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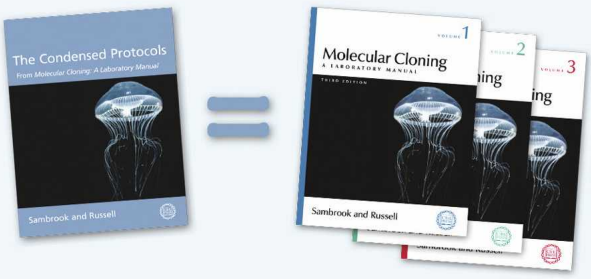

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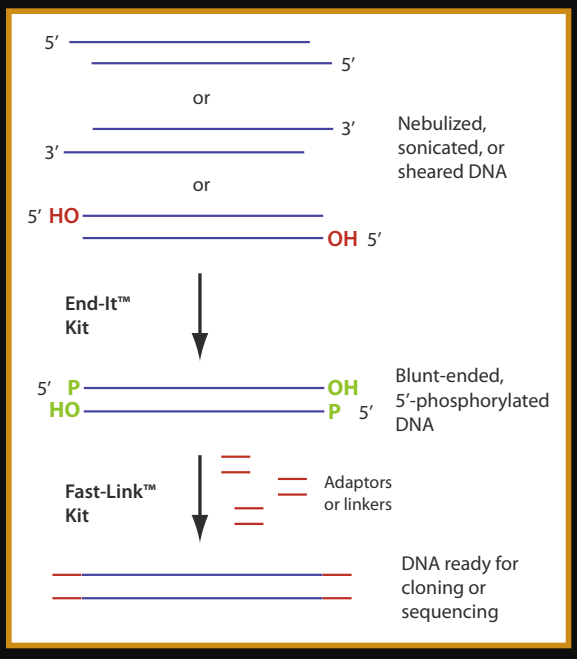

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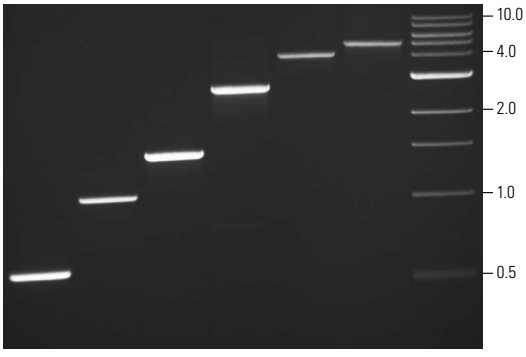

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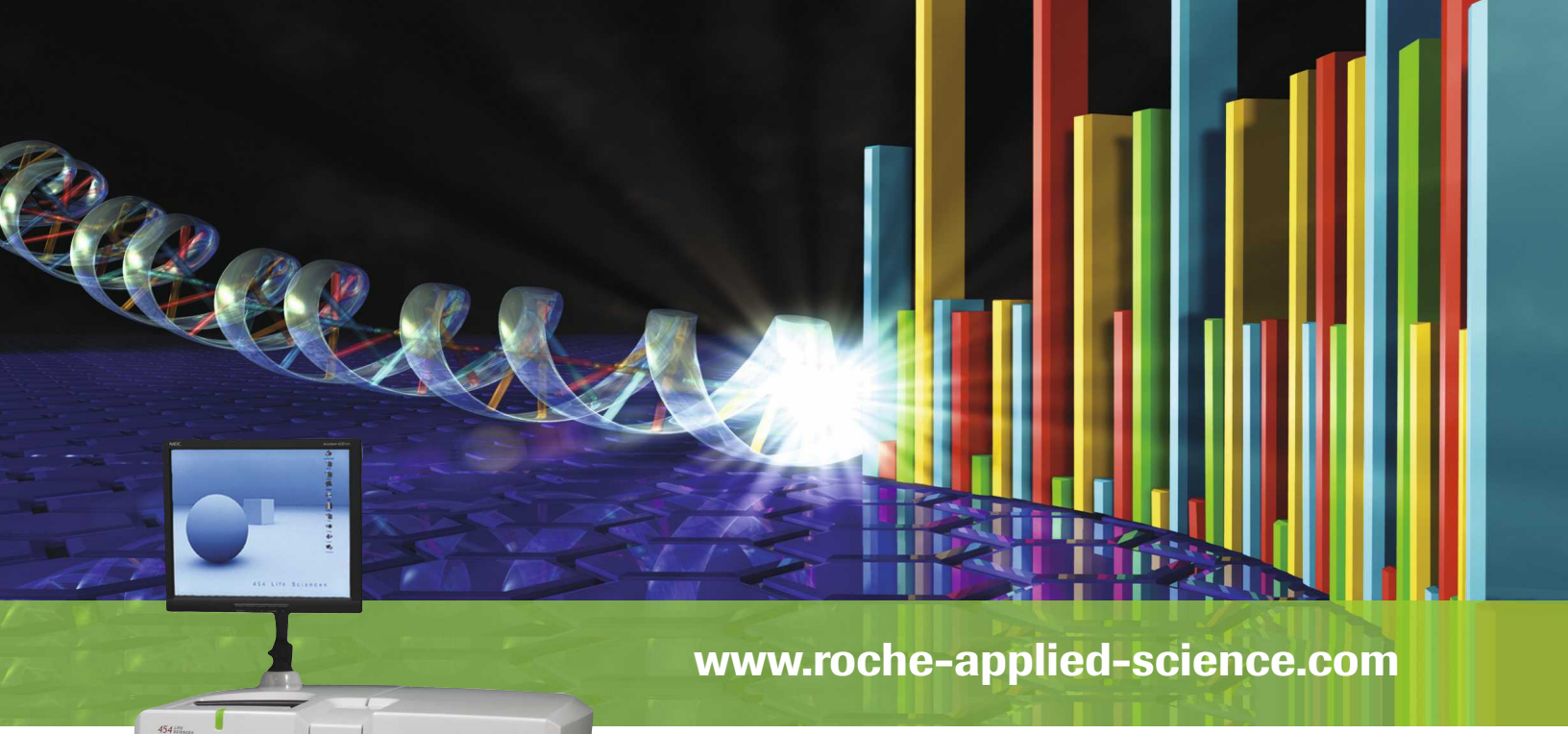

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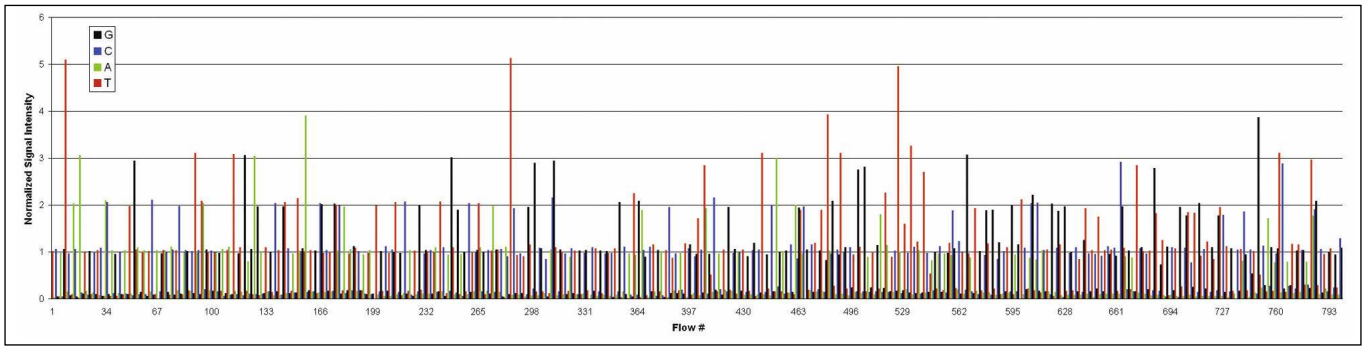

DNA Sequencing Flowgram: Each bar within the flowgram represents a discrete nucleotide (A, T, C, or G) and the height of the bar corresponds to the number of nucleotides detected. The flowgram above represents a 458-base-pair sequencing read from *E. coli* K-12.

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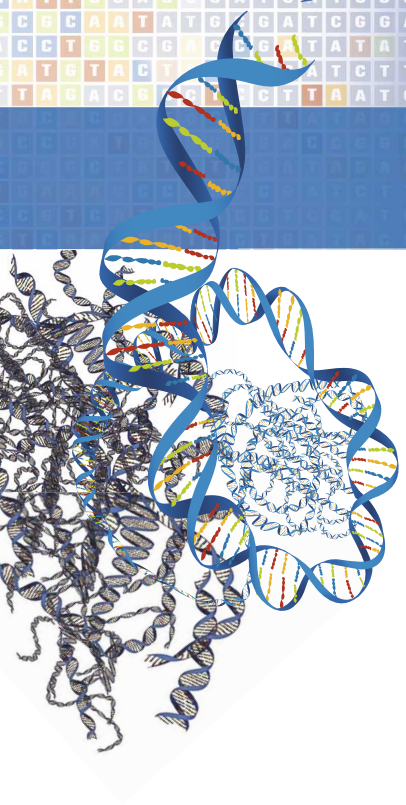

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Roche has developed NimbleGen Sequence Capture technology that enables targeted sequencing of thousands of exons or contiguous genomic loci of up to 5 Mb in a single experiment. The microarray hybridization-based NimbleGen Sequence Capture technology has considerable cost, throughput, and quality advantages when compared to PCR.

Recent technological advances in sequencing systems have rapidly increased raw sequence output. However, these next-generation sequencing systems do not currently have the throughput to sequence the whole human genome cost-effectively. Thus, they require that the complexity of genomic DNA samples be reduced to a manageable subset prior to sequencing. The prevailing method for complexity reduction has been the preparation of amplicons by parallel, multiplex, or long range PCR amplification. These PCR methods have severe cost and performance limitations when scaled to the level required to take full advantage of the capacity of currently available sequencing systems. As a result of these limitations, the bottleneck for sequencing projects has shifted to sample preparation.

To address this sample preparation bottleneck, Roche has developed the microarray hybridization-based NimbleGen Sequence Capture technology that utilizes high-density oligonucleotide microarrays as a programmable genomic selection device to allow targeted sequencing of subsets of the genome. These genome subsets can be exons, disease-associated regions, quantitative trait loci, promoters and enhancers, and other targeted regions. The revolutionary and simple workflow of NimbleGen Sequence Capture technology enables isolating megabase regions in as little as one week and eliminates the cost, labor, and infrastructure required for large-scale PCR experiments.

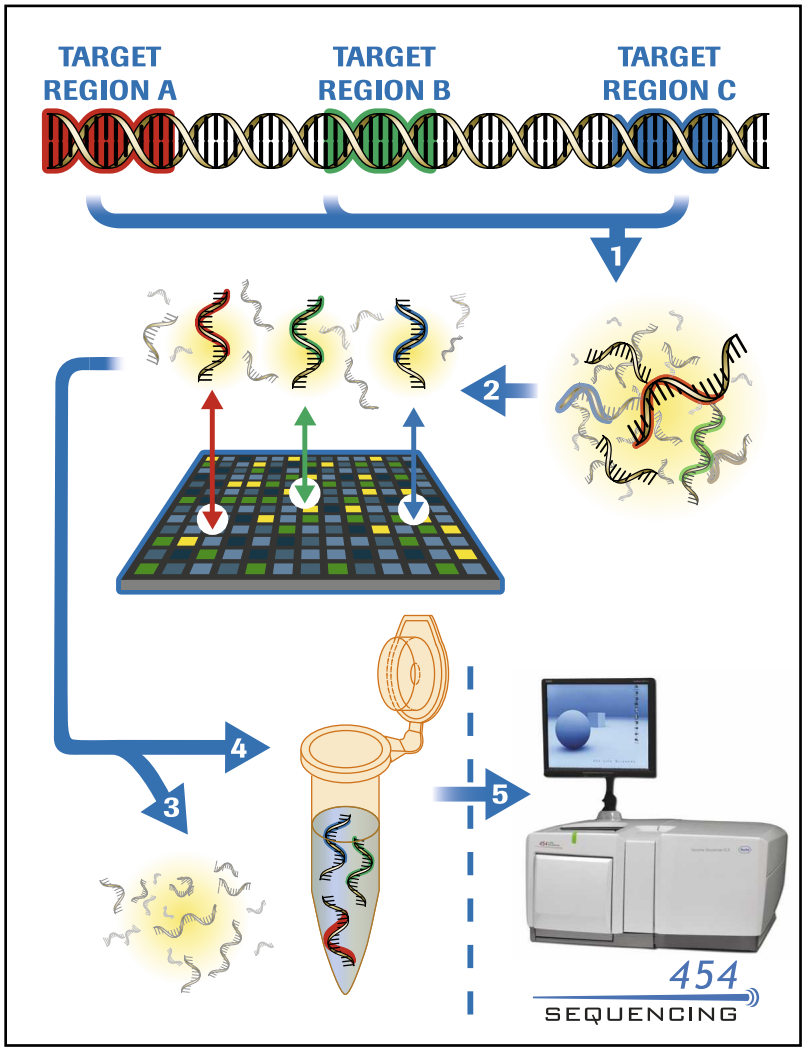


Figure 1: The NimbleGen Sequence Capture Protocol.

1. The genomic DNA sample is fragmented.
2. The sample is hybridized to a custom NimbleGen Sequence Capture array.
3. Unbound fragments are removed.
4. The target-enriched pool is eluted and amplified.
5. The enriched sample is ready for processing in the Genome Sequencer FLX sample processing workflow.

Sequence Capture Performance

The performance data shown here are derived from the first peer-reviewed publication (1) of the technology, and the NimbleGen Sequence Capture Service offers data quality equal or higher than what has been published. The actual performance depends on the sizes and exact locations of the target regions, and pilot projects involving both Sequence Capture and sequencing is recommended to accurately determine how this technology works for your region of interest. To receive the latest updates on the technology's performance, we encourage you to subscribe to NimbleGen Sequence Capture news at www.nimblegen.com/seqcap.

- With a single-array experiment covering 5 Mb target regions, followed by a single Genome Sequencer FLX run producing approximately 100 Mb total sequencing data, the majority of sequencing reads represented selected target regions (typically >70%). While the coverage typically depends on the composition of the target regions, approximately 8X median coverage can be achieved for exon-sized regions (Figure 2), and approximately 18X median coverage can be achieved for a single 5 Mb contiguous genomic region (Figure 3). The sequence coverage will increase for smaller cumulative target region sizes, if the same amount of sequencing runs is performed. For example, an experiment covering a 500 kb contiguous target region followed by a single Genome Sequencer FLX run yielded >90X median coverage. In research requiring small regions, NimbleGen Sequence Capture technology combined with 454 sequencing utilizing only a portion (1/2 to 1/16) of the picotiter plate will generate sufficient reads for sequencing applications.

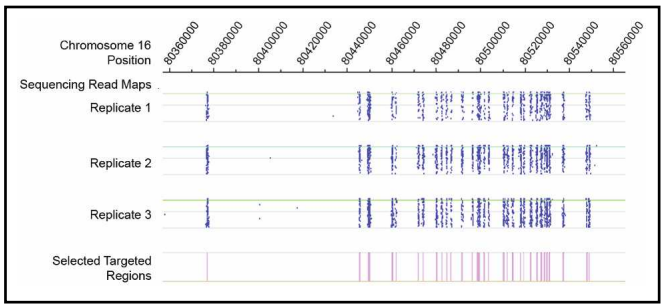


Figure 2: Sequencing Read Maps of approximately 190 kb of Chromosome 16. Sequencing capture read maps depict approximately 190 kb of chromosome 16 from three replicates of sequence capture experiments of human exons.

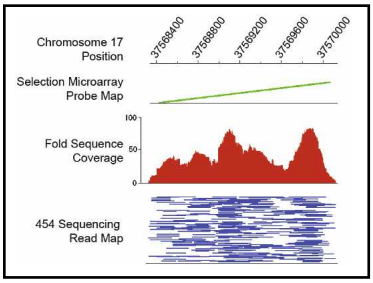


Figure 3: Sequencing Read Map of 2 kb of Chromosome 17. A sequencing read map shows 2 kb of chromosome 17 from a microarray selection of a 2 Mb contiguous region that contains the BRCA1 gene.

- Although arrays are typically designed based on the reference genome, NimbleGen Sequence Capture technology does not bias against discovery of unknown variants. As shown in Figure 4, in an experiment designed to compare the performance of this technology versus long range PCR, almost all the variants were captured with the same fidelity as PCR. In the 70 kb region targeted by both methods, 98 SNPs were identified by both, and 9 and 5 rare variants were identified by NimbleGen Sequence Capture technology and long range PCR, respectively. In addition, 22 variants in repeats were detected only by long range PCR because no probes on the array were designed for these repetitive regions.

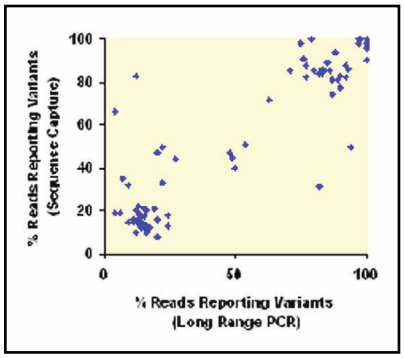


Figure 4: Sequencing Read Map of 2 kb of Chromosome 17. In this experiment, genomic DNA from a mixed cell population was used, representing both common and rare SNP variants. A 200 kb region surrounding the EGFR gene was captured using NimbleGen Sequence Capture technology, and a 70 kb region out of the 200 kb region was amplified using long range PCR. DNA samples derived from target regions using both methods were sequenced separately with the Genome Sequencer FLX instrument, and SNP discovery was performed on both data sets. The percentages of 454 sequencer reads that report variants, either from PCR or NimbleGen Sequence Capture technology, were plotted for each of the SNPs detected by both methods.

References

1. Albert TJ, et al. Direct selection of human genomic loci by microarray hybridization. Nature Methods 2007 Nov; 4(11):903-5.

2. Okou DT, et al. Microarray-based genomic selection for high-throughput resequencing. Nature Methods 2007 Nov; 4(11):907-9.

3. Hodges E, et al. Genome-wide in situ exon capture for selective resequencing. Nature Genetics 2007 Dec; 39(12):1522-7.

No More Tedious and Costly PCR Reactions

NimbleGen Sequence Capture technology has clear advantages for large-scale, targeted sequencing when compared to traditional PCR-based methods, for example:

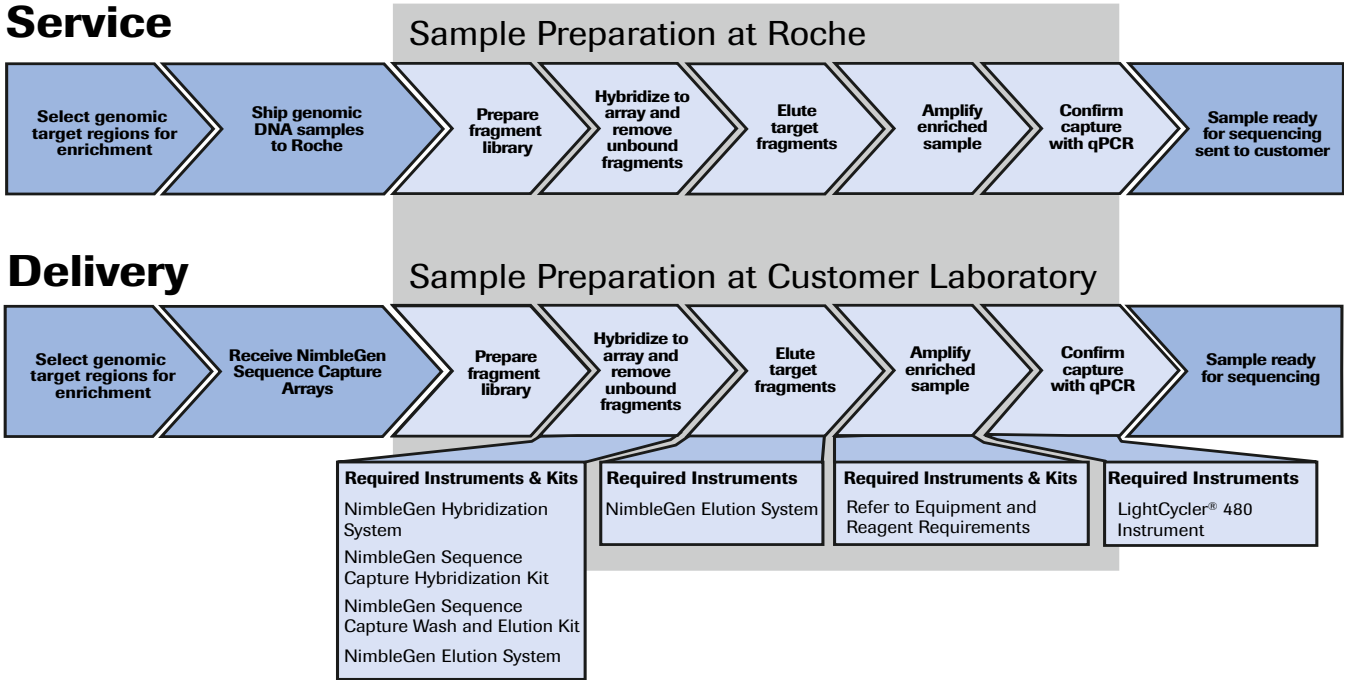
- **Select Large Genomic Regions:** For a 1 Mb disease-associated region identified by a genome-wide association study, a traditional long-range PCR approach requires 200 work days to optimize all of the PCR conditions. With NimbleGen Sequence Capture technology, this region can now be easily captured in a few weeks. In addition, you can target regions up to 5 Mb with a single array.
- **Capture Exonic Regions:** Sequencing a panel of 1,000 cancer genes with approximately 7,000 exons requires the design and synthesis of 14,000 PCR primers at a cost of >$30,000. Plus, 7,000 PCR reactions are required at a cost of >$10,000 per sample. Thus, a PCR approach is cost-prohibitive for most researchers. However, a single NimbleGen Sequence Capture array can target all 7,000 exons easily, in a single enrichment cycle, providing substantial cost and time savings.

Advantages of NimbleGen Sequence Capture

- **Target Specific Regions of Interest:** Capture up to 5 Mb total regions on a single array with high coverage and specificity.
- **Reduce Cost:** Significantly reduce time and cost compared to laborious and limiting PCR-based methods.
- **Generate Data with Confidence:** Ensure system performance prior to sequencing with built-in QC probes.
- **Customize Each Capture Design:** Specify the array design to capture contiguous genomic regions or thousands of exons in parallel.

NimbleGen Sequence Capture Arrays and Service

Perform the NimbleGen Sequence Capture workflow in your own lab or utilize the NimbleGen Service Lab. See the workflow chart below for details of each option.



"We are extremely pleased with the capabilities and efficiencies the NimbleGen Sequence Capture technology has brought to our sequencing research efforts. There are huge advantages when this technology is compared to PCR-based methods. This is the most exciting next phase in bringing genetic discovery to medicine."

Richard Gibbs, Ph.D.
Director, Human Genome Sequencing Center
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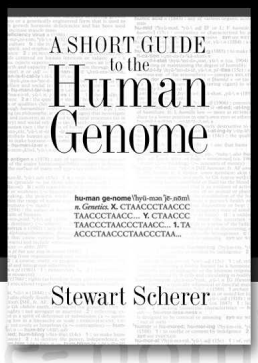

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