



Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

Phrank answers questions about SequiTherm EXCEL™ II DNA Sequencing Kits

Q: What types of tough templates can the SequiTherm EXCEL II DNA Sequencing Kit help me sequence?

A: The SequiTherm EXCEL II Sequencing reactions read through a variety of “tough” sequences, including very GC- or AT-rich regions, nucleotide repeats, hairpins and templates with extensive secondary structure.

Q: I see the SequiTherm EXCEL II DNA Sequencing Kit can be used for both cycle and isothermal sequencing... which method should I choose for my sequencing project?

A: For most DNA templates, either method works fine. Cycle sequencing reactions require about half as much template (2.0 to 2.5 µg compared to 4 to 5 µg for isothermal sequencing). If ambiguous or incomplete sequence data are obtained by cycle sequencing, then performing an isothermal sequencing reaction with end-labeled primers may generate additional or confirmatory information. Isothermal reactions generate less ambiguous data because they involve only a single synthetic step. As a consequence, isothermal reactions are also faster to complete.

Q: What labeling chemistries can be used with the SequiTherm EXCEL II Sequencing Kits?

A: You can use a number of very common dyes that are compatible with Applied Biosystems, Amersham, Beckman-Coulter, GeneSys and LI-COR®/NEN® sequencers, as long as they are attached to the primer and are

not included in the termination mixes. BigDye® terminator chemistry cannot be used with the SequiTherm EXCEL II Kits. Radiolabeled, chemiluminescent-labeled and biotin-labeled primers, as well as many other chemistries, can also be used. Users interested in sequencing with biotinylated primers should use the sequencing protocols for ³²P end-labeled primers. Contact us if you are not sure whether your specific dye can be used.

Q: Can I use SequiTherm EXCEL II sequencing reactions on an ABI PRISM® capillary or an Amersham MegaBACE™ capillary sequencing instrument? How about on an Amersham ALF™ or ABI PRISM® 373 or 377 gel-based sequencers?

A: Yes, you can. For capillary electrophoresis-based sequencers, it will be necessary to mix all four of the sequencing reaction mixes together after cycling and then load into the sample chamber of the sequencer.

With standard gel-based sequencers, you usually just add the individual sequencing reactions into the wells of the gel the same way you would for a standard manual sequencing gel. With some standard automated sequencers, the four reactions can be combined into a single well, as is done for the capillary-based sequencers.

Q: The cycling profile for my template/primer pair worked in the past with another kit, but not with the SequiTherm EXCEL II DNA Sequencing Kit... why?

A: The main reason is due to differences in the SequiTherm EXCEL II Sequencing Kit's proprietary components, compared to the reagents of other manufacturers. Simply lowering the annealing temperature of the cycle sequencing reaction should be all that is necessary.

Q: Can the SequiTherm EXCEL II DNA Sequencing Kit be used in conjunction with your EZ::TN™ and/or HyperMu™ transposon systems?

A: Yes. You just need either a radioactive primer for manual sequencing or a dye-labeled sequencing primer for automated sequencers. Radioactive primers for manual sequencing can be made by using the available transposon forward and reverse primers, and the T4 Polynucleotide Kinase available in the SequiTherm kits. Dye-labeled primers with suitable dyes for ABI PRISM®, Amersham ALF™, LI-COR®/NEN® or Beckman-Coulter CEQ™, and other automated sequencers are available from the sequencing instrument manufacturer or from a variety of custom oligonucleotide manufacturers. Consult the instrument manufacturer for details.

SEE HOW HORIZON MOLECULAR MEDICINE USES SEQUI THERM EXCEL™ II DNA SEQUENCING KITS FOR MOLECULAR DIAGNOSTICS ON PAGE 5.

info