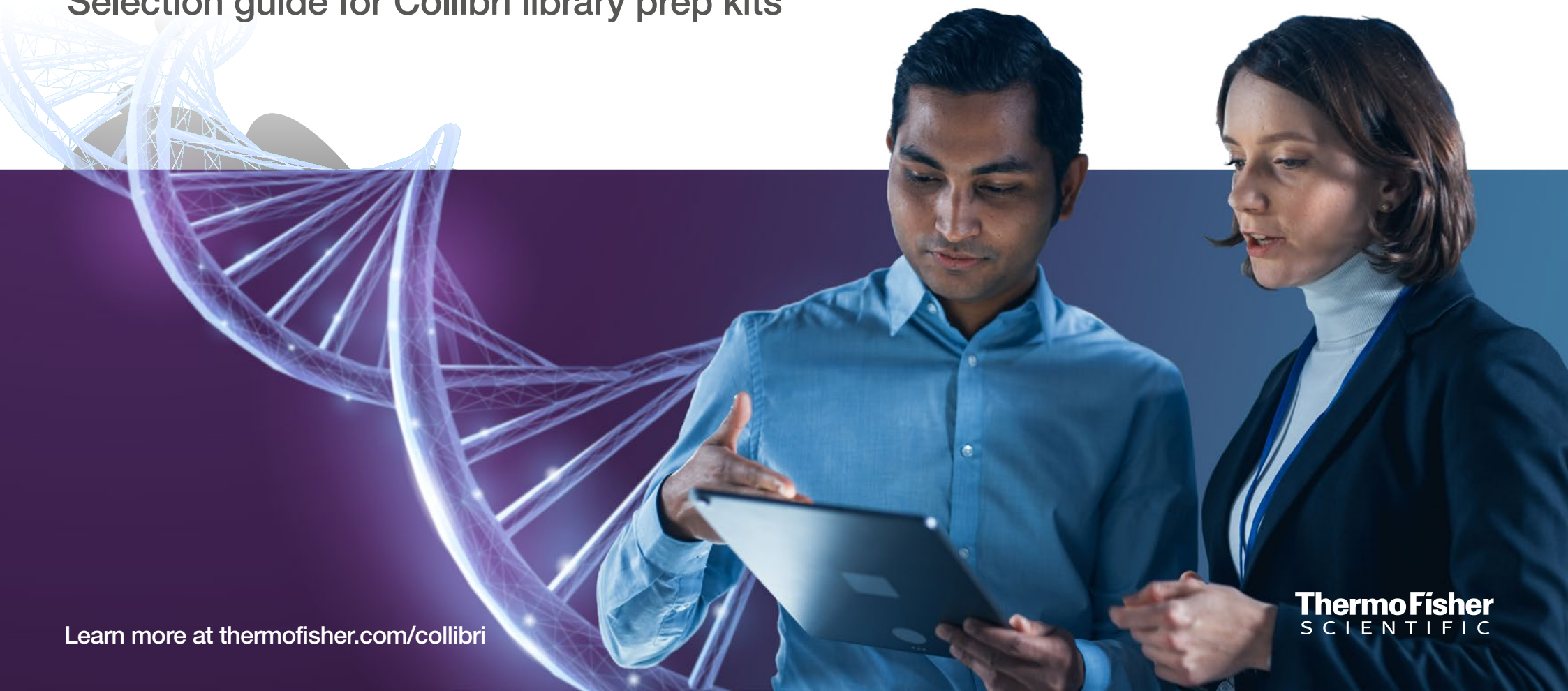
invitrogen

Take the guesswork out of NGS library prep

Selection guide for Collibri library prep kits



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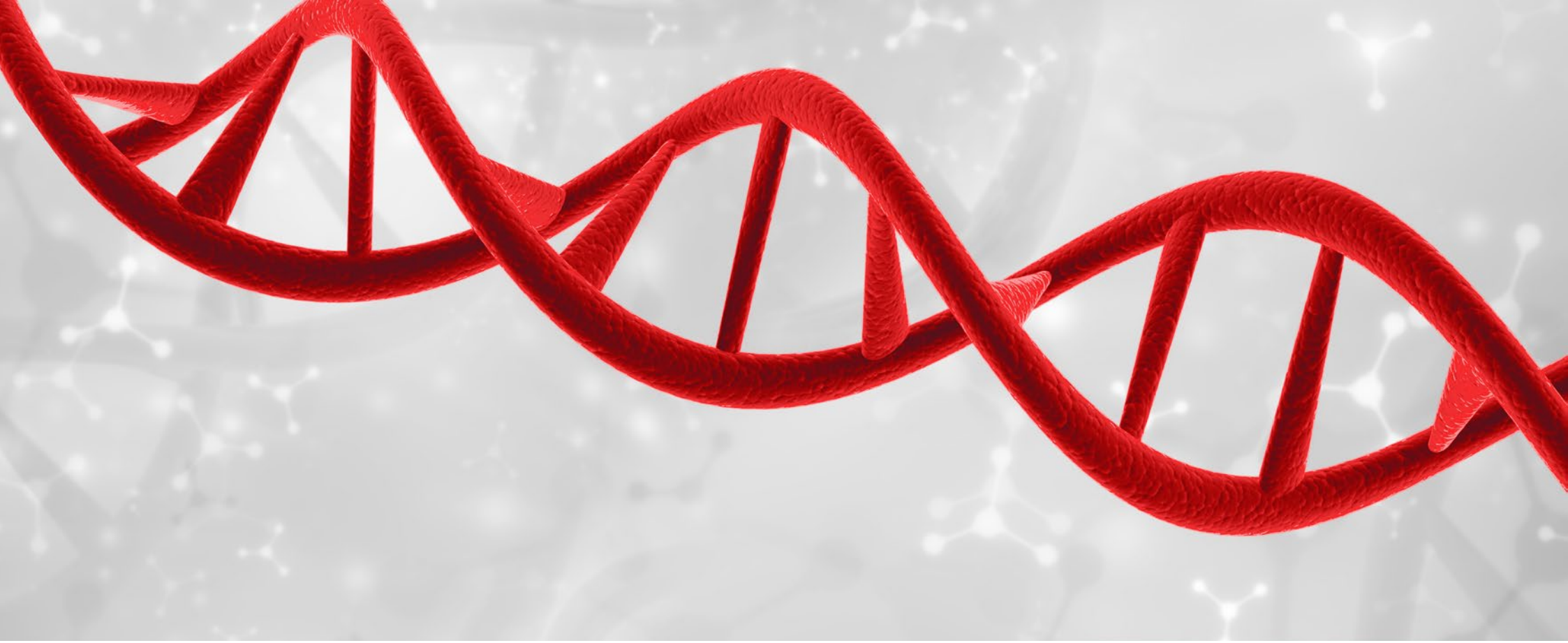
ThermoFisher SCIENTIFIC



Next-generation sequencing (NGS) continues to evolve at a rapid pace, offering new ways to unlock insights and advance global health

The research community is applying these evolving sequencing methods in areas such as food security, viral evolution, and therapeutic developments to treat cancer and genomic conditions.

This selection guide can be used in two ways:

1. Identify recommendations for NGS library prep kits and workflow accessories for a specific project.
2. Browse the guide to discover new ways to gain insights from sequencing data generated on an Illumina™ NGS system.

All recommendations in this guide are compatible with Illumina systems. We welcome your feedback, suggestions, and ideas.

Together, we can find an approach to help move your research forward.

Enabling links in this guide

Some users will need to change their PDF reader security settings to enable URL links in this guide. For instructions, go to page 32.

Contents

Starting material

Application

This NGS selection guide offers two search pathways to identify the library prep kit that best matches your project goals

A search that begins with “starting material” will allow you to explore the available options for your DNA or RNA sample. A search that begins with “Application” will allow you to choose the library prep kit that matches your research objectives, such as investigating gene expression or researching infectious diseases. Each pathway will offer you several choices to quickly identify the recommended solution.

Starting material

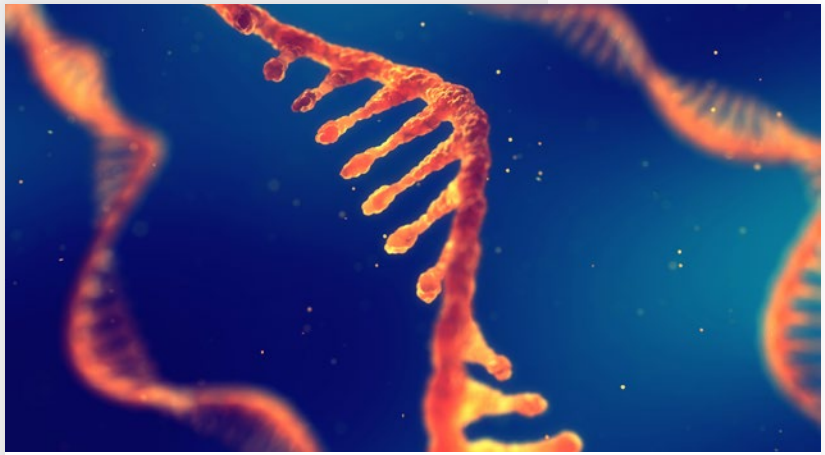
Application

Search by starting material

Library preparation options for NGS on Illumina systems often begin with the starting material. Is the sample composed of DNA or RNA? While investigation of proteins is a long-term goal of NGS systems, the current options typically begin with DNA or RNA and are refined from that starting point.

What is your starting material for library prep?

DNA

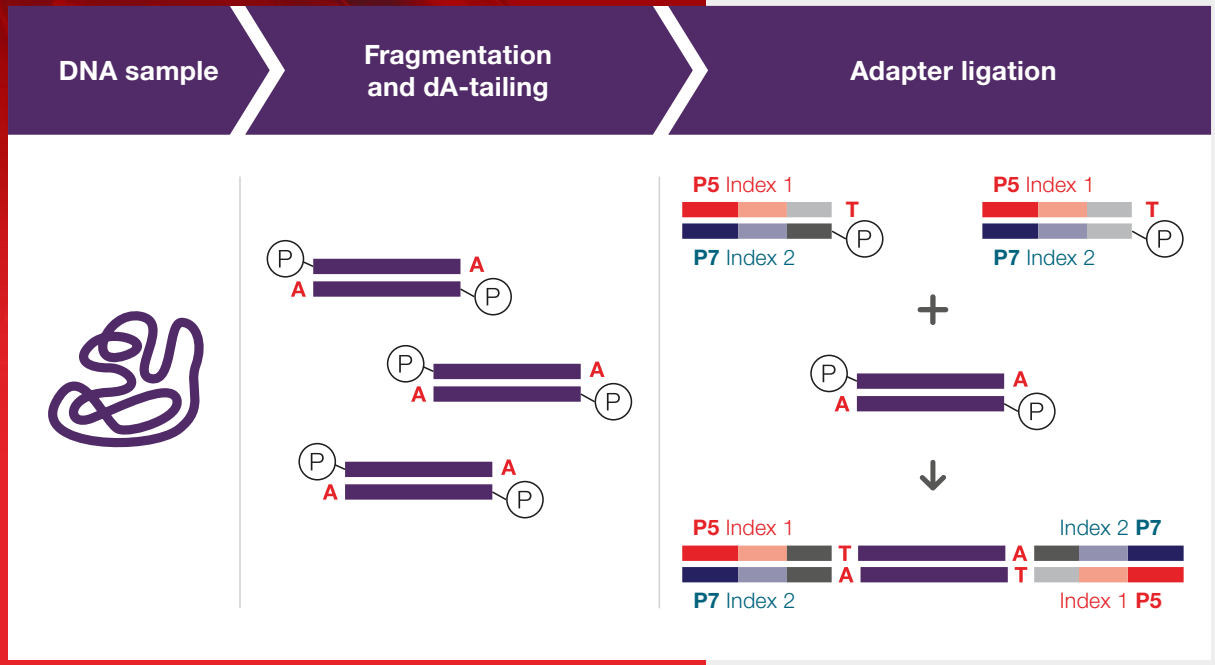


RNA

The quality and purity of starting DNA or RNA can impact the success of library preparation.

Our experienced sample prep professionals are here to help with application support, method development, or any other questions you might have about your sample prep.

Please contact techservices@thermofisher.com



Overview of the library preparation process.

The state of DNA

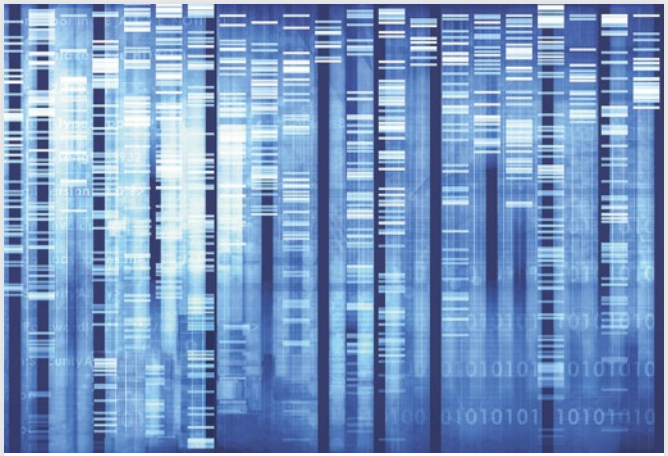
NGS has become ubiquitous in the investigation of disease at the molecular level.

This technology involves millions of nucleic acid strands being read in parallel, one base at a time. Over the past 10 years, DNA sequencing systems have evolved from instruments with a throughput of several megabases per day to instruments with a throughput of terabases per day. This expansion has required development of technologies to improve the speed, ease of use, and scalability of methods for the production of NGS libraries.

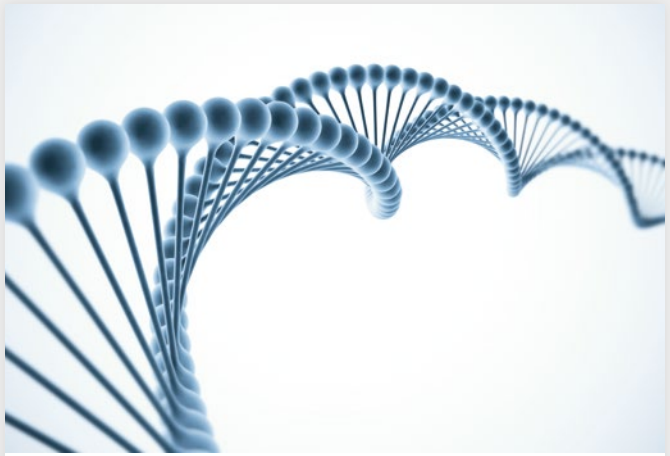
How do I choose a DNA sequencing method?

Modern NGS library prep kits are optimized for specific applications with the flexibility needed for innovation. The best approach is to select the library prep kit that matches your project goals.

What is the primary goal of your DNA sequencing project?



Best genomic coverage
(I want to see all bases in my sequencing data)



Maximal yield

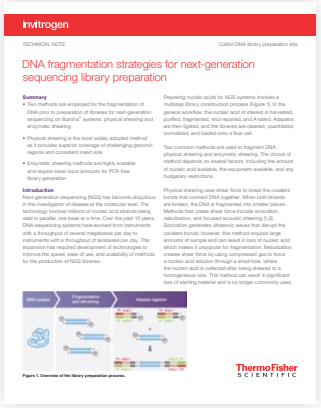
I'm not sure
(Help me choose)

How to choose a whole-genome DNA sequencing technology

Two common methods are used to fragment DNA: physical shearing and enzymatic shearing. The choice of using physically sheared (PS) DNA or enzymatically sheared (ES) DNA for NGS depends on several factors, including the amount of nucleic acid available, the equipment available, and budgetary restrictions (Table). PCR-free protocols and unique dual indexes (UDIs) are available for both fragmentation strategies.

Have more questions about choosing between physical and enzymatic shearing?

Find the answers to your questions in this technical application note.



Download

	Invitrogen™ Collibri™ PS DNA Library Prep Kits for Illumina™ Systems	Invitrogen™ Collibri™ ES DNA Library Prep Kits for Illumina™ Systems
PCR-free input	500 ng	100 ng
Input	1–1,000 ng	1–500 ng
Lowest GC bias	•	
DNA shearing method	Physical shearing (e.g., Covaris™ sonicator)	Enzymatic shearing
Species supported	All	All
PCR-free assay time	1.5 hr	1.5 hr
Assay time	2.75 hr	2.5 hr
PCR-free hands-on time	0.5 hr	0.5 hr
Hands-on time	1 hr	1 hr
Target insert size	350–550 bp	
All components included	•	•
Visual feedback	•	•

Product comparison.

View performance data

View performance data

Explore DNA library prep kit options at **thermofisher.com/collibriDNA**

Choosing a library prep kit to obtain the most consistently even coverage of the genome

Reduce bias in genomic interpretation

Coverage of challenging regions by the **Invitrogen™ Collibri™ PCR-Free Physically Sheared (PS) DNA Library Prep Kits for Illumina™ Systems** or the **Invitrogen™ Collibri™ Physically Sheared (PS) DNA Library Prep Kits for Illumina™ Systems** is consistently even among low-abundance (1 ng) and high-abundance (1,000 ng) samples. This helps to ensure higher accuracy from sequencing compared to older library prep methods from Illumina™, NEB™, or Kapa Biosystems™ kits. All components, including shearing enzymes, indexed adapters, amplification master mix (if relevant), and cleanup beads, are included for convenience and performance. Unique dual indexes (UDIs) are available for 24 or 96 reactions to filter index-hopped reads on patterned flow cells.

Enhanced coverage of challenging regions, even from FFPE samples

Consistently even GC coverage produces higher coverage of difficult genomic regions, resulting in more even whole-genome coverage (Figure). Enhanced coverage of challenging genomic regions is consistent from 1 to 1,000 ng.

Comparison between kits of normalized coverage of challenging regions. 100 ng of Horizon™ moderate damage FFPE DNA was converted to sequencing libraries using the manufacturer-recommended protocols and sequenced on an Illumina™ NovaSeq™ 6000 system with a 2 x 150 bp read length. Resulting pass filter (PF) reads were normalized to 186M reads.

Have more questions about the impact of GC bias from older library prep kits?

Find the answers to your questions in this technical application note.

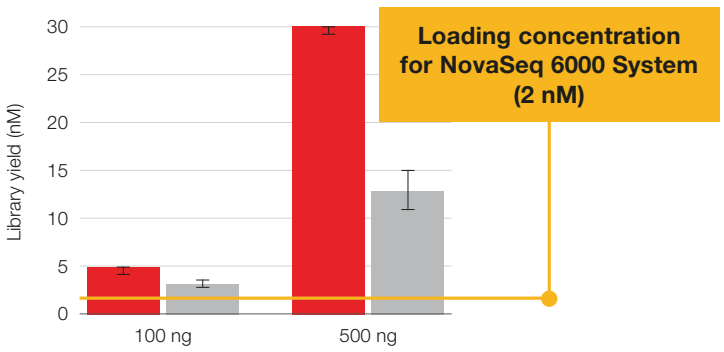
Download

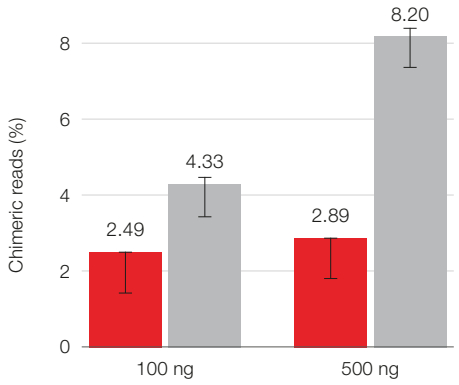
View data sets or request a sample at **thermofisher.com/collibriPSdna**

Choosing a library prep kit to maximize final yield of whole-genome libraries

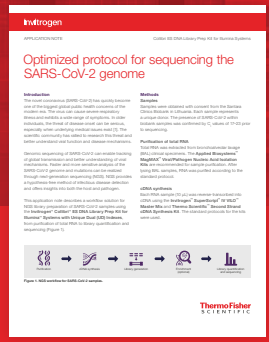
Improve library generation with high yields and strong coverage of biologically important genes with the **Invitrogen™ Collibri™ PCR-Free Enzymatically Sheared (ES) DNA Library Prep Kit for Illumina™ Systems** or the **Invitrogen™ Collibri™ ES DNA Library Prep Kit for Illumina™ Systems**. All components, including shearing enzymes, indexed adapters, amplification master mix (if relevant), and cleanup beads are included for convenience and performance. Unique dual indexes (UDIs) are available for 24 or 96 reactions to filter index-hopped reads on patterned flow cells.

The Collibri PCR-Free ES DNA Kit is optimized to convert 100 ng of DNA to a sufficient amount of library to perform quantification and more than two runs on an Illumina™ NovaSeq™ system. Resulting libraries contain fewer chimeric reads than older library prep technology (Figure).





Comparison of library yield and percentage of chimeric reads between kits. Coriell NA12878 DNA was converted to sequencing libraries using the manufacturer-recommended PCR-free protocols, and concentration was measured using the Invitrogen™ Collibri™ Library Quantification Kit. Libraries were sequenced on an Illumina NovaSeq 6000 System.



Sequencing SARS-CoV-2?

An optimized protocol using the Collibri ES DNA Library Prep Kits is available to improve coverage and variant detection for SARS-CoV-2 and other viral samples.

Download

View data sets or request a sample at **thermofisher.com/collibriESdna**

The state of RNA

RNA sequencing (RNA-Seq) is a powerful and comprehensive method for analyzing the cellular transcriptome.

This technique enables the qualitative and quantitative examination of multiple types of RNA in biological samples at specific time points. Applications for RNA-Seq are wide-ranging, from basic research on cellular structure and function to the analysis of various disease states in clinical samples. For example, gene expression patterns can be compared before and after therapeutic interventions for the presence of a disease. Posttranscriptional modifications and exon–intron boundaries can also be determined with RNA-Seq. The data obtained can provide valuable insights into basic cellular mechanisms, genome structures, disease-induced effects, and more[1].

Selecting the best library prep kit depends on your RNA-Seq project goals. What is the primary goal of your project?

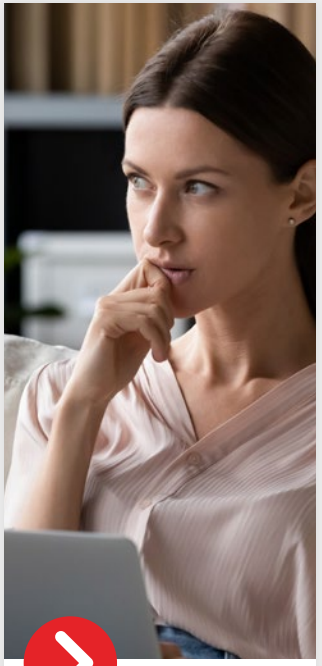
I want complete transcriptome information

- Most complete understanding of phenotype
- Detect coding and noncoding RNA
- Gene expression
- Gene and transcript abundance
- Biomarker identification

I want to understand protein-coding RNAs

- Detect coding RNA
- Gene expression

I want to study changes in gene expression



I'm not sure

(Help me choose)

1. Hrdlickova R, Toloue M, Tian B (2017) RNA-Seq methods for transcriptome analysis. *Wiley Interdiscip Rev RNA* 8(1).

How do I choose an RNA sequencing method?

Modern NGS library prep kits are optimized for specific applications with the flexibility needed for innovation. The best approach is to select the library prep kit that matches your project goals and type of sample (Table).

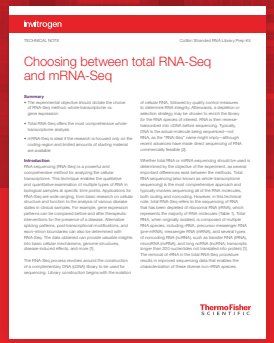
What is the primary goal of your RNA-Seq project?

- I want to maximize the transcriptome information obtained from my samples, and I have at least 100 ng of total RNA
- I want to understand protein-coding RNA activity
- I want to study changes in gene expression

Comparison of RNA-Seq methods.

	3' mRNA sequencing	mRNA sequencing	Whole-transcriptome sequencing
Applications	• Gene expression	• Detect coding RNA • Gene expression • Gene fusions • Transcript isoforms	• Most complete understanding of phenotype • Detect coding and noncoding RNA • Gene expression • Alternative gene expression • Gene and transcript abundance • Biomarker identification
Reads per sample (millions)	2–5	30	60
Hands-on time (hours)	1.75	1.5	3.5
Total time (hours)	4.5	4.5	6.5
Input range	100 pg–500 ng *	1–25 ng **	100–1,000 ng
FFPE compatible	Yes	Yes	Yes
Species compatibility	Human	All	Human, mouse, rat

*Inputs of >200 ng are recommended for efficient detection of low-abundance transcripts ** rRNA-depleted or mRNA-enriched RNA



Choosing between whole-transcriptome sequencing and mRNA sequencing?

Learn which types of RNAs are retained within the sequencing data for each option.

Download

Explore RNA sequencing options at **thermofisher.com/collibriRNA**

Choosing a library prep kit to achieve the most complete understanding of a phenotype

When performing whole-transcriptome sequencing, achieve the most complete understanding of sample phenotype with a unique technology that adds partial adapters directly to RNA to retain small RNA, coding RNA, and noncoding RNA, and makes it possible to sequence the entire transcriptome. Sequencing data represent original sample complexity to provide the most complete understanding of the sample phenotype. Applications that make use of transcriptome data generated by the Invitrogen™ Collibri™ Stranded RNA Library Prep Kits for Illumina™ Systems include:

- Studies requiring the most complete understanding of phenotype
- Detection of coding and noncoding RNA
- Gene expression analysis
- Studies of gene and transcript abundance
- Biomarker identification

Which type of sample will you investigate using whole-transcriptome sequencing?

Human, mouse, or rat

Plant or bacteria

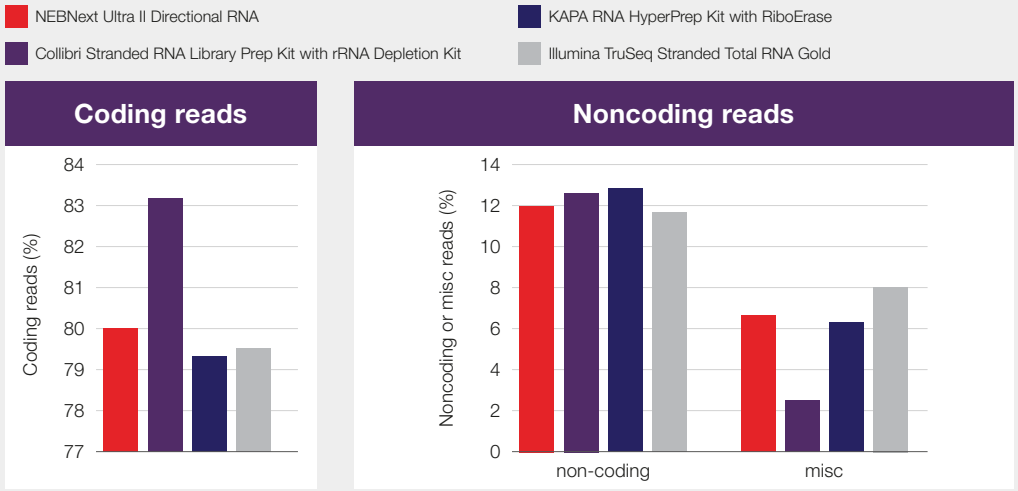
Choosing a library prep kit to achieve the most complete understanding of human, mouse, and rat sample complexity

Achieve the most complete understanding of sample phenotype with a unique technology that adds partial adapters directly to RNA to retain small RNA, coding RNA, and noncoding RNA, and makes it possible to sequence the entire transcriptome. Sequencing data represent original sample complexity to provide the most complete understanding of the sample phenotype. Depletion reagents to remove ribosomal RNA (rRNA) from human, mouse, and rat samples are included. Applications that make use of transcriptome data generated by the **Invitrogen™ Collibri™ Stranded RNA Library Prep Kit for Illumina™ Systems with H/M/R rRNA Depletion Kit** include:

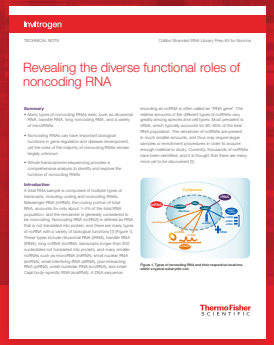
- Studies requiring the most complete understanding of phenotype
- Detection of coding and noncoding RNA
- Gene expression analysis
- Studies of gene and transcript abundance
- Biomarker discovery

Retain human, mouse, and rat sample complexity

The **Collibri Stranded RNA Library Prep Kit for Illumina Systems with H/M/R rRNA Depletion Kit** uses a unique protocol that retains the full transcriptome (Figure). Retention of both coding and noncoding RNA occurs because cDNA priming is not affected by GC content and random oligonucleotide sequences are not incorporated into cDNA, preventing false SNPs and point mutations.



Sensitive detection of coding and noncoding RNA to retain the full transcriptome. 100 ng of universal human reference RNA (UHRR) was converted to libraries following the manufacturer-recommend protocols and sequenced on an Illumina™ HiSeq™ 4000 system. Pass filter (PF) reads were normalized to 45M reads and analyzed for biotype content using the Quality of RNA-Seq Toolset (QoRTs).



Why are regulatory genes valuable for gaining insights into cancer, infectious diseases, and other biological processes?

Learn about the diverse functional roles of noncoding RNA and how they may influence human health.

Download

View data sets or request a sample at **thermofisher.com/collibriWTrna**

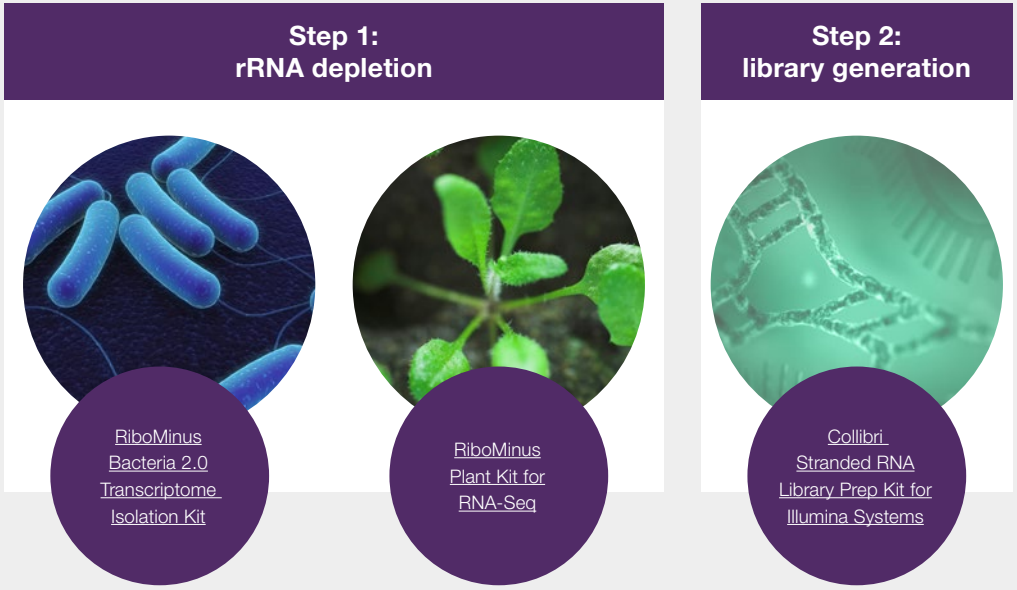
Choosing a library prep kit to achieve the most complete understanding of plant and bacterial sample complexity

Following ribosomal RNA (rRNA) depletion with your choice of technology, achieve the most complete understanding of sample phenotype with a unique technology that adds partial adapters directly to RNA to retain small RNA, coding RNA, and noncoding RNA and makes it possible to sequence the entire transcriptome. Sequencing data represent original sample complexity to provide the most complete understanding of the sample phenotype. Applications that make use of transcriptome data generated by the Invitrogen™ Collibri™ Stranded RNA Library Prep Kit for Illumina Systems include:

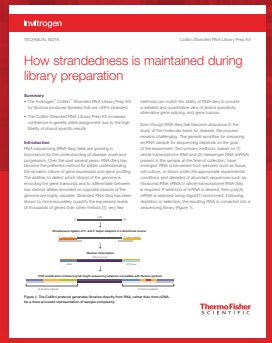
- Studies requiring the most complete understanding of phenotype
- Coding and noncoding RNA detection
- Gene expression analysis
- Gene and transcript abundance studies
- Biomarker identification

Retain plant and bacterial transcriptome diversity

Whole-transcriptome library preparation from plant and bacterial samples is a two-step process (Figure).



Recommended products for rRNA depletion and library preparation from plant and bacterial samples. In step 1, rRNA is depleted using your kit of choice. We recommend using an Invitrogen™ RiboMinus™ kit for highly efficient rRNA depletion. In step 2, RNA sample complexity is retained during library generation by using the Collibri Stranded RNA Library Prep Kit for Illumina Systems.



How is strandedness maintained during this unique workflow?

Learn how helper adapters become full-length indexed adapters during library generation.

Download

View data sets or request a sample at **thermofisher.com/collibri_plant_bacteria_rnaseq**

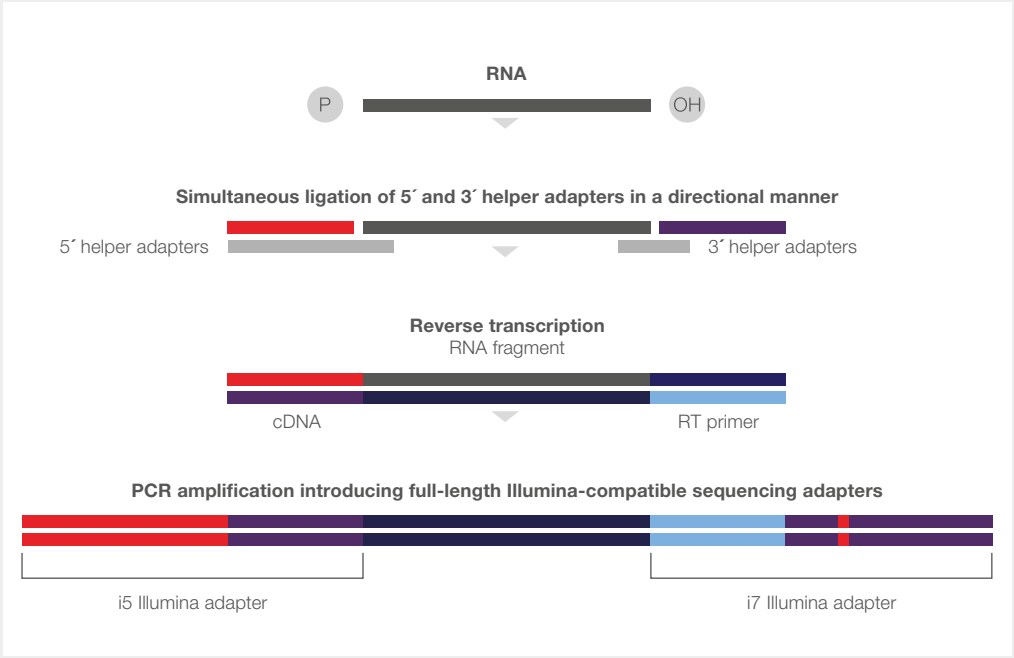
Choosing a library prep kit to study protein-coding RNA and changes in gene expression

If the research goal is to focus primarily on the coding region, mRNA-Seq represents the best choice

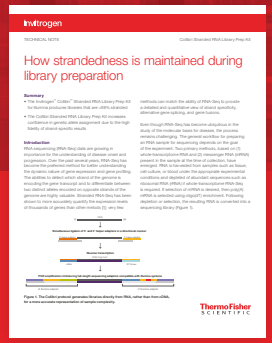
The mRNA-Seq protocol uses a selection method to enrich for RNA with a poly(A) tail. mRNA represents only a small percentage of the total RNA molecules, so enrichment for RNA with a poly(A) tail prior to library generation is the most efficient and cost-effective procedure if it meets the overall experimental goals. Following enrichment using the **Dynabeads mRNA DIRECT Purification Kit** or similar enrichment kit, a quick 4.5-hour protocol using the **Collibri Stranded RNA Library Prep Kit for Illumina Systems** generates high-quality libraries that are compatible with any Illumina NGS system. This kit is suitable for studies to discover gene fusions and alternative transcript isoforms.

Retain transcriptome diversity

The **Collibri Stranded RNA Library Prep Kit for Illumina Systems** uses a unique protocol in which adapters are added directly to RNA (Figure). The full transcriptome is retained because cDNA priming is not affected by GC content and random oligonucleotide sequences are not incorporated into cDNA, preventing false SNPs and point mutations.



Sequence the entire transcriptome for improved library complexity. Partial adapters, also known as helper adapters, are ligated directly to single-stranded RNA prior to creation of cDNA. Full-length indexed adapters are generated during PCR.

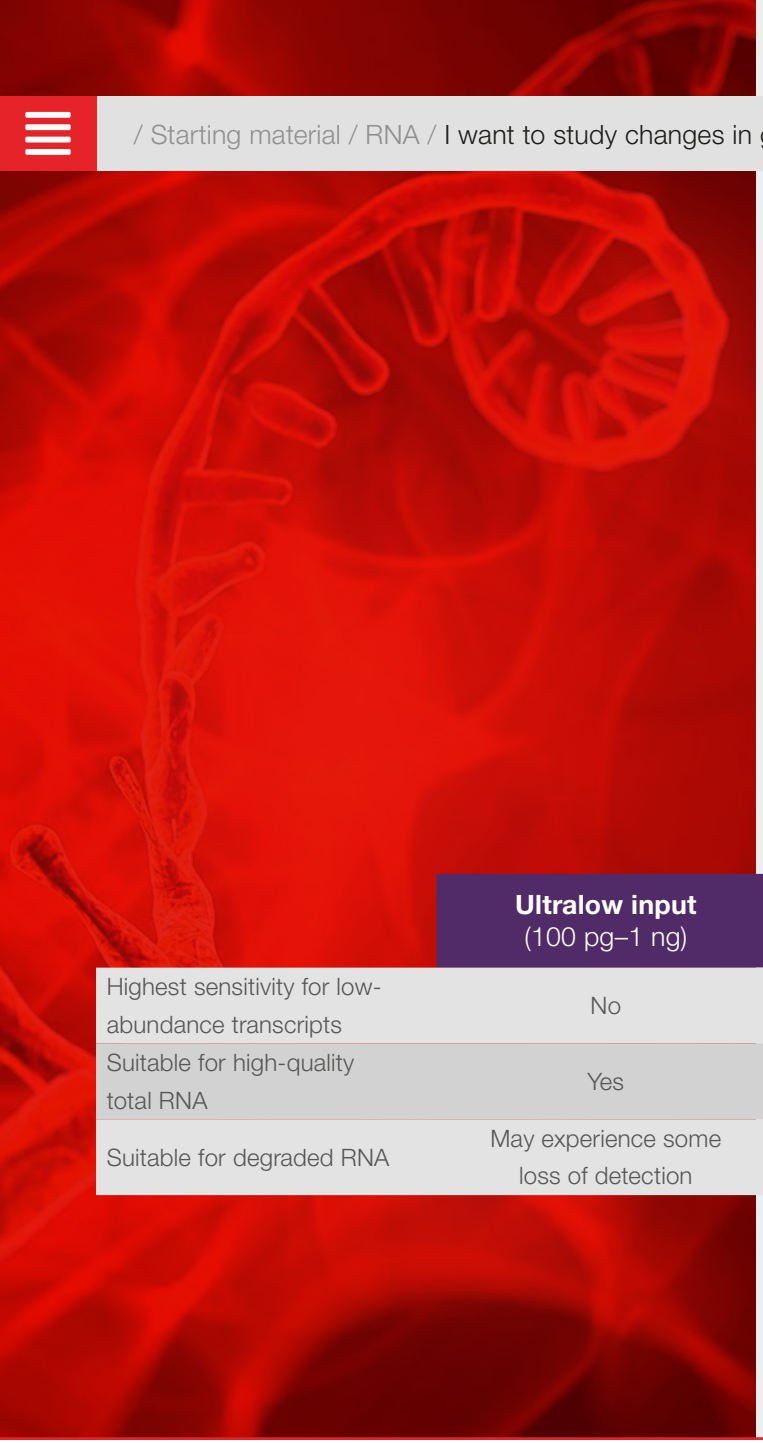


How is strandedness maintained during this unique workflow?

Learn how helper adapters become full-length indexed adapters during library generation.

Download

Request a sample at **thermofisher.com/collibrimRNA**



Choosing a library prep kit to study gene expression

If the research goal is to study gene expression, 3´ mRNA sequencing provides the lowest-cost sequencing option

	Ultralow input (100 pg–1 ng)	**Low input** (1 ng–200 ng)	**Standard input** (200 ng–500 ng)
Highest sensitivity for low-abundance transcripts	No	No	Yes
Suitable for high-quality total RNA	Yes	Yes	Yes
Suitable for degraded RNA	May experience some loss of detection	Yes	Yes

Unlock gene expression insights. Using only 2–5 million reads on any Illumina NGS system, study gene expression from low-input or low-quality total RNA samples or low-expression genes. For accurate quantitation of input RNA, we recommend using the Invitrogen™ Qubit™ RNA BR Assay Kit.

Ideal for samples ranging from 100 pg to 500 ng of total RNA, the Invitrogen™ Collibri™ 3´ mRNA Library Prep Kits for Illumina™ Systems capture the 3´ end of transcripts, enabling gene expression studies from as little as 2 million reads per sample, compared to 60 million reads for whole-transcriptome sequencing or 30 million reads for mRNA sequencing. Ribosomal depletion is not required for this method, and the Collibri 3´ mRNA kit contains an mRNA enrichment module. Globin-depletion reagents are available for projects that involve human blood samples.

What sample will you study?

Any organism

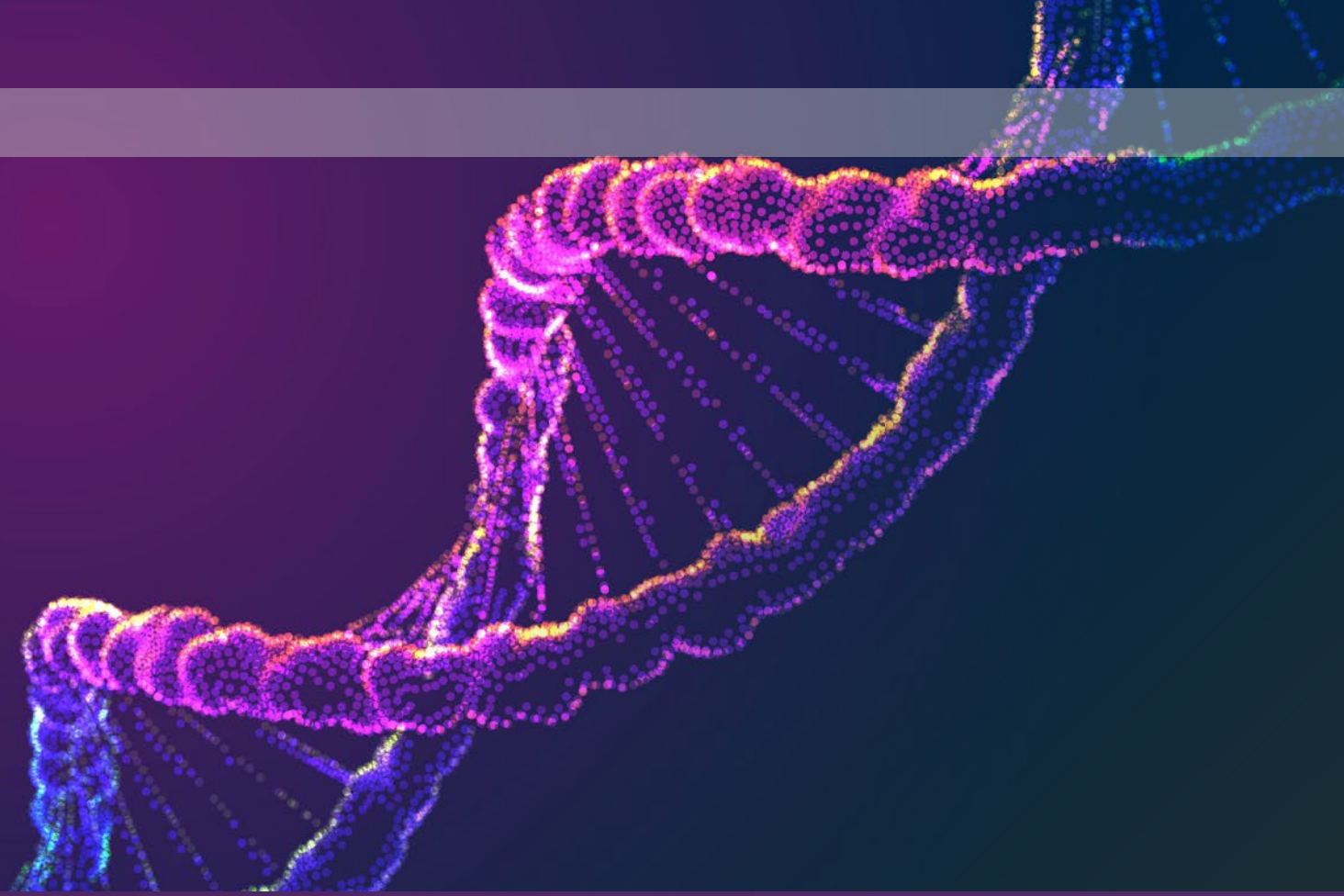
- Tissue
- Cells
- Lysate
- FFPE

Human blood

Flexible design accommodates all project goals

Each project is unique, so your library prep kit must accommodate various experimental conditions. The Collibri 3´ mRNA Library Prep Kits for Illumina Systems are compatible with a wide range of sample quality and input (Figure).

NGS



As a means to unlock the power of the genome, NGS is used in an increasing number of applications ranging from food security to advancing human health and monitoring viral evolution.

One application that has become increasingly prevalent is the use of liquid biopsies for research to investigate potential biomarkers within genetic material from tumors circulating in peripheral blood. Elevated levels of cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA) in peripheral blood are common with many cancers, due to the increased rate of apoptosis in many tumor cells.

Explore NGS applications in which Collibri library prep kits are advancing scientific understanding:

DNA

- > Resequencing
- > Population sequencing
- > cfDNA or ctDNA liquid biopsy research
- > Infectious disease research

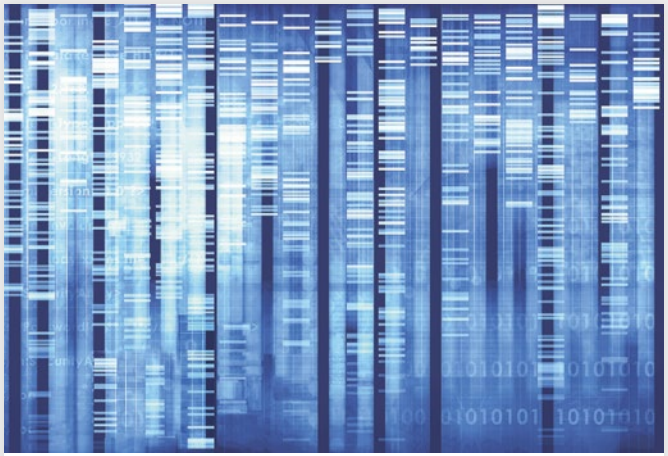
RNA

- > Biomarker discovery
- > Genomic basis for phenotypic expression
- > Gene expression
- > Role of gene fusions or alternative transcript isoforms in cancer research

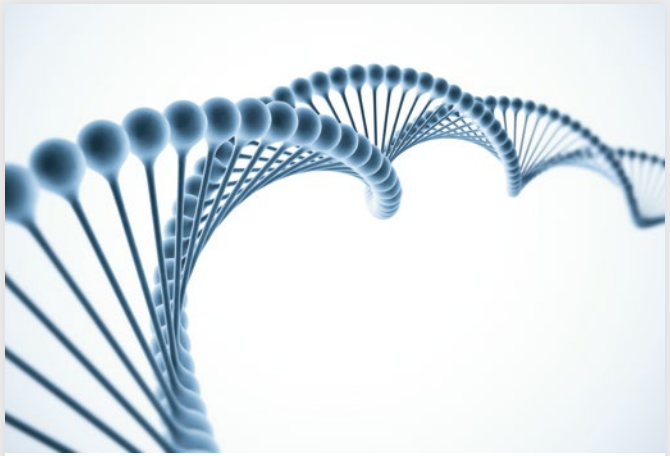
Resequencing

One of the most common sequencing applications, resequencing, involves the comparison of a specific sample genome against a reference genome to identify variations. Increasingly, the reference genome in cancer research and complex disease studies is obtained by sequencing a part of the human research subject that is not affected by the condition of study. A bone marrow cancer research project, for example, may sequence bone marrow and buccal swab (noncancerous) samples from the same person and use the buccal sample as the genomic control. As the cost of sequencing continues to decline, generating a unique reference genome for each person in a research study—which can be used for tumor/normal comparisons—is becoming routine.

The choice of library prep technology is based on the goal of the research project. What is the goal of your project?



Best genomic coverage
(I want to see all bases in my sequencing data)



Maximal yield

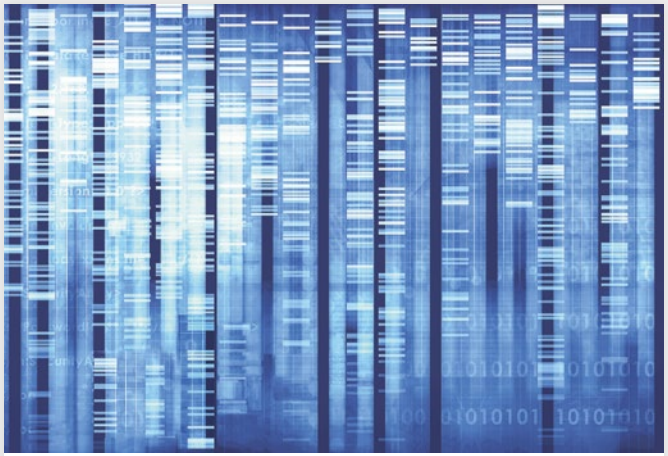
I'm not sure
(Help me choose)

Find out how Collibri DNA library prep kits improve accuracy of sequencing studies at **thermofisher.com/collibriDNA**

Population sequencing

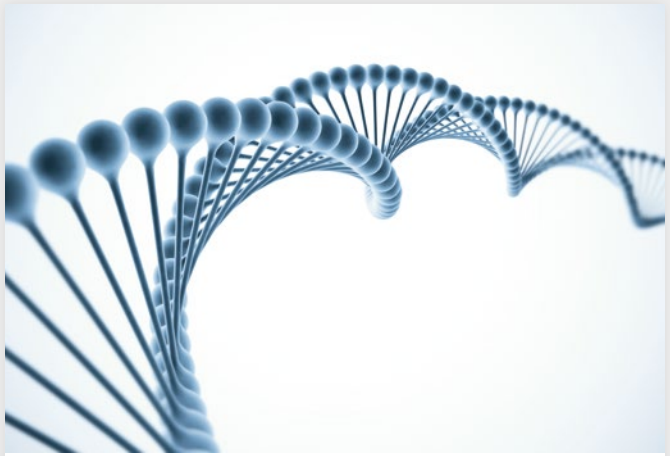
As the number of sequenced genomes increases, the ability to identify biomarkers for rare or emerging diseases increases. National health centers are increasingly looking to large cohorts of genomes in order to realize the promise of personalized medicine in the future. One example is the French Plan for Genomic Medicine 2025, developed by Inserm and its Aviesan partners, which aims to expand the use of whole-genome sequencing in clinical practice by sequencing 60,000 genomes each year until 2025, in order to strengthen personalized medicine [1].

The choice of library prep technology for population sequencing studies depends on the goal of the study. What is the goal or your project?



Best genomic coverage

(I want to see all bases in my sequencing data)



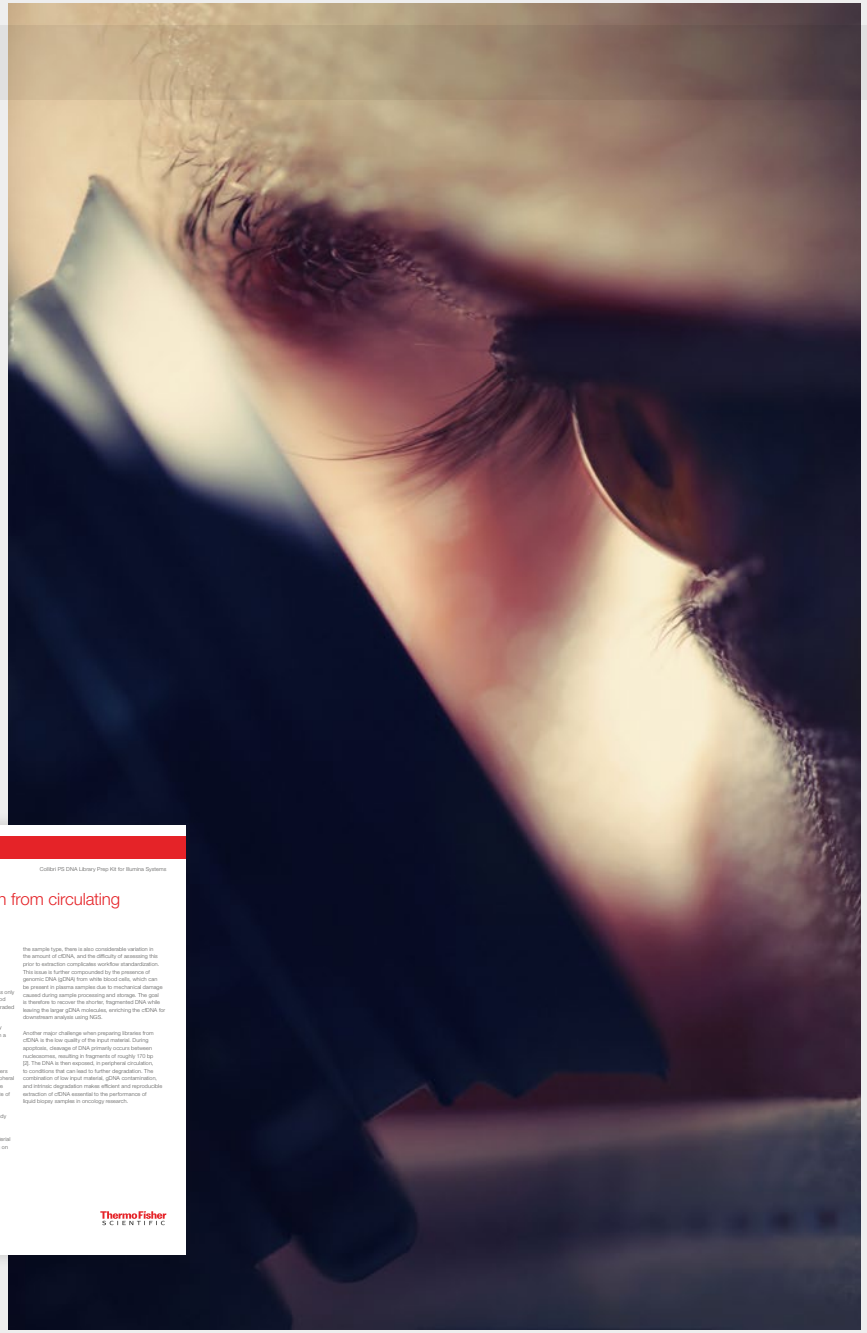
Maximal yield

I'm not sure

(Help me choose)

1. Inserm. Thematic National Plans.

cfDNA or ctDNA from liquid biopsy research samples



The use of liquid biopsies for research has become increasingly prevalent to investigate potential biomarkers within genetic material from tumors circulating in peripheral blood. Elevated levels of cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA) in peripheral blood are common with many cancers, due to the increased rate of apoptosis in many tumor cells. An **optimized protocol** for sensitive variant detection or copy number variation (CNV) detection is compatible with all Illumina™ NGS systems.

Library preparation method for cfDNA in cancer research

Researchers at the Central European Institute of Technology at Masaryk University in the Czech Republic—in collaboration with the EMBL Genomics Core Facility in Heidelberg, Germany—have recently developed a protocol for efficient cfDNA library preparation to investigate hemato-oncological disorders, mainly chronic lymphocytic leukemia (CLL). Combining this method with whole-genome sequencing (WGS), they have been able to observe genomic abnormalities that are specific to stages of disease, helping to guide their further research. This application note highlights the approach used in one of their studies.

invitrogen

APPLICATION NOTE

Collibri PS DNA Library Prep Kit for Illumina Systems

Library preparation from circulating cell-free DNA

Summary

- Circulating cell-free DNA (cfDNA) is becoming an increasingly important marker for assessing many cancer types
- cfDNA extraction and library preparation for next-generation sequencing (NGS) is challenging, as only very limited quantities of material are present in blood samples available for research, and the DNA is degraded
- The Invitrogen™ Collibri™ PS DNA Library Prep Kit for Illumina® Systems enables efficient cfDNA library generation for sequencing on Illumina systems, with a few modifications to the standard protocol

Introduction

The use of liquid biopsies for research has become increasingly prevalent to investigate potential biomarkers within genetic material from tumors circulating in peripheral blood. Elevated levels of cfDNA in peripheral blood are common with many cancers, due to the increased rate of apoptosis in many tumor cells.

Isolation of cfDNA and preparation of sequencing-ready libraries from cfDNA present a number of technical challenges. Despite cfDNA being elevated in some oncology research samples, the concentration of material available is still in the low ng/mL range [1]. Depending on the sample type, there is also considerable variation in the amount of cfDNA, and the difficulty of assessing this prior to extraction complicates workflow standardization. This issue is further compounded by the presence of genomic DNA (gDNA) from white blood cells, which can be present in plasma samples due to mechanical damage caused during sample processing and storage. The goal is therefore to recover the shorter, fragmented DNA while leaving the larger gDNA molecules, enriching the cfDNA for downstream analysis using NGS.

Another major challenge when preparing libraries from cfDNA is the low quality of the input material. During apoptosis, cleavage of DNA primarily occurs between nucleosomes, resulting in fragments of roughly 170 bp [2]. The DNA is then exposed, in peripheral circulation, to conditions that can lead to further degradation. The combination of low input material, gDNA contamination, and intrinsic degradation makes efficient and reproducible extraction of cfDNA essential to the performance of liquid biopsy samples in oncology research.

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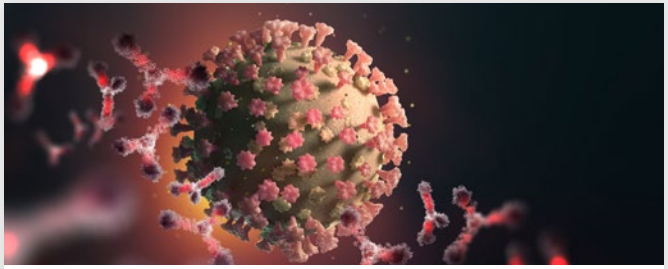
Find out how to improve results when studying cfDNA at **thermofisher.com/collibricfDNA**

Infectious disease research

While the number of available full genomes produced by NGS can grow rapidly, data sets have historically faced challenges in the quality of the sequences. High-quality sequences are required to trace epidemics, monitor for potentially emerging viruses within the population, develop potential antiviral therapies, identify targets for vaccine development, and **understand host–pathogen interactions**.

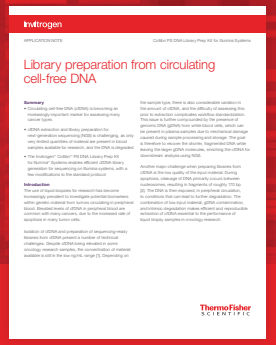
One option to improve the quality of sequences is to use library preparation kits that are designed to handle a wide variety of sample qualities and genome sizes to produce consistent, full-genome coverage. Compared to transposomic methods, the **Collibri DNA Library Prep Kits for Illumina Systems** consistently provide full coverage of pathogen and host genomes. The Collibri DNA kits are suitable for genomes of all sizes and may be used to sequence both the pathogen and the host to perform host–pathogen studies.

What is the goal of your study?



Investigate viral genomes

Study host–pathogen interactions



Sequence SARS-CoV-2 and other viral genomes

The optimized workflow to sequence SARS-CoV-2 reduces time and sequencing costs while providing flexibility to use available reagents. Generate high-quality genomic coverage from nasopharyngeal, oropharyngeal, and other diverse samples to improve accuracy in SARS-CoV-2 research studies.

Download

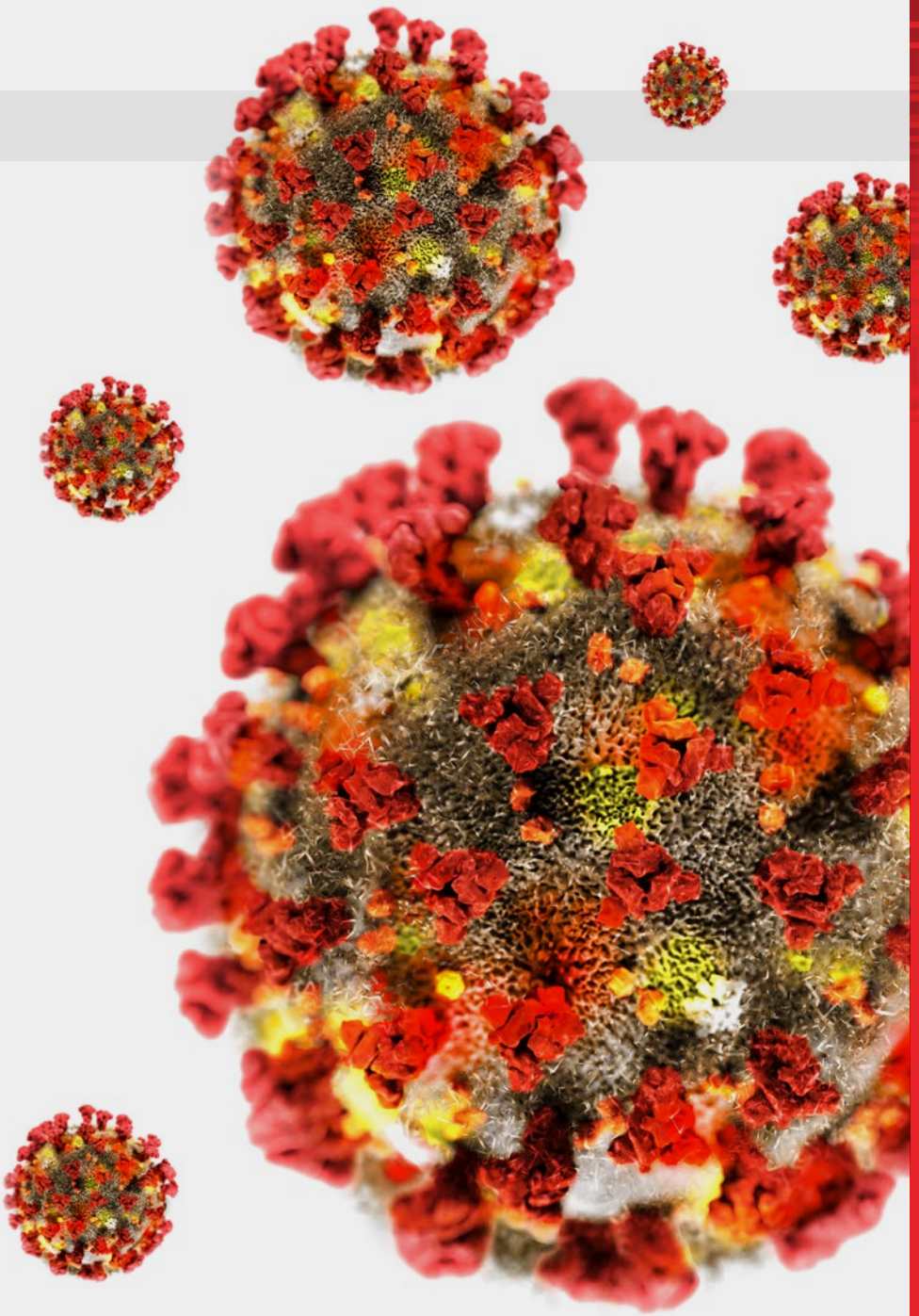
Download a protocol optimized for SARS-CoV-2 samples at **thermofisher.com/sarscov2-collibri**

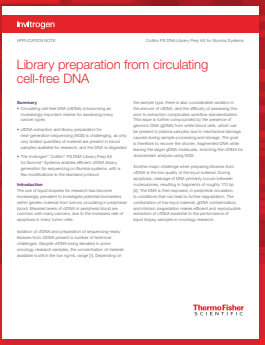
Investigate viral genomes

While the number of available viral genomes produced by NGS can grow rapidly, data sets have historically faced challenges in the quality of the sequences. High-quality sequences are required to trace epidemics, monitor for potentially emerging viruses within the population, develop potential antiviral therapies, identify targets for vaccine development, and understand host–pathogen interactions. A recent example is the use of NGS to enable monitoring of global transmission of SARS-CoV-2 to seek insights into viral evolution and pathology. The hypothesis-free analysis of SARS-CoV-2 genomes provided by Collibri DNA Library Prep Kits for Illumina Systems provides high coverage with sensitive variant detection. Faster, sensitive analysis of emerging strains allows the research community to provide insights for vaccine and therapeutic development.

A workflow optimized for the study of coronaviruses, including SARS-CoV-2, on Illumina NGS systems using Collibri ES DNA Library Prep Kits enables sensitive variant detection from genomic coverage from as few as 200,000 reads per sample.

Show me how to begin





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Download

View data and download a protocol optimized for SARS-CoV-2 samples at **thermofisher.com/sarscov2-collibri**

Unlock host–pathogen interactions

NGS 3′ mRNA gene expression analysis is a powerful, hypothesis-free tool to investigate why people respond in various ways to a pathogen, and has been used to study the impact of **Zika virus infection**. Gene expression profiles from people who become gravely ill can be compared to gene expression profiles from people who do not show symptoms (i.e., carriers) when infected with the same virus. In this approach, 3′ mRNA sequencing is used to rapidly compare gene expression profiles of many samples using simplified informatics. The method achieves simplicity and cost savings by reducing the number of required NGS reads from 30–60 million to 2–5 million per sample. The reduction in required reads provides flexibility in the choice of Illumina NGS systems and location of sequencing.

Gene expression profiles from the **Collibri 3′ mRNA Library Prep Kits for Illumina Systems** can be generated locally without the need to send samples to a distant core facility, using pre-built informatics profiles from companies such as **Genialis, Inc.**

Show me how to begin

Gene expression studies of viral infections

Rapid NGS gene expression profiling with reduced costs in sequencing chemistry is demonstrated by a study that used peripheral blood mononuclear cells from pregnant macaques acutely infected with Zika virus.

Dr. Nicholas Maness of Tulane University discusses the pros and cons of **3′ mRNA sequencing on Illumina NGS systems**, compared to whole-transcriptome sequencing, for gene expression studies.

Review case study

Biomarker discovery

As a proxy for protein production, RNA sequencing is used to discover novel biomarkers through hypothesis-free analysis of gene expression from research samples

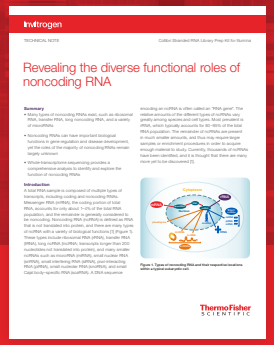
The **Collibri Stranded RNA Library Prep Kits for Illumina Systems** use a unique workflow in which helper adapters are added directly to RNA, making it possible to obtain all RNA transcripts in the resulting data without GC bias.

Retention of both coding RNA and noncoding RNA makes it possible to obtain coding and regulatory insights from each sample. The choice of an RNA-Seq library prep kit that is appropriate for a specific research project depends on the sample type.

What is the source of your sample?

Human, mouse, or rat

Plant or bacteria



Why are regulatory genes valuable for gaining insights into cancer, infectious diseases, and other biological processes?

Learn about the diverse functional roles of noncoding RNA and how they may influence human health.

Download

View data sets and find out more at **thermofisher.com/collibriWTrna**

Genomic basis for phenotypic expression

As genomic knowledge increases, it has become evident that the interaction among genes in response to environmental stimuli is a complex process

Stimuli can range from changes in exercise patterns to air pollution or stress. Coding and noncoding sequences interact to change the level and timing of gene expression, making whole-transcriptome sequencing an effective tool for investigating changes in phenotypic expression. The **Collibri Stranded RNA Library Prep Kits for Illumina Systems** use a unique workflow in which helper adapters are added directly to RNA, making it possible to obtain all RNA transcripts in the resulting data without GC bias. Retention of both coding and noncoding RNA makes it possible to obtain coding and regulatory insights from each sample. The choice of an RNA-Seq library prep kit that is appropriate for a specific research project depends on the sample type.

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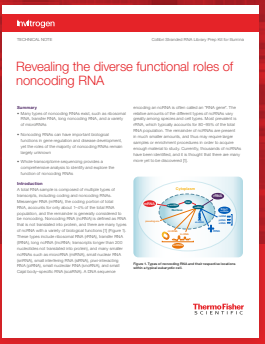

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Gene expression

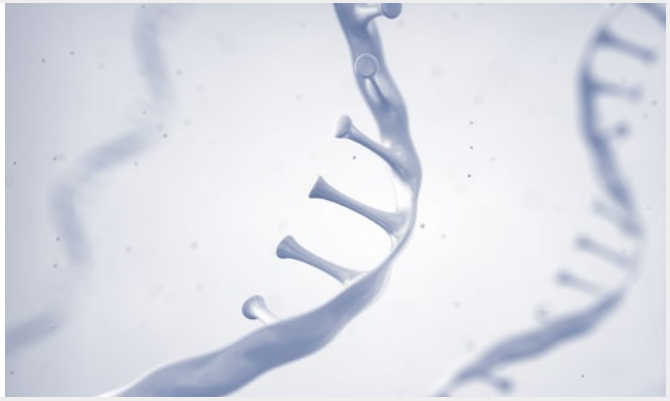
A steady cadence of insights into human health has been achieved by scientific consortia that study gene expression.

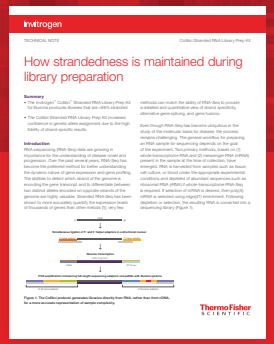
Two large international projects in particular—the **ENCODE Project** and the **GTEx Consortium**—have offered new information on regulatory sequences in the genome and their impact on gene expression. NGS techniques to investigate gene expression have expanded to provide tailored solutions to projects based on their investigation of coding sequences, noncoding sequences, or changes in gene expression in response to stimuli.

What is the goal of your project?

Analysis of gene expression

Analysis of gene expression, gene fusions, or transcript isoforms





How is strandedness maintained during this unique workflow?

Learn how helper adapters become full-length indexed adapters during library generation.

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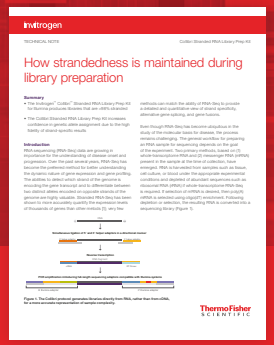
Find out more at **thermofisher.com/collibriRNA**

Role of gene fusions or alternative transcript isoforms in cancer research

Fusion genes are a class of oncogenes of interest for diagnostic and therapeutic cancer research due to their tumor-specific expression

Fusion genes have been identified in cancers ranging from hematologic to solid–tumor types and may provide attractive targets for precision medicine within oncology. Transcript isoforms with differential expression from the same gene (transcript switching) are an emerging focus of study within cancer research and therapeutics. Investigation of gene fusions or alternative transcript isoforms can be performed in hypothesis-free discovery research projects that do not require prior knowledge. The **Collibri Stranded RNA Library Prep Kits for Illumina Systems** provide sensitive detection of gene fusions and alternative transcript isoforms through library preparation that is not influenced by GC bias.

Show me how to begin



Discovery of alternative transcript isoforms requires knowledge of which strand the transcript was expressed from.

How is strandedness maintained during this unique workflow?

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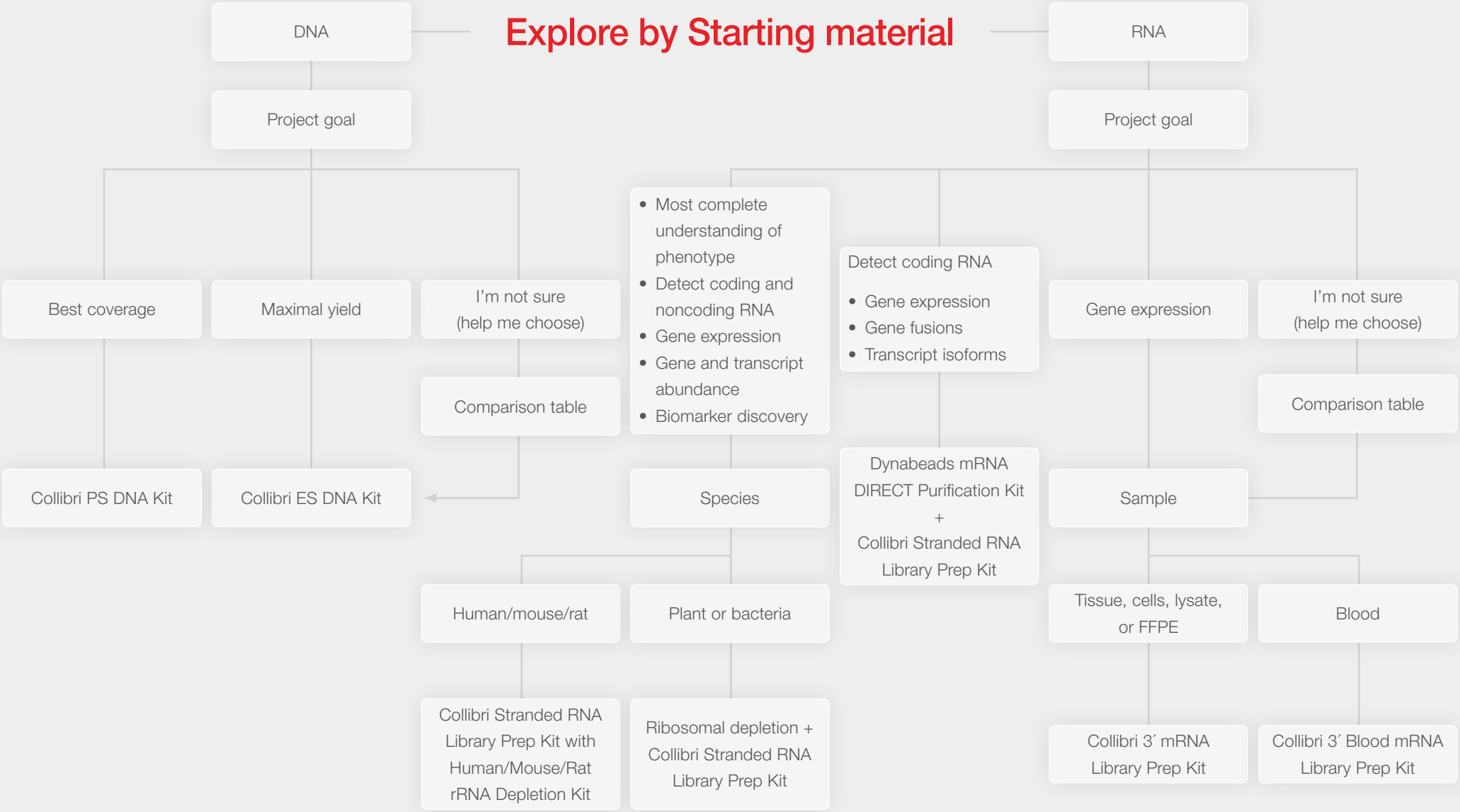
Explore by Starting material



Explore by Application

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2. In the Preferences dialog box, select **Trust Manager** in the Categories on the left and then click **Change Settings**.
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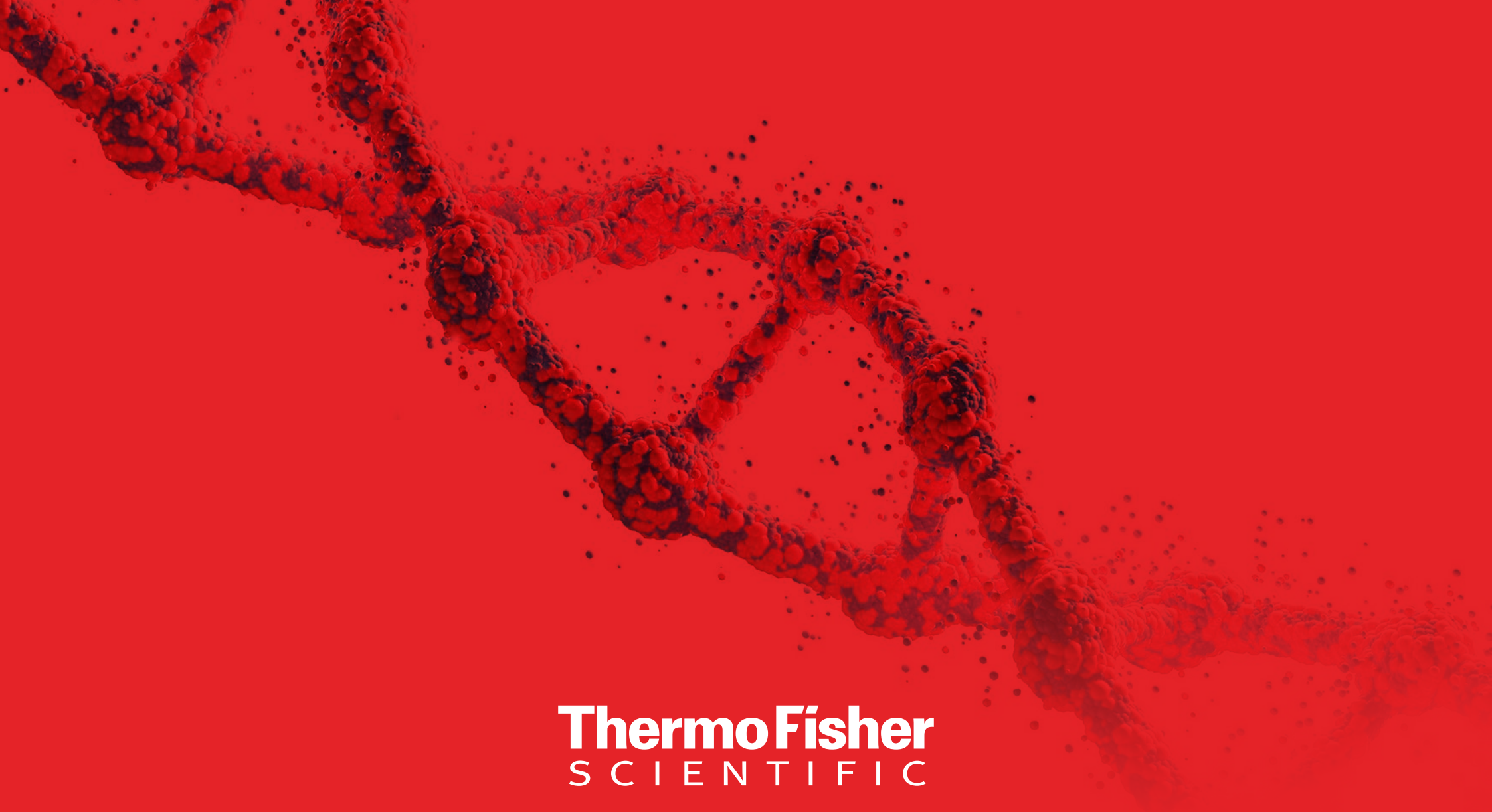

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