

SeqTarget system

For sample enrichment for next-generation sequencing

The SeqTarget system has been developed to enable long-range PCR amplification, PCR product purification, and normalization of target genomic DNA regions to meet next-generation resequencing throughput requirements. This innovative system consists of 3 components — SeqTarget Primer Select, the SeqTarget LongRange PCR Kit, and the SeqTarget Normalization Kit — providing a complete solution for sample enrichment prior to next-generation sequencing (NGS).

The SeqTarget system provides:

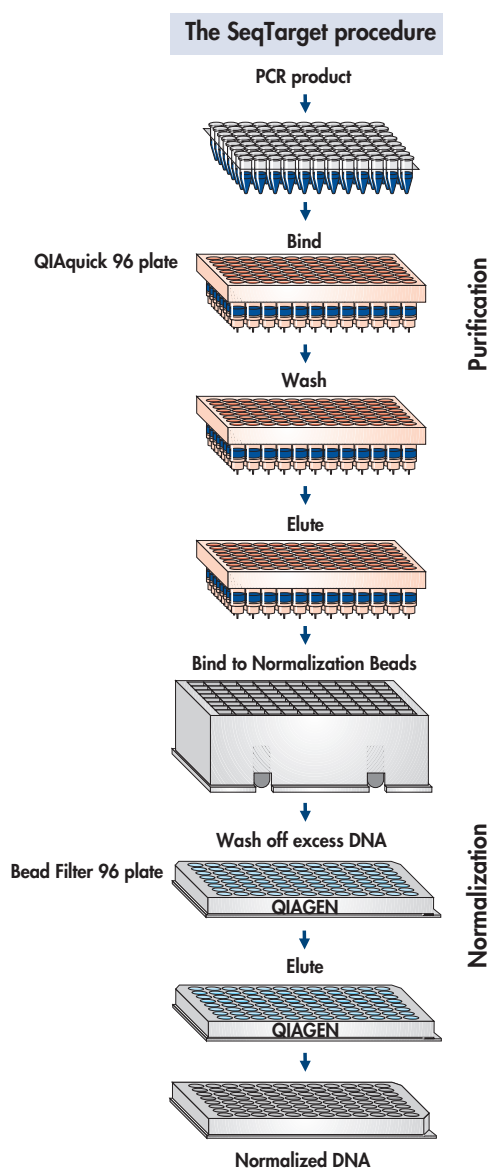
- Optimal design and ordering of primers via the GeneGlobe® Web portal
- Reliable amplification of long-range PCR fragments (up to 20 kb)
- Efficient purification and normalization of PCR fragments (>2 kb)
- Sufficient yields of normalized DNA of up to 500 ng
- Optimized kits and automatable protocols for success at the first attempt

Simplifying sample enrichment

The high demand for high-throughput sequencing has given rise to a number of second-generation sequencing technologies. However, these second-generation sequencing systems do not currently have the throughput capacity to sequence the whole human genome cost-effectively. Research areas such as medicine (e.g., cancer research and diagnostics) and population genetics often require resequencing of defined genomic regions from several samples. Therefore, a reduction in the complexity of genomic DNA samples to a manageable subset is required prior to sequencing.

The SeqTarget system has been developed to overcome these limitations, ensuring convenience, speed, and standardization by delivering ready-to-use, normalized amounts of DNA ready for library preparation and subsequent sequencing (Figure 1). Large fragments of DNA are reliably amplified and subsequently purified and normalized, and require no further adjustment or quantification prior to sequencing, eliminating the need for expensive equipment and reagents. Subsequent sequencing can be performed without bias.

Figure 1. The SeqTarget purification and normalization procedure. The simple bind–wash–elute procedure is optimized to meet different throughput requirements and to allow the use of different laboratory equipment. Purification and normalization can be performed using both centrifugation and vacuum procedures.



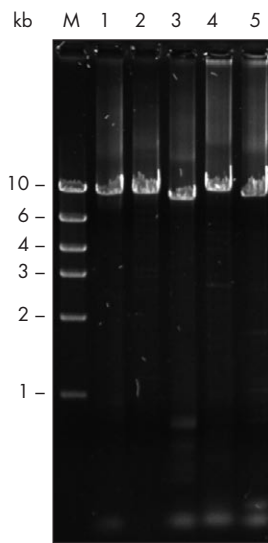


Figure 2. Agarose gel of unpurified SeqTarget long-range PCR amplicons of the human gene DDX31. Fragment sizes range from 9.8–11 kb. **M:** Marker DNA ranging from 1–10 kb. **1–5:** Five different long-range PCR products.

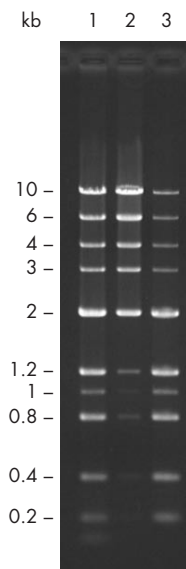


Figure 3. Successful purification of long-range PCR fragments using the SeqTarget Normalization Kit. To simulate background reduction during purification of long-range PCR fragments, a DNA molecular weight marker ranging from 100 bp to 10 kb was purified by size-selective purification using the SeqTarget Normalization Kit. DNA fragments of up to 2 kb were removed or reduced by the purification procedure. **Lane 1:** Unpurified molecular weight marker. **Lane 2:** Eluate after purification. **Lane 3:** Flow-through.

Optimal primer design and easy ordering

High-quality, well-designed primers are a prerequisite for successful PCR amplification. SeqTarget primer pairs are derived from human gene sequences contained in the ENSEMBL Reference Sequence database and are available in a convenient tube or 96-well format. Primers chosen are specific (as determined by bioinformatic validation) and are designed using algorithms optimized for long-range PCR. SeqTarget primer pairs are used to generate overlapping long fragments of ~10 kb in length (Figure 2), highly suitable for resequencing of the regions of interest (e.g., whole genes). Primers can be conveniently ordered using QIAGEN's GeneGlobe Web portal (www.qiagen.com/GeneGlobe).

High yields of long-range PCR fragments

Until recently, PCR enrichment for NGS applications has been a time-consuming and labor-intensive process. PCR amplification of long fragments of DNA can often require tedious optimization, leading to increases in both costs and time-to-result. The SeqTarget LongRange PCR Kit includes a powerful enzyme blend of thermostable DNA polymerases with enhanced processivity and proofreading ability, as well as Q-Solution[®], an innovative PCR additive that facilitates amplification of difficult templates. This ensures both a very high extension rate as well as increased fidelity, enabling reliable amplification of genomic targets longer than 20 kb. The unique PCR buffer included in the kit has a unique zwitterionic formulation which minimizes pH-driven template degradation by improving buffering of pH at high temperatures.

Ready-to-use, normalized DNA

Variations in PCR efficiency result in different yields of amplicons in a given pool. This can lead to a higher depth of sequence coverage for high-yield amplicons and results in unnecessarily low coverage for low-yield amplicons. For consistent representation, long-range PCR products need to be normalized prior to sequencing. The SeqTarget Normalization Kit offers a standardized method for purification and normalization of long-range PCR fragments (>2 kb), ready for subsequent library preparation. It is the first kit to provide yields of 500 ng ($\pm 50\%$) of normalized DNA. In addition, it eliminates the need for measurement and adjustment of DNA concentration of individual samples prior to sequencing. This simple bind–wash–elute procedure delivers equal DNA amounts. Similar-sized PCR fragments can be easily normalized and pooled together for library preparation and subsequent sequencing (Figure 1). The kit is provided with specially developed protocols optimized to meet different throughput requirements and to allow the use of different laboratory equipment, such as microplate centrifuges and vacuum manifolds. The simple procedure includes 2 steps: purification and normalization.

Purification

One main advantage of the SeqTarget Normalization Kit is the size-selective purification of long-range PCR fragments. This not only involves the removal of unincorporated primers and nucleotides, but also a significant reduction of PCR background (removal of fragments ≤ 2 kb) (Figure 3). Following the purification procedure, DNA yield can be adjusted by modifying the amount of Normalization Beads, providing increased flexibility (Figures 4A and 4B).

Normalization

The procedure involves binding of DNA to beads with limited binding capacity. Excess PCR products are simply washed off, and normalized amounts of PCR products are eluted with a mild buffer. No further quantification and normalization steps are necessary for library preparation. In addition, expensive detection devices for A_{260}/A_{280} or fluorescence measurements are not required and further adjustment to obtain the desired concentration is not needed. Subsequent sequencing can be easily performed without any sequence bias. The SeqTarget Normalization Kit ensures significant improvements in the uniformity of coverage of the entire region of interest when compared to samples that are not normalized (Figures 5 and 6).

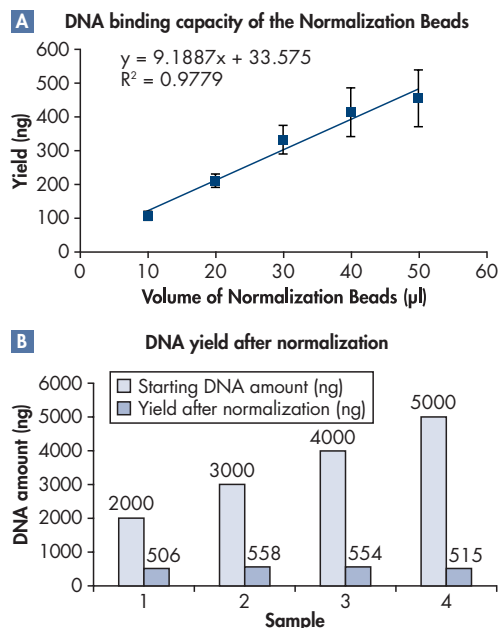


Figure 4. Typical example of the DNA-binding capacity of Normalization Beads and DNA yield after normalization. **A** DNA-binding capacity of different bead quantities was determined for 4 different SeqTarget long-range PCR fragments. **B** Example of normalization of different amounts of a DNA sample (ranging from 2–5 μ g) using a 50 μ l bead suspension. The average yield of normalized DNA was 533 ng. Normalized DNA was quantified using the Quant-iT™ dsDNA Broad-Range Assay Kit (Invitrogen).

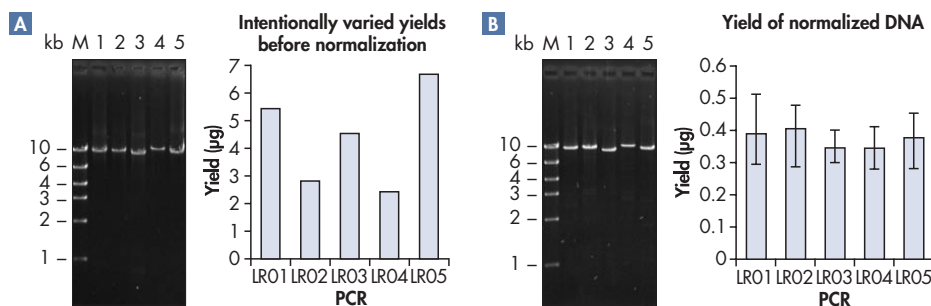


Figure 5. Yields of SeqTarget long-range PCR products before and after normalization. **A** Agarose gel of SeqTarget long-range PCR products (LRO1–LRO5) before normalization. The graph shows intentionally varied concentrations of amplicons. **B** Agarose gel of SeqTarget long-range PCR products (LRO1–LRO5) after normalization. The graph shows yields of normalized DNA samples derived from 3 independent experiments. The error bars show minimum and maximum values obtained. **Lane M:** Marker DNA ranging from 1–10 kb. **Lane 1:** LRO1. **Lane 2:** LRO2 diluted 1:2. **Lane 3:** LRO3. **Lane 4:** LRO4 diluted 1:3. **Lane 5:** LRO5.

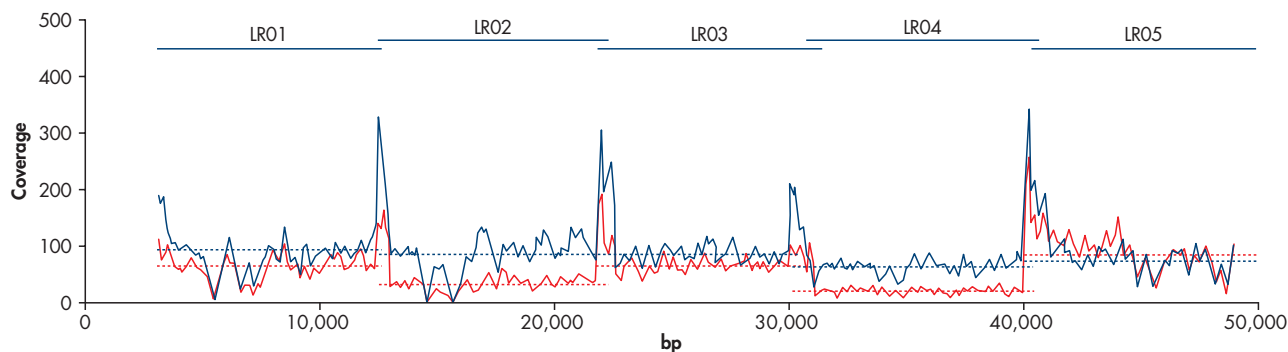


Figure 6. Depth of coverage from amplicon pools with intentionally varied or normalized concentrations. Sequence coverage of pooled amplicons after GS FLX Shotgun DNA library sequencing on a Genome Sequencer FLX (Roche) is shown. Sequence coverage of the pooled DNA fragments with intentionally varied concentrations is displayed in red. Sequence coverage of the pooled normalized DNA fragments is displayed in blue. The dotted lines indicate average sequence coverage obtained for the PCR fragments. Improvements in the uniformity of coverage is observed for normalized DNA samples. **LR01–LR05:** SeqTarget long-range PCR products.

A complete solution for sample enrichment

QIAGEN's products are recommended for library preparation by various instrument providers, such as Applied Biosystems, Illumina, and Roche, and are already part of their NGS protocols. We now offer a dedicated product range for fast and convenient sample enrichment prior to next-generation sequencing. SeqTarget products enhance sequence coverage by minimizing the risk of losing valuable sequence data. From effective primer design to reliable long-range PCR amplification and straightforward DNA normalization, SeqTarget products deliver the quality you need for successful sequencing.

Ordering Information

Product	Contents	Cat. no.
SeqTarget Primer Select	Tube format: 8.4 nmol SeqTarget Primer Select (containing a mix of lyophilized forward and reverse primers for a specific target); for 400 x 25 µl reactions	122001
SeqTarget Primer Select 96 Plate	96-well format: 8.4 nmol SeqTarget Primer Select (containing a mix of lyophilized forward and reverse primers for a specific target); for 400 x 25 µl reactions	122004
SeqTarget LongRange PCR Kit (500)	For 500 x 25 µl reactions: LongRange PCR Enzyme Mix (500 U), LongRange PCR Buffer, 5x Q-Solution, RNase-Free Water, 10 mM dNTPs, and Control Primer Pair	122117
SeqTarget Normalization Kit (480)	For 480 preps: 5 QIAquick® 96 Plates, 5 Bead Filter 96 Plates, Normalization Beads, Buffers, and Mineral Oil	122217

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Visit www.qiagen.com/goto/SeqTarget for more information!

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