

## SeqCap EZ Target Enrichment System

Discover more, sequence less





# Targeted sequencing enables genetic discovery

In life science research, understanding the human genome and the diseases associated with genetic mutations can be critical. The deeper our understanding of genetic diseases becomes, the more biological complexity is revealed.

Research has shown the majority of disease-causing mutations can occur in all coding exons in the genome or in other disease-associated regions. Identifying and targeting these specific genomic regions of interest are key to advancing our knowledge of disease and the underlying mutations present in the genome. Researchers use targeted sequencing to investigate causative mutations in disease associated regions, novel mutations present in Mendelian disease, and detecting coding variants in the exome. Targeted sequencing enables user specified enrichment to identify and 'capture' only the precise regions of interest that are main genetic drivers for disease.

## Three common approaches for targeted sequencing are:

- Sequencing all coding exons (the exome)
- Sequencing disease associated regions
- Sequencing candidate genes from disease pathways

All three approaches can be accomplished using our SeqCap EZ Probes.

#### Numerous SeqCap EZ Probe options are available, depending on your organism of choice and genomic regions of interest:

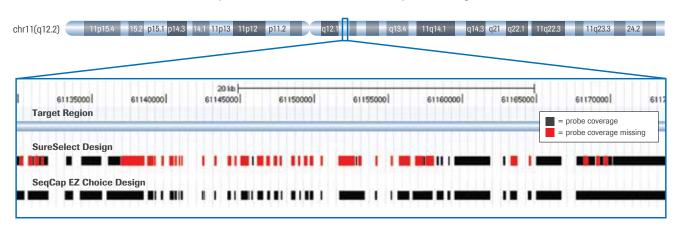
- SeqCap EZ Exome Probes enable enrichment of the whole human exome
- SeqCap EZ Choice Probes offer enrichment of specific target regions in the human genome
- SeqCap EZ Developer Probes provide enrichment of non-human genomes
- SeqCap EZ Designs offer a selection of human disease research panels and specific exomes for model organisms

SeqCap EZ Probes enable the enrichment of selected genomic regions from full complexity human genomic DNA in a single step. Developed to eliminate the necessity of setting up thousands of PCR reactions, Sequence Capture allows for parallel enrichment of target regions in a single experiment.



## Custom tools to fit your research SeqCap EZ Choice and SeqCap EZ Developer probes

#### Superior enrichment starts with superior design



SeqCap EZ Choice Probes provide superior design coverage over Agilent SureSelect. Design comparison between SeqCap EZ Choice Probes and SureSelect for a 500 Kb target on chr11 (61-61.5 Mb). SeqCap EZ Choice Probes target 20% more of the target region (372 Kb vs. 222 Kb), as illustrated by increased black areas, denoting more coverage of the target regions. Note: The SeqCap EZ Choice design was created using Roche standard design parameters; the SureSelect design was created using Agilent eArray online design tool using default design parameters.

Researchers can create their own target enrichment probe pools with Roche's custom option by utilizing SeqCap EZ Choice Probes for human targeted resequencing studies and SeqCap EZ Developer Probes for non-human targeted studies."

Good enrichment starts with good custom probe design. The Roche design algorithm selects probes where others cannot, providing you more coverage of the target area to better enable your variant discovery research.

Available Custom Design Products		
Choice	Size (Mb)	
SeqCap EZ Choice Probes	up to 7 Mb	
SeqCap EZ Choice XL Probes	>7-200 Mb	
Comprehensive Cancer Design	4 Mb	
Neurology Panel Design	1.5 Mb	
Developer	Size (Mb)	
SeqCap EZ Developer Probes	variable	
Soy Exome	85 Mb	
Mouse Exome	54 Mb	

Available custom design products and coverage sizes. SeqCap EZ Choice and Developer products are available in a wide variety of product offerings.



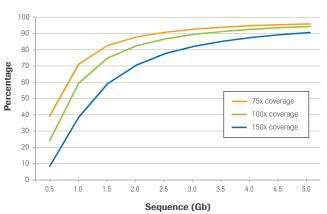
# Designed for multiplex capabilities: Target enrichment in one tube

Discover a selection of SeqCap EZ Choice and Developer probe designs available for your review, at http://sequencing.roche.com. Learn more about our Comprehensive Cancer Design, Inherited Disease Panel, VirCapSeq-VERT, and more. These are custom target enrichment designs developed in collaboration with researchers like you. Built upon our empirically optimized probe design algorithm and high efficiency uniformity of capture, SeqCap EZ Designs offer maximum performance for focused capture pools. Capitalize on our experience in developing custom design panels and discover the Roche difference.

Performance metrics of the Comprehensive Cancer Design (right), a design from the SeqCap EZ Choice probes, illustrate the percent of target bases covered at specific coverage depths as the total amount of sequencing increases which demonstrates:

- On a per sample basis only 4.5 Gb sequencing is needed to obtain 150x coverage for 90% of targeted bases
- Extensively less sequencing is needed to obtain 75x and 100x coverage over 90% of targeted bases
- Minimize sequencing costs through efficient multiplexing.
  Using an Illumina HiSeq or MiSeq instrument, multiple samples per lane are possible depending on the coverage depth required

#### Coverage per Base



#### Coverage per base comparison across three sequencing depths.

The SeqCap EZ Choice-Comprehensive Cancer Design was sequenced using Illumina 2x76 bp reads, subsampled to calculate gigabases of sequencing needed per sample for 75x, 100x and 150x coverage depths.

Sample Multiplex Capabilities		
Coverage	Illumina HiSeq (34 Gb*)	Illumina MiSeq (4 Gb*)
75x	14 samples	2 samples
100x	12 samples	1 sample
150x	7 samples	

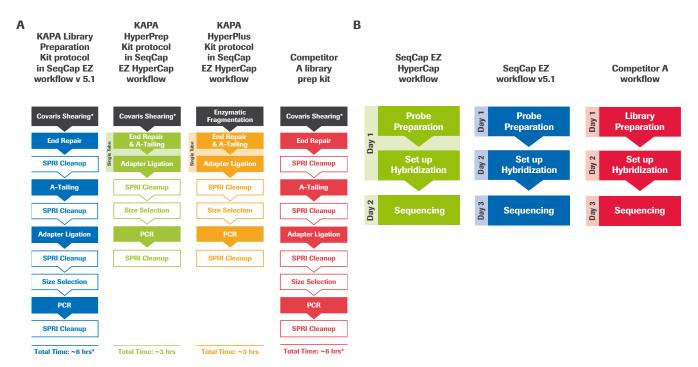
<sup>\*</sup>Estimates for sequencing throughput per lane are based on manufacturer's specifications.

Sample throughput multiplexing. This example illustrates the 75x, 100x, and 150x coverage rates with 90% bases covered using Illumina 2x76 bp reads on either a HiSeq or MiSeq instrument. Sample multiplexing estimates derived from coverage model in above graph.

# SeqCap EZ HyperCap: One enrichment workflow for all sample types and starting DNA amounts

The SeqCap EZ HyperCap workflow integrates KAPA HyperPrep and HyperPlus kits with an optimized SeqCap EZ target enrichment protocol, enabling a complete sample prep solution that can be tailored to different applications, sample types, and starting DNA amounts. This workflow reduces hands-on and overall time, user intervention, and cost, all while maintaining excellent sequence data quality.

Reduced workflow time to 1.5 days - from extracted DNA to sequencing-ready libraries.



(A) Workflow of various library preparation kits. KAPA HyperPrep and HyperPlus kits reduce library construction time by half. \*Covaris shearing time is dependent on instrument, make, model and batch size. Time estimate for the KAPA Library Preparation Kit and KAPA HyperPrep Kit do not include Covaris shearing. (B) Workflow of the SeqCap EZ target enrichment protocols.

### SeqCap EZ Exome Target Enrichment probes: Tools to fit your exome research

The human exome is critical to our genetic make-up and is widely accepted by researchers as having the greatest influence on how our genetic blueprint is utilized. The exome is defined as all coding exons in the genome and is comprised of the most functionally relevant and best understood 1% of the human genome. SeqCap EZ Exome Probes are available in numerous configurations to suit your needs.

#### **SeqCap EZ MedExome Probes**

- Exome with enhanced coverage of focus of medically relevant genes
- 47 Mb

#### **SeqCap EZ MedExome Plus Probes**

- Customizable version of MedExome
- Up to 200 Mb of custom content

#### **SeqCap EZ Exome Probes v3.0**

- Comprehensive research exome
- 64 Mb

#### SeqCap EZ Exome + UTR Probes

- Addition of UTR content to SeqCap EZ Exome v3.0
- 96 Mb

#### **SeqCap EZ Exome Plus Probes**

- Customizable version of SeqCap EZ Exome v3.0
- Up to 114 Mb

#### **HGSC VCRome**

- Exome co-developed with Baylor HGSC
- 37 Mb

#### **UTR** only

- Enriches UTR only.
  Add to existing exome data
- 50 Mb

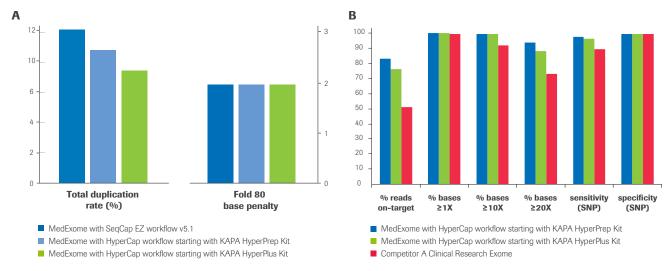
#### **Inherited Disease Panel**

- Panel of >4100 genes
- 11 Mb



## Versatile performance for maximum flexibility

- High uniformity and lower duplication rates
- Improved coverage performance over the SegCap EZ workflow v5.1
- Excellent enrichment metrics



(A) High uniformity and lower duplication rates with the SeqCap EZ HyperCap workflow. Results of testing with SeqCap EZ MedExome Probes and the Comprehensive Cancer design showed no impact to uniformity as measured by Fold 80 Base Penalty which is the fold sequencing required to bring 80% of the targeted bases to the mean. High uniformity, as indicated by a low fold 80 value, decreases the cost of sequencing and increases sensitivity for variant detection, which is a key performance indicator of enrichment. Both KAPA HyperPrep and HyperPlus kits provide lower duplication rates than the standard KAPA Library Preparation Kit, demonstrating its ability to retain molecular complexity of samples resulting in better performance metrics. (B) HyperCap workflow leads to excellent enrichment metrics. With KAPA HyperPrep and HyperPlus kits, SeqCap EZ MedExome Probes demonstrated the same excellent performance as with SeqCap EZ v5.1 workflow, providing better data quality than competitor As exome. Competitor As Clinical Research Exome (CRE) data was generated by a 3rd party service provider using 3 µg of Coriell NA 12891 DNA processed using XT kit following vendor recommendations. Data was subsampled to 60 million reads, and analyzed using the standard Roche informatics pipeline.

#### **Exon Coverage by SeqCap EZ MedExome**

Annotation Sources	Exon Coverage
CCDS	99.8%
ClinVar	99.9%
Ensembl (CDS)	99.7%
GENCODE (CDS)	99.6%
miRBase	98.7%
OMIM (disease linked)	99.7%
RefSeq (CDS)	99.8%
VEGA (CDS)	99.7%

"We are extremely pleased with the capabilities and efficiencies the Roche 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, PhD Director, Human Genome Sequencing Center Baylor College of Medicine

## SeqCap EZ Probes Options to fit your research

#### **Ordering information**

#### SeqCap EZ Exome Probes<sup>1</sup>

Roche Cat. No.	Description	Pack Size
06 465 684 001	SeqCap EZ Exome Probes v 3.0	4 rxn
06 465 692 001	64 Mb coding exons and miRNA exons	48 rxn
06 740 294 001	SeqCap EZ Exome +UTR Probes	4 rxn
06 740 308 001	64 Mb coding exons + 32 Mb UTRs	48 rxn
06 740 189 001	SegCap EZ Exome Plus Probes	12 rxn
06 740 235 001	64 Mb coding exons, plus add up to 200 Mb	48 rxn
06 740 243 001	custom capture targets	96 rxn
06 465 587 001	SeqCap EZ HGSC VCRome	12 rxn
06 465 668 001	Designed by scientists at Baylor College of Medicine, this panel covers 23,585 genes and 189,028 non-overlapping	48 rxn
06 465 676 001	exons at a total design size of 45.1 Mb capture target	96 rxn
Available Designs <sup>3</sup>	Target Regions	Reference #
SeqCap EZ Exome v 2.0	SeqCap EZ Exome v 2.0 44.1 Mb	4000016420

#### **SeqCap EZ MedExome Probes**

Roche Cat. No.	Description	Pack Size
07 676 581 001	0.0.14.15	4 rxn
07 681 330 001	SeqCap MedExome Probes	48 rxn
07 681 348 001		384 rxn
07 681 364 001	SeqCap EZ MedExome PLUS Probes	48 rxn

SeqCap EZ Developer Probes		
Roche Cat. No.	Description	Pack Size
06 740 278 001		4 rxn
06 471 684 001		12 rxn
06 471 706 001	SeqCap EZ Developer Probes	24 rxn
06 471 714 001	Target up to 200 Mb of genomic regions, contiguous or noncontiguous, for any	48 rxn
06 471 722 001	sequenced non-human genome	96 rxn
06 471 749 001		384 rxn
06 471 757 001		960 rxn
Available Designs³	Target Regions	Reference #
VirCapSeq-VERT	73 Mb	4000020060
Mouse Exome	54 Mb	9999042611
Soy Exome	85 Mb	4000007450
Mitochondrial Genome	16 kb	4000021490
Switchgrass Exome	50 Mb	4000021240
Maize Exome	110 Mb	4000020170
Barley Exome	88.6 Mb	contact your sales rep for more info
Wheat Exome	106.9 Mb	contact your sales rep for more info
Canine Exome	152 Mb	4000022470
Pig Exome	58.1 Mb	4000017630

#### **Ordering information**

#### **SeqCap EZ Choice Probes**

Roche Cat. No.	Description	Pack Size
06 740 251 001	SeqCap EZ Choice Probes Target 100 kb - 7 Mb human (HG18 or HG19) genomic regions	4 rxn
06 266 282 001		12 rxn
06 266 304 001		24 rxn
06 266 312 001		48 rxn
06 266 339 001		96 rxn
06 266 347 001		384 rxn
06 266 355 001		960 rxn
Available Designs <sup>3</sup>	Target Regions	Reference #
0 I : 0 D :	/ NAI	1000007000

Comprehensive Cancer Design	4 Mb	4000007080
Neurology Panel Design	1.5 Mb	4000007090
Human MHC	4.97 Mb	4000024240

#### **SeqCap EZ Choice XL Probes**

Roche Cat. No.	Description	Pack Size
06 740 260 001		4 rxn
06 266 363 001		12 rxn
06 266 371 001	SeqCap EZ Choice XL Probes	24 rxn
06 266 380 001	Target 7 Mb - 50 Mb human (HG18 or HG19) genomic regions	48 rxn
06 266 398 001		96 rxn
06 266 401 001		384 rxn
Available Designs³	Target Regions	Reference #
Inherited Disease Panel	11.8 Mb	4000024240
50 Mb Human UTR Design	50 Mb	400007080

### SeqCap EZ Reagents

#### **Ordering information**

#### **Probes and Reagents**

Roche Cat. No.	Description	Kit Size
06 953 212 001	SeqCap EZ Reagent Kit v2	24 rxn
06 953 247 001	SeqCap EZ Reagent Kit v2 Plus	24 rxn
06 777 287 001	SeqCap EZ HE-Oligo Kit A	96 rxn
06 777 317 001	SeqCap EZ HE-Oligo Kit B	96 rxn
07 145 594 001	SegCap EZ Accessory Kit v2	24 rxn
06 776 345 001	Sequal LZ Accessory Nit vz	96 rxn
06 977 952 001	SeqCap EZ Pure Capture Bead Probes	24 rxn
05 634 261 001	SegCap EZ Hybridization and Wash Kit	24 rxn
05 634 253 001	Sequal LZ Hybridization and Wash Nit	96 rxn
06 684 335 001	SeqCap EZ Developer Reagent	1 mL
07 141 530 001	SeqCap Adapter Kit A	96 rxn
07 141 548 001	SeqCap Adapter Kit B	96 rxn

<sup>1.</sup> SeqCap EZ Exome Probes are available in human genome build HG19.

Published by:

#### Roche Sequencing Solutions, Inc.

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#### sequencing.roche.com

SeqCap EZ Designs offers a UTR only design (see ordering information under SeqCap EZ Choice XL Probes).
 SeqCap EZ Designs offer specific panels for focused research. Use the reference number to specify the design you are ordering. More information is available on our website: http://sequencing.roche.com/products/nimblegen-seqcap-target-enrichment.html