF, pMH-10, pMH-232, PACYC 177, PSP-14, SV40, polyoma and ΦX-174 RF. In addition, inserted DNA fragments up to 0.8 kb have been isolated from their respective vectors using this same "batch" method.

References

1. Davis, R.W., Botstein, D., Roth, J.R. In *Advanced Bacterial Genetics*, pp. 116-125, 1980. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. *Focus* Vol. 3, No. 2 January, 1981.

* in 10mM Na_2EDTA, 10mM Tris-HCl (pH 7.2)

Product	BRL No.	Size	Quantity	Price
RPC-5 ANALOG	1501 AA	5 gm	each	$ 25.00
	1501 AB	50 gm	each	200.00
	1501 AC	500 gm	each	1,875.00

Enzymes for Nucleic Acids Research

New Restriction Endonucleases

Acc I 5'-GT↓($^{A}_{C}$)($^{T}_{G}$)AC-3'

BRL® *Acc* I restriction endonuclease is a catalog grade enzyme which is suitable for cloning, sequencing and mapping of DNA fragments. Of special interest is the application of this enzyme for cloning DNA fragments in M13 mp7.[1]

Number of Cleavage Sites:

ΦX174	λ	M13 mp7	Ad2	SV40	pBR322
0	7	2	8	1	2

Reference

1. Messing, J., Crea, R. and Seeburg, P. (1981) *Nucleic Acids Research* 9, 309.

Mnl I 5'-CCTC-3'

Mnl I is isolated from *Moraxella nonliquefaciens* by an unpublished procedure.[1] *Mnl* I cleaves 5 to 10 bases in the 3' direction from the recognition sequence.[2] This enzyme has been useful in fragmenting genomic DNA, and specifically in the study of the sickle cell polymorphism. The BRL unit is defined on pBR322 DNA. The current BRL preparations of *Mnl* I are state-of-the-art quality and can be used in genomic mapping experiments.

Number of Cleavage Sites:

ΦX174	λ	Ad2	SV40	pBR322
34	>50	>50	51	26

References

1. Gelinas, R. unpublished results.
2. Roberts, R.J. (1981) *Nucleic Acids Research* 9, 75.

Nci I 5'-CC($^{C}_{G}$)GG-3'

Nci I is purified from *Neisseria cinerea* by modification of a published procedure,[1] and has a very low level of contaminating exonucleases and endonucleases. The recognition sequence was deduced by a computer search of common palindromic DNA sequences that correspond to known cleavage frequencies.[1] Available for the first time from BRL, *Nci* I provides a new addition to the catalog of known restriction enzyme recognition sites.[2] *Nci* I is suitable for genomic mapping experiments.

Number of Cleavage Sites:

ΦX174	λ	Ad2	SV40	pBR322
1	>20	>15	0	10

References

1. Watson, R., Zuker, M., Martin, S. M., and Visentin, L. P. (1980) *FEBS Let.* 118, 47.
2. Roberts, R. J. (1981) *Nucleic Acids Research* 9, 75.

Pvu II 5'-CAG↓CTG-3'

Pvu II is isolated from *Proteus vulgaris* by a procedure developed at BRL. This enzyme preparation is free of detectable *Pvu* I activity. At this time, no isoschizomers of *Pvu* II are known. *Pvu* II cleaves pBR322 once at position 2068 which is situated distal to the two antibiotic resistant genes.[1,2]

Number of Cleavage Sites:

ΦX174	λ	Ad2	SV40	pBR322
0	15	22	3	1

References

1. Roberts, R. J. (1981) *Nucleic Acids Research* 9, 75.
2. Blakesley, B. (1981) personal communication.

Xma III 5'-C↓GGCCG-3'

Xma III is isolated from *Xanthomonas malvacearum* by a modification of a published procedure[1]. *Xma* III is the first restriction endonuclease known to recognize 5'-C↓GGCCG-3' which contains two CpG nearest neighbor pairs. Since this nearest neighbor pair is relatively rare in eucaryotic DNA[2], the mean fragment size following complete digestion with *Xma* III would be expected to be large[2]. In the case of *Xma* III cleavage of *Drosophila* DNA this is in fact observed[1]. Cloning vehicle pBR322 has a single *Xma* III site at position 938.

Number of Cleavage Sites:

ΦX174	λ	Ad2	SV40	pBR322
0	2	10	0	1

References

1. Kunkel, L.M., Silberklang, M. and McCarthy, B.J. (1979) *J. Mol. Biol.* 132, 133.
2. Swartz, M.N., Trautner, T.A. and Kornberg, A. (1962) *J. Biol. Chem.* 237, 1961.

Product	BRL No.	Size	Quantity	Price
Acc I	5415SA	70 units	each	$ 55.00
	5415SB	350 units	each	210.00
Mnl I	5418SA	60 units	each	$ 55.00
	5418SB	300 units	each	210.00
Nci I	5411SA	100 units	each	$ 55.00
	5411SB	500 units	each	210.00
Pvu II	5412SA	1500 units	each	$ 55.00
	5412SB	7500 units	each	210.00
Xma III	5414SA	20 units	each	$ 55.00
	5414SB	120 units	each	210.00

Meetings and Courses

• **August 2-7.** *Fifth International Congress for Virology.* Strasbourg, France, Professor L. Hirth, Chairman. National Host Committee, Institute of Molecular and Cellular Biology, CNRS, 15 Rue Descartes, 67000 Strasbourg France.

• **August 30-September 12.** *Control and Process in the Biosynthesis of Macromolecules.* Island of Spetsai, Greece. The Spetsai Summer School Secretary, Department of Chemistry, Aarhus University, Langelandesgabe 140, 8000 Aarhus C, Denmark.

• **August 31-September 4.** *International Symposium on Comparative Research on Leukemia and Related Diseases (10th).* Los Angeles, Calif. Dr. David S. Yohn, Secretary General, Suite 357 McCampbell Hall, 1580 Cannon Dr., Columbus, OH 43210.

• **September 20-23.** *A COGENE Symposium* "From Genetic Experimentation to Biotechnology. The critical transition." Dr. W. J. Whelan, Biochemistry-UMED, P.O. Box 016129, Miami, Florida 33101.

• **September 24-26.** *International Specialized Symposium on Yeast: Cell Walls (VIIth).* Valencia, Spain. VIIth U.S.S.Y. Secretariat, Facultad de Farmacia, Departmento de Microbiologia, Avenida Blasco Ibanez, 13, Valencia-10, Spain.

• **October 8-10.** *International Symposium on Medical Virology.* [illegible]heim, Calif. Luis M. de la Maza. Dept. of Pathology, University of California, Irvine Medical Center, Orange, CA 92668.

• **October 12-24.** *A COGENE Practical Course on Recombinant DNA Techniques.* Ljubljana, Yugoslavia. W.J. Whelan, COGENE, P.O. Box 016129, Miami, FL 33101.

• **October 13-16.** *Recent Advances in Medical Microbiology.* New York, NY. Victor Lorian, The Bronx-Lebanon Hospital Center, Albert Einstein College of Medicine, Fulton Ave. at 169th St., Bronx, NY 10456.

• **November 4-6.** *Interscience Conference on Antimicrobial Agents and Chemotherapy (21st).* Chicago, Ill., R. W. Sarber, ASM, 1913 I St., N.W., Washington, DC 2006.

• **November 9-12.** *International Conference on Streptococcal Genetics.* Sarasota, Fla. Don B. Clewell, Dept. of Oral Biology, University of Michigan School of Dentistry, Ann Arbor, MI 48109; D. Leblanc, Bldg. 30, Room 530, NIH, Bethesda, MD 20205; R. W. Sarber, ASM, 1913 I St., N.W., Washington, DC 20006.

Anyone wishing to list meetings in *Focus* should send all pertinent information to Ms. Nancy Hampar, Director of Communications, P.O. Box 577, Gaithersburg, MD 20877.

Special Announcement:

The 14th Miami Winter Symposium

From Gene to Protein: Translation into Biotechnology

January 11-15, 1982 Miami, Florida

The 14th Miami Winter Symposium will be devoted to the frontier areas of genetic experimentation with special reference to the translation of laboratory processes into practical applications e.g. cell culture techniques, monoclonal antibodies, in vitro synthesis of DNA and the generation of protein analogs, increasing the levels of gene expression, the biological activities of cloned gene products and horizons in biotechnology.

BRL is pleased to sponsor the 1982 Feodor Lynen Lecture. The Lynen Lecturer will be Cesar Milstein, Nobel Laureate.

The poster sessions will concentrate on developments in the above areas and biotechnology in general.

Inquiries and requests for registration forms and for details on poster presentations should be sent to:

Miami Winter Symposium
P.O. Box 016129
Miami, Florida 33101, U.S.A.
(Phone: Mrs. Sandra Black, 305-547-6265)

Applicants will be placed on the mailing list for the full program, to be distributed in August 1981.

Look for BRL at the ASCB Meeting
in Anaheim, California — November 9-13, 1981

Tools for the Molecular Biologist

The BRL Sequence Service

For DNA sequence determination and analysis that is:

- **Cost-Effective**
- **Rapid**
- **Accurate**

Utilize the Proven Expertise of Our Knowledgeable, Experienced Staff

- More than twelve years cumulative experience in *all* sequencing methodologies
- Two years successful service to the research community
- Operates active in-house sequencing program
- Trains researchers of major universities, institutes and biotechnology companies *via* BRL Sequence Analysis Workshops

Access the Complete Array of Rapid Sequencing Technologies

- Maxam/Gilbert Method[1]
- Sanger 'Dideoxy' Chain Termination Procedure[2]
- MCS*/Universal Primer Cloning-Sequencing System[3]

Select Tailored Sequencing Strategies

- Precise answers to *your* experimental question
- Collaborative protocol development available
- "Go/No Go" options; feasibility studies, partial sequence data acquisition

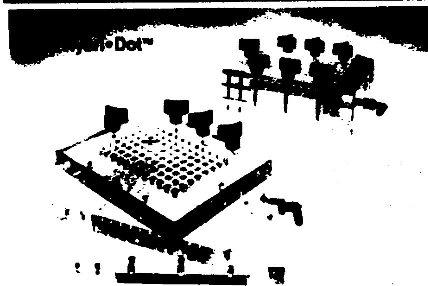
*Multiple Cloning Site

References

1. Maxam, A.M. and Gilbert, W. (1980) *Methods in Enzymology* 65, 499-560.
2. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
3. Heidecker, G., Messing, J. and Gronenborn, B. (1980) *Gene* 10, 68-73.

For further information, please write the Sequencing Contracts Manager or call Toll Free (800) 638-4045.

Also Available from the BRL Sequence Service-Computerized Sequence Data Analysis



5′ DNA Terminus Labeling System

The 5′-phosphoryl terminus of DNA or RNA can be radiolabeled *in vitro* with T4 polynucleotide kinase either by a reaction which first requires a dephosphorylation event with alkaline phosphatase prior to the labeling step or by an exchange reaction which takes advantage of the reversibility of the T4 polynucleotide kinase reaction[1,2].

The BRL® 5′ Terminus Labeling System consists of a number of high quality reagents that individually pass stringent quality control assays prior to use in the labeling system. Included in the BRL System are T4 polynucleotide kinase, buffers, bacterial alkaline phosphatase, test DNA, and an extensive protocol.

Product	BRL No.	Size	Quantity	Price
5′ DNA Terminus Labeling System	8060SA	10 assays	each	$125.00
	8060SB	20 assays	each	210.00
T4 Polynucleotide Kinase	8004SA	100 units	each	$ 55.00
	8004SB	500 units	each	210.00

References

1. Richardson, C.C. (1965) *Proc. Natl. Acad. Sci. USA* 54, 158.
2. Chaconas, G. and Van de Sande, J. (1980) in *Methods in Enzymology* Grossman and K. Moldave, eds.) 1st Ed., Vol. 65, p. 75.

BRL Hybri•Dot™ System

For low cost, time saving hybridization to "dots" of target DNA and RNA, BRL has designed a 96-well manifold that simplifies scale-up of the sensitive "dot hybridization" procedure for detection of specific DNA and RNA sequences[1,2]. In this procedure, as little as 1 picogram of a specific nucleic acid sequence, bound to nitrocellulose as a small spot, can be detected using ^{32}P-labeled probe ($\sim 10^8$ dpm/μg). Dot hybridization has been used to detect viral DNA in lymphocytes[3] and in serum[4].

The 3 mm diameter sample wells of the BRL Hybri•Dot™ System are arranged to precisely match the array of a standard 96-well microtiter plate. This simplifies and accelerates procedures such as the large-scale screening of bacterial colonies for recombinant plasmids[5].

Our Hybri•Dot™ System is precision-machined from rugged cast acrylic so that it seals without gaskets, O-rings, or grease. It can be assembled in less than a minute and comes supplied with precut nitrocellulose sheets.

References

1. Kafatos, F.C., Jones, C.W., Efstradiatis, A. (1979) *Nucleic Acids Res.* 7, 1541.
2. Thomas, P.T. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5201.
3. Brandsma, J. and Miller, G. (1980) *Proc. Natl. Acad. Sci. USA* 77, 6851.
4. Berninger, M. (1980) Unpublished observations.
5. Grunstein, M. and Hogness, D.S. (1975) *Proc. Natl. Acad. Sci. USA* 72, 396

Product	BRL No.	Quantity	Price
Hybri•Dot	1050MM	1 unit	$350.00
		2-5 units	330.00
		6+	310.00

M13 and Its Applications

Dr. Joachim Messing
Department of Biochemistry
University of Minnesota,
Minneapolis-St. Paul

A Report on the M13 Cloning and Sequencing Course and the Workshop on Eukaryotic Genes, September 15-19, 1980.

Since the development of M13 as a vehicle for random cloning of genes (e.g., the histidine operon control region) and the subsequent sequencing of these DNA clones by the dideoxy method, a more advanced M13-*lac* vector/host system has been developed. During the last 2-1/2 years M13 mp2, and *Eco* RI cloning phage, M13 mp5, *Hind* III cloning phage, pHM232 (master primer), JM101 (host strain) and a protocol for the use of this system have been sent out to numerous researchers interested in DNA sequencing, single-stranded DNA hybridization probes and *in vitro* site specific mutagenesis. To handle the increasing demand of detailed technical information, a course in cloning and sequencing with M13 mp2 and its derivatives was organized in September, 1980 at the University of Minnesota with 25 scientists from around the country participating.

The course curriculum included three major areas: (1) handing of the phage, template preparation, dideoxy sequencing, and gel reading; (2) care of the host strain, host infection, and preparation of the double-stranded form (RF) of the phage for cloning; (3) cutting, ligation, transformation of host cells, DIGE (direct gel electrophoresis) of recombinant phage.

Participants in the course received a kit including master primer, M13 mp5, M13 mp7, JM103, M13 mp7 RF (prepared during the course) and an updated manual of the use of M13 mp2 and derivatives. The same kit and information except M13 mp7 RF was sent out to the other applicants who were not accepted due to lack of available space.

Most of the course and workshop expenses were covered by tuition. Additional financial aid was provided by the University of Minnesota and the Bethesda Research Laboratories in the form of reagents required for the course.

As an addition to the course, a small workshop was held on M13 and its application toward the study of eukaryotic gene expression. Richard Gelinas from the Fred Hutchison Cancer Research Center in Seattle presented a comprehensive overview on the structure of globin genes and an introduction to the use of computer programs in the evaluation of DNA sequencing data. Daniel Vapnek from the University of Georgia in Athens gave a seminar on the structure of the dehydroquinase gene from *N. crassa*. DNA sequence data were derived from a template bank of overlapping fragments. The vector was M13 mp2, the fragments were adapted with *Eco* RI "linkers", and the synthetic oligonucleotide described recently was used as a master primer. Dan talked in his second seminar about the various approaches of site specific *in vitro* mutagenesis using single-stranded templates of M13 recombinant phages. Stephen Anderson from Cambridge, England reported on the mitochondria DNA work done in Fred Sanger's laboratory. A large portion of the DNA sequences were derived using M13 mp2 clones of *Eco* RI adapted DNA fragments and a master primer, in this case the 96 bp primer. Steve's second talk dealt with a new approach. This involves the random cleavage of DNA with DNAse I, the subsequent enrichment of fragments of an average size of 300 bp, and the cloning of these fragments in M13 mp2 with *Eco* RI "linkers". Since DNA is fragmented randomly, the entire sequence can be reconstructed by overlaps and complementary sequences. James Roberts from Columbia University in New York used an M13 clone as a probe to study expression of the ACTH-endorphin gene system. He also discussed the technical details of preparing highly labeled single-stranded DNA probes with M13. Peter Seeburg presented the work on the interferon gene structure and expression in *E. coli* done by him and his colleagues at Genentech, Inc. in San Francisco. A nucleotide sequence of various interferon clones was derived rapdily using a new M13 cloning vector, M13 mp7. He presented in a second seminar the approach of using M13 mp7 in shotgun DNA sequencing. Participants had the opportunity to discuss their work with the speakers through most of the week.

I wish to thank everyone who helped make the course successful, especially my secretary, Bonnie Allen, and my students, Jeff Vieira and Lynn Corcoran. Due to the enthusiasm and response to this year's course, the Second Annual M13 Workshop is planned for September and is announced on page 12 of this issue.

Meetings and Courses

Second Annual M13 Workshop

Approaches to Understanding DNA Structure

To be held at the University of Minnesota, September 14 - 18, 1981 in conjunction with the M13 cloning and sequencing course.

The use of the M13 sequencing technique leads to an enormous accumulation of DNA sequencing data. In order to discuss the translation of this data into possible secondary structure or features which can be related to genetic functions, this year five colleagues have agreed to come and contribute to this discussion.

Jurgen Brosius, presently in Walter Gilbert's laboratory (Harvard University), has determined the ribosomal DNA sequence in *E. coli* during his stay in Harry Noller's lab. Carl Woese's work on 16s ribosomal RNA's in numerous organisms has allowed Jurgen and his colleagues in collaboration with Carl Woese's laboratory to determine the secondary structure of the 16s ribosomal RNA due to evolutionary diversity.

David Pribnow, presently in Larry Gold's laboratory (University of Colorado) is using a rich source of mutant messenger RNA's to relate mRNA structure to the initiation frequency of translation. This work relates back to extensive genetics done with the rIIB cistron of bacteriophage T4.

Peter Czernilowsky, now working in Austrian Academy of Sciences (Salzburg), has determined the DNA sequence of the Rous sarcoma virus "src" gene during his stay in Mike Bishop's laboratory (University of California, San Francisco). Since the function of "src" can be tested in tissue culture, it is a good model to use *in vitro* mutagenesis in order to relate DNA structure to function.

David Mount, working in the Department of Microbiology at the University of Arizona, is studying the effect of chemical mutagenesis *in vivo* and how specific changes can occur with different types of mutagens. This knowledge consequently allows us to define how specific mutational changes can affect expression of eucaryotic DNA's in *E. coli*.

Mark Zoller, working in Mike Smith's laboratory (University of British Columbia), is using the chemical synthesis of DNA to introduce defined mutations into the DNA. This technique provides the ultimate method of creating mutations in positions of DNA where functions can be related to a particular nucleotide or nucleotide sequence.

Tuition for the course and workshop will be $275. Applications should contain a brief resume of research interests and must be received by Dr. Joachim Messing, Department of Biochemistry, University of Minnesota, Minneapolis-St. Paul, MN 55108, no later than August 20, 1981.

Expanded Content!

DNA Sequence Analysis Workshops

In response to many requests, we have enlarged the scope of our popular 3-day DNA Sequence Analysis Workshop. In addition to learning the theory and practice of Maxam-Gilbert and Sanger 'dideoxy' sequencing technologies, Workshop participants will be introduced to the M13 cloning methodology.[1,2] Developed by Messing and coworkers,[3] use of M13 coliphage cloning vehicles significantly increases the speed and flexibility of the Sanger method. Don't miss this opportunity to learn this latest improvement in sequencing methodology. The current schedule for the BRL Sequence Analysis Workshops is:

August 18, 19, 20	**McGill University** **Montreal, Quebec, Canada**
August 25, 26, 27	**University of California at San Diego** **La Jolla, California**
September 9, 10, 11	**University of Toronto,** **Toronto, Ontario, Canada**
September 21, 22, 23	**Case Western Reserve School of** **Medicine, Cleveland, Ohio**

BRL plans to conduct more workshops during the fall, but exact dates have not yet been determined.

If you want information and applications for one of these workshops, if you'd like to be informed of future dates as they are scheduled, or if you want to discuss sponsoring a workshop in your own laboratory, write Mary Fraker, the BRL Workshop Coordinator, or call her at (800) 638-8992.

1 Messing, J., Crea, R. and Seeburg, P. (1981) *Nucleic Acids Res.* 9.309
2 Heidecker, G., Messing, J. and Gronenborn, B (1980) *Gene 10*, 69
3 For a complete review of M13 cloning sequencing system applications, write to the BRL Communications Department and request the M13 booklet

BRL Position Available in Germany

Announcing: Position in Germany: Bethesda Research Laboratories is now seeking an individual for the position of Director of Technical Services—European Operations to be based in Neu Isenburg, West Germany. Applicants must have a strong background in nucleic acid enzymology, protein chemistry and cell biology. Major responsibilities will involve developing a technical interaction between European customers and our laboratories in both Europe and the USA.

Please direct your inquiries to:

Dr. Tonu M. Wali
BRL GmbH
Offenbacher Strasse 113
6078 Neu Isenburg
West Germany (Frankfurt area)