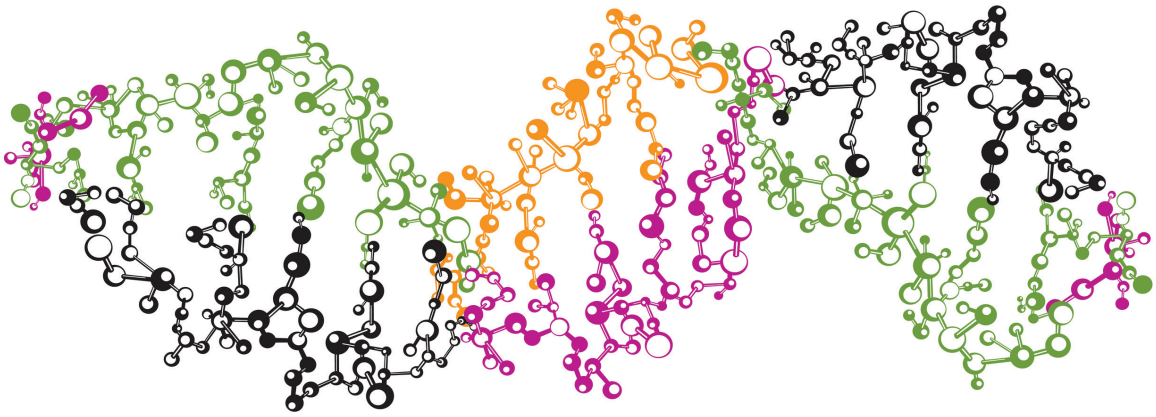

Next Generation Sequencing Experiments Using Takara Bio Reagents

Selected Citations from the Literature

More and more researchers are using high-throughput sequencing to efficiently answer a variety of biological questions. Although there are an array of next generation sequencing technologies and platforms, all NGS experiments require high-quality reagents for upstream sample preparation.

The following peer-reviewed references show examples of how scientists have used Takara Bio products for NGS experiments.

Massively Parallel Sequencing | *TaKaRa LA Taq*® DNA Polymerase

Mondal *et al.* (2012) Excess variants in AFF2 detected by massively parallel sequencing of males with autism spectrum disorder. *Hum. Mol. Genet.* 21(19):4356-64.

In an effort to understand the genetic basis of autism spectrum disorder, Mondal and colleagues sequenced the AFF2 locus in 202 male probands. AFF2 is silenced in patients with Fragile X Syndrome, a disease that manifests with many phenotypic similarities to autism spectrum disorder. *TaKaRa LA Taq*® DNA Polymerase was used to prepare target DNA prior to sequencing on an **Illumina HiSeq system**. The authors state that "LA Taq facilitated long PCR (LPCR) amplification of the AFF2 genomic region, with standard LA Taq buffer used for amplicons with normal %GC composition and 1X GC Buffer used for amplicons with high GC content."

Pyrosequencing Ultra-Low Concentration DNA Samples | *TaKaRa LA Taq*® HS DNA Polymerase

Duhaime *et al.* (2012) Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. *Environmental Microbiol.* 14(9):2526–2537.

DNA is often limiting in metagenomic studies, where total DNA available may be less than 1 ng. Duhaime *et al.* developed modified linker-amplified shotgun library (LASL) techniques for metagenomic analyses on **Roche 454 pyrosequencing systems**, and found that *TaKaRa LA Taq*® HS DNA Polymerase outperformed Pfu DNA polymerase in applications where detection of extremely low abundance sequences is desirable. The authors state, "Based on sensitivity, TaKaRa [LA Taq HS] outperformed Pfu for microbial 16S samples and an isolate genome dilution series, while the opposite held true for DNA extracted from a varied collection of ocean virus concentrates. However, the sensitivity of Pfu came at a cost, as this enzyme amplified a 'no template control' at 30 and 35 cycles, while TaKaRa [LA Taq HS] did not. Further, in select samples, TaKaRa [LA Taq HS] yielded more product and with a broader size range than Pfu. Finally, while Pfu was more sensitive, amplifying some samples that could not be amplified by TaKaRa [LA Taq HS], the LA TaKaRa HS enzyme enriched for sequences that were of extremely low abundance, 'rares', in the original sample … the use of LA TaKaRa HS polymerase may be of particular interest to studies targeting components of the rare biosphere."

Multi-Sample Pooling for NGS | *TaKaRa LA Taq*® HS DNA Polymerase

Margraf *et al.* (2010) Multi-Sample Pooling and Illumina Genome Analyzer Sequencing Methods to Determine Gene Sequence Variation for Database Development. *J. Biomol. Tech.* 21(3):126–140.

While assessing methods for determining sequence variation within a genetic locus, researchers analyzed a pool of 10 samples with 13 known unique variants in the RET proto-oncogene on an **Illumina genome analyzer (GA) system**. *TaKaRa LA Taq*® HS DNA Polymerase was used to amplify all experimental samples and one control (wild-type) sample prior to analysis using with an Illumina GA system. The authors suggest that the "methods presented in this report could be used for any gene or small genome, where nonbar-coded, multiple sample pooling on the Illumina GA is used to determine population sequence variation."

Targeted Resequencing | *TaKaRa LA Taq*® DNA Polymerase

Dames *et al.* (2010) Comparison of the Illumina Genome Analyzer and Roche 454 GS FLX for Resequencing of Hypertrophic Cardiomyopathy-Associated Genes. *J. Biomol. Tech.* 21(2):73–80.

This study enriched a single human genomic DNA sample using long-range PCR with *TaKaRa LA Taq*® DNA Polymerase, then re-sequenced 16 genes associated with hypertrophic cardiomyopathy using either an **Illumina Genome Analyzer** or a **Roche 454 GS FLX system**. Of the 67 LR-PCR amplifications, 84% reproducibly yielded single bands of the correct length.

Targeted Resequencing | *TaKaRa LA Taq*® DNA Polymerase with GC Buffers

Paola Benaglio and Carlo Rivolta (2010) Ultra High Throughput Sequencing in Human DNA Variation Detection: A Comparative Study on the NDUFA3-PRPF31 Region. *PLoS ONE.* 5(9):e13071.

To study the use of Ultra High Throughput Sequencing (UHTS) technologies in targeted resequencing of candidate genes or of genomic intervals from genetic association studies, the authors tested the three most widespread UHTS platforms (**Roche/454 GS FLX Titanium, Illumina/Solexa Genome Analyzer II**, and **Applied Biosystems/SOLiD System 3**) on a well-studied region of the human genome containing many polymorphisms and a very rare heterozygous mutation.

In this study, *TaKaRa LA Taq*® DNA Polymerase with GC Buffer I was used for long range PCR to amplify a 31 kb genomic region of NDUFA3-PRPF31 in four individual reactions. Samples comprising all four amplified regions were subsequently pooled and sequenced on **Roche/454, Illumina/Solexa GA II**, or **ABI/SOLiD platforms**. The aim of this work was to provide human molecular geneticists with a tool to evaluate the best UHTS methodology for efficiently detecting DNA changes, from common SNPs to rare mutations.

Targeted Sequencing | *TaKaRa LA Taq*® DNA Polymerase with GC Buffers

Vinturini *et al.* (2012) CNOT3 Is a Modifier of PRPF31 Mutations in Retinitis Pigmentosa with Incomplete Penetrance. *PLoS Genetics.* 8(11):e1003040.

Mutations in the PRPF31 gene cause Retinitis pigmentosa (RP), an inherited, degenerative eye disease. This study describes the identification of a modifier gene involved in penetrance of PRPF31 mutations. To accomplish this, a 34 kb region of interest was amplified from samples from 10 individuals in 3 overlapping fragments using *TaKaRa LA Taq*® DNA Polymerase with GC Buffer I, then subjected to sequencing on an **Illumina HiSeq system**. This analysis identified a polymorphism within CNOT3 that associated with RP.

Pyrosequencing | PrimeSTAR® GXL DNA Polymerase

Koyanagi *et al.* (2011) Pyrosequencing survey of the microbial diversity of 'narezushi', an archetype of modern Japanese sushi. *Letters in Applied Microbiology.* 53:635–640.

Narezushi is a traditional form of fermented sushi. The fermentation processes that leads to unique flavors of narezushi had remained largely a mystery, because fermentation does not involve a starter culture and the microbiota varies tremendously from batch to batch. Koyanagi and colleagues used pyrosequencing to analyze the microbiota of different narezushi samples. 16S rRNA regions were amplified by PrimeSTAR® GXL DNA Polymerase and analyzed using a **Genome Sequencer FLX System (Roche)**. Over 700 different operational taxonomy units were found in all samples, and while the microbiota of each product differed, the microbial populations consisted of over 90% Lactobacillales in all samples.

Whole Genome Transcriptome Analysis | PrimeScript™ Reverse Transcriptase, Mighty Mix DNA Ligation Kit, and *TaKaRa* DNA Polymerases

Djebali et al. (2012) Landscape of transcription in human cells. Nature. 489(7414):101-108.

In this publication, scientists working at 19 different institutions used RNA-Seq and RNA-PET (RNA Paired End Tags) to study the transcriptomes of 15 different human cell lines. Multiple Takara Bio reagents were used to perform the experiments, including PrimeScript™ Reverse Transcriptase, Mighty Mix DNA Ligation Kit, and *TaKaRa LA Taq*® DNA Polymerase as well as *TaKaRa Ex Taq*® DNA Polymerase for RNA-PET library preparation. The results of Djebali *et al.* suggest that over 74% of the human genome is covered by primary transcripts. The finding that three-fourths of the genome is transcribed greatly reduces the length of 'intergenic' regions, and thereby "…prompts a redefinition of the concept of a gene," state the authors.

High-Throughput Pyrosequencing | *TaKaRa Ex Taq*® DNA Polymerase

Zhou *et al.* (2011) BIPES, a cost-effective high-throughput method for assessing microbial diversity. *The ISME Journal.* 5:741–749.

To address the need for low-cost, high-throughput pyrosequencing method for assessing microbial diversity in environmental samples, the authors developed a barcoded Illumina paired-end (PE) sequencing (BIPES) method to analyze 16S rRNA variable tags. In BIPES, sequences are determined via each 16S V6 tag from both ends on the **Illumina HiSeq 2000**, and the PE reads are then overlapped to obtain the V6 tag. *TaKaRa Ex Taq*® DNA Polymerase was used for amplification prior to pooling and sequencing on an **Illumina HiSeq 2000**.

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