

How to Use Primers Ordered with the Primer Designer™ Tool

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Note: Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

The Primer Designer™ Tool allows you to search our database of more than 300,000 pre-designed primer pairs, which are for use with a Sanger sequencing workflow (from PCR-to-cycle sequencing). This Quick Reference provides guidance on preparing your primer stocks for PCR/Sanger cycle sequencing.

- 1** **Locate the assay ID and catalog number of your primer** Find the assay ID and catalog number on the tube label, Certificate of Analysis (provided in the same package containing the primers), or shipping slip.
 - Each assay ID number (for example, Hs00263589_CE) represents one amplicon.
 - Each catalog number represents each specific primer details:

Cat. no.	Full name	Short description	Primer 5'-tail option	Purification method	Synthesis scale	Minimal spec yield
A15629	Human, Forward PCR/SEQ primer, desalted	Human, FWD primer, Ds	Non-tailed	Desalted	25 nmole	8 nmole
A15630	Human, Reverse PCR/SEQ primer, desalted	Human, REV primer, Ds	Non-tailed	Desalted	25 nmole	8 nmole
A15631	Human, Forward PCR/SEQ primer, HPLC	Human, FWD primer, HPLC	Non-tailed	HPLC	50 nmole	900 pmole
A15632	Human, Reverse PCR/SEQ primer, HPLC	Human, REV primer, HPLC	Non-tailed	HPLC	50 nmole	900 pmole
A15633	Human, Forward PCR primer, M13-tailed, desalted	M13 Human, FWD primer, Ds	M13-tailed	Desalted	25 nmole	8 nmole
A15634	Human, Reverse PCR primer, M13-tailed, desalted	M13 Human, REV primer, Ds	M13-tailed	Desalted	25 nmole	8 nmole
A15635	Human, Forward PCR primer, M13-tailed, HPLC	M13 Human, FWD primer, HPLC	M13-tailed	HPLC	50 nmole	900 pmole
A15636	Human, Reverse PCR primer, M13-tailed, HPLC	M13 Human, REV primer, HPLC	M13-tailed	HPLC	50 nmole	900 pmole
A15637	M13 (-21) Forward sequencing primer	M13 (-21) seq FWD primer, HPLC	N/A	HPLC	50 nmole	900 pmole
A15638	M13 Reverse sequencing primer	M13 seq REV primer, HPLC	N/A	HPLC	50 nmole	900 pmole

- Each forward and reverse primer will be provided in its own tube (meaning, a primer pair will be provided in 2 tubes).

- 2** **Evaluate the yield of your primer** The yield can be found on the tube label and the Certificate of Analysis.

Note:

 - All primers will be received dried-down after desalting or purification.
 - The synthesis scale is not equal the actual yield for the primer you receive.

- 3** **Make the primer stocks and working solutions based on your needs**
 - a. Make 100- μ M stock solutions.
 - We recommend resuspending dried-down primers in sterile, 1X TE buffer with low amounts of EDTA (Cat. no. 12090-015).
 - Alternatively, dried-down primers may be resuspended in UltraPure™ DNase/RNase-free Distilled Water (Cat. no. 10977-023).

Note: Be sure the water you use is at neutral pH to avoid depurination.
 - b. Make working solutions from the 100- μ M stock solutions based on your PCR or sequencing needs.

Storage conditions:

- Dried-down primers are stable at -25 to -15°C for at least 1 year.
- 100- μ M stock solutions of primers are stable for 1 year at -25 to -15°C .
- Working solutions of primers are stable for 6 months at -25 to -15°C or 2 to 8°C in the absence of nucleases.

Note: Do not store primers in water at 2 to 8°C .

4 Proceed with your PCR protocol and Sanger cycle sequencing protocol Only the non-tailed PCR primers (Cat. nos. A15629–A15632) can be used for both PCR and cycle sequencing reactions. See the following table for more details:

Cat. no.	Full name	Short description	Primer 5'-tail option	Can be used as PCR primers†	Can be used for BigDye® cycle sequencing primers†
A15629	Human, Forward PCR/SEQ primer, desalted	Human, FWD primer, Ds	Non-tailed	✓	✓
A15630	Human, Reverse PCR/SEQ primer, desalted	Human, REV primer, Ds	Non-tailed	✓	✓
A15631	Human, Forward PCR/SEQ primer, HPLC	Human, FWD primer, HPLC	Non-tailed	✓	✓
A15632	Human, Reverse PCR/SEQ primer, HPLC	Human, REV primer, HPLC	Non-tailed	✓	✓
A15633	Human, Forward PCR primer, M13-tailed, desalted	M13 Human, FWD primer, Ds	M13-tailed	✓	x‡
A15634	Human, Reverse PCR primer, M13-tailed, desalted	M13 Human, REV primer, Ds	M13-tailed	✓	x‡
A15635	Human, Forward PCR primer, M13-tailed, HPLC	M13 Human, FWD primer, HPLC	M13-tailed	✓	x‡
A15636	Human, Reverse PCR primer, M13-tailed, HPLC	M13 Human, REV primer, HPLC	M13-tailed	✓	x‡
A15637	M13 [-21] Forward sequencing primer	M13 [-21] seq FWD primer, HPLC	N/A	x	✓‡
A15638	M13 Reverse sequencing primer	M13 seq REV primer, HPLC	N/A	x	✓‡

† PCR and sequencing reactions may require different primer concentrations.

‡ M13[-21] Forward sequencing primers (5' TGTA AACGACG GCCAGT 3', Cat. no. A15637) and M13 Reverse sequencing primers (5' CAGGAAACAGCTATGACC 3', Cat. no. A15638) are designed for those PCR products amplified from M13-tailed PCR primer pairs.

All the PCR products are compatible with BigDye® chemistry v1.1 and v3.1. The PCR products amplified by non-tailed PCR primers can not be used with BigDye® Direct chemistry. See the following table for more details:

Cat. no.	Short description	Primer 5'-tail option	BigDye® Terminator v1.1 or v3.1 Cycle Sequencing Kits	BigDye® Direct Cycle Sequencing Kit
A15629	Human, FWD primer, Ds	Non-tailed	✓†	x
A15630	Human, REV primer, Ds	Non-tailed	✓†	x
A15631	Human, FWD primer, HPLC	Non-tailed	✓†	x
A15632	Human, REV primer, HPLC	Non-tailed	✓†	x
A15633	M13 Human, FWD primer, Ds	M13-tailed	Use with M13 sequencing primers (Cat. nos. A15637 & A15638)‡	Use with specific M13 primers provided in the BigDye® Direct Kit§
A15634	M13 Human, REV primer, Ds	M13-tailed	Use with M13 sequencing primers (Cat. nos. A15637 & A15638)‡	Use with specific M13 primers provided in the BigDye® Direct Kit§
A15635	M13 Human, FWD primer, HPLC	M13-tailed	Use with M13 sequencing primers (Cat. nos. A15637 & A15638)‡	Use with specific M13 primers provided in the BigDye® Direct Kit§
A15636	M13 Human, REV primer, HPLC	M13-tailed	Use with M13 sequencing primers (Cat. nos. A15637 & A15638)‡	Use with specific M13 primers provided in the BigDye® Direct Kit§

† You can use the same primers for both PCR and cycle sequencing reactions; however, different concentrations of primers may be necessary.

‡ M13[-21] Forward sequencing primers (5' TGTA AACGACG GCCAGT 3', Cat. no. A15637) and M13 Reverse sequencing primers (5' CAGGAAACAGCTATGACC 3', Cat. no. A15638) are designed for those PCR products amplified from M13-tailed PCR primer pairs.

§ BigDye® Direct Cycle Sequencing Kits include M13 sequencing primers that are specifically modified to be compatible with the BigDye® Direct Cycle Sequencing workflow. (Primers A15637 and A15638 are NOT compatible with the BigDye® Direct Cycle Sequencing workflow.)

Note: To learn more about DNA sequencing, go to www.lifetechnologies.com and click on Product & Services ▶ Sequencing ▶ Sanger Sequencing ▶ Learn About DNA Sequencing

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