DepleteX Human DNA Depletion Kit

KIT2020

Contents

Contact Us

If you have any questions, contact Technical Support at support@jumpcodegenomics.com

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Product overview

Human microbiome sample types are diverse and derived from many tissues, including saliva, gut and skin among others. Samples often consist of a mixture of human cells and microbes. Depending on the sample type and research goal, complex human-derived samples can be a challenge to analyze due to the large human host DNA content that obscures data from the microbial sequences of interest.

The Human DNA Depletion Kit leverages Cas9 depletion and exonuclease activity to efficiently remove human DNA from samples with high human content and reassign sequencing reads to microbial content. As a result, the kit allows users to generate more human microbiome data at lower sequencing costs.

Description	
Assay time	8 hours
Hands-on time	2.5 hours
Sample type	Human microbiome samples
DNA input	250 ng to 3 ug
DNA fragment size	>2 kb for efficient depletion
Method	CRISPR-Cas9 mediated depletion for shotgun metagenomics
Designed to deplete	Human DNA fragments
Short-read library prep recommendation	The final DNA yield of this protocol is low. It is important to use library preparation kits that support DNA inputs below 5 ng. This protocol has been validated on the Illumina DNA Prep (previously known as Nextera DNA Flex).
Sequencing platform	This protocol is compatible with short read sequencing platforms. For long read sequencing compatibility, please reach out to support@jumpcodegenomics.com for more information.

Workflow

This host depletion protocol is designed for a short-read sequencing workflow. The host depletion workflow is applied after DNA extraction but before DNA library preparation. High molecular weight DNA is highly recommended for maximum depletion efficiency.

The protocol takes approximately 8 hours, with 2.5 hours of hands-on time and 2 safe stopping points. Once complete, the host-depleted product is ready for use in NGS library generation. Detailed protocol steps are listed below.

The protocol consists of two main stages. The first stage involves DNA repair and end protection. The second stage involves CRISPR Cas-mediated cleavage of human DNA and subsequent exonuclease-mediated degradation of the DNA cut by the CRISPR Cas enzyme. The combination of CRISPR and exonuclease activity results in removing fragments containing human sequences.

1. DepleteX Human DNA Depletion Kit

   Step A:: 22kb Bead Cleanup

   Step B: Template DNA Repair and Protection

   ☐ Hands-on time: 75 min | Total time: ~4 hrs

   1. DNA Damage Repair
   2. Adapter Ligation
   3. Bead Cleanup
   4. Exonuclease Treatment
   5. Bead Cleanup

2. Safe Stopping Point

   Step C: Depletion of Human DNA

   ☐ Hands-on time: 70 min | Total time: ~4 hrs

   1. Ribonucleoprotein Complex Formation
   2. CRISPR Digestion
   3. Bead Cleanup
   4. Exonuclease Treatment
   5. Bead Cleanup

   Safe Stopping Point

3. DNA Library Prep

4. Sequencing

Kit Contents & Storage

The DepleteX Human DNA Depletion Kit contains enough material for the depletion of 24 samples. The kit contents and storage temperatures are indicated in the tables below.

ASY-1082-001: Human DNA Depletion Kit (24 Samples)

Stored at 4°C

Kit contents	Part number	Quantity per bag
Cleanup Beads	REA1121-001	1 tube

ASY-1062: Human DNA Depletion Kit (24 Samples)

Stored at -20°C

Kit contents	Part number	Quantity per box
Nuclease-Free Water	REA1027	2 tubes
Repair Buffer	REA1028	1 tube
Repair Enzyme	REA1029	1 tube
Proteinase K	REA1030	1 tube
End Prep Enzyme	REA1031	1 tube
Adapters	REA1034	1 tubes
Ligation Buffer	REA1032	1 tube
Ligation Enzyme	REA1033	1 tube
Tris Buffer	REA1038	1 tube
Cleanup Enzyme Buffer	REA1036	1 tube
Cleanup Enzyme	REA1035	1 tube
10x Cas9 Buffer	REA1001	1 tube
RNase Inhibitor	REA1007	1 tube
Cas9	REA1026	1 tube

ASY-1063: Human DNA Depletion Kit (24 Samples)

Stored at -80°C

Kit contents	Part number	Quantity per bag
Guide RNA for Human DNA Depletion	REA1037	1 tube

Required materials and equipment provided by the user

Type	Item	Supplier
Plastics	0.5 mL, 1.5 mL DNA LoBind Tubes	Eppendorf 022431021
	0.2 mL thin wall PCR tubes	General Lab Supplier
	Low-Retention Filtered Sterile Tips (10 µl, 20 µl, and 1000 µl)	General Lab Supplier
Reagents	AMPure XP Beads	Beckman Coulter A63881
	Absolute Ethanol, 200 Proof	General Lab Supplier
	Qubit dsDNA HS Assay Kit	ThermoFisher Scientific Q32854
Equipment	Single Channel Pipettes (10 µl, 20 µl, 200 µl, and 1000 µl)	General Lab Supplier
	Multichannel Pipettes (10 µl, 20 µl, and 200 µl)	General Lab Supplier
	Vortex Mixer	General Lab Supplier
	Microcentrifuge	General Lab Supplier
	PCR Magnetic Rack or Stand for use with tubes	General Lab Supplier
	Ice Bucket	General Lab Supplier
	PCR Thermal Cycler with block volume up to 100 µl	General Lab Supplier
	Qubit Fluorometer	ThermoFisher Scientific Q33238
	Automated electrophoresis, such as Agilent TapeStation	General Lab Supplier

Best Practices

General

- Periodically, optimizations and revisions are made to the kit components and user manual. Therefore, following the protocol included with or appropriate for the kit in question is important. This can be done by comparing the name and version number of the product to the name and version number of the protocol. If you need further assistance in this regard, please contact support@jumpcodegenomics.com
- Do not use the kit beyond 6 months after the manufacturing date. The date of manufacturing is present on the kit box.
- Maintain a laboratory temperature of 20°–25°C for room temperature incubations.
- Follow good laboratory practices throughout the protocol, including RNA-safe procedures when handling RNA. Ensure all pipette tips, microcentrifuge tubes, and other consumables are DNase- and RNase-free.
- Use properly calibrated pipettes, as low-volume transfers are sensitive to pipetting error. Use low-retention, filtered, sterile pipette tips throughout the protocol and avoid vortexing to minimize shearing of the DNA.
- When undertaking the protocol, always proceed immediately to the next step. If a stop is necessary, safe stopping points are available. Refer to the workflow schematic on page 3.

Reagent Handling

- Do not remove Cas9, RNase Inhibitor or other enzymes from storage until before use. Maintain on ice during reaction setup. Return to -20°C immediately after use.
- Do not remove the guide RNA from storage until immediately before use. Maintain on ice during reaction setup. Return to -80°C immediately after use. Avoid more than 3 freeze-thaw cycles of the guide RNA. Prepare aliquots of the guide RNA when you first use it if you believe you will use the kit contents more than 3-4 times.
- Do not freeze Size Selection Beads or AMPure XP beads. Beads should always be stored at 2°C to 8°C. Allow beads to come to room temperature before use. A minimum of 20 minutes at room temperature is recommended before use. Vortex and/or invert mix the beads several times immediately before each use to ensure homogenous resuspension.
- Ensure complete pipette mixing of the sample with Size Selection Beads or AMPure XP beads when beads are added to the sample. Failure to mix appropriately can lead to inconsistent results.

Equipment Handling

- Thermal cycling should be performed with a heated lid, except where specified otherwise. Thermal cycler(s) must support uniform heating up to 100 uL sample volume.
- Use magnetic stands appropriate for PCR tubes.

Revision log

Version	Date	Description
V1.0	August 20233	Product Release

Input DNA

This protocol is optimized for 250 ng – 3 ug of high molecular weight DNA. No shearing (either mechanical or enzymatic) of DNA is required. Inputs of >250 ng gDNA are required because the protocol is PCR-free and higher input amounts can improve the boost in microbial reads after depletion of contaminating human DNA.

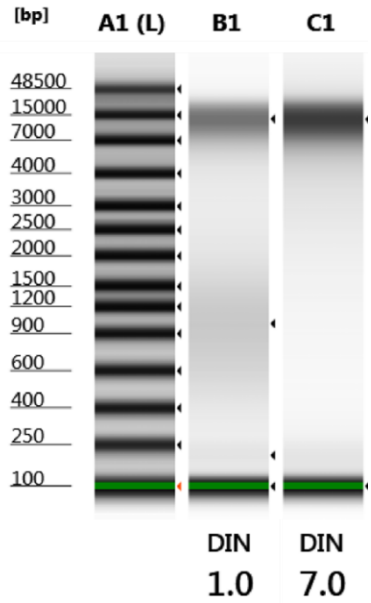
Prior to beginning the protocol, it is essential to evaluate DNA quantity and quality. The success of human DNA depletion is significantly improved with high molecular weight DNA size selected for >2 kb fragments. Even if the sample seems to meet the 2 kb size criteria, it is recommended that bead-based size selection using the provided Size-Selection Beads be used to select for >2 kb fragments before starting the protocol. Electrophoresis (either standard gel-based electrophoresis or pulse-field gel electrophoresis) may also be used for the size selection of long fragments and removal of smaller fragments.

Accurate gDNA quality control is recommended to maximize depletion efficiency. Validate input DNA quantity using a fluorometric-based method, such as the Qubit Fluorometer. **Evaluate quality using an automated electrophoresis method, such as the Agilent TapeStation.**

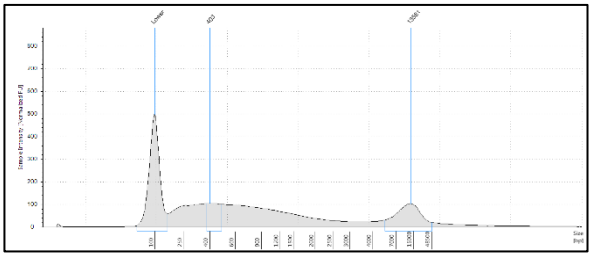
Step A: 2kb Bead Cleanup

1. Adjust sample volume to 100 µL using Tris Buffer or Nuclease-Free Water
2. Add 90 µL (1X) of thoroughly resuspended, room temperature Size Selection Beads to the sample. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 10 minutes.
4. Place the tube on a magnet until the solution clears (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 70% ethanol to the tube while still on the magnet. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnet while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, return it to the magnet and remove any traces of ethanol. Do not dry the beads.
8. Remove the tube from the magnet and immediately add 20 µL of Tris Buffer to the beads. Slowly pipette ~10 times to resuspend. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
9. Incubate the sample for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
10. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
11. Place the tube on the magnet. Allow the solution to clear and transfer the eluted DNA supernatant to a new Eppendorf DNA LoBind tube. This is a >2 kb size-selected DNA sample. It can be used as input material in the host DNA depletion protocol.
12. Quantify the output DNA. Qbit is recommended to quantify the quantity and Fragment Analyzer is recommended to obtain the fragment size range going into depletion (please see the figure below for example traces before and after bead size selection).

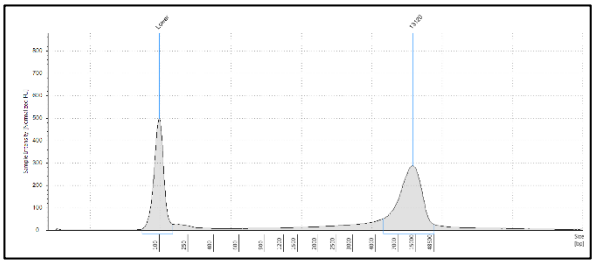
TapeStation profile of sample that has undergone bead-based size selection of >2 kb fragments:



TapeStation ScreenTape Image



Lane B1: No size selected material



Lane C1: Bead size selected for >2kb

Protocol

Step B: Template DNA Repair and End-Protection

☐ Hands-on time: 75 min | Total time: ~4 hrs

Reagents preparation

Item	Storage	Handling
Human DNA (50ng to 1 ug)	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
Repair Enzyme		
Proteinase K		
End Prep Enzyme		
Ligation Enzyme		
Cleanup Enzyme		
Repair Buffer	-20°C	Thaw at room temperature. Vortex briefly and spin down. Keep on ice.
Ligation Buffer		
Adapters		
Cleanup Enzyme Buffer		
Tris Buffer	-20°C	Bring to room temperature. Vortex and invert mix.
AMPure XP Beads	4°C	
80% Ethanol	Room Temperature	Prepare fresh.

B1: DNA Damage Repair

1. Obtain the Tris Buffer and AMPure XP beads (required for downstream steps) from the refrigerator and place them on the laboratory bench to bring them to room temperature. A minimum of 20 minutes at room temperature is recommended before use. Vortex the beads immediately before use to fully resuspend them in the buffer.

2. Combine the following reagents in the order listed below in a 0.2 mL Eppendorf DNA LoBind tube (or similar tube) on ice:

Cleaned up sample from Step A (50ng to 1 ug)	Up to 46 µL
Repair Buffer	7 µL
Repair Enzyme	2 µL
Nuclease-Free Water	Use to make up to total volume
Total Volume	55 µL

3. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube. **DO NOT VORTEX.**
4. Incubate the reaction for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C. Hold at 4°C or transfer the tube to ice immediately after that.
5. On ice, add 2 µL of Proteinase K to the reaction.
6. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
7. Place the tube in a thermal cycler wit a heated lid set t o ≥75°C. Run the following program:

Temperature	Cycle Time
37°C	15 min
65°C	5 min
4°C	HOLD

9. Transfer the tube to ice. Add 3 µL of End Prep Enzyme to the reaction.
10. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube. **DO NOT VORTEX.**
11. Place the tube in a thermal cycler with a heated lid set to ≥75°C. Run the following program:

Temperature	Cycle Time
20°C	30 min
65°C	30 min
4°C	HOLD

12. Proceed immediately to the next step (Adapter Ligation).

B2: Adapter Ligation

1. On ice, add the following reagents in the order listed below to the product of DNA damage repair (from the previous step):

Adapter Ligation Mix	Volume
Repair & End Prep Reaction product	60 µL (already present in tube)
Adapter	5 µL
Ligation Buffer	40 µL
Ligation Enzyme	5 µL
Total Volume	110 µL

2. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube. **DO NOT VORTEX.**
3. With the heated lid off, Incubate the reaction for 15 minutes at 20°C in a thermal cycler (see Best Practices), then hold at 4°C.
4. Proceed immediately to the next step (Bead Cleanup).

B3: Bead Cleanup

1. Add 110 µL (1X) of thoroughly resuspended, room temperature AMPure XP beads to the sample. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
2. Incubate the sample at room temperature on the laboratory bench for 5 minutes.
3. Place the tube on a magnet until the solution is clear (3-5 minutes). Discard the supernatant without disturbing the beads.
4. Add 200 µL of freshly prepared 80% ethanol to the tube while on the magnet. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnet while performing the ethanol wash.
5. Repeat the previous wash step.
6. Briefly spin the tube, return to the magnet and remove any traces of ethanol. Do not allow the beads to air dry. Overdrying the beads affects elution yield.
7. Remove the tube from the magnet and immediately add 20 µL of Tris Buffer to the beads. Slowly pipette mix ~10 times to resuspend. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
8. Incubate the sample for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C.
9. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
10. Place the tube on the magnet. Allow the solution to clear and transfer the eluted DNA supernatant to a new 0.2 mL Eppendorf DNA LoBind tube (or similar tube).
11. Proceed immediately to the next step (Exonuclease Treatment).

B4: Exonuclease Treatment

1. On ice, add the following reagents in the order listed below to the tube containing the adapter-ligated product (from the previous step):

Exonuclease Reaction Mix	Volume
Adapter Ligation product	20 µL (already present in tube)
Nuclease-Free Water	22 µL
Cleanup Enzyme Buffer	5 µL
Cleanup Enzyme	3 µL
Total Volume	50 µL

Note: The Cleanup Enzyme should be maintained on ice during this step and transferred back to the -20°C freezer immediately after use.

2. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the reaction for 30 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C, then hold at 4°C. Proceed immediately to the next step (Bead Cleanup).

B5: Bead Cleanup

1. Add 50 µL of Tris Buffer to the sample.
2. Add 100 µL (1X) of thoroughly resuspended, room temperature AMPure XP beads to the sample. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 5 minutes.
4. Place the tube on a magnet until the solution clears (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube while still on the magnet. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnet while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, return to the magnet and remove any traces of ethanol. Do not allow the beads to air dry. Overdrying the beads affects elution yield.
8. Remove the tube from the magnet and immediately add 17 µL of Tris Buffer to the beads. Slowly pipette mix ~10 times to resuspend. Briefly spin the tube in a microcentrifuge to collect all of the liquid at the bottom of the tube.
9. Incubate the sample for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C.
10. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
11. Place the sample on the magnet. Allow the solution to clear and transfer the eluted DNA supernatant to a new Eppendorf DNA LoBind microcentrifuge tube (or similar tube). This is your “End-Protected DNA.”
12. Quantify 1 µL of the sample using a dsDNA fluorescent dye method, such as with a Qubit dsDNA HS kit. Multiply the concentration (ng/µL) by the elution volume to determine the total yield (ng).

SAFE STOPPING POINT

The tube can be safely stored at -25°C to -15°C.

Step C: Depletion of Human DNA

☐ Hands-on time: 70 min | Total time: ~4 hrs

Reagents preparation

Item	Storage	Handling
Step B "End-Protected DNA"	-20°C	If frozen, thaw on ice. Finger-tap mix and spin down. Keep on ice. Return to freezer immediately after use.
RNase Inhibitor		
Cas9		
Cleanup Enzyme		
Guide RNA for Human DNA Depletion	-80°C	
Nuclease-Free Water	-20°C	Thaw at room temperature. Vortex briefly and spin down. Keep on ice.
10X Cas9 Buffer		
Cleanup Enzyme Buffer		
Tris Buffer	-20°C	Bring to room temperature. Vortex and invert mix
AMPure XP Beads	4°C	During incubation, the AMPure XP beads required for subsequent size selection steps may be removed from the refrigerator and placed on the laboratory bench to bring them to room temperature. A minimum of 20 minutes at room temperature is recommended before use.
80% Ethanol	Room Temperature	Prepare fresh.

C1: Ribonucleoprotein Complex Formation

1. Review concentration measurements of the End-Protected DNA in Step B5-12 (previous step).
   a. If the yield of the End-Protected DNA is >50 ng, dilute it with Nuclease-Free Water to 50 ng in 15 µL. Set the quantity aside for the CRISPR Digestion step (Step C2-1).
   b. If the End Protected DNA yield is ≤50 ng, set aside the entire 15 µL volume of End-Protected DNA for the CRISPR Digestion step (Step C2-1).
2. At room temperature, combine the following reagents in the order listed below in a new 0.2 mL Eppendorf DNA LoBind tube or similar tube:

"RNP Complex Formation" Reaction Mix	Volume
Nuclease-Free Water	4.57 µL
10X Cas9 Buffer	1.00 µL
RNase Inhibitor	1.00 µL
Cas9 (diluted 1:2 in 1X Cas9 buffer immediately before use)	1.04 µL
Guide RNA for Human DNA Depletion	0.89 µL
Total Volume	8.50 µL

Note: The Cas9 and RNase Inhibitor reagents should be maintained on ice during this step and transferred back to the -20°C freezer immediately after use. The guide RNA should be completely thawed on ice before use and returned to the -80°C freezer immediately after use.

3. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
4. Incubate the reaction at room temperature for 10 minutes.
5. This tube contains the ribonucleoprotein complex (RNP). Proceed immediately to the next step (CRISPR Digestion).

C2: CRISPR Digestion

1. Combine the following reagents in the order listed below with the sample from the previous step (Step C1-5):

CRISPR Digestion Reaction Mix	Volume
Product of the previous step (ribonucleoprotein complex)	8.5 µL (already present in tube)
10X Cas9 Buffer	1.5 µL
End-Protected DNA (from Step A5-12)	15 µL
Total Volume	25 µL

2. Mix by pipetting ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Place the tube in a thermal cycler with a heated lid set to ≥75°C. Run the following program:

Temp	Cycle Time
42°C	60 min
65°C	5 min
4°C	HOLD

Note: During incubation, the Tris Buffer and AMPure XP beads required for subsequent size selection steps may be removed from the refrigerator and placed on the laboratory bench to bring to room temperature. A minimum of 20 minutes at room temperature is recommended before use. Vortex the beads immediately before use to fully resuspend them in the storage buffer.

C3: Bead Cleanup

1. Add 25 µL of Tris Buffer to bring the volume of the CRISPR digestion reaction to 50 µL.
2. Add 50 µL (1X) of thoroughly resuspended, room temperature AMPure XP beads to the reaction. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 5 minutes.
4. Place the tube on a magnet until the solution clears (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube while still on the magnet. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnet while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, return it to the magnet and remove any traces of ethanol. Do not allow the beads to air dry. Overdrying the beads affects elution yield.
8. Remove the tube from the magnet and immediately add 20 µL of Tris Buffer to the beads. Slowly pipette mix ~10 times to resuspend. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.

9. Incubate the sample for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C.
10. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
11. Place the sample on the magnetic stand. Allow the solution to clear and transfer the eluted DNA supernatant to a new 0.2 mL Eppendorf DNA LoBind tube (or similar tube).
12. Proceed immediately to the next step (Exonuclease Treatment).

C4: Exonuclease Treatment

1. On ice, add the following reagents in the order listed below to the sample from the previous step:

Exonuclease Reaction Mix	Volume
Product from previous section (Step C3-11)	20 µL (already present in tube)
Nuclease Free Water	22 µL
Cleanup Enzyme Buffer	5 µL
Cleanup Enzyme	3 µL
Total Volume	50 µL

Note: During this step, the Cleanup Enzyme should be maintained on ice or in a benchtop cooler and transferred back to the -20°C freezer immediately after that.

2. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the reaction for 30 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C, then hold at 4°C.
4. Proceed immediately to the next step (Bead Cleanup).

C5: Bead Cleanup

1. Add 50 µL of Tris Buffer to the product of exonuclease treatment (from the previous step).
2. Add 100 µL (1X) of thoroughly resuspended, room temperature AMPure XP beads to the reaction. Mix by pipetting up and down ~10 times. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
3. Incubate the sample at room temperature for 5 minutes.
4. Place the tube on a magnet until the solution clears (3-5 minutes). Discard the supernatant without disturbing the beads.
5. Add 200 µL of freshly prepared 80% ethanol to the tube while still on the magnet. Wait 30 seconds, then remove and discard the ethanol. Do not remove the tube from the magnet while performing the ethanol wash.
6. Repeat the previous wash step.
7. Briefly spin the tube, return it to the magnet and remove any traces of ethanol. Do not allow the beads to air dry. Overdrying the beads affects elution yield.
8. Remove the tube from the magnet and immediately add 15 µL of Tris Buffer to the beads. Slowly pipette mix ~10 times to resuspend. Briefly spin the contents of the tube in a microcentrifuge to collect the liquid at the bottom of the tube.
9. Incubate the sample for 15 minutes at 37°C in a thermal cycler with the heated lid set to ≥50°C.
10. Briefly spin the contents of the tube in a microcentrifuge again to collect the liquid at the bottom of the tube.
11. Place the tube on the magnet. Allow the solution to clear and transfer the eluted DNA supernatant to a new Eppendorf DNA LoBind tube. This is the human DNA-depleted metagenomic sample. The depleted sample tube can be safely stored at -25°C to -15°C.

SAFE STOPPING POINT

Next Steps:

The human DNA-depleted sample is ready for library preparation and subsequent short-read sequencing.

DNA quantification: Depending on initial DNA input and the relative quantity of human DNA in the sample, the DNA concentration of the sample may or may not be quantifiable by standard quantification methods, such as the Qubit fluorometer or Agilent TapeStation. However, even if no significant DNA is detected, it is recommended that the user continues with NGS library preparation. It is also recommended that the user employ the entire CRISPRclean depleted sample (13 – 14 µL) for library preparation when working with non-quantifiable samples.

DNA library preparation: Unless using high initial DNA inputs (>1 µg), the final DNA yield of this protocol will be low. It is essential to use library preparation methods that support DNA inputs below 5 ng and, potentially, as low as 1 ng. We recommend the Illumina DNA Prep (formerly Illumina Nextera Flex) for library preparation.

Contact support@jumpcodegenomics.com for additional information.