CB 2/98

# QuickPoint™

Rapid DNA Sequencing System


## TECHNICAL GUIDE

NOVEX™
NOVEL EXPERIMENTAL TECHNOLOGY

This technical guide contains questions commonly asked about the QuickPoint™ Rapid DNA Sequencing System and is intended to supplement the QuickPoint Rapid DNA Sequencing System Instruction Booklet (IM4500) and the QuickPoint Sequencing Cell Instructions (IM4505). Complete protocols for setting up the Sequencing Cell, sample preparation, buffer preparation, denaturing gel electrophoresis, and preparing gels for autoradiography are provided in these instruction booklets. Additionally, a laminated Quick Reference Guide of the QuickPoint protocol is also available (IM4515). To request this Quick Reference Guide, or for additional information, please call NOVEX at **1-800-456-6839.** For Technical Service, call **1-800-55-NOVEX** (1-800-556-6839), between 7:00AM and 5:00PM Pacific Time.

# Table of Contents

# I. Introduction

The NOVEX™ QuickPoint Rapid DNA Sequencing System is a revolutionary neutral pH, pre-cast denaturing polyacrylamide DNA sequencing system. The system provides a fast, easy-to-use, pre-cast DNA sequencing gel capable of sequencing a minimum of 60 bases in only 15 minutes. Researchers requiring short read lengths, such as those found with epitope libraries, phage display, random display, and other site-directed mutagenesis applications, will find the QuickPoint system to be more convenient and less time-consuming than conventional manual sequencing systems. The neutral pH environment during electrophoresis of the DNA contributes to better gel performance, band resolution, and gel stability, providing you with increased confidence in your sequence results. The QuickPoint Rapid DNA Sequencing System consists of the following components:

## QuickPoint Pre-Cast Sequencing Gel*

- 6% polyacrylamide denaturing gel in a 12.5cm x 10cm glass cassette
- 16 pre-formed wells
- Gel is bound to one glass plate and is easier to handle and manipulate than conventional-sized sequencing gels, making gel breakage virtually impossible
- Glycerol tolerant—accommodates all types of sequencing reactions
- 4 gels per box
- 6-month shelf life

## QuickPoint Buffers

- QP Sample Loading Buffer—uniquely formulated* to give the highest band resolution on QuickPoint gels
- 50X QP Running Buffer—no need to make up buffers

## QuickPoint Sequencing Cell

- Includes the lower buffer chamber, buffer core, wedges, buffer dam, and lid/leads designed specifically to accommodate 12.5cm x 10cm QuickPoint gels
- Accommodates 2 sequencing gels per Sequencing Cell, allowing for a total of 8 different sample templates per 15 minute run

* Patents are pending for the QuickPoint™ Rapid DNA Sequencing System including all buffers and gel formulations.

# II. QuickPoint Sequencing Gels and Buffer System

**Why are QuickPoint gels better than conventional TBE-Urea sequencing gels?**

QuickPoint gels offer a number of advantages over conventional pour-your-own TBE-Urea gels. Besides the obvious advantage of a pre-cast gel, QuickPoint gels have a 6-month shelf life, a run time of only 15 minutes, and are strictly quality-controlled to ensure reproducible batch-to-batch performance. The unique, neutral pH chemistry of the QuickPoint System enables the user to sharply resolve a minimum of 60 bases on a gel measuring only 12.5cm x 10cm. The smaller format of the QuickPoint gel and Sequencing Cell is a lot easier to handle and set-up than the conventional TBE-Urea pour-your-own format.

**What if I want to pour my own QuickPoint sequencing gel?**
**Do you have a reference that I can follow to make comparable gels?**

The QuickPoint System is a proprietary gel system developed at NOVEX. While there are references discussing advantages of neutral pH gel systems, we cannot provide a reference for the actual gel matrix or the chemical composition of the buffering system. Patents are pending.

**Can I recycle the QuickPoint glass plates and use them to pour my own sequencing gel?**

No. The glass plates have been treated with proprietary materials during the manufacturing process. The polyacrylamide has been chemically bound to one glass plate, making it difficult to clean and re-use. NOVEX does not guarantee acceptable band resolution and performance with gel matrices other than that of the QuickPoint System. NOVEX does not sell the glass plates, spacers, or combs separately.

# II. QuickPoint Sequencing Gels and Buffer System (cont'd)

### Are the QuickPoint gels glycerol tolerant?

Yes, QuickPoint gels are glycerol tolerant! The boric acid present in conventional TBE sequencing gels interacts with glycerol and results in severe band distortion. Since QuickPoint gels are made without boric acid, higher concentrations of glycerol can be present in the sequencing reaction mixture, and subsequently tolerated on QuickPoint gels without causing band distortion. DNA polymerases are stabilized by the higher concentrations of glycerol necessary for achieving longer labeling reactions or labeling reactions at higher temperatures. Higher temperature labeling reactions can be beneficial for better primer specificity. Up to 8% glycerol in the final reaction sample can be tolerated on QuickPoint gels as determined using Sequenase™ Version 2.0 and ThermoSequenase™ kits by USB/Amersham, shown in the following figure.

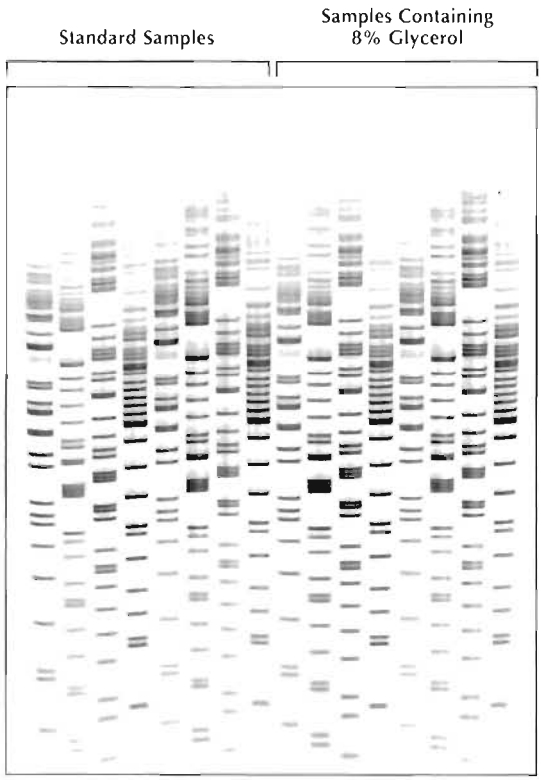



**Figure A**
QuickPoint gels are glycerol tolerant!

# II. QuickPoint Sequencing Gels and Buffer System (cont'd)

**How long are the QuickPoint gels stable for?**

NOVEX guarantees the stability of QuickPoint gels for a minimum of 6 months from the date of shipment when stored at 4°C.

**How long are the QuickPoint buffers stable for?**

The 50X QP Running Buffer is guaranteed to be stable for a minimum of 1 year from the date of shipment when stored at 4°C. The QP Sample Loading Buffer is guaranteed for a minimum of 6 months from the date of shipment when stored at -20°C.

**What if my QuickPoint gels and/or buffers are left out at room temperature?**

QuickPoint gels and buffers can tolerate only short term storage at room temperature. Exposure of the gels and/or the buffers to room temperature for greater than 24 hours may result in decreased gel performance. Please store the QuickPoint gels and the QP Running Buffer at 4°C. Store the QP Sample Loading Buffer at -20°C.

**The QP Sample Loading Buffer is very viscous and difficult to pipette. Why?**

The QP Sample Loading Buffer is highly concentrated, making it viscous and difficult to pipette at temperatures below room temperature. By heating the QP Sample Loading Buffer to 72°C for no more than 2 minutes, vortexing, and spinning briefly, the buffer becomes more manageable. Pipette the buffer slowly to ensure all liquid is dispensed from the pipette tip.

**Can the QuickPoint gels and/or buffers be frozen for long term storage?**

No! All QuickPoint gels and buffers should be stored at 4°C except for the QP Sample Loading Buffer which is stored at -20°C. Freezing the gels will destroy the polyacrylamide matrix. Do not use frozen gels. The 50X QP Running Buffer composition may be altered if frozen. We cannot recommend using the 50X QP Running Buffer or any dilution of this buffer that has been previously frozen.

# III. Sample Preparation and Sequencing Reaction

### Why do I need to use $Mn^{2+}$ in the labeling reaction?

When using Sequenase™ Version 2.0 to make sequencing reactions, the addition of $Mn^{2+}$ in the labeling reaction is one method used in order to emphasize the sequence very close to the primer. This method is easily employed when running QuickPoint gels in order to increase the intensity of the bands from short fragments of DNA, hence allowing one to read more bases. Using the general conditions without $Mn^{2+}$ will generate sequencing ladders which are faint or absent for nucleotides close to the primer when limited amounts of DNA (eg. 1µg M13) are used for the reaction. The addition of $Mn^{2+}$ ions to the normal ($Mg^{2+}$) sequencing reaction (with fixed deoxy to dideoxy ratios) reduces the average length of DNA synthesized in the termination step, intensifying bands corresponding to sequences close to the primer. With the addition of $Mn^{2+}$, approximately 80 nucleotides can easily be read in a single run on a QuickPoint Rapid DNA Sequencing Gel even with reduced amounts of template. For a more detailed explanation on the use of $Mn^{2+}$, please consult the Sequenase™ Version 2.0 Instruction Manual and the following reference:

Tabor, S. and Richardson, C.C., *Proc. Nat. Acad. Sci. USA*, 86:4076-4080, 1989.

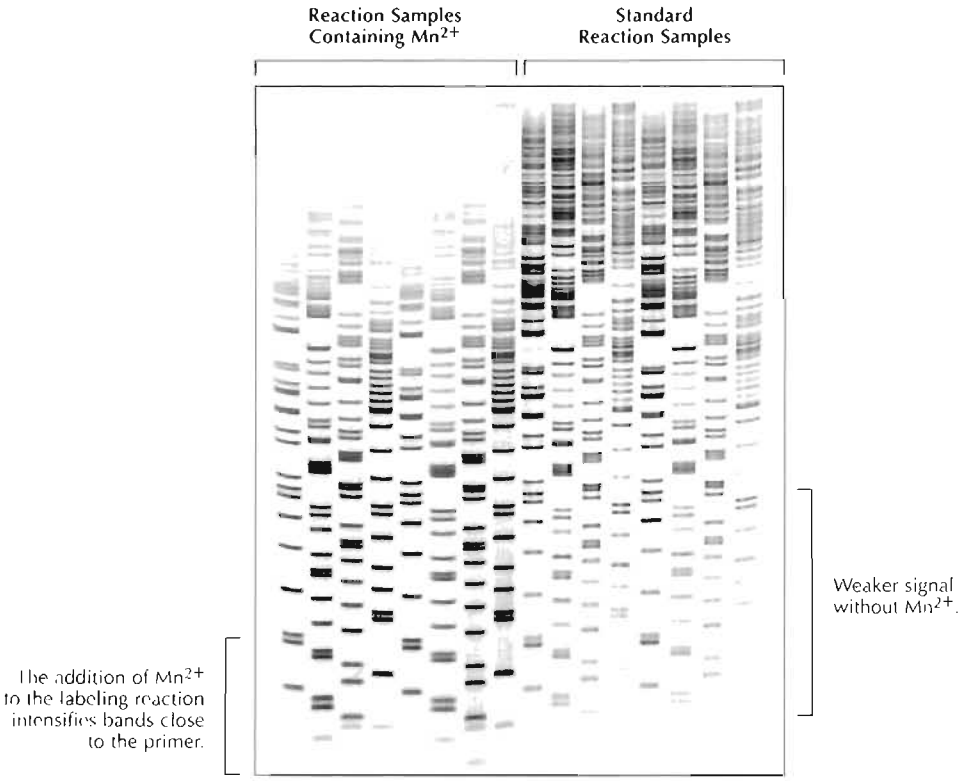



**Figure B**

# III. Sample Preparation and Sequencing Reaction (cont'd)

### What happens if I use sub-standard quality DNA in the sequencing reaction?

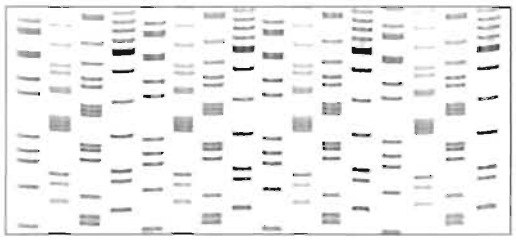
Template DNA of good purity is essential for excellent band resolution and optimal read lengths. Several methods have been published for preparing both single-stranded and/or double-stranded DNA from phage or plasmid DNA for the purpose of sequencing. These procedures involve denaturization and purification of the template by one of several methods including either boiling mini-prep method, PEG precipitation, alkaline lysis, and/or phenol-chloroform extraction. Please refer to the following references for protocols:


1. Amersham Life Science, *USB Sequenase™ Version 2.0 DNA Sequencing Kit Manual, 9th Edition*, 1994.
2. Epicentre Technologies Corporation, *DNA Sequencing Methods and Protocols, 2nd Edition*, 1997.
3. Ausbel, Frederick M., ed., et. al., *Current Protocols in Molecular Biology, Volume 2*, 1994.
4. Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual, 2nd Edition*, 1989.


### Why do I have to use the QuickPoint Sample Loading Buffer?

The QuickPoint Sample Loading Buffer is uniquely formulated and designed to be used with the neutral pH buffering system present in the QuickPoint Rapid-DNA Sequencing System. Additionally, its high density allows the samples to be pipetted easily and conveniently into the QuickPoint gels without the need for special flat gel loading tips. If sample buffers other than the QuickPoint Sample Loading Buffer are used, band resolution may be sacrificed due to difficulty in pipetting the samples.

Samples loaded with QP Sample Loading Buffer



Samples loaded with Sequenase™ Stop Buffer

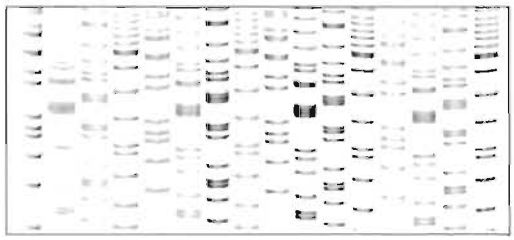


**Figure C**

Note that the sequence is clear and consistent when loaded from left to right with QP Sample Buffer. Samples get progressively more diffuse when loaded from left to right with Sequenase™ Stop Buffer.

# III. Sample Preparation and Sequencing Reaction (cont'd)

**Can I use manual sequencing systems other than Sequenase™ Version 2.0 (example: Cycle Sequencing, High Temperature Isothermal Sequencing, or end-labeled primers)?**

Yes. The following table (Table 1) summarizes the different sequencing methods and corresponding QuickPoint sequence results and recommendations.

**TABLE 1**

| **Sequencing Kit** | **Sequenase™ Version 2.0** (Amersham #US70770) | **ThermoSequenase™** (Amersham #US79750) | **SequiTherm EXCEL™ II DNA Sequencing Kit** (Epicentre Technologies #SEM7902) | **Silver Sequence DNA Sequencing System** (Promega #Q4130) |
|---|---|---|---|---|
| **Polymerase** | Modified T7 DNA Polymerase | ThermoSequenase™ DNA Polymerase | SequiTherm EXCEL™ II DNA Polymerase | Sequencing Grade Taq DNA Polymerase |
| **Labeling Method** | a. 5' end labeling with [$\gamma$-$^{33}$P]-ATP<br>b. internal labeling with [$\alpha$-$^{35}$S]-dATP<br>c. internal labeling with [$\alpha$-$^{32}$P]-dATP | 3' end labeling with [$\alpha$-$^{33}$P]-ddNTP | a. 5' end labeling with [$\gamma$-$^{33}$P]-ATP<br>b. internal labeling with [$\alpha$-$^{35}$S]-dATP | No radioactive label needed. Detect by silver staining. |
| **Sequencing Method** | Manual Method at 37°C | Cycle Sequencing | Isothermal High-Temperature Cycle Sequencing | Cycle Sequencing |
| **Template** | ssDNA (M13)<br>dsDNA (puc18) | ssDNA (M13 + fd)<br>dsDNA (puc18) | ssDNA (M13)<br>dsDNA (pSAD2 control) | ssDNA (M13)<br>dsDNA (pGEM) |
| **Modified Nucleotide** | dITP<br>7-deaza-dGTP | None | 7-deaza-dGTP | None |
| **Results with QuickPoint System** | Excellent | Excellent | a. Ok—can easily read sequence close to primer but band resolution not as good as with Sequenase™.<br>b. Weak signal for DNA fragments less than 60 bases. Need protocol modifications in order to read close to the primer. | Poor—very weak or no signal detected! |
| **Recommended For Use with QuickPoint?** | Yes | Yes | If necessary | No—the concentration of DNA in a band on QuickPoint Gels is too low to be detected by silver staining methods. |
| **Suggestions For Use with QuickPoint** | Use $Mn^{2+}$ buffer. Use QP Sample Loading Buffer as recommended in the QuickPoint instruction manual. | Follow ThermoSequenase™ instruction manual. Use QP Sample Loading Buffer as recommended in the QuickPoint instruction manual. | Use end-labeling method or internal labeling method with modified protocol for sequencing DNA close to the primer. Use QP Sample Loading Buffer as recommended in the QuickPoint instruction manual. | Silver staining methods not recommended. |

# IV. Loading and Running QuickPoint Gels

### What happens if I accidentally forget to pre-run the gel for 5 minutes?

Bands may appear slightly diffuse if the gel is older than 4 months. Otherwise, there is no visible difference with fresh gels.

### What happens if I do not heat the 5X QP Running Buffer to 45°C–55°C?

Complete denaturization of the DNA during electrophoresis is essential for sharp resolution. Maintenance of denaturization is optimal at 45°C to 55°C. Band sharpness and resolution is sacrificed at temperatures below 45°C. The buffer should be heated approximately 5°C higher than desired to accommodate the temperature loss during buffer transfer to the Sequencing Cell.

### What happens if the 1X QP Running Buffer accidentally boils while heating?

Band diffusion and distortion will result. Do not heat 1X QP Running Buffer higher than 65°C. No adverse effects are noticed if the buffer is boiled and then allowed to cool down to 50°C. If the buffer is not allowed to cool down to 50°C, and is used at temperatures greater than 65°C, poor band resolution and band diffusion is prominent. Do not use 1X QP Running Buffer at temperatures higher than 65°C.

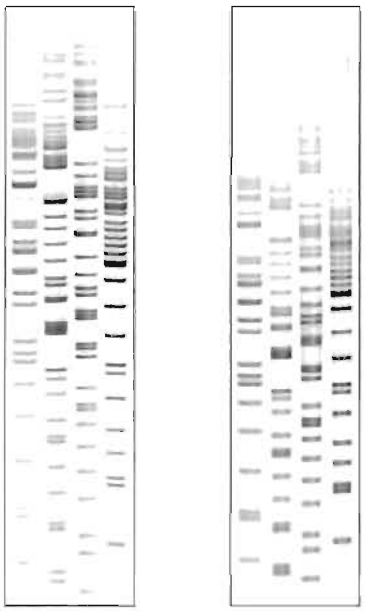


**Figure D**

Left: 1X QP Running Buffer was allowed to cool to 50°C before electrophoresis.

Right: Electrophoresis proceeded with the 1X QP Running Buffer at ~75°C, which resulted in diffusion of the bands.

# IV. Loading and Running QuickPoint Gels (cont'd)

**What happens if I accidentally dilute the 50X QP Running Buffer incorrectly or put the wrong dilution of the buffer in either the upper or the lower buffer chambers?**

Bands will appear diffuse and/or distorted. For maximum gel performance, the minimum concentration of the QP Running Buffer required in the upper buffer chamber is 5X QP Running Buffer. The minimum concentration of QP Running Buffer required in the lower buffer chamber is 1X QP Running Buffer. Concentrations up to 5X QP Running Buffer in either chamber will yield equivalent and acceptable sequence information but will deplete the 50X QP Running buffer stock faster than necessary.

**I can't see the back gel when loading my samples. What can I do?**

Use an overhead lighting source. Position yourself so that the light refraction is optimal. Alternatively, mark the outline of the wells on the glass with an indelible black pen before assembling gels into the Sequencing Cell and loading.

**If I want to read at a particular base from the primer, how far, and corresponding to which dye front should I run the QuickPoint gel?**

Please refer to the following table (Table 2). Results were obtained using the QuickPoint recommended protocols for reaction preparation of purified M13 ssDNA, gel electrophoresis, and autoradiography.

TABLE 2

| Run Time (approximate) | Readable Starting Base Position—Primer Included (approximate) | Tracking Dye | Run Time Required for Dye to Reach Bottom of Gel (approximate) | Corresponding Base Position of Dye Front (approximate) |
|---|---|---|---|---|
| 20 minutes | 116 | | | |
| 18 minutes | 103 | Xylene Cyanol | 19 minutes | 110 |
| 16 minutes | 75 | | | |
| 14 minutes | 69 | | | |
| 12 minutes | 47 | Pink Dye | 13 minutes | 60 |
| 10 minutes | 32 | Bromophenol Blue | 10 minutes | 26 |

NOTE: This information reflects approximate run times and base positions obtained using M13 ssDNA and the -40 primer. Actual run times and base positions may vary slightly depending upon the template sequence, specific labeling reaction used, initial buffer temperature, and run parameters. Typically, 80 ± 20 bases total can be read in a single run on a QuickPoint gel. Note that the Bromophenol Blue dye front migrates slightly ahead of the first readable base.

# IV. Loading and Running QuickPoint Gels (cont'd)

**My Sequencing Cell wedges cannot be secured, or pop up during the run causing the chambers to leak. What can I do?**

Make sure gels are positioned correctly, wedges are in place, and that the buffer core electrodes are seated properly. When assembling the QuickPoint Sequencing Cell and gels, firm pressure should be applied to rear wedge after adding the heated 1X QP Running Buffer. Make sure the Sequencing Cell is thoroughly washed of all residual buffers between uses. If not washed thoroughly, the urea from the gel diffuses and tends to build up on the Sequencing Cell causing the plastic to get slick. Alternatively, we have found that fine-grit sandpaper applied to the contact areas of both the front and rear wedges allows the wedges to make a tight seal, thus eliminating popping wedges and leaking chambers.

**How fast can I run a QuickPoint gel?**

Including the 5 minute pre-run, QuickPoint gels are generally run at temperatures between 45°C and 55°C at 1200 volts constant for 15 minutes or until the Bromophenol Blue dye front reaches the bottom of the gel. By increasing the buffer temperature or the voltage, faster run times may be achieved but band sharpness and/or resolution may be sacrificed. Do not exceed 1500 volts. Power limit should be set to 70 watts when using voltages that exceed 1200 volts or when using 1X Running Buffer at temperatures exceeding 60°C.

**CAUTION:** Band distortion, damage to gel, and/or damage to Sequencing Cell may result with power greater than 70 watts.

**Can I run other NOVEX pre-cast gels in my QuickPoint Sequencing Cell?**

No. There are subtle differences between the QuickPoint Sequencing Cell and the XCell II Mini-Cell (which accomodates all other NOVEX pre-cast gels).

1. The QuickPoint Buffer Core is designed specifically to accomodate the longer QuickPoint gels (12.5cm x 10cm).
2. The QuickPoint Lower Buffer Chamber includes an additional gold-plated standoff on the electrode in order to accommodate the longer buffer core.
3. The gold-plated bracket underneath the electrode on the QuickPoint Lower Buffer Chamber has been modified to prevent "arcing" at 1200 volts.
4. The blue QuickPoint lid includes higher-rated electrode leads in order to run the QuickPoint gels at the required 1200 volts.

**Can I run my QuickPoint gels in either the XCell II™ or the ThermoFlow™ Mini-Cells?**

No. The QuickPoint cassettes measure 12.5cm x 10cm, whereas the regular NOVEX pre-cast gels measure only 10cm x 10cm. Consequently, QuickPoint gels are not compatible with the shorter buffer cores of the XCell II and ThermoFlow units.

**CAUTION:** Improper use of either the QuickPoint Sequencing Cell, XCell II Mini-Cell, or ThermoFlow Mini-Cell may result in safety hazards.

# V. Preparing Gels for Autoradiography

**What happens if I don't fix, wash, or dry gel long enough with fresh solutions?**

Bands may appear faint and diffuse. Film may stick to the gel during exposure and cause the autorad to develop with dark blotches.

**What happens if I leave my gel in the Fix or Wash longer than 20 minutes?**

Weak bands will result on the autorad.

**What happens if I dry my gel longer than recommended in the oven or microwave?**

Drying the gel longer than 1 hour in the 80°C oven causes the band signal to weaken. Increased drying times in the microwave may cause the glass plate to crack. Drying time in the microwave should be determined by 30-second interval testing on half-power prior to selecting a permanent microwave drying time.

# VI. Detection and Sequence Results

### Can I read further from the primer than 60 bases?

Yes. Please refer to the following table (Table 2). Results were obtained using the QuickPoint recommended protocols for sample preparation of purified M13 ssDNA, gel electrophoresis, and autoradiography.

**TABLE 2**

| **Run Time** (approximate) | **Readable Starting Base Position—Primer Included** (approximate) | **Tracking Dye** | **Run Time Required for Dye to Reach Bottom of Gel** (approximate) | **Corresponding Base Position of Dye Front** (approximate) |
|---|---|---|---|---|
| 20 minutes | 116 | Xylene Cyanol | 19 minutes | 110 |
| 18 minutes | 103 | | | |
| 16 minutes | 75 | | | |
| 14 minutes | 69 | Pink Dye | 13 minutes | 60 |
| 12 minutes | 47 | | | |
| 10 minutes | 32 | Bromophenol Blue | 10 minutes | 26 |

**NOTE:** This information reflects approximate run times and base positions obtained using M13 ssDNA and the -40 primer. Actual run times and base positions may vary slightly depending upon the template sequence, specific labeling reaction used, initial buffer temperature, and run parameters. Typically, 80 ± 20 bases total can be read in a single run on a QuickPoint gel. Note that the Bromophenol Blue dye front migrates slightly ahead of the first readable base.

# VI. Detection and Sequence Results (cont'd)

### Can I read more bases if I run multiple loads?

Yes. Various consecutive triple loads of M13 ssDNA were run on a single QuickPoint gel in order to determine how much total readable sequence could be obtained from a single gel. The best results were obtained by using the recommended $Mn^{2+}$ buffer in the labeling reaction and running each consecutive load until the Bromophenol Dye migration was 2cm from the bottom of the glass cassette. Run time for each consecutive load was 7.5 minutes at approximately 50°C. With these parameters, 171 total bases were readable. Additional data with various parameters was obtained resulting in less sequence information as outlined in the following table (Table 3).

**TABLE 3**

| Sample | Total Readable Bases * | First Load | Second Load | Third Load | Bromophenol Blue Location | Run Time at 50°C @ 1200V (approx.) |
|---|---|---|---|---|---|---|
| $Mn^{2+}$ | 171 | 114–171 | 47–129 | 1–86 | 2cm from bottom | 7.5 minutes |
| $Mg^{2+}$ | 167 | 107–167 | 42–111 | 1–79 | 2cm from bottom | 7.5 minutes |
| $Mn^{2+}$ | 152 | 74–152 | 21–111 | 1–69 | 4cm from bottom | 5.5 minutes |
| $Mg^{2+}$ | 152 | 74–152 | 19–100 | 1–44 | 4cm from bottom | 5.5 minutes |

* Readable start base position for the M13 sequence (primer included) is at position 32 in the sequence. Greater or lesser read lengths may be obtained depending upon specific primers and/or sequences used.

### What is the maximum performance NOVEX has been able to achieve on a QuickPoint gel in a single run?

While the maximum sequence information obtainable is highly dependent on the specific sequence, quality of DNA, reaction conditions, and run parameters, NOVEX has successfully achieved up to 110 bases in a single run with puc18 dsDNA using the QuickPoint recommended protocols.

### My samples are "narrowing" to a V-shape at the bottom of the gel. What can I do to eliminate this?

Make sure all blank wells are filled with 0.5µl of QP Sample Loading Buffer. This will eliminate the conductance difference throughout the gel during electrophoresis and ultimately eliminate the narrowing.

### Can I use a phosphor-imaging device to detect my sequence on a QuickPoint gel?

Yes. Although we have limited experience in this area, reports from the field indicate that results from QuickPoint gels can be obtained in significantly less time when detected with phosphor-imaging devices. Follow the manufacturer's recommended protocol for exposure times.

# VII. Isotope Considerations

Sequencing reactions are typically labeled by either attaching a radioactive phosphate to the 5' end of the sequencing primer (end labeling) or by incorporating ∝-labeled nucleotides directly into the growing DNA chain (internal labeling). There are various factors that should be considered when choosing a labeling method including the following:

1. Required sequence information vs. position of first labeled nucleotide
2. Eliminating sequencing artifacts associated with sub-standard or minimal amounts of template DNA
3. Increasing band intensity, sharpness, and resolution
4. Eliminating exposure variations throughout the DNA ladder
5. Reducing the exposure time required to read the autorad
6. Expense
7. Stability of the reaction products
8. Exposure risks

With these factors in mind, NOVEX prefers and routinely uses $^{35}S$ with the QuickPoint Rapid DNA Sequencing System. The sharpest and most clearly resolved autorads have been obtained using $^{35}S$ with an overnight exposure.

For a more detailed explanation of the advantages and disadvantages of the various labeling methods, please consult the following references:

1. Amersham Life Science, *USB Sequenase™ Version 2.0 DNA Sequencing Kit Manual, 9th Edition,* 1994.
2. Epicentre Technologies Corporation, *DNA Sequencing Methods and Protocols, 2nd Edition,* 1997.
3. Ausbel, Frederick M., ed., et. al., *Current Protocols in Molecular Biology, Volume 2,* 1994.
4. Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual, 2nd Edition,* 1989.

# VII. Isotope Considerations (cont'd)

Please refer to the following table (Table 4) when choosing the isotope and labeling method to be used in conjunction with the QuickPoint System.

TABLE 4

| | INTERNAL LABELING | | END LABELING | | |
|---|---|---|---|---|---|
| | [∝-$^{35}$S]-dATP | [∝-$^{32}$P]-dATP | 3' End Labeling with [ß-$^{32}$P]-dATP | 5' End Labeling with [ß-$^{32}$P]-ATP | 3' End Labeling with [∝-$^{32}$P]-ddNTP |
| **Amount of Template Required** | Average | Average | Minimal | Average | Average |
| **Overall Band Resolution** | Best | Good | Adequate | Good | Good |
| **Exposure Time Required** | Overnight | Fast (<8 hours) | Fast (<4 hours) | Fast (<8 hours) | Overnight |
| **Expense** | Medium | Medium | Low | Medium | High |
| **Readability in Upper Portion of Gel** | Excellent | Good | Poor | Good | Excellent |
| **Stability of Reaction Products** | Several weeks | 2–3 days | 2–3 days | 1 week | 1 week |
| **ß-Emission and Exposure Risks** | Low | Moderate to High | High | Moderate | Moderate |

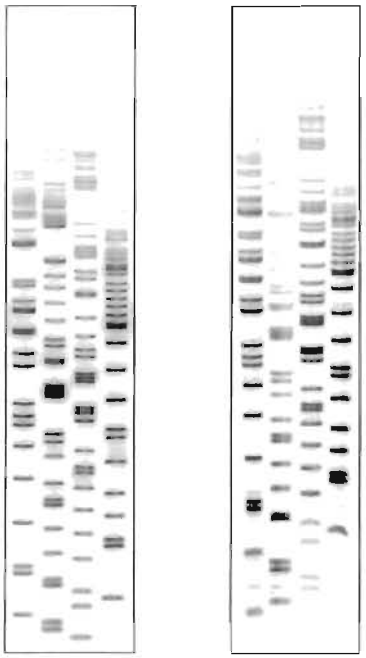


**Figure E**

Left: [∝-$^{35}$S]-dATP internal labeled M13 ssDNA.
Right: [∝-$^{32}$P]-dATP internal labeled M13 ssDNA.

# VIII. Troubleshooting Guide

Common sequencing problems—possible causes and solutions:

**Film blank or nearly blank:**

- Incorrect exposure. Make sure correct sides of both the gel and the film are exposed. If using single-sided film, the emulsion side must be placed facing the dried gel. Check the age and quality of developing reagents.
- DNA preparation may be sub-standard. Purify problem templates by ethanol precipitation or phenol extraction: ethanol precipitation. Purify PCR products to remove residual primer and dNTPs. Try control DNA known to sequence well.
- Specific activity of labeled nucleotide has decreased. Use isotope within the specified time limits.
- Some component missing in the sequencing reaction.
- Enzyme has lost activity. Polymerases will loose activity if left at temperatures greater than -20°C for extended periods.
- Priming reaction may be sub-standard. Try control DNA.
- Insufficient template or primer. Quantify DNA template by recommended methods and confirm the primer concentrations. Consult sequencing protocol for recommended ratio in reaction.
- If using end labeled primers, primers may be poorly labeled. Check labeling efficiency by TCA precipitation. Try additional primer purification prior to labeling.
- Check template for RNA contamination.
- Poor annealing of primer to template. Verify sequence and orientation of primer. Super-coiling of the template can make the primer binding site inaccessible to the primer. Nick or linearize the DNA template prior to sequencing.

**Bands appear smeared, fuzzy, or diffuse on autorad:**

- DNA prep may be sub-standard. Try control DNA
- Gel run too cold or too hot. For optimal resolution, QuickPoint Rapid DNA Sequencing gels should be run between 45°C and 55°C. Optimal voltage is 1200 volts. Voltage should not exceed 1200 volts since this will increase the gel temperature. Additionally, voltages lower than 1100 volts may lead to decreased resolution and poor band quality.
- Samples were not denatured to completion. Samples should be heated to 72°C for at least 2 minutes and no longer than 4 minutes prior to loading on gel. Avoid excessive heating which can degrade the sample.
- Gel/glass plate may have shifted during exposure of film. NOVEX recommends a metal cassette holder to insure proper tight fit of gels/glass plates during exposure.

# VIII. Troubleshooting Guide (cont'd)

- QP Sample Loading Buffer was not used to terminate sequencing reaction. The QuickPoint Sequencing System utilizes a unique neutral pH buffering system which requires that the final sequencing reactions be diluted in the QP Sample Loading Buffer. If sample buffers other than the QuickPoint Sample Loading buffer are used, band resolution may be sacrificed due to difficulty in pipetting the samples consistently.
- Samples were not loaded quickly enough. Samples should be loaded within 10 minutes onto pre-run QuickPoint gel.
- QuickPoint gel was older than 4 months and was not pre-run for 5 minutes at 1200 volts constant.
- Correct pipette tips and pipettor were not used. NOVEX recommends using the RT-10 10µl pipette tips from RAININ Instruments Co. with a RAININ™ P-2 pipettor for the best, most accurate and evenly distributed dispensing of samples into the wells of the QuickPoint Rapid DNA Sequencing Gel.
- Wells were not rinsed out thoroughly. Rinse wells immediately before loading to remove urea and unpolymerized acrylamide.
- Excess salt in sample. Ethanol precipitate and wash template with 70% ethanol.
- Incorrect buffer composition. Make sure upper and lower buffers are diluted correctly from the stock 50X QP Running Buffer.
- Gels were not fixed to completion. Fixed gels produce sharper bands. NOVEX recommends fixing QuickPoint gels in fresh fixing solution containing 10% ethanol and 10% acetic acid in water, for 10 minutes.

**Bands appear dark and not fully resolved:**

- Too much radioactivity was used or too much volume was loaded onto the gel. NOVEX recommends using 0.5µl of $^{35}S$ in the sequencing reaction and loading 0.3µl to 0.5µl of the final diluted sample onto QuickPoint gels. When the isotope is fresh, limit the volume loaded to 0.3µl. Volume can be increased as the isotope decays and/or the sample ages.
- Film was over-exposed. Reduce exposure time.

**Sequence faint near the primer:**

- Insufficient DNA in sequencing reaction. Check your sequencing reaction protocol for the recommended minimum quantity of DNA needed to perform the sequencing reaction.
- $Mn^{2+}$ buffer was not used in the Sequenase™ sequencing reaction. See discussion on page 4 on the use of $Mn^{2+}$ in the reaction mix for the QuickPoint System.
- Insufficient primer. Check your sequencing reaction protocol for minimum primer concentrations necessary.

# VIII. Troubleshooting Guide (cont'd)

**Miscellaneous sequencing anomalies:**

For further information on other various sequencing anomalies, please refer to your particular sequencing reaction protocol and troubleshooting guide. Troubleshooting information specifically related to the sequencing reactions and DNA structure is beyond the scope of the QuickPoint Technical Guide. The following references are suggested for additional information on DNA sequencing:

1. Amersham Life Science, *USB Sequenase™ Version 2.0 DNA Sequencing Kit Manual, 9th Edition,* 1994.
2. Epicentre Technologies Corporation, *DNA Sequencing Methods and Protocols, 2nd Edition,* 1997.
3. Ausbel, Frederick M., ed., et. al., *Current Protocols in Molecular Biology, Volume 2,* 1994.
4. Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual, 2nd Edition,* 1989.

# IX. Ordering Information

| *Catalog No.* | *Description* |
|---|---|
| EI9700 | QuickPoint Rapid DNA Sequencing Cell |
| QP9731 | QuickPoint Rapid DNA Sequencing Gels, 16 well (box of 4) |

**Replacement Parts**

| | |
|---|---|
| EI9703 | Lower Buffer Chamber for EI9700 QuickPoint Sequencing Cell |
| EI9012 | Rear Wedge (w/screw hole) for EI9700 |
| EI9013 | Front Wedge for EI9700 |
| EI9704 | Lid with Cables for EI9700 |
| EI9701 | Buffer Dam for EI9700 |
| EI9702 | Buffer Core for EI9700 |
| EI9742 | Curved-Tip Syringe |
| EI8016 | Silicone Sponge Gaskets |
| EI9021 | Repair Kit for Sequencing Cell Buffer Core:<br>platinum wire, 0.01" x 12", wire insulation, post, nut, washer |
| EI9022 | Buffer Core replacement wire, platinum, 0.010" x 12" |

**Pre-Mixed Liquid Buffers**

| | |
|---|---|
| QP9732 | 50X QP Running Buffer |
| QP9733 | QP Sample Loading Buffer |

# X. Additional NOVEX Literature

| *Lit. No.* | *Description* |
|---|---|
| EP001 | NOVEX Product Catalog |
| EP031 | Current *NOVEX News* Newsletter<br>Past Newsletter (quote volume and issue) |
| EP060 | QuickPoint Rapid DNA Sequencing System Brochure |
| IM4500 | QuickPoint Rapid DNA Sequencing System<br>Gel Instruction Manual |
| IM4505 | QuickPoint Rapid DNA Sequencing Cell<br>Instruction Manual |
| IM4515 | QuickPoint Quick Reference Card |


NOVEX • 11040 Roselle Street • San Diego, CA 92121
Tel: (619) 452-6634 / (800) 456-6839 • Fax: (619) 452-6635
E-mail: nvxinfo@novex.com • Website: http://www.novex.com

**Technical Service: 1-800-55-NOVEX** (1-800-556-6839)



IM4510 REV.A 2/98