

**my** NGS Guides™

## **MitoDeplete™ Kit**

Removal of mitochondrial DNA-derived sequences from NGS libraries

**Manual**

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**FOR RESEARCH USE ONLY. Not intended for diagnostic use.**



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**CONTENTS**

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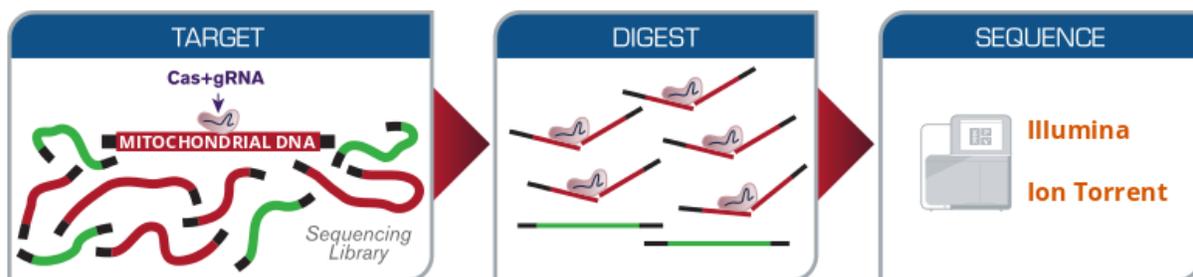
<b>INTRODUCTION</b>	
Specifications	3
Product compatibility	4
Kit components and stability	4
<b>REQUIREMENTS AND RECOMMENDATIONS</b>	
Materials required	4
Library specifications	4
<b>PROCEDURE</b>	5
<b>TYPICAL RESULTS</b>	6
<b>APPENDIX</b>	
A1 Frequently asked questions	7
A2 Predicted off-target effects	7

## INTRODUCTION

The MitoDeplete kit facilitates removal of mitochondrial DNA molecules from the sequenceable fraction of a DNA sequencing library (e.g. ATAC-Seq library). It can be used on a pool of libraries immediately prior to loading on the sequencing platform.

Before using this kit, a sequencing library must first be made using a validated protocol and quantified for total mass, preferably using library quantitative PCR. Then library is mixed with a ribonucleoprotein (RNP) complex of *S. pyogenes* Cas9 nuclease and sgRNA molecules to specifically digest mitochondrial DNA targets, rendering them non-amplifiable and non-sequenceable.

Pre-designed panels of species-specific Cas9 single guide RNAs are available for human and mouse. Cas9 guide RNAs of any kind can also be coupled with this kit with minimal procedure modifications - contact us for any scale of custom sgRNA synthesis.



## Specifications

<b>Description</b>	Reagents and protocol to cleave mitochondrial DNA templates in a user-provided sequencing library pool
<b>Procedure Time</b>	2 hours
<b>Hands-On Time</b>	15 minutes
<b>Input Material</b>	100 ng sequencing library (see Appendix - FAQ for more information)
<b>Depletion Rate</b>	~ 70-95% mtDNA reads removed
<b>Material Recovery</b>	~ 10-40 ng, depending on starting mtDNA proportion

## Arbor Biosciences products compatible with this manual

Catalog #	Name	Description
901008	myNGS Guides MitoDeplete - Human	Kit for depleting human mitochondrial DNA molecules from sequencing libraries
902008	myNGS Guides MitoDeplete - Mouse	Kit for depleting mouse mitochondrial DNA molecules from sequencing libraries
909008	myNGS Guides MitoDeplete - Custom	Kit for depleting customized species mitochondrial DNA molecules from sequencing libraries

Contact [info@arborbiosci.com](mailto:info@arborbiosci.com), or visit our website, for product manuals not listed above.

## Kit components and stability

Box	Reagent	Cap Color	Volume (8 rxn)
<b>Box 1</b> <i>Store at -20 °C</i>	Cas9 Enzyme	Purple	10 µL
	10x Buffer C	Yellow	20 µL
	ProK	Green	20 µL
	Nuclease Free Water	White	200 µL
<b>Box 2</b> <i>Store at -80 °C</i>	Guides - Human	Blue	20 µL
	- or - Guides - Mouse	- or - Orange	

At the recommended storage temperatures, the MitoDeplete kit components have a shelf life of 6 months from the date of receipt.

## REQUIREMENTS AND RECOMMENDATIONS

### Materials required

- Nuclease-free (**NF**) 0.2 mL tubes
- Pipettors and tips capable of pipetting 1 µL – 20 µL
- Thermocycler
- Silica-column PCR purification system (e.g. QIAquick® PCR Purification Kit from QIAGEN®)

### Library specifications

- 100 ng sequencing library in a total of up to 9 µL.
- Multiple individual libraries can be pooled into a single depletion reaction.

## PROCEDURE

1. Thaw the Guides on ice. In a 0.2 mL tube, combine the following to generate the “**RNP Mix**”:

 ***RNases will destroy guide RNAs! Use RNase-free equipment and bench technique.***

Guides	2 $\mu$ L
Cas9 Enzyme	1 $\mu$ L
10x Buffer C	1 $\mu$ L
Nuclease-Free Water	6 $\mu$ L
<b>TOTAL</b>	<b>10 <math>\mu</math>L</b>

2. Mix by pipetting up and down 5 times and incubate the “**RNP mix**” at room temperature for 10 minutes. During this time, complete step 3.

3. In a separate 0.2 mL tube, combine the following to generate the “**Library Mix**”

Sequencing library (100 ng in 9 $\mu$ L)	9 $\mu$ L
10x Buffer C	1 $\mu$ L
<b>TOTAL</b>	<b>10 <math>\mu</math>L</b>

4. When the “**RNP Mix**” room temperature incubation has completed, transfer 10  $\mu$ L “**RNP Mix**” to 10  $\mu$ L “**Library Mix**” and homogenize by pipetting up and down 5 times (avoid generating bubbles). This is the “**Depletion Reaction**”.

5. Incubate the “**Depletion Reaction**” at 37°C for 1 hour in a thermocycler (without heated lid).

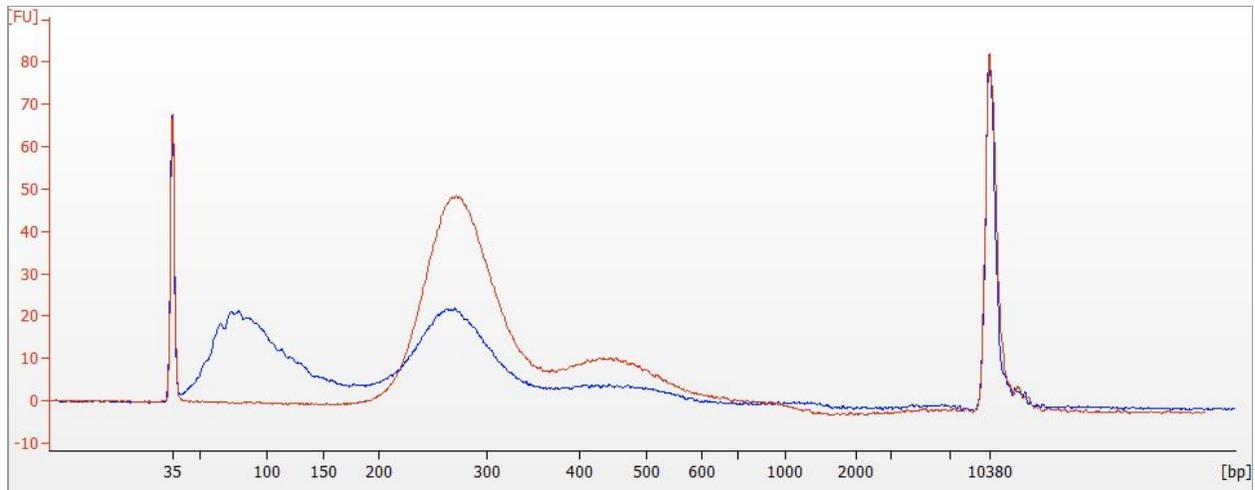
 ***Incubation time is critical (see FAQ). Proceed immediately to the next step.***

6. Add 2  $\mu$ L of ProK to the “**Depletion Reaction**” and mix by pipetting up and down 5 times. Then incubate for 15 min at 37°C in the thermocycler (without heated lid).

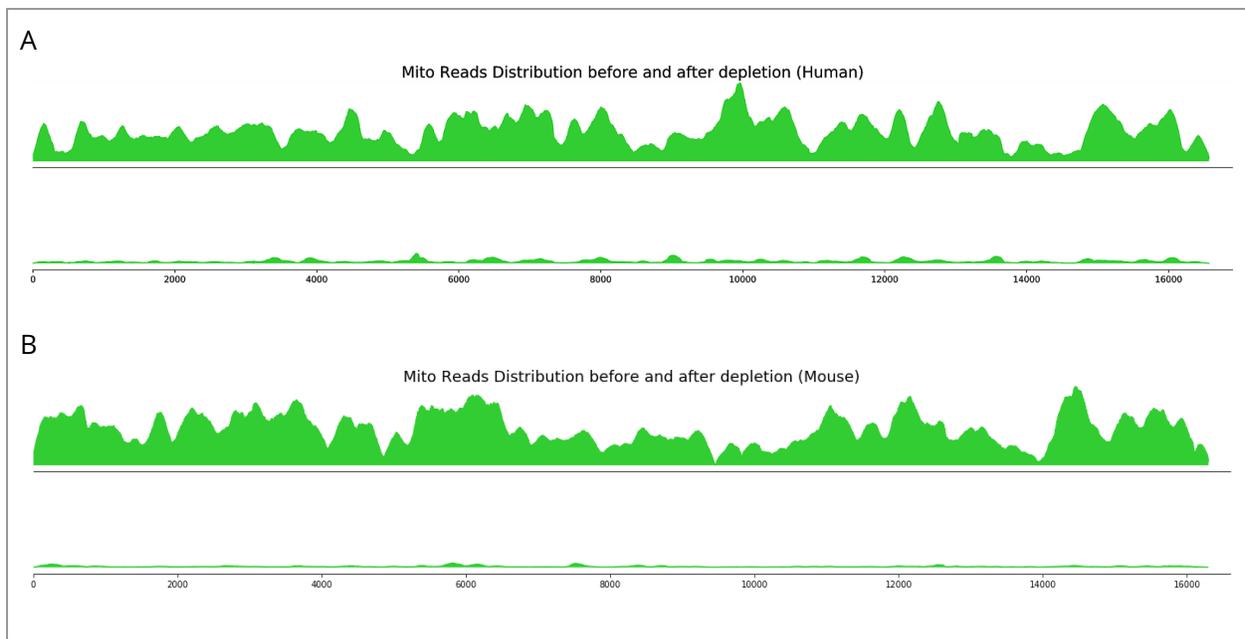
7. Purify the final 22  $\mu$ L “**Depletion Reaction**” using silica columns according to the manufacturer's instructions.

8. Quantify amplifiable DNA in the eluate by library qPCR. Use the eluate directly for sequencing. Further amplify the library only if necessary.

## TYPICAL RESULTS



**Figure 1.** Bioanalyzer® trace using Agilent dsDNA high sensitivity chip of sequencing library both before (in Red) and after (in Blue) depletion. Input 148ng of library with 33.8% Mito; incubated 1h at 37°C, column purified.



**Figure 2.** Normalized read coverage of human (A) and mouse (B) libraries on their mitochondrial genomes before and after depletion. Depletion reactions were performed on 100 ng human sequencing library containing 56% starting mtDNA, and 140 ng mouse sequencing library containing 36% mtDNA. After treatment, mitochondrial reads were reduced by 92% and 94%, respectively.

## APPENDIX I - FAQ

### **Can I pre-build and then store my RNP Mix prior to performing depletion?**

No, we recommend using RNP Mix immediately after the 10 minute room temperature incubation.

### **With what kinds of libraries is the MitoDeplete kit compatible?**

The kit is directly compatible with Illumina® and Ion Torrent® NGS libraries. However, when treating Oxford Nanopore® or PacBio® libraries, short fragments should be removed after depletion to prevent residual fragments from occupying space on the platform flow cell.

### **Can I use MitoDeplete with more/fewer than 100 ng sequencing library?**

Each MitoDeplete reaction can accommodate as few as 30 ng and as many as 150 ng input library under standard conditions. However the rate of nonspecific digestion depends on the total amount of mtDNA in the library, therefore optimization of the digestion time may be required for best results.

### **The off-target site list overlaps with my region(s) of interest. What should I do?**

We can design and synthesize custom myNGS Guides panels that avoid your target region(s) of interest while still depleting your desired non-target molecules. Please contact [info@arborbiosci.com](mailto:info@arborbiosci.com) for more information.

### **Can I use SPRI beads to clean up the depletion reaction?**

Yes. You can clean up the “Depletion Reaction” by using SPRI beads according to the manufacturer’s instructions.

### **How can I improve the rate of mtDNA depletion?**

Depletion rates are influenced by the input library amount and length distribution, the relative fraction of mitochondrial templates, the amount of Guides and Cas9, and the time and temperature of the digestion.

- For low starting mitochondrial DNA (<25%), consider reducing the incubation time from 1 hour to 30 minutes.
- For high starting mitochondrial DNA (>80%), considering increasing the incubation time from 1 hour to 2 hours.
- For long-insert libraries (>500 bp average length), consider reducing the Guides to 0.5 µL per reaction and Cas9 Enzyme to 0.25 µL per reaction. Pre-dilution of both components may be required for accurate pipetting.

## APPENDIX II - PREDICTED OFF-TARGET EFFECTS

The sgRNA pools included with MitoDeplete have been assayed *in silico* for specificity to the mitochondrial genome. Predicted off-target site lists for both human and mouse kits are available upon request to [info@arborbiosci.com](mailto:info@arborbiosci.com).