

Agilent SureSelect NGS panels

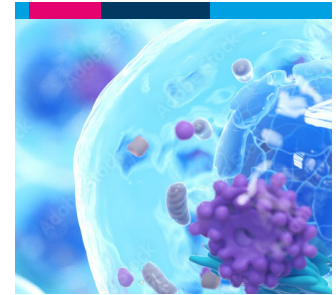
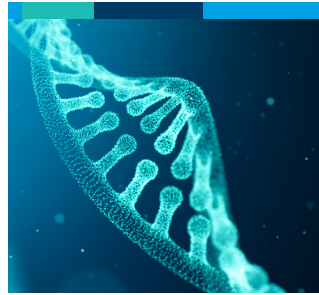
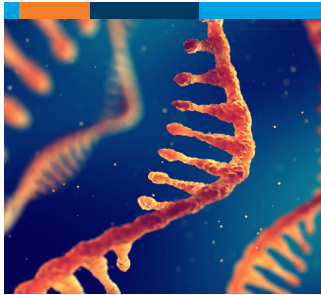
# Enriching Targets to Enhance Your NGS Data: Customized for Any Application

Application Compendium





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# Using Your Vision, Your Design, and Our Expertise to Address Evolving Research Needs

Since the inception of SureSelect custom probes in 2009, Agilent has supported researchers in their endeavors to deliver scientific advancements using our SureSelect next-generation sequencing (NGS) portfolio and comprehensive NGS solutions. The Agilent NGS workflow includes nucleic acid quality control (QC) instruments, SureSelect library preparation kits, SureSelect target enrichment catalog and custom panels, and automation with the Bravo NGS workstation and Magnis NGS prep systems. With these integrated components, our SureSelect panels have played a key role in work that has led to thousands of publications.

With Agilent SureDesign, our next-generation web design portal, you can leverage sophisticated probe design algorithms to develop your own panel in a matter of hours. In addition, with the new Agilent design and verification process, you can free up valuable lab resources by verifying your custom panel design before target enrichment probes are even shipped to you. Get the reliability and precision your research requires using Agilent SureSelect custom target enrichment panels, manufactured with recent improvements including:

- Probe design powered by machine learning
- Dual-stranded oligo printing
- New probe recovery process

The following sections showcase how we have helped enable labs around the globe to accelerate their important assay development in multiple applications using custom and catalogue SureSelect panels.

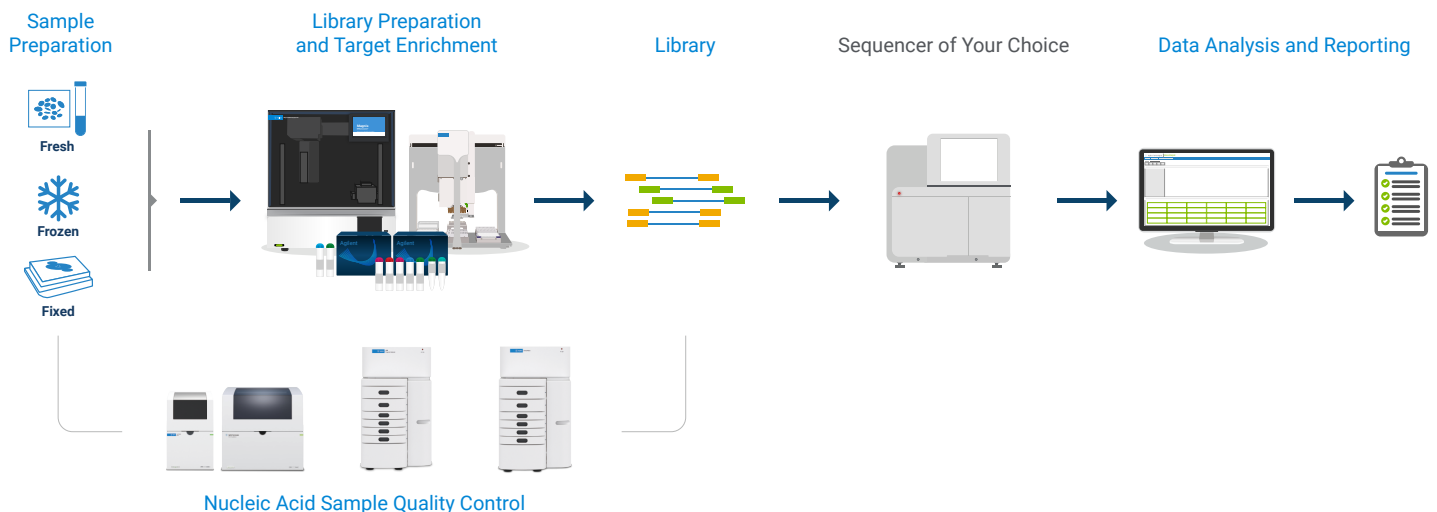
# Agilent Community Designs

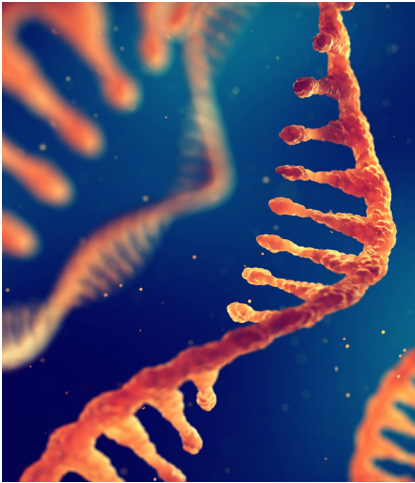
Agilent also offers NGS panels designed by our customers with application expertise under the Agilent Community Design program. Agilent Community Designs for next-generation sequencing (NGS) are targeted sequencing panels established in collaboration with subject matter experts in different research fields. These NGS designs are available as custom, made-to-order panels that provide you with robust and cost-effective sequencing results that focus only on your genes of interest. Not only does our community designs program save time designing target enrichment probe libraries and arrays, but it will also give access to the experience and expertise of our established customer base. These expert-validated designs have been tested in their labs for unique applications and will now be available to all Agilent customers.

For more information please click the link below.

[www.agilent.com/en/product/next-generation-sequencing/community-designs-ngs](http://www.agilent.com/en/product/next-generation-sequencing/community-designs-ngs)

## Agilent NGS Workflow





# RNA Transcriptomics

The application of high-throughput sequencing to transcriptomics (RNA-Sequencing, RNA-Seq) has not only facilitated global gene expression profiling, but has also added precise information on splice variants, fusion transcripts, post-translational editing, and allele-specific expression. This allows a comprehensive understanding of protein coding transcripts within a wide variety of tissue types, pathogenic states, and organisms.

## Study 1

Neurofibromatosis type 1 (*NF1*) is one of the most common autosomal dominant tumor-predisposition disorders caused by a loss-of-function mutation in the tumor-suppressor gene *NF1*. To investigate *NF1* loss-of-function in RNA purified from lymphocytes, researchers at the Maastricht University Medical Center<sup>1</sup> used a custom SureSelect RNA target enrichment panel designed to detect pathogenic RNA splicing and associated pathogenic DNA variants. The study concluded that a targeted RNA-Seq approach, in combination with the authors' specific computational pipeline, successfully detects and quantifies pathogenic *NF1* splicing events driven by intronic or missense variants.

## Study 2

The grafting of fat tissue is important for repair of soft tissue damage. Researchers at the Yonsei University College of Medicine<sup>2</sup> and their collaborators used a mouse model to investigate Notch ligand Delta-like ligand 4 (DII4) in angiogenesis within grafted fat, its effect on graft retention, and the impact of DII4 inhibition on adipose-derived stem cells (ASCs). The underlying mechanism of DII4 inhibition on ASC-supplemented fat grafts was investigated using transcriptome analysis. For this, the authors used the Agilent SureSelect XT Mouse All Exon kit to capture sequencing libraries prepared with the Agilent SureSelect RNA Direct kit.<sup>2</sup> Transcriptomic analysis showed the synergistic effect of DII4 inhibition on cell-assisted lipotransfer in fat grafts and that this may be associated with enhanced vascular integrity, relieving inflammation and increasing anti-inflammatory immune cells.

## Study 3

Researchers at Keimyung University School of Medicine and Dongsan Hospital<sup>3</sup> investigated whether circulating tumor DNA (ctDNA) from bile was potentially feasible for investigating patients with biliary tract cancers. As part of this study, the authors performed transcriptomic sequencing of one set of paired bile and FFPE samples. They used the Agilent SureSelect XT Human All Exon V6 + UTR bait set combined with the SureSelect XT RNA Direct workflow to determine expression level of *KRAS*-associated signaling oncogenes in the bile. Tissue samples showed a strong positive correlation; these results suggest that bile is an effective biopsy fluid for ctDNA analysis.



# DNA Methylation

Epigenetics and epigenomics help explain the mechanisms by which our environment affects our phenotype. Of all the epigenetic markers, CpG methylation is the most studied and best characterized. Methylation status is associated with why genes are expressed at a given level and therefore how they have a role in various complex diseases. These include cancers, psychiatric disorders, neurological conditions, and autoimmune disorders.

## Study 1

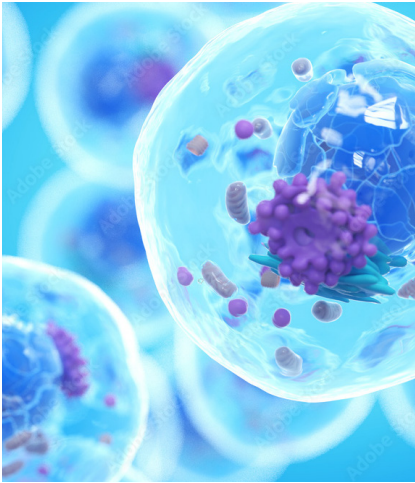
Epigenetic reprogramming that favors survival and results in stunted growth has been postulated to be a consequence of environmental stress in infancy. Researchers at the University of Cambridge<sup>4</sup> and their collaborators examined differential DNA methylation patterns using targeted methyl sequencing of regions regulating gene activity in groups of rural Gambian infants. Analysis with the Agilent SureSelect XT Methyl-Seq target enrichment panel allowed the authors to conclude longitudinal differences in methylation patterns may arise from methylation adjustments, changes in cellular composition of blood, or both. The researchers indicate these effects continue during the critical postnatal growth period. The authors note, *“greater coverage of the SureSelect targeted sequencing approach of key gene regulatory elements (adjacent and distant, proximal or distal) to genes they control, offers the opportunity to identify additional methylation marks not necessarily scored by the array-based platforms.”*<sup>4</sup>

## Study 2

Researchers at Baylor College of Medicine<sup>5</sup> and their collaborators used a custom SureSelect panel with the SureSelect XT Methyl-Seq target enrichment panel to perform the first large-scale NGS assessment of methylation quantitative trait loci (mQTL). The assays were performed on human genomic regions specifically selected for interindividual variation in CpG methylation and are known as correlated regions of systemic interindividual variation (CoRSIVs). The authors integrated methylation, genome sequence, and gene expression data on these same individuals. They showed methylation profiling in peripheral blood at CoRSIV regions could be used to make inferences about epigenetic regulation in various organs of the body. The researchers also showed genetic influences on CoRSIV methylation indicate an unprecedented level of mQTL at these regions. Analyses of GWAS summary statistics show that mQTL polymorphisms at CoRSIVs are associated with metabolic and other classes of human disease.

## Study 3

In mammals CpG methylation is not limited to CpG islands.<sup>5</sup> In this study, researchers at the University of Southampton<sup>6</sup> analyzed this in the wider methylome across multiple tissues from the same individuals to better understand non-CpG methylation distribution within different tissues and individuals. The authors note one of the strengths of this study includes the increased coverage of the methylome provided by SureSelect data compared to array-based methods.



# High-Throughput Chromatin Conformation Capture

Knowing how DNA is arranged in the nucleus, how it folds, and how it can facilitate interaction with genetic regions that are not associated in cis is crucial to our understanding of transcriptional regulation. High-throughput chromatin conformation capture (Hi-C) is a technique used to generate genome-wide chromatin architecture maps. These maps, when acquired at high resolution, can reveal unique details of chromatin organization and dynamics. Capturing these interactions with SureSelect pull-downs allows you to focus your in-depth investigation to regions that are most important to you.

## Study 1

In this study, clinical researchers at the National Cancer Institute<sup>7</sup> and their collaborators first used genome-wide association studies (GWAS) to fine-map melanomas and identify four independent sets of candidate causal variants. They designed a capture Hi-C probe set using a SureSelect library design targeting specific restriction fragments encompassing two significant 7p21 signals. A capture Hi-C study of primary melanocytes identified physical interactions between two causal markers and the promoter of the aryl hydrocarbon receptor (*AHR*) gene. Subsequent studies reinforced the findings of capture Hi-C, that *AHR* is a likely causal melanoma gene. They concluded the linking of the 7p21 melanoma susceptibility region to *AHR* suggests signalling through the *AHR* pathway could highlight a potential role for the larger *AHR* pathway in melanoma risk.

## Study 2

Systemic sclerosis (SSc) is a complex autoimmune disease with a strong genetic component. However, most of the causal genes and variants are still unknown. Researchers at the Institute of Parasitology and Biomedicine, Spanish National Research Council<sup>8</sup> and colleagues used a custom SureSelect bait library designed to capture transcription start sites from 18,755 protein coding genes. This was used to perform promoter capture Hi-C (pCHi-C) on samples of CD4<sup>+</sup> T cells and CD14<sup>+</sup> monocytes from SSc patients and healthy controls. The group linked SSc-associated loci to 39 new potential target genes and seven previously assigned genes. The authors also identified 15 potential drug targets already found in similar, immune-mediated diseases that could be repurposed for SSc treatment.

## Study 3

SARS-CoV-2 infection results in a broad spectrum of COVID-19 disease symptoms and severity. Severity has been associated with the magnitude of the adaptive immune response to SARS-CoV-2 infection. Clinical researchers at the Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania<sup>9</sup> took single nucleotide polymorphisms (SNPs) associated with severe SARS-CoV-2 response and used HiC in harvested immune cells to map these disease-associated elements to their effector genes. The SureSelect Custom Capture HiC library included 36,691 RNA baited fragments through the genome. Variant-to-gene mapping of genes involved in COVID-19 severity identified *GART* as a novel target whose activity could potentially be promoted for better anti-viral humoral immune responses or inhibited as a potential treatment for systemic autoimmune disease.



# Model Organisms

Basic research is often performed using model organisms with discoveries that are then applied to the biological processes of genetically-similar species. The sequencing of entire exomes or a focus on biologically relevant regions enables these research efforts while being more cost-effective than whole-genome sequencing. Understanding the species-specific genetic architecture of complex traits has many applications in evolution, ecology, conservation biology, and plant and animal production systems. The hybridization kinetics of Agilent SureSelect panels enable cross-species capture and thus further enhance the studies that can be performed using a single species bait set.

## Study 1

In aquaculture, the progress of genetic study of many species has been slowed due to a lack of genetic information. To circumnavigate this issue in the Dusky kob, a Sciaenid finfish and emerging aquaculture species, researchers at Stellenbosch University<sup>10</sup> used its homology to zebrafish, another well-studied model species. The Agilent SureSelect Zebrafish All Exon predesigned probe-set capture was used to enrich DNA samples from kob before massive parallel sequencing on the Ion Torrent platform. Despite the evolutionary divergence between zebrafish and kob, more than 55,000 single nucleotide polymorphisms (SNPs) could be reliably identified and genotyped to the individual level. Using SNP differences between cohorts of large fish and cohorts of small fish, a number of candidate genes associated with growth were identified for future investigation. These findings contribute to the growing body of evidence demonstrating the utility of a cross-species capture approach in the development of important genomic resources for understanding traits of interest in species without reference genomes.

## Study 2

In this study, researchers at the Wellcome Sanger Institute<sup>11</sup> and their collaborators *Wong et al* performed a cross species comparison of mucosal melanomas in human, equine and canine models. To perform whole-exome sequencing within these species they used Agilent SureSelect Custom panels to design whole-exome capture baits covering all protein-coding transcripts annotated in the equine reference genome EquCab2.0. For the canine cases they used Agilent SureSelect custom baits designed using transcripts in the canine reference genome CanFam3.1. Sequencing libraries for human cases were generated using the Agilent SureSelect All Exon V5 platform. The group identified recurrently mutated driver genes such as *NRAS*, *FAT4*, *PTPRJ*, *TP53* and *PTEN*, in all species. Further study showed similar genetic changes in mucosal melanomas between species but also distinctly different paths to tumorigenesis.

## Study 3

Understanding viral integration into host genomes is important. It is a hallmark of retrovirus infection, can be a predisposition to cancer, and can play a role in gene augmentation therapy. Adeno-associated virus (AAV)-mediated gene augmentation therapy is considered the gold standard for treating inherited retinal dystrophies (IRDs). In this study, researchers from the University of Buffalo and the University of Utah<sup>12</sup> investigated the integration of recombinant AAV into the retinal genome. The Agilent SureSelect XT HS2 DNA library preparation was combined with Agilent SureSelect Custom baits designed to hybridize the pAAV-AcGFP virus, was used in a mouse model that had been infected with pAAV-AcGFP. They found 904 to 8,195 unique integration sites per animal, verified by RT-PCR. However, there was little overlap between integration sites in different animals, or between eyes of the same animal, suggesting AAV integration is largely random. Random integration opens the possibility that sites may occur in deleterious regions and is an avenue for further investigation.



## Ag/Bio

Crop breeding programs have modernized to rely heavily on the assay of quantitative trait loci/markers and molecular breeding. For crop research it is vital to understand which genes and genetic regions result in the phenotypes of interest. Next-generation sequencing (NGS) has facilitated rapid advancements in this field and led to faster identification of genomic variants, allowing phenotype-to-genotype correlations of agronomic traits and pathogen resistance. Targeted enrichment has enabled crop breeders and researchers to focus on these critical genomic regions and interrogate them in a more cost-effective manner.

### Study 1

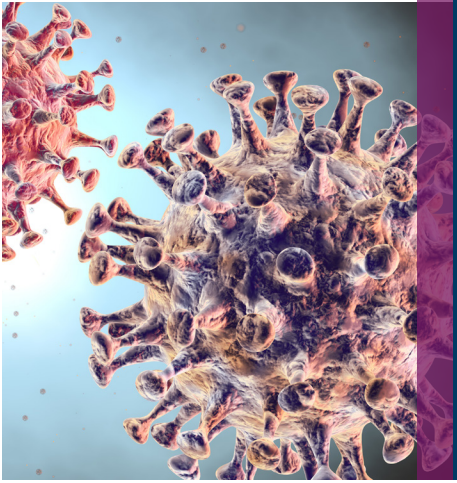
Chickpea (*Cicer arietinum L.*) is the third most important grain legume crop after soybean and pea. This is due to its high protein content, and therefore chickpea serves as an important protein source in the vegetarian diet of many developing nations. It is important to maximize the yield of this crop from agricultural land. Development of varieties resistant to biotic and abiotic stresses is the most effective and viable option for ongoing sustainability and has been one of the major aims for chickpea breeding programs across the world. Advances in NGS technologies have provided opportunities to enrich genomic and genetic resources for chickpea. Researchers at the Centre for AgriBioscience<sup>13</sup> and their collaborators used a SureSelect custom design to target 11,222 SNPs selected based on uniform distribution across the chickpea genome and identify genomic regions conferring resistance to *Ascochyta* blight. They concluded the capture-based method of genotyping was robust, reproducible, and scalable. This could be applied to applications such as trait dissection, association mapping, and genomic selection (GS) in crop plants.

### Study 2

Nucleotide-binding site leucine-rich repeat (NLR) genes is one of the most abundant gene families in plant genomes. Studies of their genetic diversity and expression have showed that NLR proteins are associated with disease resistance via involvement in effector-triggered immunity. Researchers at the University of Florida<sup>14</sup> and their collaborators designed capture probes for NLR genes in loblolly pine (*Pinus taeda*). They used a SureSelect custom workflow to identify and map SNPs in NLR regions associated with resistance to fusiform rust disease. The authors concluded that, "*the geographic diversity of NLR genes provides evidence of NLR gene family evolution in loblolly pine. The SNPs associated with rust resistance provide a resource to enhance breeding and deployment of resistant pine seedlings.*"<sup>14</sup>

### Study 3

Carotenoids are involved in many various functions in plants. Approximately 115 different carotenoids have been reported in citrus fruits. The color of the fruit and peel are caused by carotenoid accumulation and content, and composition varies among citrus varieties. In the Japanese citrus breeding program, the enrichment of carotenoids with health-promoting properties, especially  $\beta$ -cryptoxanthin, is an important objective with the aim of expanding citrus fruit consumption. Researchers at the Institute of Fruit and Tea Tree Science<sup>15</sup> and their collaborators explored the genetic makeup of carotenoid in key founders of a citrus breeding population. To enable this, SNPs of target carotenoid metabolic genes in 13 founders of the Japanese citrus breeding population were assayed by targeted NGS using SureSelect custom enrichment. Results showed the composition of specific alleles highly influence the carotenoid composition in citrus fruits. This would allow breeders to use this molecular genetic information to enrich key carotenoids in citrus fruits.



# Pathogen Research

After the SARS-CoV-2 pandemic in 2020, there has been intensive interest in using NGS techniques to investigate and monitor the genetics of specific pathogen outbreaks or infection. Sequence data can be used for public health monitoring and treatment decisions. Targeted capture is an ideal methodology as it negates the need for culture, purifies the pathogen DNA from any host sequence, and utilizes the same method for this investigation. The technique is also agnostic of template size or pathogen in question, whether a virus, bacterium, protozoan, or fungus.

## Study 1

Chlamydia is the most prevalent cause of bacterial, sexually transmitted infections (STIs) worldwide. The causative bacterium, *Chlamydia trachomatis* is not easily cultured, and so comparative genomic studies are limited. This restricts the understanding of strain diversity and emergence among populations globally. Researchers at the Centers for Disease Control<sup>16</sup> and their collaborators designed a SureSelect XT target enrichment RNA bait library based on 85 different genomes. The library was designed for whole-genome enrichment and sequencing of *C. trachomatis* directly from clinical urine, vaginal, conjunctival, and rectal samples. The group concluded the SureSelect workflow provides a robust approach for discerning genomic diversity and advancing our understanding of the molecular epidemiology of contemporary *C. trachomatis* STIs across sample types, geographic populations, and sexual networks.

## Study 2

Antiretroviral therapy (ART) is vital in the treatment of HIV-1 carriers in West Africa. There has been an emergence in genetic patterns of resistance in West African HIV-1 subtypes which can result in first-line virological failure and have negative implications for future antiretroviral options. Researchers at the University College London<sup>17</sup> and their collaborators used SureSelect target enrichment with an HIV-1 specific custom bait to facilitate deep sequencing of HIV-1 sequences by NGS in 101 study participants. Mutations detected at  $\geq 2\%$  frequency were analyzed and compared by subtype. The authors concluded that extensive drug resistance had accumulated in people with West African HIV-1 subtypes, prior to second-line ART; however, deep sequencing with the SureSelect workflow significantly increased the detection of resistance-associated mutations.

## Study 3

Human respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection in young children globally, but little is known about within-host RSV diversity. Researchers at the University of Oxford<sup>18</sup> and their collaborators designed a SureSelect probe set targeting more than 100 pathogenic bacteria and viruses, including both RSV-A and RSV-B. Differences in within-host virus populations were identified, including variants that would affect efficacy of RSV monoclonal antibody treatment. This emphasizes the importance of monitoring for vaccine efficacy and may help to explain the difference in prevalence of monoclonal antibody-escape mutants in patient groups.

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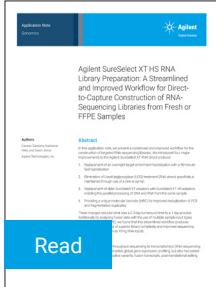
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# Additional Resources

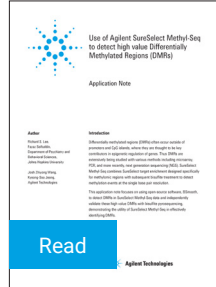
Further enhance your understanding of the power of SureSelect for different research applications and discover more with our Agilent published application notes.

## RNA / Transcriptomics



Agilent SureSelect XT HS RNA Library Preparation: A Streamlined and Improved Workflow for Direct-to-Capture Construction of RNA-Sequencing Libraries from Fresh or FFPE Samples

## DNA Methylation



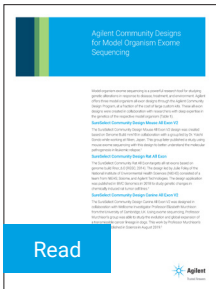
Use of Agilent SureSelect Methyl-Seq to detect high value Differentially Methylated Regions (DMRs)

## Chromatin Conformation



Utilizing Agilent SureSelect XT HS2 Target Enrichment and Arima-HiC to Improve Resolution of High Throughput Chromatin Conformation Capture

## Model Organism



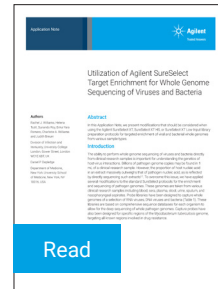
Agilent offers three model organism all-exon designs through the Agilent Community Design program.

## Ag/Bio



The Agilent SureSelect XT CD Medicinal Genomics StrainSEEK panel identifies genes of interest to accelerate cannabis and hemp breeding programs.

## Pathogen Research



Utilization of Agilent SureSelect Target Enrichment for Whole Genome Sequencing of Viruses and Bacteria

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