febit

READ, WRITE, UNDERSTAND THE CODE OF LIFE

HybSelect™

SEQUENCE CAPTURE FOR NEXT GENERATION SEQUENCING

Genomic DNA | Library preparation | Hybridization | Elution | Sequencing

HYBSELECT

For Convenient Targeted Re-Sequencing

- **HYBSELECT**

  is a new product available from febit for targeted, hybridization-based DNA capture. Our technology enables the enrichment of specific regions of interest from complex mixtures such as large eukaryotic genomes. The recovered DNA is ideal for targeted re-sequencing on next generation sequencing systems.

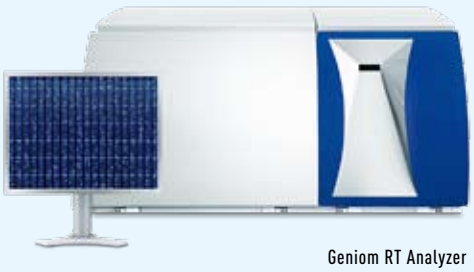
  HybSelect has successfully been applied to human, mouse and microbial samples in combination with the Illumina Genome Analyzer II (GAII).

  HybSelect takes advantage of the Geniom platform, wherein the fluidics of the Geniom Biochip and the Geniom RT Analyzer instrument provide a robust, easy to use and highly automated enrichment environment for DNA fragments.

  Validated protocols and HybSelect performance data from the Illumina Genome Analyzer (GAII) are available. Protocols for the ABI SOLID and Roche-454 FLX will soon follow.

  We currently offer two HybSelect options:
  a) Full HybSelect services from febit, and,
  b) Installation of a Geniom RT Analyzer in your lab.

- **FULLY AUTOMATED SOLUTION FOR SEQUENCE CAPTURE**
- **DEEP SEQUENCE COVERAGE**
- **EXCELLENT SNP DETECTION AND PROVEN ABSENCE OF ALLELIC BIAS**
- **FLEXIBLE AND FAST SCIENTIFIC BIOINFORMATICS FOR DESIGN AND ANALYSIS**
- **FAST TURN AROUND TIME**



Geniom RT Analyzer

HYBSELECT

The Power of Detection

THE FIRST FULLY AUTOMATED SEQUENCE CAPTURE SOLUTION

It is easy to capture your region of interest automatically using the Geniom RT Analyzer in your laboratory. The HybSelect protocol only takes 30 minutes of hands-on time using the Geniom RT Analyzer. The streamlined HybSelect protocol is simple to implement.

The entire HybSelect sequence capture process is enabled by febit's microfluidic Biochips. Just insert your Biochip, load your samples, and walk-away. Sample loading, hybridization, washing and temperature control are fully automated in the Geniom RT Analyzer.

You can work with our catalog Biochips, or with Biochips custom-designed to capture your region of interest. Customized HybSelect Biochips are quickly designed by our bioinformatics team.

FAST TURN AROUND TIME

Two days are required for one HybSelect run on the Geniom RT Analyzer. Even without multiplexing, you can process up to 16 samples per week per instrument.

Our full HybSelect service, including Custom Biochip Design, Sequence Capture, Next-Gen Sequencing, and Bioinformatics Report takes 4 – 6 weeks.

POWER OF DETECTION

The HybSelect technology offers an excellent depth of coverage of your genomic region of interest: typically several hundred-fold for targets up to 2 Mb. This capability can be used for accurate detection of mutations in disease- related genes. For example 98.1 % (455/464) of heterozygous SNP's were detected and called correctly from a 1.5 Mb region of interest in a HapMap CHB reference sample (NA18561). HybSelect gives you an excellent power of detection for finding the mutations in your region of interest.

FLEXIBLE AND FAST BIOINFORMATICS DESIGN AND ANALYSIS

We have the fastest and most flexible bioinformatics support available for targeted sequence capture. Just specify the genes that you want to capture and we will design and synthesize a customized HybSelect Biochip for you within days.

If you choose our HybSelect Service, you will also receive a concise and complete Bioinformatics Report. We can assist in the analysis and interpretation of your sequencing data. If you need additional bioinformatics support, please contact us.

HYBSELECT

Two Convenient Alternatives

FULL SERVICE

Specify the genes which you want to capture, and then ship your genomic DNA samples to febit. We will sequence your region of interest on an Illumina Genome Analyzer (GAII).

We will provide you a clear and publishable bioinformatics report based on the sequencing results.

HYBSELECT IN YOUR LAB

You don't need to be a microarray hybridization expert to use HybSelect. With HybSelect and a Geniom RT Analyzer, you can easily perform sequence capture in your own laboratory.

HYBSELECT WORKFLOW

1. **Library preparation**
   Sequencing library is prepared according to the Illumina protocol from genomic DNA.
2. **Hybridization**
   A Biochip designed to specifically capture your region of interest is inserted into the Geniom RT Analyzer. Your samples are automatically loaded and hybridized to the HybSelect Biochip.
3. **Array Washing**
   The Biochip is washed according to automated programs on the Geniom RT Analyzer.
4. **Elution**
   The captured DNA is eluted from the Biochip.
5. **Amplification**
   The captured DNA is amplified employing 10 cycles of PCR.
6. **Sequencing**
   Paired end sequencing is performed on the Illumina Genome Analyzer (GAII).
7. **Mapping and data analysis**
   The NGS data is aligned to the reference sequence by febit. Then, the results and data analysis are summarized in a Bioinformatics report.

PREPARED AT FEBIT

select region of interest → ship DNA samples to febit → Illumina GA library preparation → hybridization and washing → elution of enriched sample → next-gen sequencing → Bioinformatics report

PREPARED ON YOUR GENIOM RT ANALYZER

select region of interest → Illumina GA library preparation → hybridization and washing → elution of enriched sample → next-gen sequencing → Bioinformatics report

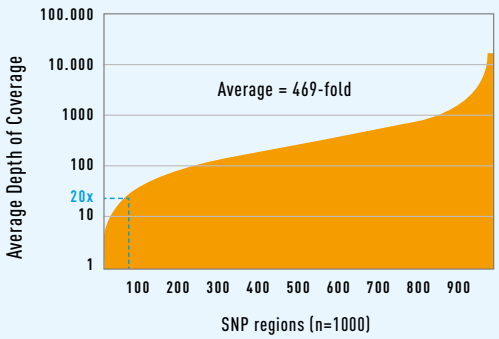
HYBSELECT

Unmatched Depth of Coverage

TARGETED CAPTURE OF SNP REGIONS USING HYBSELECT

One thousand 500 bp regions with a centrally located SNP were HybSelected from a HapMap reference sample. The ranked average depth of coverage for the 1000 individual 500 bp regions is shown in the figure below. The average depth of coverage was 469 fold.

Depth of Coverage Across 1,000 SNP regions (500 bp)
(500 kb Total Target Region)



HYBSELECT DETECTION PERFORMANCE

The table below shows results of the sequence capture of 1000 HapMap SNPs out of a 1.5 Mb region of interest. The results demonstrate HybSelect's detection power. 91.2 % of the 1000 SNP positions were covered at ≥20 x, and 98.6 % of those were concordant with the reference sequence. There was no evidence of allelic bias, and 98.1 % of the heterozygotes were detected and called correctly.

HYBSELECT	PERFORMANCE
Region of Interest	1.5 Mb
HybSelected Region	0.5 Mb
Average Coverage	469 fold
1 x Consensus Coverage	97.5 %
10 x Consensus Coverage	90.5 %
20 x Consensus Coverage	88.5 %
Enrichment	1062

HYBSELECT™ SEQUENCE CAPTURE

Bioinformatics Definitions

REGION OF INTEREST
The summed length (bp) of specific locations within the genome from which the researcher wants sequence information.

HYBSELECTED REGION (HR)
The summed length (bp) of the ROI for which the HybSelect Biochip is designed to capture minus the regions (bp) that are deliberately excluded (e. g., repetitive elements).

AVERAGE DEPTH OF COVERAGE
Number of base pairs from sequence reads within the HR divided by the total number of base pairs of HR.

CONSENSUS COVERAGE
Percentage of the HR for which there is at least 1 x Depth of Coverage.

ENRICHMENT
Percentage of filtered bases from NGS which map to the HR, divided by the percentage of the genome represented by HR.
(number of bases mapped to HR/ number of filtered bases from NGS) / (size of HR in bp/size of genome in bp).

Catalog Order Numbers

HYBSELECT FULL SERVICE – Library preparation, Sequence Capture, Next-Generation Sequencing and Bioinformatics Report	S0008
BIOINFORMATICS CONSULTING	S0080
GENIOM® RT ANALYZER – Instrument, software package, PC, screen, installation, training, service package	P0001

EUROPE

febit biomed gmbh
Im Neuenheimer Feld 519
69120 Heidelberg / Germany
Phone +49 6221 6510-300
Fax +49 6221 6510-329
info@febit.eu
www.febit.com

USA

febit inc.
99 Hayden Avenue, Suite 620
Lexington, MA 02421 / USA
Phone +1 781 391 4360
Fax +1 781 391 4362
Toll-free 877-GENIOM1 (or 877-436-4661)*
info@febit.com
www.febit.com

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