NEXTFLEX® HT Agrigenomics Low-Pass WGS Kit

(For Illumina® & Element® Platforms)

KIT CONTAINS: 24 or 384 RXNS

USER MANUAL FOR:

#NOVA-5141-70 (24 rxn, Barcodes 1-24)
#NOVA-5141-71 (384 rxn, Barcodes 1-384)
#NOVA-5141-72 (384 rxn, Barcodes 385-768)
#NOVA-5141-73 (384 rxn, Barcodes 769-1152)
#NOVA-5141-74 (384 rxn, Barcodes 1153-1536)

NEXTFLEX® HT Agrigenomics Low-Pass WGS Kit

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This product is for research use only.
Not for use in diagnostic procedures.

GENERAL INFORMATION

Product Overview

The NEXTFLEX® HT Agrigenomics Low-Pass WGS kit is designed for the highly efficient conversion of DNA, from both high- and low-quality samples, into Illumina® and Element® compatible libraries. The chemistry has been optimized to produce libraries from 1 ng – 500 ng DNA while minimizing bias and artifacts.

The integrated Fragmentation, End-Repair, and Adenylation simplifies workflow and shortens hands-on library construction time to ~3.5 h. In addition, the bundle of the library prep with up to 1,536 different Unique Dual Index adapter barcodes facilitates high-throughput applications.

There are three main steps involved in preparing DNA for sequencing: DNA fragmentation/end repair/adenylation, adapter ligation, and post-adapter ligation PCR amplification. The NEXTFLEX® HT Agrigenomics Low-Pass WGS kit contains the necessary material to take the user's extracted nucleic acid sample through preparation and amplification for loading onto flow cells for sequencing.

Kit Contents, Storage & Shelf Life

The NEXTFLEX® HT Agrigenomics Low-Pass WGS kit contains enough material to prepare 24 or 384 samples for Illumina® and Element® sequencing. The shelf-life of all reagents is at least 6 months when stored properly. The Nuclease-free Water can be stored at room temperature. The NEXTFLEX® NGS Cleanup Beads should be stored at 4°C, and all other components should be stored at -20°C.

Kit Contents	24 rxn	384 rxn	Storage Temp.
NEXTFLEX® HT Agri Frag Buffer	48 µL	768 µL	-20°C
NEXTFLEX® HT Agri Frag Enzyme	72 µL	1,152 µL	-20°C
NEXTFLEX® HT Agri Ligation Mix	240 µL	3.84 mL	-20°C
NEXTFLEX® HT Agri PCR Mix	300 µL	4.8 mL	-20°C
NEXTFLEX® HT Agri Primer Mix	60 µL	960 µL	-20°C
NEXTFLEX® UDI Barcodes (25 µM)	2.5 µL per well		-20°C
Nuclease-free Water	2 mL	30 mL	Room Temp.
NEXTFLEX® NGS Cleanup Beads	1.5 mL	22 mL	4°C

Required Materials Not Provided

- 1ng – 500 ng of DNA in up to 20 µL nuclease-free water
- Ethanol 100% (room temperature)
- 96 well PCR Plate Non-skirted (Phenix Research, Cat # MPS-499) or similar
- Adhesive PCR Plate Seal (BioRad, Cat # MSB1001)
- Magnetic Stand -96 (Thermo Fisher Scientific, Cat # AM10027) or similar
- Thermal Cycler
- 2, 10, 20, 200 and 1000 µL pipettes / multichannel pipettes
- Nuclease-free barrier pipette tips
- Vortex

Warnings and Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, contact us at https://www.revvity.com/contact-us/technical-support and chose the "Next Gen Sequencing" category.

- Do not use the kit past the expiration date.
- Ensure pipettes are properly calibrated as library preparations are highly sensitive to pipetting error.
- Do not remove enzymes from -20°C until immediately before use; return to -20°C immediately after use.
- Do not freeze NEXTFLEX® NGSCleanup Beads and vortex beads until they are a uniform suspension before use.
- Thermal cycling should be performed with a heated lid (103°C-105°C) except where specified.
- Maintain a laboratory temperature of 20°–25°C (68°–77°F).
- DNA sample quality may vary between preparations. It is the user's responsibility to utilize high quality DNA. DNA that is heavily nicked or damaged may impact results or cause library preparation failure. Absorbance measurements at 260 nm are commonly used to quantify DNA and 260 nm / 280 nm ratios of 1.8 - 2.0 usually indicate relatively pure DNA. The user should be aware that contaminating RNA, nucleotides, and single-stranded DNA may affect the amount of usable DNA in a sample preparation.
- Presence of EDTA in starting input DNA can alter final library size. For optimal results, input DNA should be resuspended in ultrapure water or low TE buffer.

Revision History

Version	Date	Description
V25.01	January 2025	Product Launch

SAMPLE PREP WORKFLOW

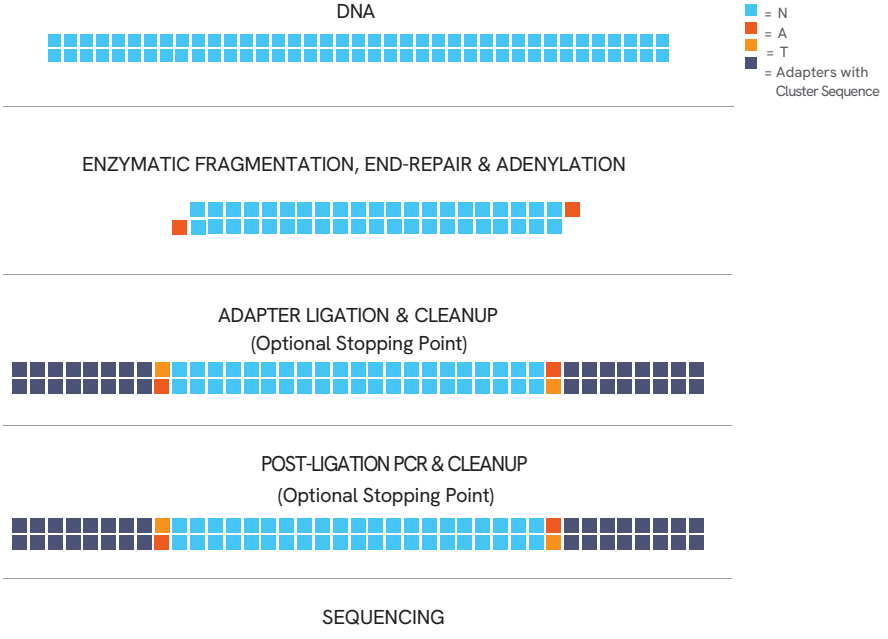


Figure 1. Sample flow chart showing the different steps of the protocol.

LIBRARY PREP PROTOCOL

Starting Material

The NEXTFLEX® HT Agrigenomics Low-Pass WGS kit has been optimized and validated using high- and low-quality genomic DNA inputs ranging from 1 ng - 500 ng.

Reagent Preparation

1. Briefly spin down each component to ensure material is not lodged in the cap or side of tube. Keep on ice and vortex each NEXTFLEX® component except NEXTFLEX® HT Agri Frag Enzyme, NEXTFLEX® HT Agri Ligation Mix, and NEXTFLEX® HT Agri PCR Mix prior to use. Nuclease-free Water should be stored at room temperature. NEXTFLEX® NGS Cleanup Beads should be stored at 4°C but equilibrated to room temperature prior to use.
2. DTT in buffers may precipitate after freezing. If precipitate is seen, vortex buffer for 1-2 minutes or until the precipitate is in solution. The performance of the buffer is not affected once the precipitate is in solution.
3. The 80% ethanol for bead washing steps should be freshly made prior to use.
4. Allow NEXTFLEX® NGS Cleanup Beads to come to room temperature and vortex the beads until homogenous.

SAMPLE PREP PROTOCOL

STEP A: Fragmentation, End-repair & Adenylation

MATERIALS

- NEXTFLEX® HT Agri Frag Buffer
- NEXTFLEX® HT Agri Frag Enzyme

User Supplied

- DNA in 20 µL (or less) nuclease-free water, 10 mM Tris-HCl, or Low TE (0.1 mM EDTA)
- Thermal Cycler
- 96 well PCR Plate
- Adhesive PCR Plate Seal
- Microcentrifuge

! NOTE: The presence of EDTA in the DNA sample can result in larger sized fragments in the final library. We strongly recommend the removal of EDTA from the sample prior to fragmentation.

1. Set the thermocycler to 4°C paused while performing the following steps:
2. For each sample, prepare Fragmentation Master Mix combine the following reagents on ice

   2 µL NEXTFLEX® HT Agri Frag Buffer

   3 µL NEXTFLEX® HT Agri Frag Enzyme

3. Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.
4. For each sample, add the fragmentation master mix as specified below on a 96 well PCR plate:

_ µL	Nuclease-free water
_ µL	DNA (1 ng - 500 ng)
5 µL	Fragmentation Master Mix
25 µL	TOTAL

5. Vortex each reaction to homogenize the reaction. The consistency of this mixing step from tube to tube and experiment to experiment is important for consistent fragmentation results between samples. Briefly centrifuge to collect the sample at the bottom of the tube, then place back on ice.

! NOTE: Complete mixing is critical to achieve desired fragment lengths. Mixing by pipetting is also acceptable. On ice, pipette 12 µL of the reaction up and down 10 times to mix completely.

6. Transfer plate to thermal cycler at 4°C (paused).
7. Apply adhesive PCR plate seal and incubate on a thermal cycler using the program below. Lid temperature is 105°C:

HOLD	4°C
1 min	4°C
5 min*	30°C
30 min	65°C
hold	4°C

*Here we describe optimized conditions for plant WGS applications. These times are recommendations only, and incubation time may need to be optimized for different sample inputs and types to obtain desired mode fragment size.

NOTE: The final library size will be approximately 120 bp larger than the fragment size.

8. Proceed immediately to Step B.

STEP B: Adapter Ligation

MATERIALS

- NEXTFLEX® HT Agri Ligation Mix
- NEXTFLEX® NGS Cleanup Beads
- NEXTFLEX® UDI Barcodes
- Nuclease-free Water

User Supplied

- 25 µL of Fragmented, End Repaired, and Adenylated DNA (from STEP A)
- Thermal Cycler
- Adhesive PCR Plate Seal
- 80% Ethanol, freshly prepared (room temperature)
- Magnetic Stand

Adapter Ligation

1. Invert the Ligation Mix 5 times to homogenize (DO NOT VORTEX) and place on ice.
2. Each sample will require 2.5 µL of barcoded adapter to be added. Combine the following in the PCR plate and mix thoroughly by pipette:

   *For starting input ≤10 ng, dilute NEXTFLEX® Barcoded Adapter 1:2 with ultrapure water.

25 µL	Fragmented, End Repaired & Adenylated DNA (from Step A)
10 µL	NEXTFLEX® HT Agri Ligation Mix
2.5 µL	NEXTFLEX® UDI barcodes
37.5 µL	TOTAL

! Adapter should not be premixed to prevent excess adapter dimer formation.

3. Apply adhesive PCR plate seal and run the following program on a thermal cycler with heated lid turned off or open:

30 min*	20°C
*hold	4°C

*Ligation time may be extended to a maximum of 16 h. Library quality decreases with overnight ligation.

Post-Ligation Clean-up

1. Remove plate from thermal cycler.
2. Add 30 µL of NEXTFLEX® NGS Cleanup Beads to the 37.5 µL of Adapter Ligated DNA.
3. Incubate at room temperature for 5 minutes.
4. Place the 96 well PCR plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
5. Remove and discard clear supernatant, taking care not to disturb beads. Some liquid may remain in well.
6. With the plate on the stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
7. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.
8. With the plate on the magnetic stand, let beads air dry at room temperature for 3 minutes. Do not over dry beads or yield may be negatively impacted.
9. Resuspend dried beads with 12 µL of Nuclease-Free Water. Mix thoroughly until homogenized.

   If with the bead pellet is difficult to resuspend at this volume, increase resuspension volume to 20 µl of Nuclease-Free Water, transfer 10 µL in step 12 and increase PCR by 1 cycle. To accommodate for the lost material, use the maximum recommend PCR cycles for the starting input.
10. Incubate sample at room temperature for 2 minutes.
11. Place the 96 well PCR plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
12. Do not discard the sample in this step. Transfer 10 µL of clear sample to a new well. Remove the 96 well PCR plate from the magnetic stand.
13. Proceed immediately to Step C.

STEP C: Post-Ligation PCR

MATERIALS

- NEXTFLEX® HT Agri PCR Mix
- NEXTFLEX® HT Agri Primer Mix

User Supplied

- 10 µL of Adapter Ligated DNA (from STEP B)
- Thermal Cycler
- Adhesive PCR Plate Seal
- 96 Well PCR Plate
- 80% Ethanol, freshly prepared (room temperature)
- Magnetic Stand

! *Thaw NEXTFLEX® HT Agri PCR Mix on ice. Once thawed invert several times or swirl to vigorously mix (DO NOT VORTEX).

1. For each sample, combine the following reagents on ice in the PCR plate. Mix thoroughly.

10 µL	Adapter Ligated DNA (from Step B)
12.5 µL	NEXTFLEX® PCR Master Mix *
2.5 µL	NEXTFLEX® Primer Mix*
25 µL	TOTAL

*These components can be premixed and added in a single step.

2. Apply adhesive PCR plate seal and place in thermal cycler for the following PCR cycle. Lid temperature is 105°C s:

Time	Temperature	Cycles
45 sec	98°C	1
15 sec	98°C	
30 sec	60°C	See Table
30 sec	72°C	
1 min	72°C	1
HOLD	12°C	1

Genomic DNA input (ng)	PCR Cycles
1	8-10
5	6-8
10	5-6
50	2-3
100	1-2
500	0

3. Post-PCR, proceed to Step D Post-Amplification Cleanup.

STEP D: Post-Amplification Cleanup

1. Add 20 µL of NEXTFLEX® NGS Cleanup Beads to the 25 µL of PCR-amplified libraries.
2. Incubate at room temperature for 5 minutes.
3. Place the 96 well PCR plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
4. Remove and discard clear supernatant taking care not to disturb beads. Some liquid may remain in well.
5. With the plate on the stand, add 200 µL of 80% ethanol to each magnetic bead pellet and incubate plate at room temperature for 30 seconds. Carefully remove ethanol by pipette.
6. Repeat previous step for a total of 2 ethanol washes. Ensure all ethanol has been removed.
7. With the plate on the magnetic stand, let beads air dry at room temperature for 3 minutes. Do not over dry beads or yield may be negatively impacted.
8. Resuspend dried beads with 22 µL of Nuclease-Free Water. Mix thoroughly until homogenized.
9. Incubate sample at room temperature for 2 minutes.
10. Place the 96 well PCR plate on the magnetic stand at room temperature for 2 minutes or until the supernatant appears completely clear.
11. Do not discard the sample in this step. Transfer 20 µL of clear sample to a new well. Remove the 96 well PCR plate from the magnetic stand.
    Quantification of each library can be performed using fluorometric methods [recommended: Qubit] to determine concentration.

    ! NOTE: qPCR is recommended to quantify DNA library template for optimal cluster density. This can be performed using any qPCR quantification kit for Illumina platforms and the NEXTFLEX® Primer Mix as needed.
12. Examine your libraries by electrophoresis to ensure proper library sizing [recommended: LabChip® GX Touch instrument (Revvity)].

    ! NOTE: Library size should be close to 425bp. If user does not have electrophoresis capabilities, this size may be used.
13. The library is now ready for cluster generation per the standard Illumina® protocol. Proceed to cluster generation or seal with adhesive PCR plate seal and store at - 20 °C.

Library Validation

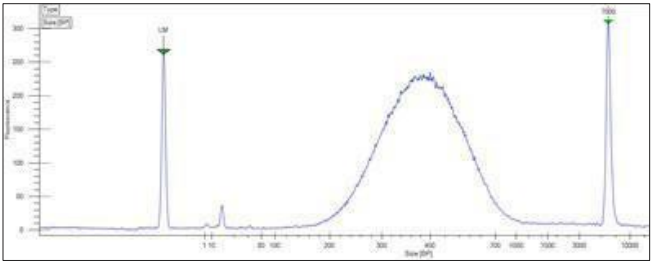


Figure 1: Libraries were analyzed using the LabChip® GX Touch™ instrument (Revvity)

APPENDIX A

Oligonucleotide Sequences

NEXTFLEX®	Sequence (5′ → 3′)
PCR Primer 1	AATGATACGGCGACCACCGAGATCTACAC
PCR Primer 2	CAAGCAGAAGACGGCATACGAGAT
NEXTFLEX® UDI Barcode	AATGATACGGCGACCACCGAGATCTACAC<u>XXXXXXXXXX</u>[1]ACACTCTTTCC CTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCAC<u>XXXXXXXXXX</u>[2]ATCTCGT ATGCCGTCTTCTGCTTG

<u>XXXXXXXXXX</u>[1] denotes the P5 index region of adapter. The index sequences contained in each adapter are listed below.

<u>XXXXXXXXXX</u>[2] denotes the P7 index region of the adapter. The index sequences contained in each adapter are listed below.

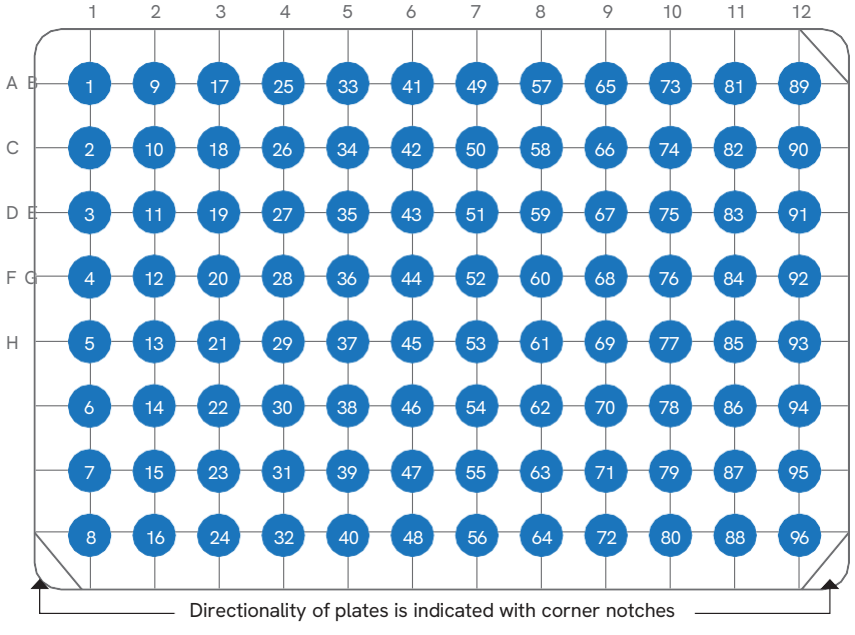
For a digital copy of indices, please visit our website or this address: https://www.revvity.com/content/nextflex-udi-barcodes-sequences-1536-set, or contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.

When entering index sequences for the Illumina® MiniSeq®, NextSeq®, HiSeq® 3000 or HiSeq® 4000 platforms, enter the P5 Index Reverse Complement. For all other Illumina® platforms, enter the P5 Index in the first column.

APPENDIX B

Plate Format

All barcodes in 96-well plate; 2.5 µL (1 reaction) / well
Representative Orientation



NOVA-5151-70: Contains only Barcodes 1-24 listed in column 1-3

Rest of part numbers contain 4x96-well plates with barcodes arrayed in columns as in the picture

NOVA-5151-71: Contains Barcodes 1-384
NOVA-5151-72: Contains Barcodes 385-768
NOVA-5151-73: Contains Barcodes 769-1152
NOVA-5151-74: Contains Barcodes 1153-1536

APPENDIX C

Curio Genomics Ultra-Efficient Data Analysis Platform

The CURIO™ platform, from Curio Genomics, uses proprietary ultra-parallelized cloud architecture to enable unprecedented data processing speed of large and complex genomic data sets. Through the platform's intuitive user interface, breeders can process, organize, visualize, and interpret their data independently, resulting in faster, more informed breeding decisions.

Figure 1: NEXTFLEX® HT Agrigenomics Low-Pass WGS Kit to CURIO™ Imputation analysis workflow

Breeder Friendly Next-Generation Bioinformatics

- From raw reads through VCF in minutes
- Transparent imputation with best-in-class algorithms (Beagle, Glimpse2, etc.)
- Works with any species, and supports building reference panels
- Easily visualize, filter, and compare imputed SNPs
- Faster iterations to optimize library prep & sequencing depth
- Automated & integrated for high throughput processing

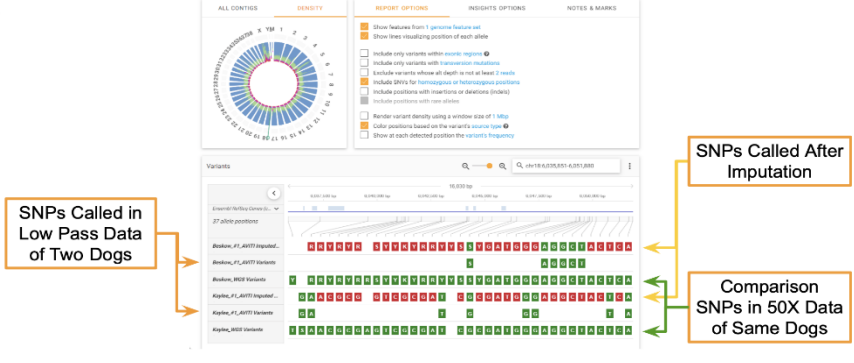
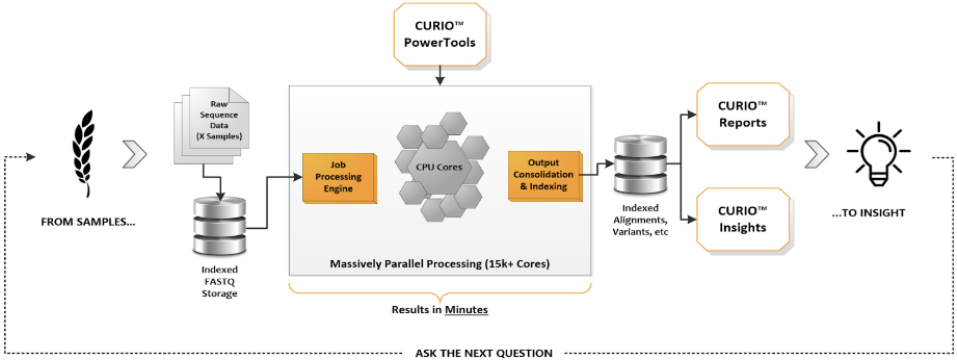


Figure 2: Comparing variant calls found in high coverage (50X) dog samples to variant calls found in low pass samples (1X), before and after imputation using the Beagle algorithm, which is one of the algorithms available within Curio. Curio highlights in green the variants found in the sequencing data, and shown in red are those imputed from the reference panel

CURIO's Proprietary Cloud-Based Architecture

CURIO provides rapid and scalable solutions that streamline the sequencing process for large populations. This efficiency is crucial for breeders who need to analyze extensive genomic data to make timely, data-driven decisions on breeding programs, ultimately enhancing desired traits.



Speed - Results in minutes

Versatility - Any species with reference

Flexibility - Massively scalable

Convenience - DIY reference builder

Efficiency - Automated workflows

Transparency - Open algorithms

From sample to insights, Revvity + CURIO enables simpler, more efficient agrigenomics with better results for more informed data-driven breeding decisions.

Learn more about Revvity + CURIO today
https://curiogenomics.com/revvity

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