Roche

Next-Generation Sequencing

Targeted Sequencing

Shared Design Panel

ANTEC Bioscience Inc.

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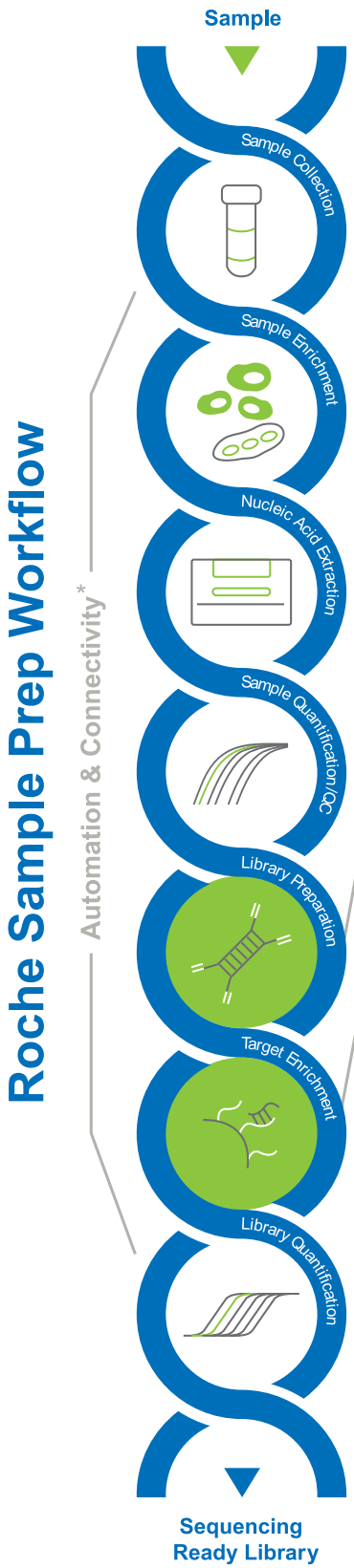


Library Preparation

Shared Design Panel

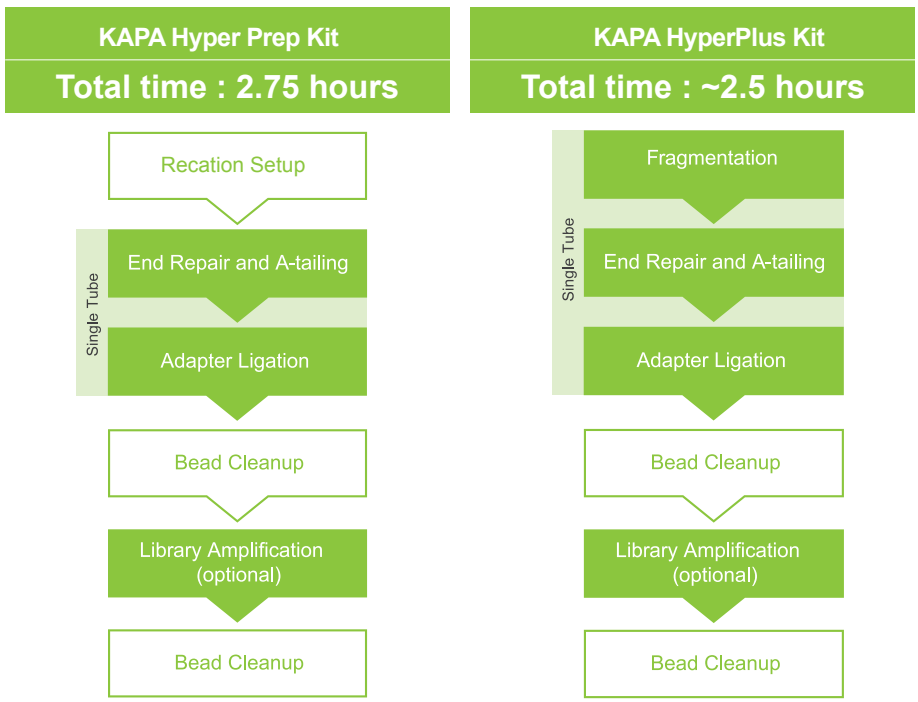
KAPA Hyper Prep Kit & HyperPlus kit

創新單管設計，搭配優化的配方以及經由 "directed evolution" 技術篩選過的酵素，能夠使 adapter-ligated library 獲得高產率並且降低 amplification bias。特別是針對 FFPE 與 low-input 樣本可以獲得更高的 library 多樣性、降低 duplication rate 與更均勻的覆蓋率

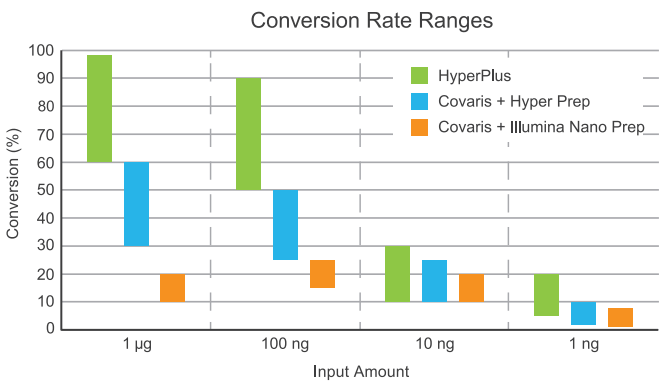
快速、簡便的流程，輕鬆完成 library 構築

- **One-tube** 流程縮短操作時間
- **PCR-free** 流程 **<2** 小時，含 **PCR** 的流程 **<3** 小時即可完成
- 更少的處理步驟可改善一致性與再現性

置備流程



不同廠牌的 conversion rate 比較



KAPA HyperPlus Kit 在 high-input DNA 以及 low-input DNA 均可獲得較高的 conversion rate

conversion rate 是指有多少百分比的 input DNA 轉化成可定序的 library。adapter-ligated library 是 構築 library 的重要指標，其影響 library 的多樣性與品質

Human Genetics

SeqCap EZ Prime Exome Design

- Organism : Human
- Genome Build : HG38
- Specifications/Details : SeqCap EZ Prime Exome has enhanced uniformity and offers comprehensive exome coverage with low sequencing requirements. The SeqCap EZ Prime Exome is tiled with probes that cover 98.1% of the coding regions from the CCDS Release 20 (Sept. 8, 2016) resulting in a 37 Mb capture target size. It also provides coverage of 340 sample-tracking SNPs found in numerous commercial kits, to facilitate sample identification throughout sample processing.

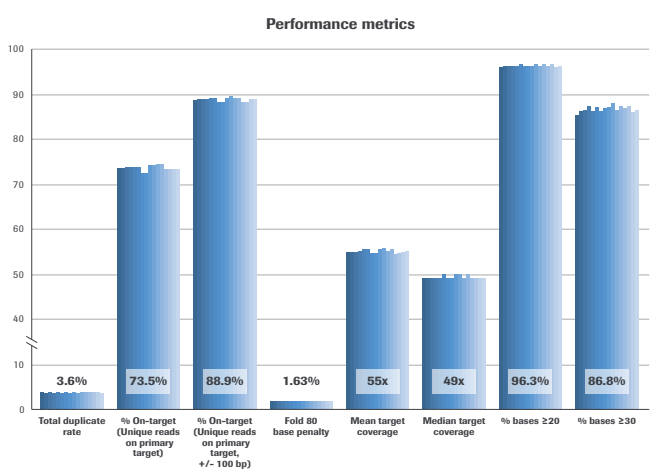
Inherited Disease Panel

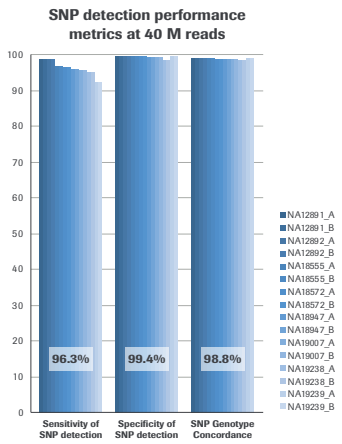
- Genome Build : HG38
- Collaborator : Dr. Jason Park
- Specifications/Details : The SeqCap EZ Inherited Disease Panel covers over 4100 genes and regions associated with inherited disease and was designed in by scientists at Roche Sequencing and Dr. Jason Park. Total design size (hg38) is 9.0 Mb primary target and 11.8 Mb capture target.

Human MHC Design

- SeqCap EZ Design : Human MHC Design
- Organism : Human
- Genome Build : HG19
- Collaborator : BGI
- Specifications/Details : Targets the MHC region (3.37Mb), 1.6Mb of regions surrounding the MHC, and 8 known haplotypes with a total design of 4.97Mb.

SeqCap EZ Prime Exome exhibits high on-target rates with greater coverage depth



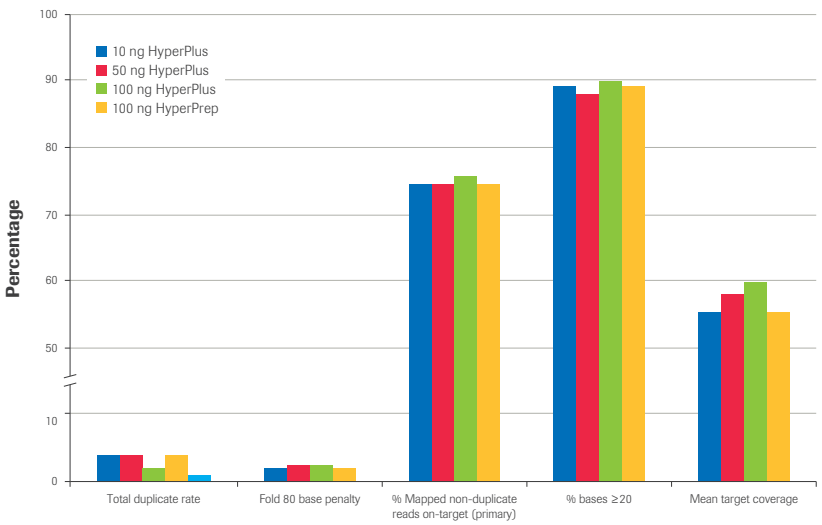


Oncology Research

Human Oncology Design

- Organism : Human
- Genome Build : HG38
- Design Files : Human Oncology Design
- Demo Data: available, please contact your local Roche office
- Specifications/Details : The SeqCap EZ Human Oncology Panel covers 981 genes with a primary target size of 2.75Mb. It was designed using the following sources:
- Publications/Information
  - ∗ Cancer Gene Census (Sanger) : http://www.sanger.ac.uk/genetics/CGP/Census
  - ∗ NCBI Gene Tests : http://www.ncbi.nlm.nih.gov/sites/GeneTests
  - ∗ ClinVar Cancer Genes : https://www.ncbi.nlm.nih.gov/clinvar
  - ∗ TARGET db : http://archive.broadinstitute.org/cancer/cga/target
  - ∗ Cancer Literature (PMID:26392535; PMID:25109877)
  - ∗ Customer Input
  - ∗ CDS gene coordinates obtained from Ensembl http://ensembl.org, RefSeq https://www.ncbi.nlm.nih.gov/refseq, CCDS https://www.ncbi.nlm.nih.gov/CCDS

SeqCap EZ Human Oncology Panel Sequencing Metrics

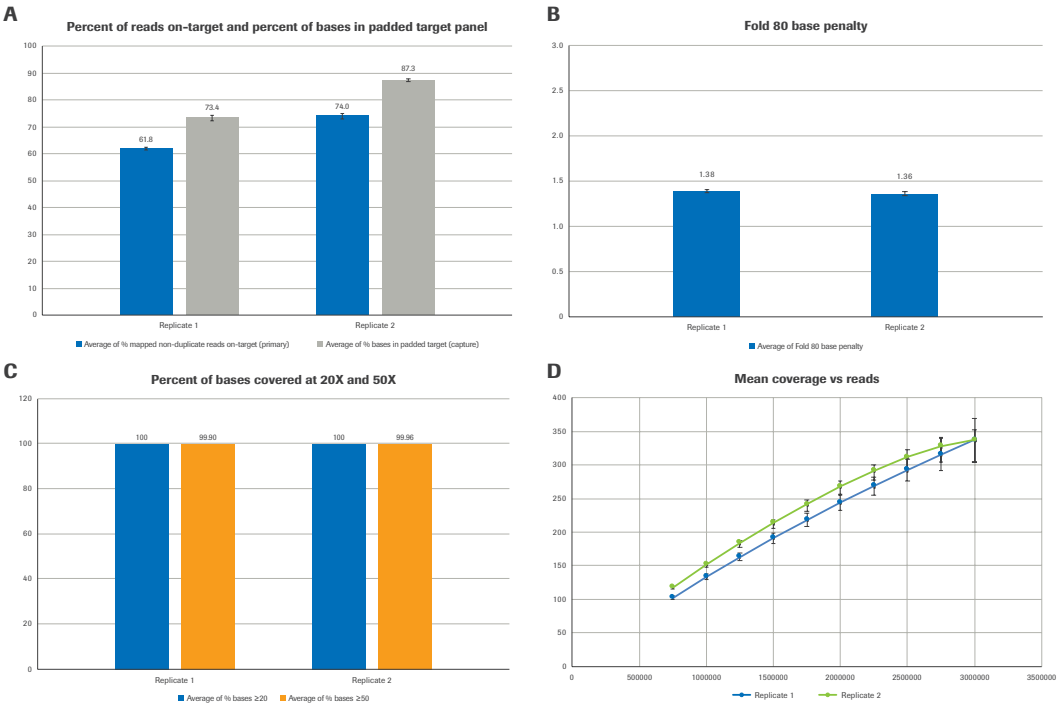


Consistent high performance across varying DNA input amounts.
With input amounts as low as 10 ng, the SeqCap EZ Human Oncology Panel exhibited consistently low duplication rates, high on-target rates, and excellent coverage depth. The experiment was conducted using 10 ng, 50 ng, and 100 ng Coriell samples with the KAPA HyperPlus and HyperPrep Kits. This design is compatible with the new streamlined HyperCap Workflow v2.0.

Cardiology Research

Cardiomyopathy Design

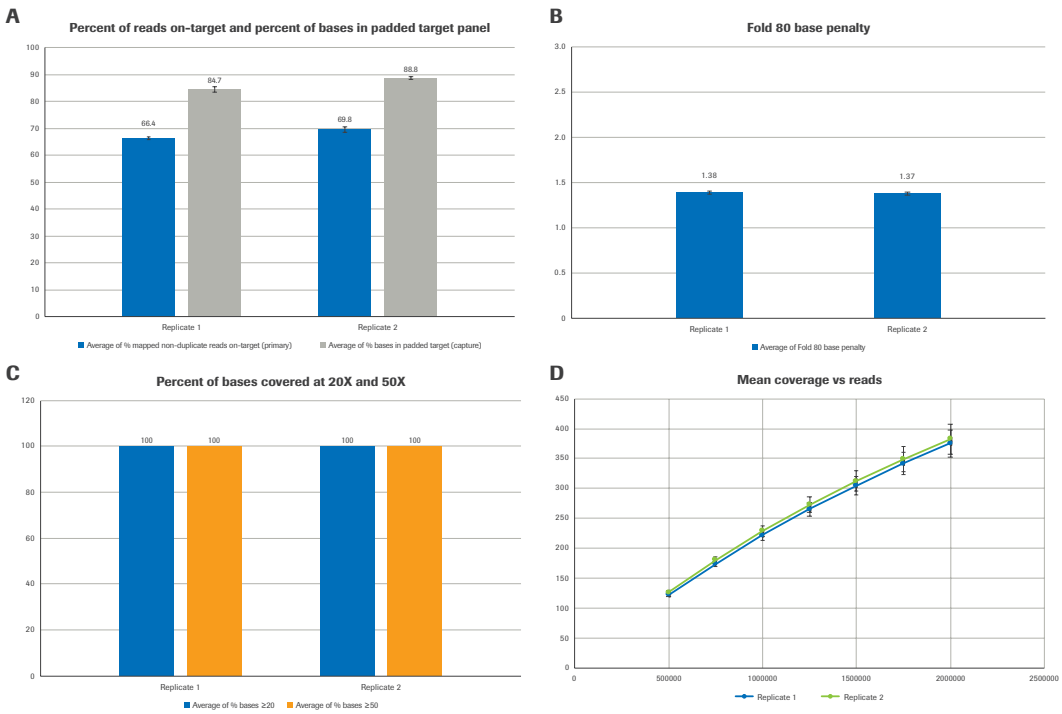
- Organism : Human
- Genome Build : HG38
- Specifications/Details : The SeqCap EZ Cardiomyopathy Panel covers 76 genes and regions associated with cardiovascular conditions with a primary target size of 372 kb. It was designed by scientists at Roche Sequencing with guidance from publications and experts.



Cardiology Research

Channelopathy and Arrhythmias Design

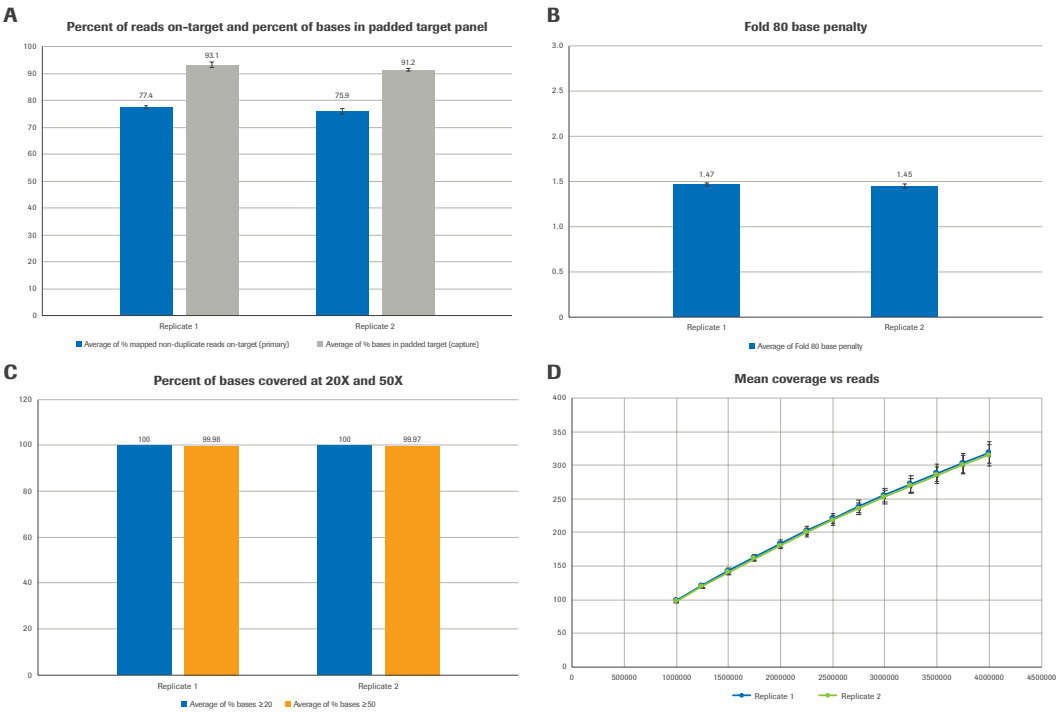
- Organism : Human
- Genome Build : HG38
- Specifications/Details : The SeqCap EZ Channelopathy and Arrhythmias Panel covers 54 genes and regions associated with cardiovascular conditions with a primary target size of 203 kb. It was designed by scientists at Roche Sequencing Solutions with guidance from publications and experts.



Cardiology Research

Sudden Cardiac Death Design

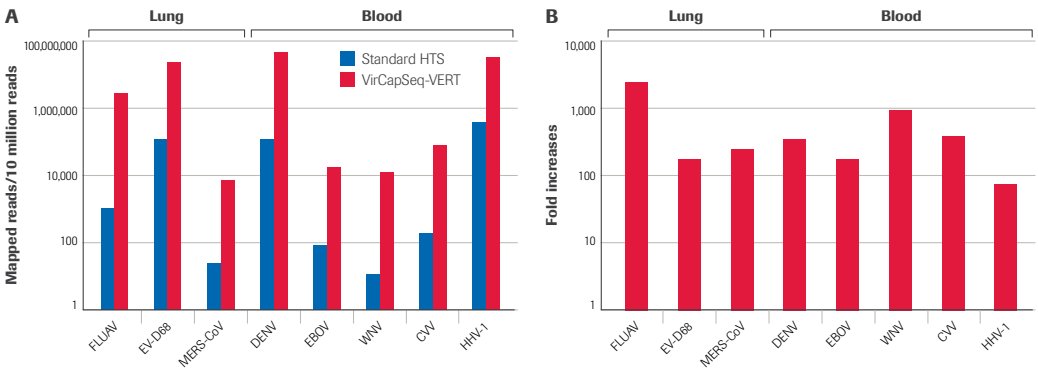
- Organism : Human
- Genome Build : HG38
- Specifications/Details : The SeqCap EZ Sudden Cardiac Death Panel covers 140 genes and regions associated with cardiovascular conditions with a primary target size of 610 kb. It was designed by scientists at Roche Sequencing Solutions with guidance from publications and experts.



Virology

VirCapSeq-VERT Design

- Organism : Vertebrate Infecting Viruses
- Genome Build : EMBL Coding Domain Sequence Database release 122 (December 2014)
- Collaborator : Dr. Ian Lipkin
- Publication :
  Virome Capture Sequencing Enables Sensitive Viral Diagnosis and Comprehensive Virome Analysis
  mBio (2015), e01491-15
- Specifications/Details : The SeqCap EZ VirCapSeq-VERT Capture Panel covers the genomes of 207 viral taxa known to infect vertebrates (including humans) and enables detection of viral sequences in complex sample types. The panel was designed in collaboration by scientists at Roche Sequencing and Professor Ian Lipkin using a database of 401,716 representative sequences spanning all virus sequence records, excluding bacteriophages. A list of all virus genera known to infect vertebrates was generated from the master species list of the International Committee on Taxonomy of Viruses (ICTV; http://talk.ictvonline.org/files/ictv_documents/m/msl/5208.aspx).
  Through cross-referencing of protein IDs with NCBI taxonomy IDs, a set of 342,438 coding sequence records was identified for the selected virus genera.



VirCapSeq-VERT enhances the performance of high-throughput sequencing by increasing the number of mapped viral reads recovered from high-background specimens. Eight different viral NAs were quantitated by qPCR and used to spike a background of lung-derived (3 viruses) or blood-derived (5 viruses) NA extracts. Samples were split in two and processed by standard HTS (blue) or with VirCapSeq-VERT (red). FLUAV, influenza A virus; EVD-68, enterovirus D68; MERS-CoV, MERS coronavirus; DENV, dengue virus; EBOV, Ebola virus; WNV, West Nile virus; CVV, Cache Valley virus; HHV-1, human herpesvirus 1.

Metabolic Disease Research

Human Metabolic Disease Methylome Design

- SeqCap Epi Developer XL Design : 131010_HG19_EG_met_EPI
- Organism : Homo sapiens (human)
- Genome Build : GRCh37/hg19
- Collaborator : Dr. Elin Grundberg, McGill University and Genome Quebec Innovation Centre, Department of Human Genetics, McGill University, Montreal, Quebec, Canada
- Specifications/Details : The panel targets 156Mb of sequence spanning ~4.5 million unique CpGs (~9 million total CpGs) and ~3 million autosomal SNPs from dbSNP137 allowing researchers to do simultaneous epigenome- and genome wide association studies focusing on metabolic diseases and related traits.
  - ∗ The regions targeted by the panel aim to cover the functional methylome including:
    - → CpGs contained within low and unmethylated regions identified from large-scale whole-genome bisulfite sequencing data from human adipose tissue and associated with enhancers and promoters, respectively.
    - → Additional CpGs located within human adipocyte regulatory elements (H3K4me1 and H3K4me3 marking enhancers and promoters, respectively) profiled by the NIH Roadmap Epigenomics Mapping Consortium.
    - → All ~ 480,000 CpGs from the Illumina 450K array.
  - ∗ Of the ~3 million SNPs covered by the panel, the following variants are included:
    - → About 30,000 regions covering metabolic disease associated GWAS loci from the NHGRI GWAS catalog (9 January, 2014)
    - → About 250,000 highly-informative genome-wide tag SNPs found across diverse world populations from the Illumina HumanCore BeadChip.

Mitochondrial Genome Design

- SeqCap EZ Design : Mitochondrial Genome Design
- Organism : Human
- Genome Build : HG38
- Specifications/Details : This is a 16 Kb capture design that targets the entire mitochondrial genome. The design is based on hg 38 annotation source.
- Publications/Information
  - ∗ Tech Note : How To Target the Mitochondrial Genome with MedExome?

Gene Regulation

50 Mb Human UTR Design

- SeqCap EZ Design : 50 Mb Human UTR Design
- Organism : Human
- Genome Build : HG19
- Specifications/Details : UTRs are untranslated regions on the 5' and 3' side of genes. The 3' UTR contains regulatory elements that are involved in the control of expression of many genes. This design is a 50Mb capture library targeting these regions.

> 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

Model Organisms

Mouse Exome Design

- SeqCap EZ Design : Mouse Exome Design
- Organism : Mouse
- Genome Build : C57BL/6J, NCBI37, MM9
- Collaborator : Roche NimbleGen/Mouse Consortium
- Specifications/Details : The coding sequence selected for the mouse exome probe pool design includes 203,225 exonic regions, including microRNAs, and collectively comprises over 54.3 Mb of target sequence (C57BL/6J, NCBI37/mm9). The design was based on a unified, Mouse Genome Database-curated gene set, consisting of non-redundant gene predictions from the National Center for Biotechnology Information (NCBI), Ensembl and The Vertebrate Genome Annotation (VEGA) database. To manage the size of the probe pool and to avoid non-uniquely mappable regions, we excluded olfactory receptors and pseudogenes from the target sequence.In cases where an exon contained both UTR and coding sequence, the UTR sequence was included in the design.
- Publications/Information
  - ∗ Mutation discovery in mice by whole exome sequencing.
  - ∗ Massively parallel sequencing of the mouse exome to accurately identify rare, induced mutations : an immediate source for thousands of new mouse models

Canine Exome Design

- SeqCap EZ Design : Canine Exome
- Organism : Canis familiaris
- Genome Build : CanFam3.1
- Collaborators : Bart Broeckx, Christophe Hitte, Thomas Derrien, Jessica Nieuwerburgh, Dieter Deforce
- Specifications/Details : The Canine Exome design combines the exons of the CanFam 3.1 Ensembl annotation, more recently discovered protein coding exons and a variety of non-coding RNA regions (microRNAs, long non-coding RNAs and antisense transcripts), leading to a total size of approximately 152 Mb. This design originated from a collaboration between Ghent University, Université Rennes1, Broad Institute of MIT, Harvard University and Uppsala University. For additional information, see Broeckx et al. (2015).
- Publications/Information
  - ∗ Improved canine exome designs, featuring ncRNAs and increased coverage of protein coding genes
  - ∗ Development and performance of a targeted whole exome sequencing enrichment kit for the dog (Canis Familiaris Build 3.1)

Pig Exome Design

- SeqCap EZ Design : Roslin Pig Exome Design Version 1
- Organism : Sus scrofa (pig)
- Genome Build: Sscrofa10.2 with additional sequences from GenBank in provided file
- Collaborator : Mick Watson, Edinburgh Genomics, The Roslin Institute, University of Edinburgh
- Specifications/Details : The Ensembl gene annotations for the pig from release 71 were used as a starting point for the design, corresponding to assembly Sscrofa10.2 and the May 2012 genebuild (patched Oct 2012). The file Sus_scrofa.Sscrofa10.2.71.gtf.gz was downloaded from the Ensembl ftp site, and the lengths of non-overlapping genomic regions corresponding to exons of protein-coding genes were summed. Having found that the pig "exome" is smaller than that of human and mouse, ESTs from build 42 of UniGene Sus scrofa were mapped to the pig genome. The file Ssc.seq.uniq, representing the longest, best quality single sequence from each cluster, was downloaded from the NCBI FTP site, and used as input for NCBI BLASTN. High-scoring segment pairs (HSPs) at least 50 bp in length and >90% identical were chosen; HSPs that mapped more than 200 times in the genome filtered out; and overlapping HSPs merged. The resulting regions summed to 22.5Mb (mega-bases). These regions were merged with the Ensembl gene annotation using BEDTools. The final set of target genomic regions sums to 58.1 Mb.
- Publications/Information :
  ∗ Design and development of exome capture sequencing for the domestic pig (Sus scrofa)

Agriculture

Maize Exome Design

- SeqCap EZ Design : Maize Exome
- Organism: Maize
- Genome Build : Maize (B73 and Mo17)
- Collaborator : Dr. Patrick Schnable and Dr. Nathan Springer
- Specifications/Details : The Maize Exome design is a collaboration by Roche NimbleGen, Dr. Patrick Schnable (Iowa State University) and Dr. Nathan Springer (University of Minnesota). It is based on a comprehensive collection of 110 Mb of exome content derived from the B73 reference genome and expressed non-B73 sequences identified from the founder inbreds of the NAM population and teosinte via RNA-Seq.
- Publications/Information :
  - Webinar-Efficient SNP Discovery for Crop Genomes through Exome Sequencing
  - Extreme-phenotype genome-wide association study (XP-GWAS) : a method for identifying trait-associated variants by sequencing pools of individuals selected from a diversity panel.

Barley Exome Design

- SeqCap EZ Design : Barley Exome
- Organism : Barley
- Genome Build : Hordeum vulgare (various), see provided file barley_mapping_sequence.fa
- Collaborator : Wheat Barley Exome Consortium (WBEC)
- Specifications/Details : The Barley Exome design is a consortium derived design, based on the mRNA coding exome from multiple sources with a total design size of 88.6 Mb. The Wheat Barley Exome Consortium is a collaboration of researchers from the University of Liverpool, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), James Hutton Institute, Kansas State University, University of Minnesota, University of Saskatchewan, and BIOGEMMA.
- Publications/Information :
  - Barley whole exome capture: a tool for genomic research in the genus Hordeum and beyond
  - A physical, genetic and functional sequence assembly of the barley genome
  - Webinar-Efficient SNP Discovery for Crop Genomes through Exome Sequencing

Wheat Exome Design

- SeqCap EZ Design : Wheat Exome
- Organism : Wheat
- Genome Build : Triticum aestivum (various), see provided file wheat_mapping_sequence.fa
- Collaborator : Wheat Barley Exome Consortium (WBEC)
- Specifications/Details : The Wheat Exome design is a consortium derived design, comprised of 106.9 Mb of genomic DNA sequence from multiple wheat varieties.The Wheat Barley Exome Consortium is a collaboration of researchers from the University of Liverpool, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), James Hutton Institute, Kansas State University, University of Minnesota, University of Saskatchewan, and BIOGEMMA.
  - Publications/Information:
  - Webinar - Efficient SNP Discovery for Crop Genomes through Exome Sequencing

Switchgrass Exome Design

- SeqCap EZ Design : Switchgrass Exome
- Organism : Switchgrass
- Genome Build : Two sets of transcripts were used for probe design. The PlantGDB (Duvick et al., 2008) assembly of P. virgatum Sanger-derived expressed sequence tags (Release 181a; 120 524 unique transcripts) and a custom assembly of P. virgatum pyrosequencing-derived transcript sequences downloaded from NCBI (SRR064785-SRR064802).
- Collaborator: Shawn Kaeppler and Steph Chow, University of Wisconsin, Madison
- Specifications/Details: Of the annotated genes, the probe set represents a total of 50,038,805 bp composed of 168,961 exons in 44,873 genes in Release 0 of the AP13 reference genome.
- Publications :
  - Nucleotide polymorphism and copy number variant detection using exome capture and next-generation sequencing in the polyploid grass Panicum virgatum The Plant Journal, (2014), 79, 993-1008

Soy Exome Design

- SeqCap EZ Design : Soy Exome Design
- Organism : Soy
- Genome Build : Joint Genome Institute
- Collaborator : Roche NimbleGen
- Specifications/Details : The Soy Exome is an 85.3Mb design that targets 95.3% of the bases associated to the exon of 46,367 soy genes. We recommend the use of SeqCap EZ Developer Reagent (Cat. No.06684335001) for effective enrichment of the soybean exome.
- Publications/Information:
  - Soybean Exome Capture-Technical Note

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DNA Library Preparation

Target Enrichment

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