

# revvity

## Single Cell Sequencing with DepleteX<sup>®</sup> Getting Started

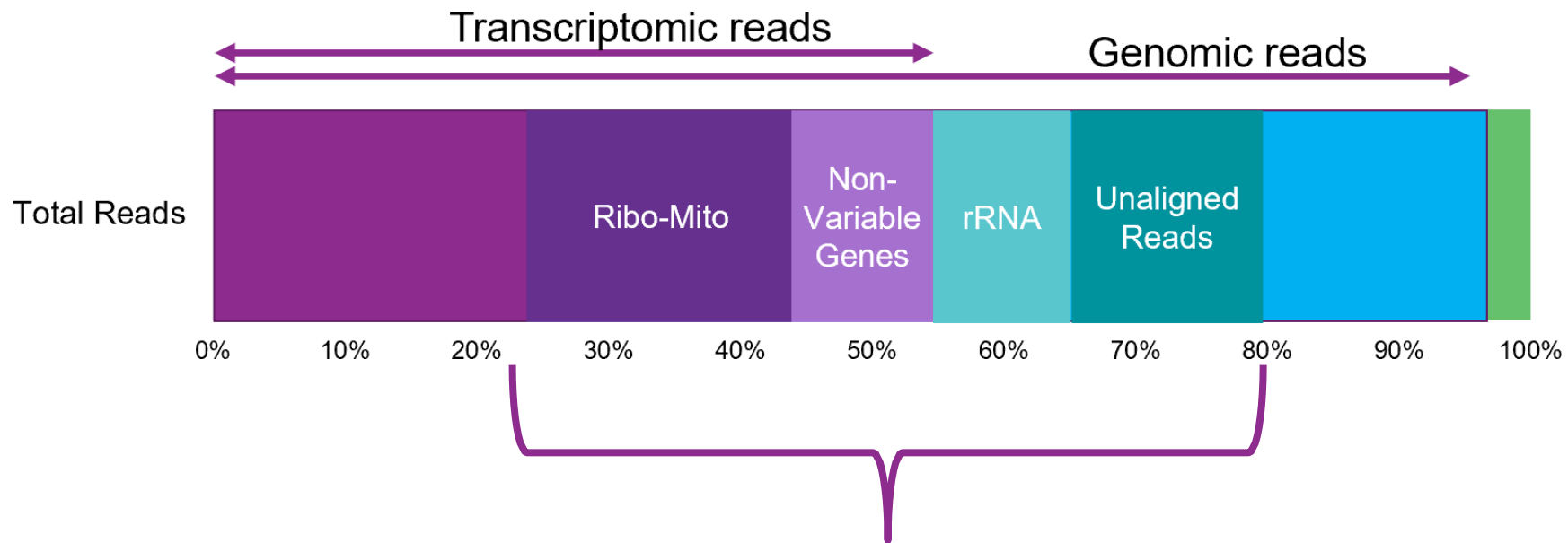


# Goals

To ensure customer success on their first single cell experiment using DepleteX<sup>®</sup> technology:

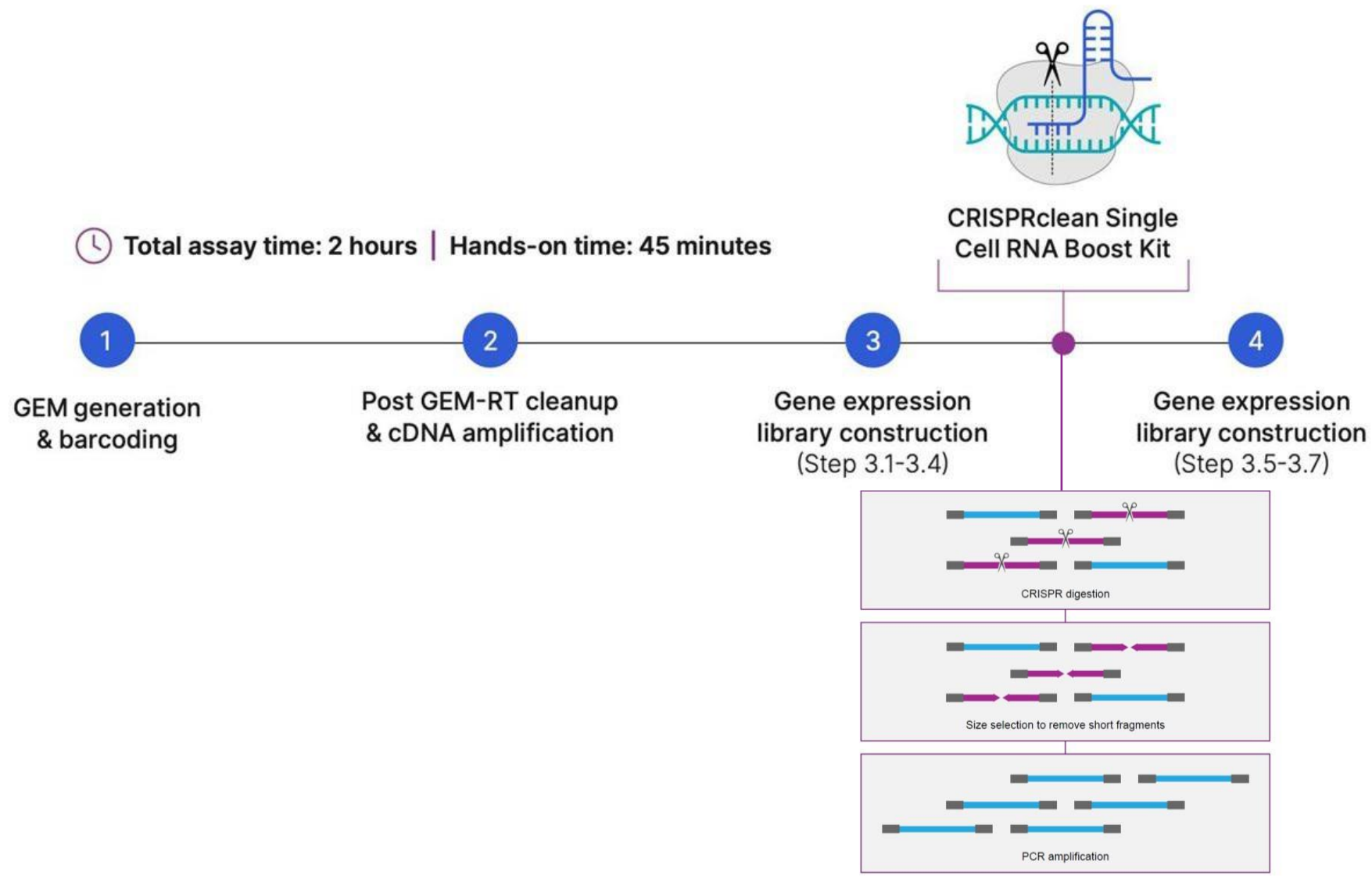
- Provide a high-level overview of the process
- Answer any customer questions including FAQ
- Provide point of contact for support in case any issues arise during the experiment or analysis

# What are we removing?

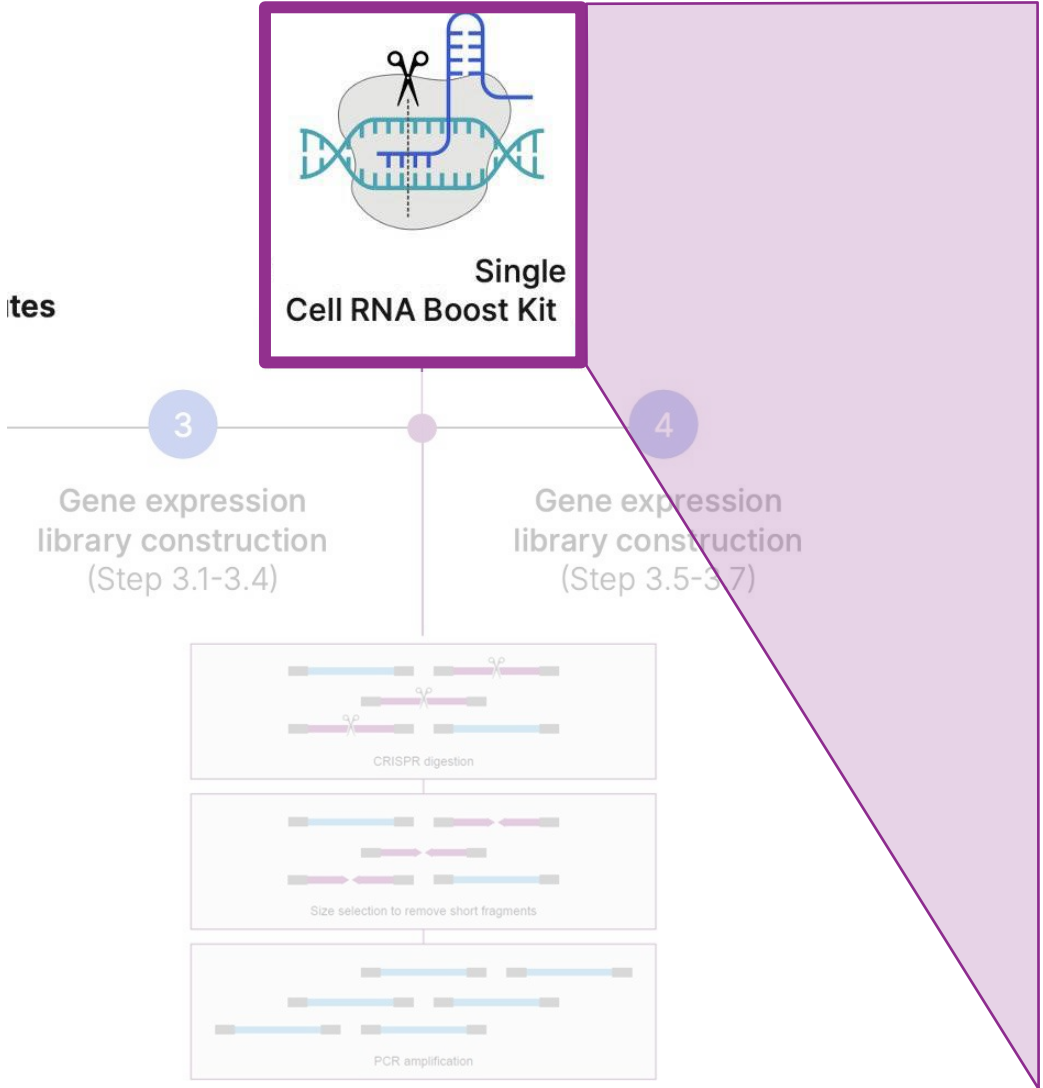


Typically uninformative for secondary analysis;  
Often removed informatically by Seurat / Scanpy

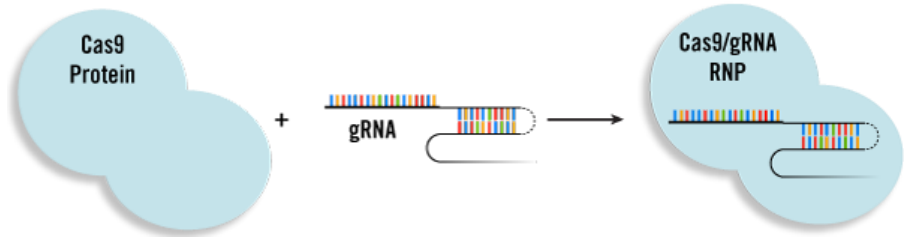
# Overview of DepleteX<sup>®</sup> workflow with 10x 3' Gene Expression



The entire process can be inserted into any single cell application and only takes 45 minutes of hands-on time

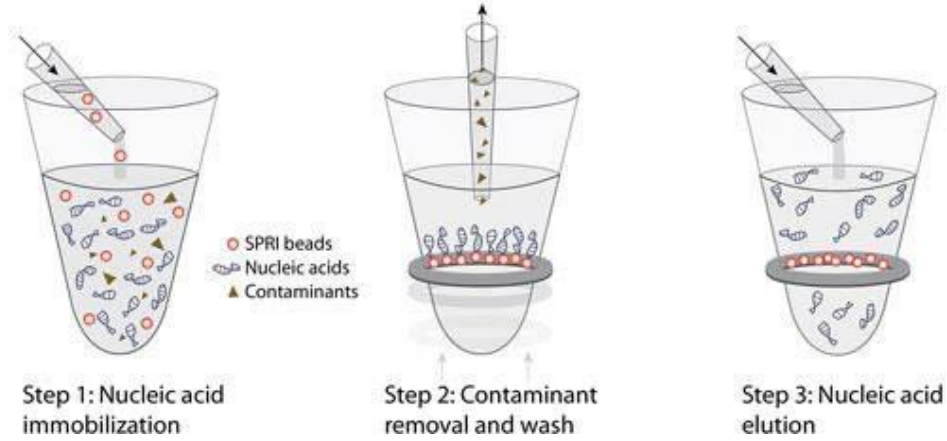


**1. Make RNP:** combine guides with Cas9 (15 min)

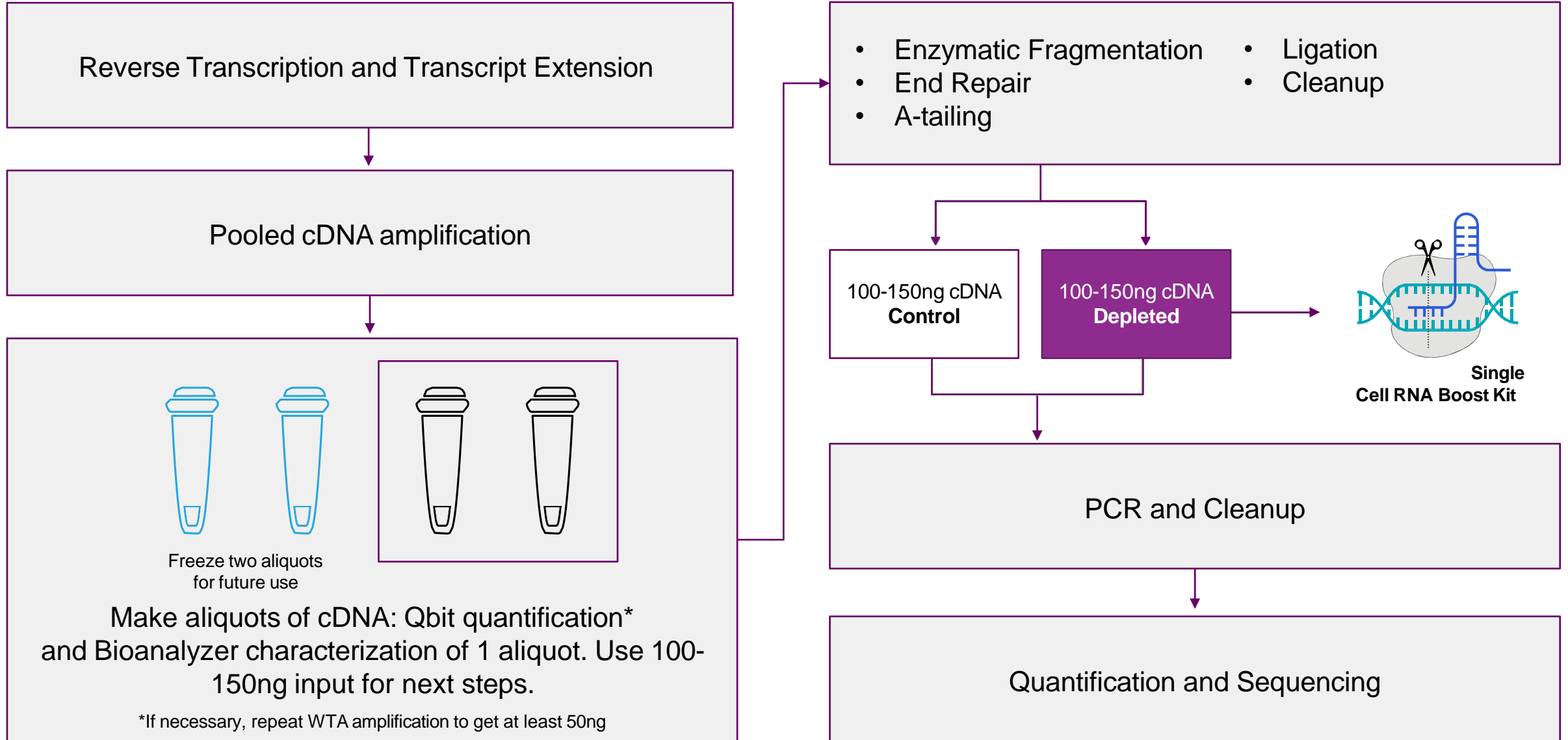


**2. Incubate** with cDNA at 37°C - 1 hour incubation

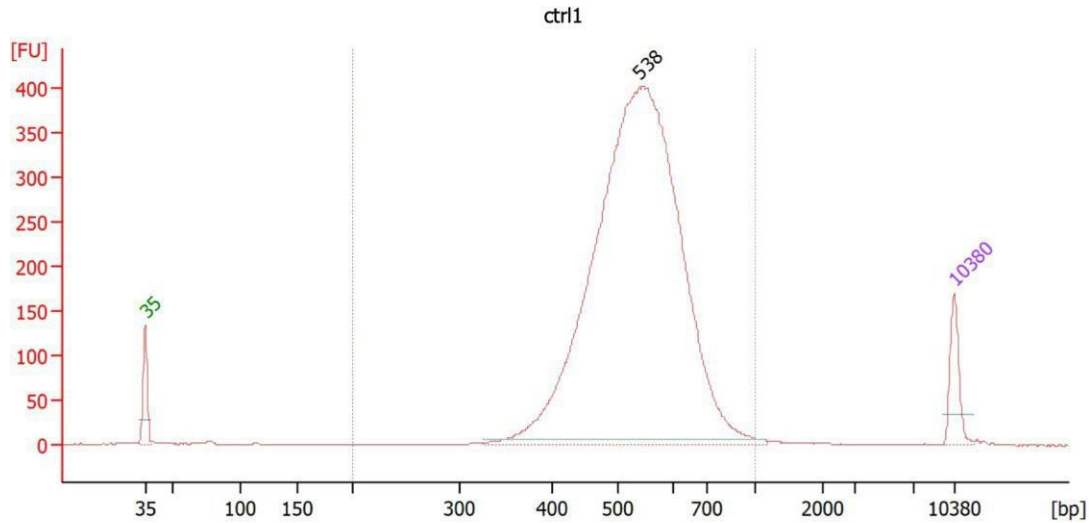
**3. Bead clean** to remove cut fragments (30 min)



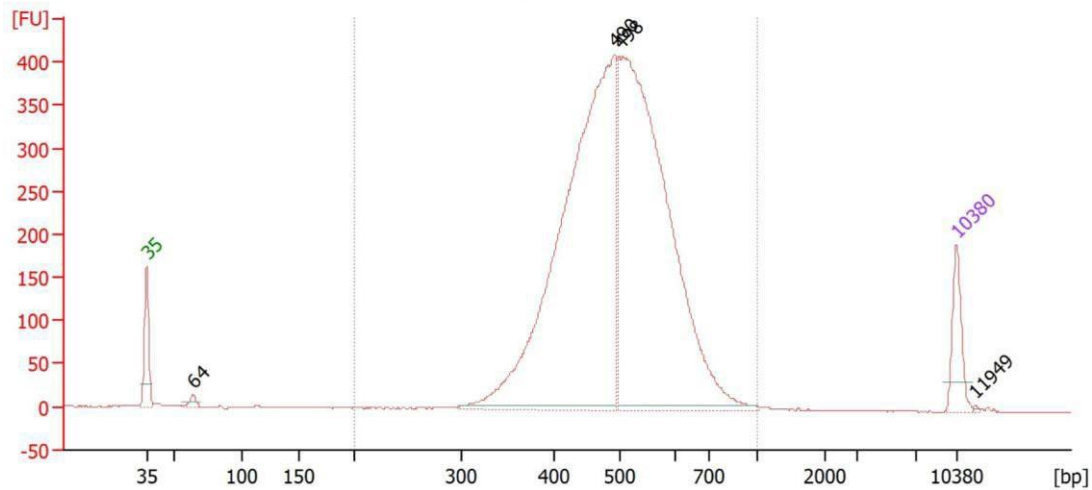
# Process workflow



# Bioanalyzer traces



- Traces of the control vs depleted condition for libraries treated with DepleteX®.
- The depleted condition is around 50bp shorter in size in this example, however, it is normal for the size shift to increase or decrease.



# Assay FAQs

## Can I use DepleteX on mouse samples?

- Yes, but the depletion rate is lower since the guides were designed for human samples. We sell mouse-specific ribo-mito depletion guides.

## Does DepleteX use all of the cDNA from my 10X prep?

- No, only 1 tube of the available cDNA from a given 10X prep is used in general. The customer is free to customize this as needed to hit the 100-150ng input recommendation.

## Is this kit compatible with the 5' Chromium assay? What about Visium spatial?

- Yes, it is compatible with both kits. Our depletion panel is designed to tile across the full length of transcripts.

## What is the sequencing depth recommendation for the depleted condition?

- In general, single cell users use between 25K – 50K reads/cell depending on their application. For the depleted condition, users can choose to sequence 12.5K – 25K reads/cell to see the same information or sequence at the same depth to see more information.

## Can I still use mitochondrial reads to separate “intact” cell from “compromised” cells?

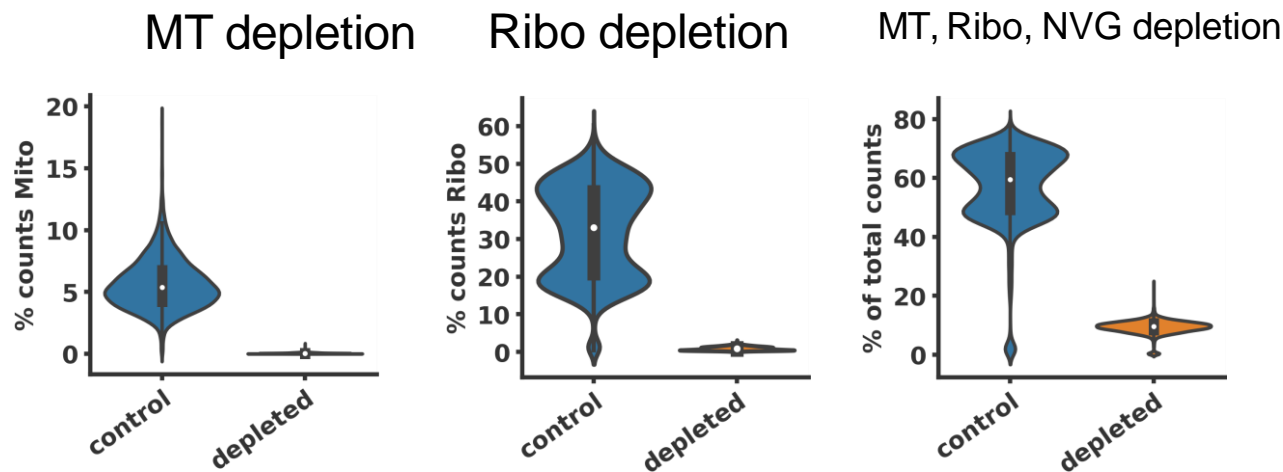
- Yes, mitochondrial reads are targeted by DepleteX but removal is not complete. They can be used to separate intact from compromised cells. Instead of setting an arbitrary 10% threshold, alternative approaches are described in the Python and R guides provided (see next slide).



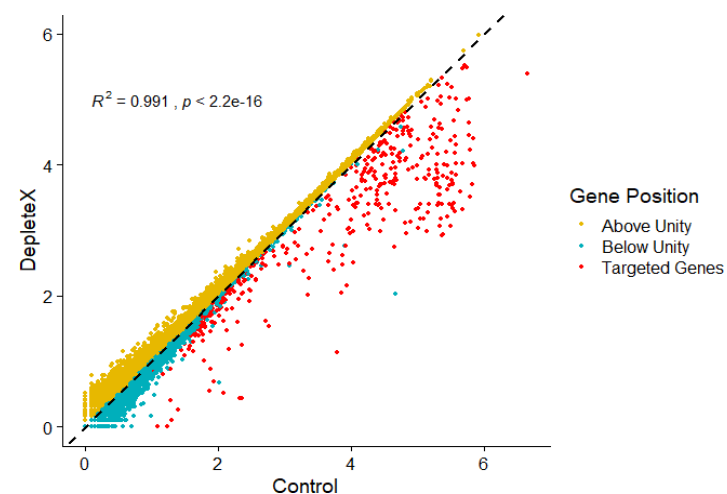
# Data Analysis guidelines

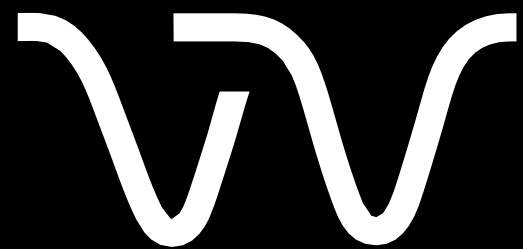
We have multiple options to support your analysis needs:

- [Cell Ranger](#) is recommended to compare the control to the depleted sample to see the benefit of depletion in sequencing saturation metrics.
- [Python](#) or [R](#) step-by-step guide to show you how to analyze your data and see the benefits of depletion in secondary analysis, including a boost in UMI/cell and genes/cell.



R-Squared shows only Targeted Genes are being depleted





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