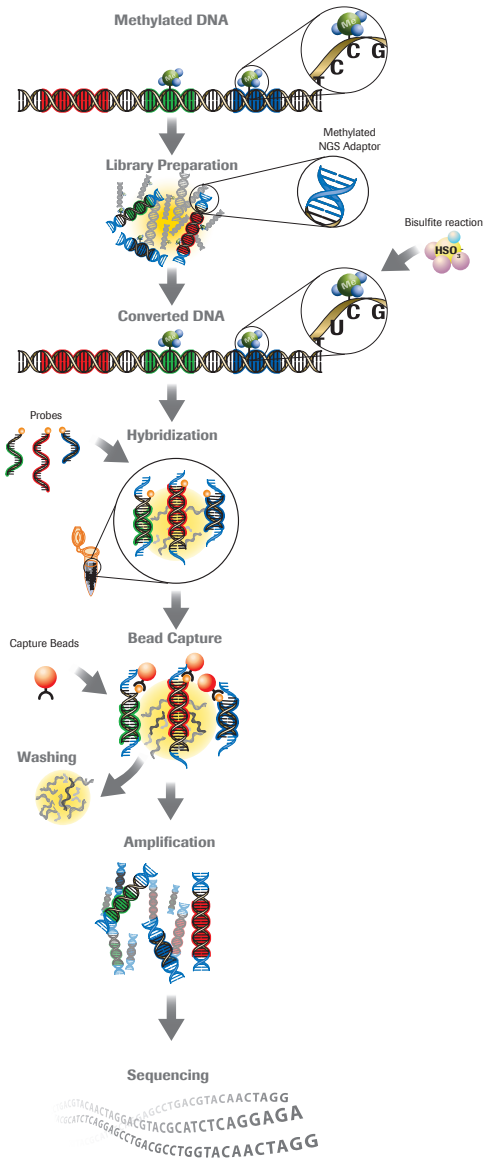
SeqCap Epi Enrichment System

Revolutionize your epigenomic research



Target Enrichment Solution for Bisulfite Treated DNA

The SeqCap Epi System is a set of target enrichment tools for DNA methylation assessment at single-base resolution. This system includes a fixed content epigenome-wide design and a full range of custom target offerings, suitable for both broad discovery and focused research applications.

Built on Roche NimbleGen's proprietary probe design and manufacturing technologies, the SeqCap Epi system will enable you to revolutionize your epigenetics research. Discover more with the breadth, depth, or throughput more advanced than other technologies available commercially.

- **Discover differential methylation efficiently.** Target ***your*** epigenomic region of interest with the first custom enrichment solution for bisulfite sequencing using a capture technology to deliver massive time and cost savings over whole genome or fixed content sequencing approaches.
- **Reveal hidden epigenetic modifications.** Innovative probe design and manufacture allows for the capture of both strands enabling the detection of complex or rare methylation events.
- **Experience superior performance.** Inherent flexibility of the system and the optimized bisulfite-then-capture workflow offer unprecedented resolution, sensitivity and sequencing sample capacity.

Learn more about these advantages at
www.nimblegen.com/SeqCapEpi

For life science research only.
Not for use in diagnostic procedures.

Join the revolution in next-gen methylation studies using the SeqCap Epi Enrichment System

In life science research, it is critical to understand the association between diseases or traits and modifications to the genome. Epigenetic modifications, which are functionally relevant changes to the genome that do not involve a change in the nucleotide sequence, are an essential part of genomics. A major epigenetic modification is DNA methylation.

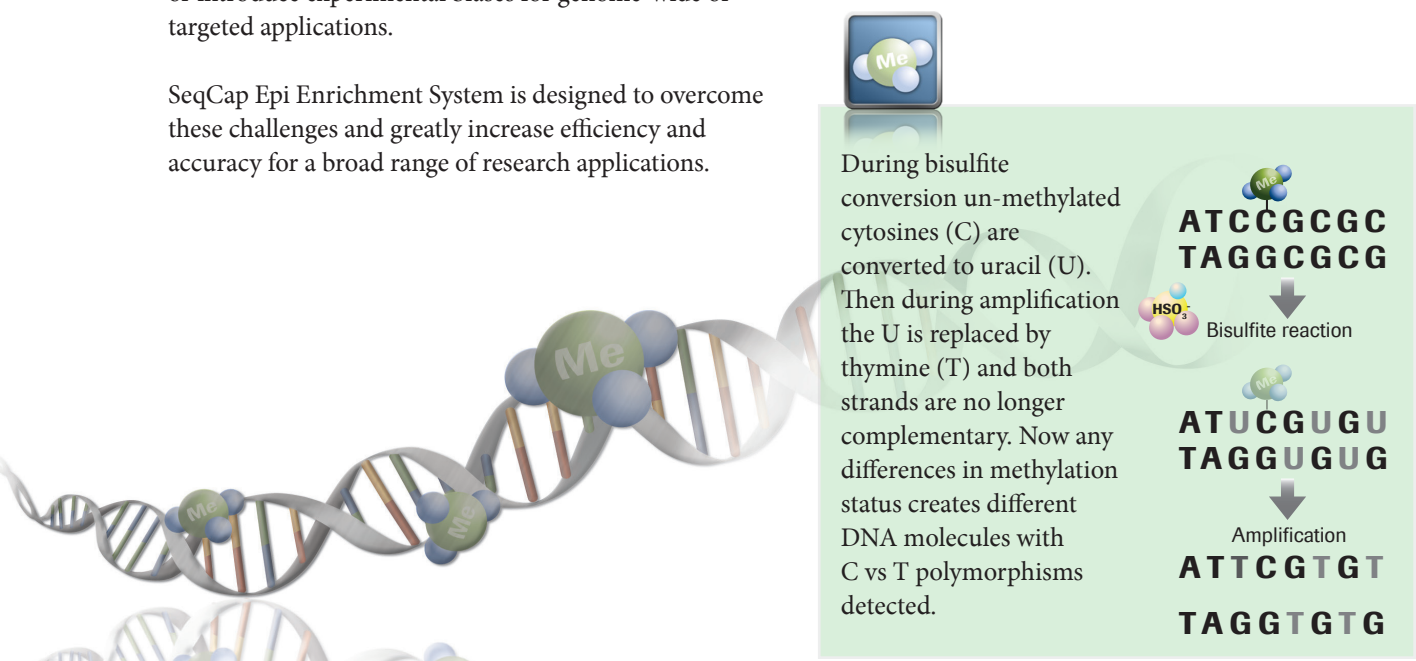
DNA methylation has been shown to play an important role in a wide variety of biological processes, including silencing of transposable elements, stem cell differentiation, embryonic development, genomic imprinting and inflammation. Alteration of methylation patterns has been identified in many diseases including cancer, diabetes, cardiovascular disease, inflamation and neurological disorders.

Researchers have been using a number of approaches for methylation assessment, utilizing next-generation sequencing or microarray technologies. However, current research tools either provide limited efficiency or introduce experimental biases for genome-wide or targeted applications.

SeqCap Epi Enrichment System is designed to overcome these challenges and greatly increase efficiency and accuracy for a broad range of research applications.

Applying concepts similar to those demonstrated by the best-in-class[1] technical performance of the SeqCap EZ products, Roche NimbleGen has pioneered target enrichment of bisulfite treated DNA. Depending on your organism of choice and genomic region of interest, a series of SeqCap Epi products are available to you:

- **SeqCap Epi CpGiant Enrichment Kits** offer researchers a solution to epigenome-wide discovery and easy transition from microarrays to a sequencing based workflow.
- **SeqCap Epi Choice Enrichment Kits** offer customized enrichment of your regions of interest in the human genome ranging in size up to 90 Mb.
- **SeqCap Epi Developer Enrichment Kits** provide enrichment of any organism and allow for custom designs up to 210 Mb.



[1] Clark M, *et al. Nat. Biotech.* 2011 Sep 25;29(10):908-14. doi: 10.1038/nbt.1975; Bodi K, *et al. J Biomol Tech.* 2013 Jul;24(2):73-86. doi: 10.7171/jbt.13-2402-002.

Targeted bisulfite sequencing enables focused epigenetic discovery

Whole genome shotgun bisulfite sequencing (WGBS) has been the traditional method used by many researchers, as it provides DNA methylation status at base pair resolution and allows for the assessment of percent methylation at each position in the genome. However, WGBS is time-consuming and costly due to large amount of sequencing and data analysis, when generally 65% of the reads do not contain any CpGs[2], and only a subset of the genome is of interest to most researchers. Targeted bisulfite sequencing will enable users to specifically capture the precise regions of interest associated with a disease or phenotype.

The SeqCap Epi Enrichment system enables the reproducible targeting of selected genomic regions, for regions less than 210 Mb, from bisulfite treated genomic DNA in a single workflow (Table 1). It was developed for researchers to more efficiently study a variety of regions, including:

- Select differentially methylated regions (DMRs)
- Promoter & enhancer regions from disease pathways
- All potentially methylated regions of the genome, also known as the methylome

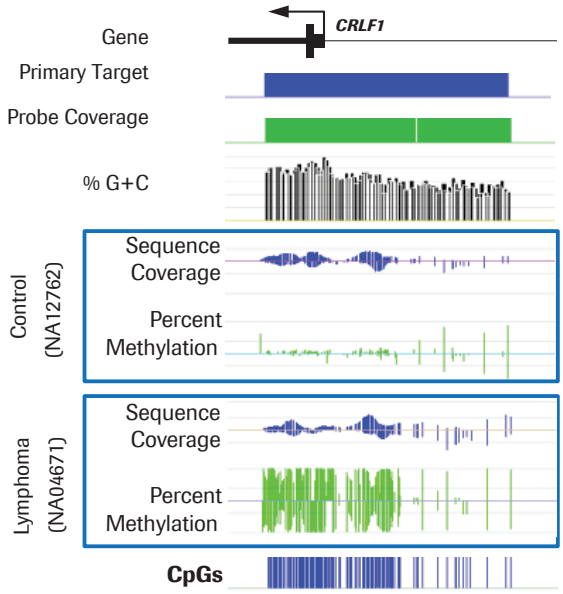
To illustrate this, a 3.2 Mb SeqCap Epi Choice design was used to capture bisulfite converted DNA from two cell lines. After sequencing on a single MiSeq run, a DMR can easily be seen covering the CRLF1 locus (Figure 1). Data produced on an 83.9 Mb design also demonstrates excellent correlation with WGBS data (Figure 2) for confidence in converting to targeted resequencing.

Low Technical Variation in Methylation Data

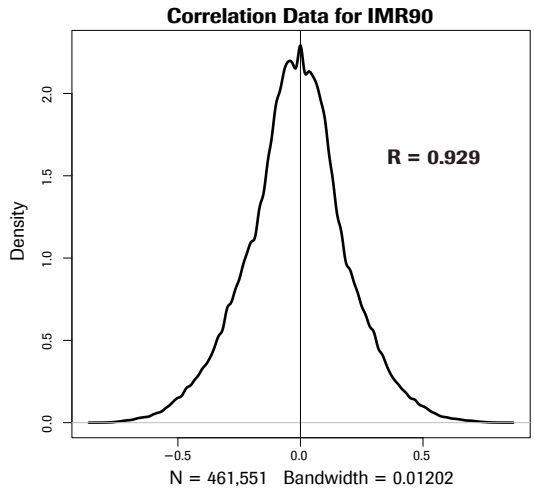
Sample	PF Reads Aligned	Reads on Target (%)	Fold Enrichment	Duplicate Reads (%)
NA04671_#1	4,427,673	53.1%	654.78	2
NA04671_#2	4,732,482	47.3%	583.32	5
NA04671_#3	4,990,449	51.3%	632.84	3
NA04671_#4	5,401,533	52.4%	646.29	7
Correlation of methylation data between samples is 0.98 (R^2)				

▲ **Table 1: Low technical variation in methylation data.** Four independent captures for the same sample show high reproducibility using a 3.2 Mb SeqCap Epi Choice Design.

[2] Ziller M, *et al. Nature.* 2013 Aug 22;500(7463):477-81. doi: 10.1038/nature12433. Epub 2013 Aug 7.



▲ **Figure 1: SeqCap Epi can identify Differentially Methylated Regions (DMRs).** Differential methylation can be seen by the large increase in percent methylation in the Burkitt's lymphoma cell line (NA04671) compared to a normal control cell line (CEPH; NA12762) in a 3.2 Mb SeqCap Epi Choice design.



▲ **Figure 2: SeqCap Epi shows high correlation to WGBS from an IMR90 cell line.** The left-right symmetry of the density plot of difference between WGBS and an 83.4 Mb SeqCap Epi Choice design percent methylation indicates little systematic bias on 2x100bp HiSeq sequencing. Correlation is for all common data points where read depth ≥ 5X. Data points with values of 0% or 100% are not shown. Inter replicate correlation of methylation occupancy between three replicates was between 1 and 0.95 (data not shown).

Double-stranded design delivers balanced coverage of methylation events for unparalleled results

Bisulfite treatment brings a series of challenges to variant analysis. The SeqCap Epi Enrichment System can overcome these challenges by utilizing a design algorithm that creates probes against the possible methylation configurations on both strands from a bisulfite converted genomic template. This is made possible through Roche NimbleGen's ability to manufacture millions of DNA probes in parallel.

Such designs enable you to capture bisulfite treated DNA and get sequencing and methylation pattern for both strands, something no other technology yet can do (Table 3). This will allow for better determination of SNPs vs. methylation status events, plus preservation of sequencing data where one strand may not have sufficient coverage (Figure 3). The SeqCap Epi product was more uniform, had better coverage statistics, and assayed approximately 440,000 more CpGs (Table 2).

As shown on the cover page, in the SeqCap Epi protocol, the bisulfite conversion is conducted before the capture step. This is a more efficient workflow to conserve molecular complexity of your epigenome, ultimately allowing you to:

- Detect strand-specific methylation and interrogate more CpG's than other enrichment platforms
- Start with a lower sample input amount
- Create your own custom designs

Coverage Comparison of IMR90 Cell Line

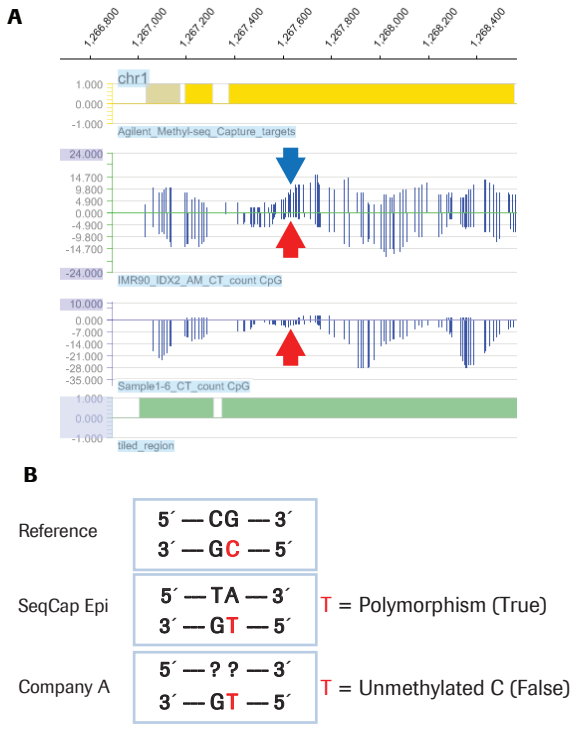
Platform	SeqCap Epi Choice	Company A Enrichment System
Primary Capture Target Size	83.9 Mb	84 Mb
Median Depth of Coverage	29	34
Target Covered >1X (%)	99.228	98.133
Target Covered >10X (%)	90.322	86.696
Uniformity (Fold 80 Penalty)	2.03	3.07
Total CpG's Assayed	3,438,562	3,028,258

▲ **Table 2: Comparison of a SeqCap Epi Choice Design created to capture all targets covered by the alternate enrichment system.** An IMR90 cell line DNA was captured and sequenced on an Illumina HiSeq (2 x 100 bp reads), subsampled to 55 million reads.

Design comparison

Platform	Customizable	Target Size	DNA Input	Strand Coverage
SeqCap Epi CpGiant	No	80.5 Mb	1 µg	Both
SeqCap Epi Choice	Yes	up to 90 Mb	1 µg	Both
SeqCap Epi Developer	Yes	up to 210 Mb	1 µg	Both
Company A	No	84 Mb	3 µg	Single

▲ **Table 3: SeqCap Epi design metrics and comparison with an alternate enrichment system for methylation detection.**



▲ **Figure 3: Capturing both DNA strands for methylation analysis using the SeqCap Epi Enrichment System.** Sequence coverage over both DNA strands provides important advantages for analysis: A) In regions where one strand exhibits shallow coverage by both products (red arrows), the 83.9 Mb SeqCap Epi Choice design can still provide good coverage of the other strand (blue arrow); B) Distributing sequence coverage over both strands allows SeqCap Epi can to distinguish "**T**"s resulting from bisulfite conversion, from "**T**"s that are existing polymorphisms in the sample relative to the reference.

Upgrade your epigenetic research from microarray to targeted sequencing for broader discovery

Microarrays have been another popular platform for DNA methylation analysis. However, arrays have problems with incorporating genetic information like SNP detection, discovery of new loci, and providing allele specific patterns. What's more, array probes are susceptible to batch effects and can potentially cross-hybridize with non-targeted DNA, confounding results and requiring secondary confirmation.

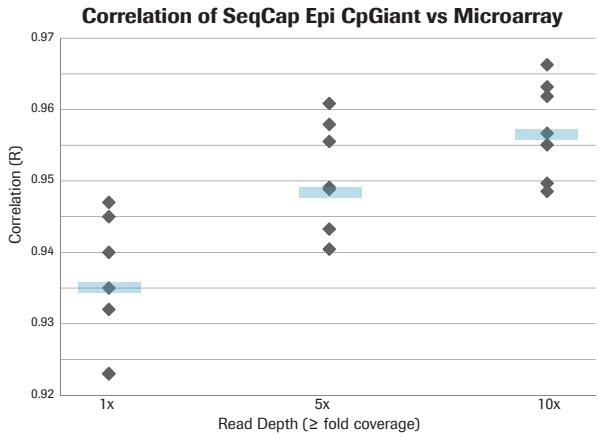
As a fixed epigenome-wide design, the SeqCap Epi CpGiant Enrichment Kit provides a new platform for discovery screening, without the array based drawbacks and with all the NGS based benefits (Table 4). CpGiant design targets more than 5.5 million CpG's, or 12x more CpG's than a commonly used microarray platform for DNA methylation detection (Table 4). Its coverage is highly correlated with the microarray data allowing for your NGS data to be easily compared with your existing array data sets (Figure 4).

SeqCap Epi CpGiant is also cost effective. Based on empirical coverage statistics, to achieve mean coverage of 30x across the entire target, a researcher could fit 4 samples in one lane of a HiSeq 2000 using 2 x 100bp reads (Figure 5).

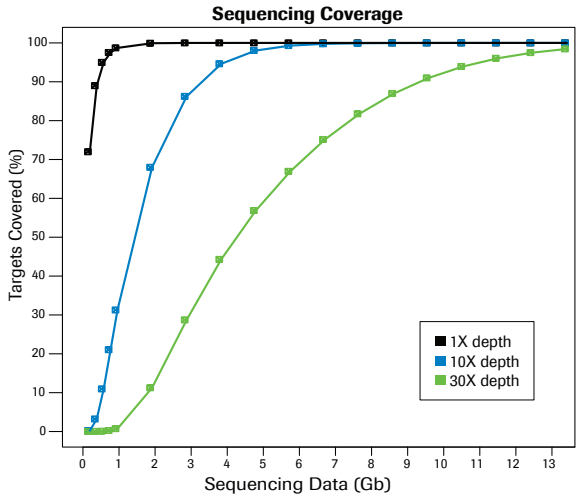
Product comparison

Platform	SeqCap Epi CpGiant	Company B microarray
CpG's Targeted	> 5.5 Million	485,512
Discern SNPs from Methylation?	Yes	N/A
Discover Novel CpG's?	Yes	N/A
Providing Allelic Specificity?	Yes	N/A
Scalable	Process up to 96 samples at once	12 samples per array

▲ **Table 4: SeqCap Epi CpGiant comparison against a commonly used microarray platform.**



▲ **Figure 4: Same site CpG methylation is strongly correlated between the 80.5 Mb SeqCap Epi CpGiant design and microarray data.** DNA methylation status was measured at CpG sites in seven buccal DNA samples using a microarray and the SeqCap Epi CpGiant design, at 500 ng sample DNA input. The mean value is indicated by the blue bars. Increased read coverage depth filtering for the SeqCap Epi CpGiant data results in improved correlations.



▲ **Figure 5: Coverage per base comparison across three sequencing depths.** The 80.5 Mb SeqCap Epi CpGiant design was sequenced using Illumina 2x100 bp reads, subsampled to calculate gigabases of sequencing needed per sample for 1x, 10x and 30x mean coverage depths. Based on these results 4 samples could be sequenced in one lane of a HiSeq2000 to achieve 30x mean coverage.

Target any region of interest in any genome with SeqCap Epi Choice and Developer Kits

Sequencing specific genomic regions of interest is vital to advancing our knowledge of epigenetic variation. Targeted bisulfite sequencing helps accelerate epigenetic discovery by allowing for higher depths of coverage, increased sample throughput, and lower costs compared to WGBS.

For example, with the same amount of sequencing, a 3 Mb human target design gives you the opportunity to achieve up to 1,000 fold of enrichment, or up to 1,000 times of sample throughput compared to WGBS. Targeted bisulfite sequencing leverages the depth of coverage available from next-gen sequencing, providing a solution that was previously impractical in DNA methylation occupancy estimations.

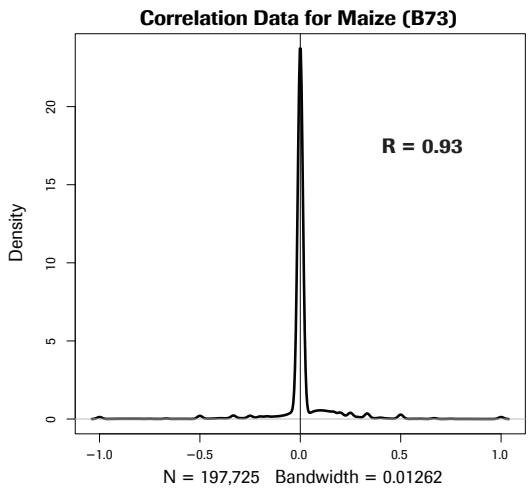
SeqCap Epi Choice Enrichment Kits are designed for human targeted bisulfite sequencing studies, for regions of interest up to 90 Mb. SeqCap Epi Developer Enrichment Kits offer a solution to study any organism, or any human genomic region larger than 90 Mb and up to 210 Mb.

Targeted bisulfite sequencing with the SeqCap Epi Choice kits enables discovery in human genome regions not covered by other enrichment products (Figure 6). SeqCap Epi Developer is also the first product enabling targeted bisulfite sequencing in non-human species, with excellent correlation with WGBS (Figure 7).

Available Custom Products

Product	Size (Mb)
SeqCap Epi Choice S Enrichment Kit	Up to 30 Mb
SeqCap Epi Choice M Enrichment Kit	30 - 60 Mb
SeqCap Epi Choice L Enrichment Kit	60 - 90 Mb
SeqCap Epi Developer S Enrichment Kit	Up to 30 Mb
SeqCap Epi Developer M Enrichment Kit	30 - 60 Mb
SeqCap Epi Developer L Enrichment Kit	60 - 90 Mb
SeqCap Epi Developer XL Enrichment Kit	90 - 210 Mb

▲ **Figure 6: The SeqCap Epi Choice Kit's custom options allow you to study your regions for focused research.** A 3.2 Mb SeqCap Epi Choice design was created to cover known DMRs. Illustrated is a region not covered by the alternative target enrichment product, which is representative of many regions with limited coverage.



▲ **Figure 7: This 4.9 Mb SeqCap Epi Developer design shows high correlation with Whole Genome Bisulfite Sequencing (WGBS) results for Maize.** Density plots of differences in methylation value measurements relative to WGBS data are shown for DNA isolated from the B73 strain of Maize. The left-right symmetry of the plot indicates little systematic bias in % methylation measurements compared to the WGBS data. The correlation between platforms is: R = 0.93

Maximize your workflow with reagents optimized for your research

The SeqCap Epi Enrichment System is available with a complete set of reagents for your target enrichment workflow. Optimized reagents are maximized to work for your targeted bisulfite sequencing with Roche NimbleGen.

All-in-One Kits:

- **SeqCap Epi Reagent Kit**
  SeqCap HE-Oligo Kit A, SeqCap Epi Accessory Kit, and SeqCap EZ Hybridization and Wash Kit
- **SeqCap Epi Reagent Kit Plus**
  SeqCap HE-Oligo Kit A, SeqCap Epi Accessory Kit, SeqCap EZ Hybridization and Wash Kit, plus SeqCap EZ Pure Capture Bead Kit

Individual Kits:

- **KAPA Library Preparation Kits**
  Reagents for library preparation
- **SeqCap Adapter Kit A**
  Library adapters for 1-12 samples
- **SeqCap Adapter Kit B**
  Library adapters for for 13-24 samples
- **SeqCap HE-Oligo Kit A**
  Barcode oligos for 1-12 samples
- **SeqCap HE-Oligo Kit B**
  Barcode oligos for 13-24 samples
- **SeqCap Epi Accessory Kit**
  Reagents for processing SeqCap Epi Enrichment System; includes bisulfite capture enhancer, bisulfite conversion control and PCR reagents
- **SeqCap EZ Hybridization and Wash Kit**
  Reagents for hybridization and washing

Ordering Information

SeqCap Epi CpGiant Enrichment Kits

Product	Cat. No.	Pack Size
SeqCap Epi CpGiant Enrichment Kit	07 138 881 001	4 reactions
	07 138 911 001	48 reactions
	07 138 920 001	384 reactions

SeqCap Epi Choice Enrichment Kits

Product	Cat. No.	Pack Size
SeqCap Epi Choice S Enrichment Kit *Target up to 30 Mb of genomic regions*	07 138 938 001	12 reactions
	07 138 946 001	48 reactions
	07 138 954 001	384 reactions
SeqCap Epi Choice M Enrichment Kit *Target 30 - 60 Mb of genomic regions*	07 138 962 001	12 reactions
	07 138 989 001	48 reactions
	07 138 997 001	384 reactions
SeqCap Epi Choice L Enrichment Kit *Target 60 - 90 Mb of genomic regions*	07 139 004 001	12 reactions
	07 139 012 001	48 reactions
	07 139 039 001	384 reactions

SeqCap Epi Developer Enrichment Kits

Product	Cat. No.	Pack Size
SeqCap Epi Developer S Enrichment Kit *Target up to 30 Mb of genomic regions*	07 139 071 001	12 reactions
	07 139 080 001	48 reactions
	07 139 098 001	384 reactions
SeqCap Epi Developer M Enrichment Kit *Target 30 - 60 Mb of genomic regions*	07 139 101 001	12 reactions
	07 139 128 001	48 reactions
	07 139 578 001	384 reactions
SeqCap Epi Developer L Enrichment Kit *Target 60 - 90 Mb of genomic regions*	07 139 586 001	12 reactions
	07 139 594 001	48 reactions
	07 139 608 001	384 reactions
SeqCap Epi Developer XL Enrichment Kit *Target 90 - 210 Mb of genomic regions*	07 139 624 001	12 reactions
	07 139 659 001	48 reactions
	07 139 667 001	384 reactions

Individual Kits and Reagents

Product	Cat. No.	Pack Size
KAPA Library Preparation Kit	07 137 923 001	10 reactions
	07 137 974 001	50 reactions
KAPA High-Throughput Library Preparation Kit	07 138 008 001	96 reactions
SeqCap Adapter Kit A	07 141 530 001	96 reactions
SeqCap Adapter Kit B	07 141 548 001	96 reactions
SeqCap HE-Oligo Kit A	06 777 287 001	96 reactions
SeqCap HE-Oligo Kit B	06 777 317 001	96 reactions
SeqCap Epi Accessory Kit	07 145 519 001	24 reactions
	07 185 707 001	96 reactions
SeqCap EZ Pure Capture Bead Kit	06 977 952 001	24 reactions
SeqCap EZ Hybridization and Wash Kit	05 634 261 001	24 reactions
	05 634 253 001	96 reactions

Complete Sets of Kits and Reagents

Product	Cat. No.	Pack Size
SeqCap Epi Reagent Kit	07 185 715 001	24 reactions
Includes SeqCap HE-Oligo Kit A, SeqCap Epi Accessory Kit, SeqCap EZ Hybridization and Wash Kit		
SeqCap Epi Reagent Kit Plus	07 185 723 001	24 reactions
Includes SeqCap EZ HE-Oligo Kit A, SeqCap EZ Accessory Kit, SeqCap EZ Hybridization and Wash Kit, plus SeqCap EZ Pure Capture Bead Kit		

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