UniSeq FAQ

1. What kind of templates can be used with UniSeq?

A: UniSeq has been successfully performed on PCR-products up to 5.5 kb and plasmids up to 6.5 kb. In principle, there is no limit to the template size providing the primer binding site occurs only once in a template. Given that the UniSeq extended S-primer is an 11-mer, each priming site should only be found once every 4^{11} (~4,000,000) base pairs.

2. Is there a difference in the sequence quality or read length obtained using UniSeq as compared with a standard reaction?

A: No, once the S-primer is generated UniSeq is essentially identical to a standard sequencing reaction.

3. What is the success rate of UniSeq?

A: Using high purity templates and multiple UniSeq primer sets, Nucleics has observed a success rate of greater than 85%. Application of UniSeq under production condition (microbial genome sequencing) with templates of highly variable quality yielded a success rate of approximately 70%.

4. How many UniSeq reactions has Nucleics performed?

A: We have performed thousands of successful reactions.

5. What sequencing chemistries have been tested?

A: We have optimised UniSeq for use with Applied Biosystems BigDye™ vers. 1 & 3 and Amersham Biosciences DYEnamic™ Terminator chemistries. Other chemistries can be used but may require optimisation. Please contact Nucleics if you would like to use other sequencing chemistries.

6. How much can UniSeq save me on the cost of a genome sequencing project?

A: This is highly dependent on the level of integration of UniSeq into your project. Simulation studies by Nucleics suggest that saving in excess of 80% can be obtained with full integration.

7. Can UniSeq be applied to projects other than genome sequencing?

A: Yes. UniSeq can be a cost effectively technology for other sequencing projects. Examples include EST sequencing, transposon-mutant sequencing, individual BAC sequencing, etc.

8. How many EO and how many TO primers are in the library?

A: There are 256 EO and 512 TO primers in the UniSeq library.

9. Can I use other sequencing diluents or additives with UniSeq?

A: We do not recommend using other diluents or additives other than those provide with the UniSeq kit.

10. What is the reaction modifier and reaction enhancer?

A: They are trade secrets.

11. What is the shelf life of the system?

A: All components will last for at least 12 months if stored at –20°C.

12. Can I use other volumes for the library than specified in the manual?

A: Nucleics can provide more or less concentrated primer libraries on request.

13. What operating system does the UniSelect software work on?

A: The UniSelect software has been written in ANSI C and has been ported to Redhat Linux, MacOS X and Windows 2000. Binaries for other platforms can be provided on request.

14. What is the input file format(s) does UniSelect accepts?

A: The PHRED PHD format is the preferred format, however, UniSelect can also process files in the FASTA format.

15. What format is the UniSelect output file in?

A: A Tab delimited text file.

16. How long does UniSelect take to select a primer pair?

A: Less than a second (benchmarked on Intel Pentium III 800Mhz running Redhat 7.3).

17. What user-defined parameters can I change in the UniSelect software?

A: The UniSelect software allows the user to control a wide range of parameters including: the input file(s) and directory(s), the output file(s) and directory(s), user defined RegEx file parsing, directory recursion, primer energy thresholds, template secondary structure thresholds, template quality thresholds, and the extent of read overlap. For further details please see the UniSelect users manual.

18. Can I feed the UniSelect output into my robot software?

A: Yes. The Tab delimited text file can be parsed to provide "cherry picking" instructions for most robotic systems. Nucleics has developed scripts for controlling the Biomek 2000 robotic system. Nucleics will work with your scientists to integrate UniSelect into other robotic systems on request.