JUMPCODE GENOMICS

USER MANUAL

KIT2002

# DepleteX® Mito DNA Depletion Kit for the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay

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## Contact Us


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

Find us at our website: jumpcodegenomics.com

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## Product overview

Mitochondrial RNA is a big challenge in single cell RNA Seq studies especially in low viability samples. This contamination can result in unnecessary sequencing of mitochondrial genes that may be undesired. Combining the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay with Jumpcode's DepleteX assay provides improved data quality and biological insights in challenging samples exhibiting high mitochondrial reads. Depletion can be added to the BD Rhapsody™ WTA Assay protocol where high mitochondrial reads in challenging samples can limit sensitivity to detect biologically interesting mRNA expression.

| Description | |
|---|---|
| Catalog | KIT2002 |
| Samples per kit | 24 |
| Assay time | 2 hours |
| Hands-on time | 45 min |
| Sample type | Human-derived single cell samples with high mitochondrial content |
| Designed to deplete | Mitochondrial DNA or RNA |


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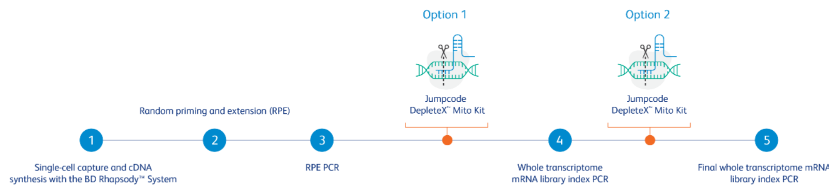
# Workflow

Users have two options for implementing mitochondrial depletion with Jumpcode DepleteX® in the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol (ID: 23-24117):

1) After the RPE PCR step (Step 15 in RPE PCR cleanup and quality check section), or

2) After the WTA index step (Step 21 in WTA index PCR cleanup and quality check section).

After the Jumpcode DepleteX® protocol is complete, the user resumes the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol (ID: 23-24117) from step 1 in performing WTA index PCR section for both workflow options 1) depleting after RPE PCR, and 2) depleting after WTA Index).



Total assay time: 2 hours | Hands-on time: 45 minutes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# Protocol Overview:

**Step A**

Option 1: Mitochondrial Depletion using WTA RPE PCR product

1. Perform RPE PCR and the purifying RPE PCR amplification product (single-sided cleanup) up to step 15 in section 'RPE PCR cleanup and quality check' from the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol (ID: 23-24117).
2. Using the RPE PCR product quantify on a Bio Analyzer and take ~ 50-100 ng in 15 µl.
3. Proceed to **step B**.

Option 2: Mitochondrial Depletion using WTA Index product

1. Perform library preparation up to and including Step 21 in the section 'WTA index PCR cleanup and quality check' from the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol (ID: 23-24117)
2. Using the WTA index PCR product quantify on a Bio Analyzer and take ~ 50-100 ng in 15 µl.
3. Proceed to **step B**.

**Step B**

Follow the DepleteX Mito DNA Depletion Kit protocol. (page 7)

1. Ribonucleoprotein complex formation
2. CRISPR digestion
3. Size selection

**Step C**

Continue with the BD Rhapsody™ System mRNA Whole Transcriptome Analysis (WTA) Library Preparation Protocol (ID: 23-24117) until the end with the preparation of sequencing-ready libraries.

1. Performing WTA index PCR
2. WTA index PCR cleanup and quality

Deleted:


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## Kit contents, storage, and shelf life

DepleteX® Mitochondrial DNA Depletion Kit contains enough material to deplete 24 libraries. The products listed below are sold separately. Kit contents and storage temperatures are indicated in the tables below.

DepleteX® Mitochondrial DNA Depletion Kit depletion reagents
24 samples
Stored at -20°C

| Kit contents | Part number | Quantity |
|---|---|---|
| Cas9 | REA1000 | 1 |
| 10x Cas9 Buffer | REA1001 | 1 |
| RNase Inhibitor | REA1007 | 1 |
| Nuclease-free Water* | REA1023 | 2 |

* Note: Store at 4°C after first use.

DepleteX® Mitochondrial DNA Depletion Kit
24 samples
Stored at -80°C

| Kit contents | Quantity |
|---|---|
| DepleteX Mito Guides | 1 |


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## Required materials provided by the user

| Material Type | Description | Supplier and Part Number |
|---|---|---|
| Plastic | 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, 200 µl and 1000 µl) | General Lab Supplier |
| Reagents | Purified RPE PCR amplification product/Purified WTA index PCR product from BD Rhapsody™ WTA Protocol (ID: 23-24117) | BD |
| | 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) I go | 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 | General Lab Supplier |
| | Qubit Fluorometer | ThermoFisher Scientific Q33238 |
| | Automated electrophoresis such as TapeStation | General Lab Supplier |
| | DNA analysis instrument, such as the Agilent 2100 Bioanalyzer® System | General Lab Supplier |

## Best Practices

**General:**


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- Periodically, optimizations and revisions are made to the kit components and user manual. Therefore, it is important to follow the protocol included with or appropriate for the kit in question. If you need further assistance in this regard, contact support@jumpcodegenomics.com.
- Use properly calibrated pipettes as library preparation is sensitive to pipetting error.
- Ensure that all pipette tips, microcentrifuge tubes, and other consumables are DNase- and RNase-free.
- Follow good laboratory practices throughout the protocol, including RNA-safe procedures when handling RNA.

**For specific reagents**

- Do not remove Cas9 and RNase Inhibitor from -20°C until before use. Return to -20°C immediately after use.
- Store the Guide RNA at -80°C. Do not remove the reagent from -80°C until time of use. Return it to -80°C immediately after use.
- We recommend a maximum of 3 freeze-thaw cycles for the Guide RNA. The Guide RNA tube contains material for 24 samples. It is strongly recommended that multiple smaller aliquots of the Guide RNA be prepared when the reagent is first thawed in order to reduce the number of freeze-thaw cycles affecting the Guide RNA.
- Do not freeze AMPure® XP beads.
- Allow AMPure® XP beads to come to room temperature for 30 minutes before use.
- Vortex AMPure® XP beads immediately before use. Ensure that they are in a uniform suspension before use. Use magnetic stands appropriate for the size of tubes

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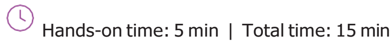
# Protocol

**Step A: Ribonucleoprotein complex formation**


Hands-on time: 5 min | Total time: 15 min

Materials provided

- 10X Cas9 Buffer
- RNase Inhibitor
- DepleteX Mito Guides
- Cas9

Required materials provided by the user

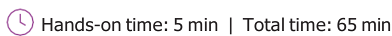
- Nuclease-free microcentrifuge tubes

1. Combine the following reagents in the order listed below in a 0.2 mL or 0.5 mL nuclease-free microcentrifuge tube.

| Component | Volume |
|---|---|
| 10X Cas9 Buffer | 1.0 µL |
| RNase Inhibitor | 1.0 µL |
| Mitochondrial Guide RNA | 2.68 µL |
| Cas9 | 1.16 µL |
| Nuclease-free Water | 2.36 µL |
| Total Volume | 8.2 µL |

2. Mix the contents gently by pipetting up and down. Centrifuge briefly to collect the contents at the bottom of the tube.
3. Leave the tube on the laboratory bench for 10 minutes at room temperature. This is the ribonucleoprotein complex (RNP).
4. Proceed immediately to Step B below: CRISPR digestion.

**Step B: CRISPR digestion**


Hands-on time: 5 min | Total time: 65 min

Materials provided.

- 10X Cas9 Buffer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- Ribonucleoprotein complex (RNP) from Step A

Required materials provided by the user

- Purified RPE PCR amplification product from step 15 in the section 'RPE PCR cleanup and quality check',

  or

- Purified WTA index PCR product from Step 21 in the section 'WTA index PCR cleanup and quality check'
- Nuclease-free DNA low bind microcentrifuge tubes
- Thermal cycler or heating block
- AMPure® XP beads (stored at room temperature)
- Ice

1. Combine the following reagents in a 1.5 mL nuclease-free microcentrifuge tube:

| Component | Volume |
|---|---|
| 10X Cas9 Buffer | 1.5 µL |
| Purified RPE PCR amplification product / Purified WTA index PCR product | 15 µL |
| Ribonucleoprotein complex (RNP) from Step A | 8.2 µL |
| Total Volume | ~25 µL |

2. Mix the CRISPR digestion reaction gently by pipetting up and down. Centrifuge briefly to collect the contents at the bottom of the tube. Incubate the tube at 42°C for 60 minutes.

> Note: During this incubation period, remove the AMPure® XP beads from the refrigerator and place them on the laboratory bench to bring them to room temperature. A minimum of 30 minutes at room temperature is recommended before use.

3. After the incubation is complete, transfer the tube to ice for ~2 minutes.

4. Briefly centrifuge the tube to collect the contents at the bottom of the tube. Transfer the tube to the laboratory bench. Immediately proceed to Step C: Size Selection.

**Step C: Size Selection**

Materials provided

- DepleteX® Mito DNA Nuclease-Free Water

Required materials provided by the user

- CRISPR-digested product from Step B


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- AMPure® XP beads (brought to room temperature prior to use)
- 80% Ethanol, freshly prepared (room temperature)
- Nuclease-free microcentrifuge tubes
- Magnetic stand

1. Add 25.3 µl of Nuclease-Free Water to the CRISPR digestion reaction.
2. Mix gently by pipetting up and down several times. Place the tube on the laboratory bench at room temperature.
3. Add 30 µl of well-resuspended, room-temperature AMPure® XP beads, pipette up and down several times to mix and incubate the tube at room temperature for 10 minutes. Mix the sample halfway through the incubation by pipetting up and down several times.
4. Place the tube on the magnetic stand. Allow the solution to clear (3–5 minutes). Remove and discard the supernatant without disturbing the beads.
5. Add 200 µl of freshly prepared 80% ethanol to the tube.
6. After 30 seconds, remove and discard the ethanol. Leave the tube on the magnetic stand during this step.
7. Repeat the wash step (previous two steps) with 200 µl of 80% ethanol. Remove as much ethanol as possible after the second wash.
8. Open the cap of the tube while it is on the magnetic stand and allow the beads to air dry for 5 minutes.
9. Add 31 µl of DepleteX® Mito DNA Nuclease-Free Water to the beads.
10. Remove the tube from the magnetic stand. Mix the contents by pipetting up and down several times to fully resuspend the beads in the liquid.
11. Incubate the tube at room temperature for 10 minutes. Mix the sample halfway through the incubation (5 minutes) by pipetting up and down several times.
12. Place the tube on the magnetic stand. Allow the solution to clear.
13. Transfer 30 µl of the supernatant to a new microcentrifuge tube. Place the tube on ice. This tube contains the DepleteX® Mito DNA-depleted library.
14. Proceed to WTA indexing following the BD Rhapsody™ WTA Assay protocol (Doc ID: 23-24117) from step 1 in 'WTA index PCR' section.
15. After digestion of the product (either RPE PCR product or the WTA index PCR product): Run a Bio Analyzer and determine the concentration based on the base pair sizes from 230 bp to 600 bp.
    - The BD Rhapsody™ WTA Assay protocol recommends calculating the concentration (nM) from 200 bp, but digestion


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might cause some small molecular weight byproducts (<230 bp) that will not be indexed and therefore should not be included in the quantification of the library.

16. Proceed to WTA indexing following the BD Rhapsody™ WTA Assay protocol beginning from step 1 in 'WTA index PCR' section.

Note: For the sample started with the WTA index PCR product make sure to use the same index primers for the final index PCR step.

Note: If the final Index PCR did not yield a sufficient amount of product for sequencing needs, it is possible to go back to the remaining digest product and increase the PCR cycle number accordingly.


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## Library validation

It is recommended that the user assess library yield using a dsDNA-specific fluorescence-based method (such as a Qubit fluorometer) and library fragment profile on an Agilent Bioanalyzer 2100 or equivalent instrument before sequencing. DepleteX® Mito DNA-depleted libraries have a similar fragment profile to those of standard BD Rhapsody™ WTA Assay single cell libraries.

It is also recommended that qPCR quantification be performed to ensure optimal cluster density on an Illumina sequencing instrument.

Once the library has been quantitated, it is ready for cluster generation on an Illumina instrument. Please follow standard Illumina protocols for the loading of the library and for cluster generation on the instrument. If the library needs to be stored before sequencing, please store it at -20°C.

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