HIVE CLX Troubleshooting Guide

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//// Overview

This document is to serve as a resource for users for troubleshooting during use of HIVE CLX™ scRNAseq. For additional help, submit a support request at support.honeycomb.bio or email support@honeycomb.bio

SAMPLE PREPARATION & CAPTURE

Issue: Cell viability is low (recommend >90% viability)

Potential Cause:

- Poor quality sample
- Physical damage due to sample prep method

Recommendation:

- Use dead cell removal kit
- Improve sample dissociation method
- Use wide-bore pipette tips when pipetting single cell suspensions, and pipette slowly and gently to reduce shearing forces

Issue: Cell aggregates are visible

Potential Cause:

- Some cell types form clumps when kept in suspension for an extended period of time
- Tissue was not fully dissociated
- DNA aggregates from lysing cells are causing clumping

Recommendation:

- Run the sample through a filter
- Improve sample dissociation method

Issue: During sample loading, cell media bubbles up out of the HIVE Collector port

Potential Cause:

- If the port is wet, media will not enter the HIVE Collector smoothly

Recommendation:

- Remove excess media from the surface, pat port dry and try again with a fresh pipet tip

Issue: When adding cells in media, the media does not spread evenly around the HIVE

Potential Cause:

- The array of the HIVE Collector dried out from being left without liquid for too long
- Media without protein was used for cell loading

Recommendation:

- Avoid leaving the HIVE without liquid for too long. If HIVE is dry, start again with a new HIVE Collector
- Use media containing 0.1% BSA or 1% FBS for sample loading

Issue: High red blood cell contamination in blood-derived sample

Potential Cause:

- Red blood cell removal method was not sufficient

Recommendation:

- Repeat red blood cell depletion method. We recommend the EasySep RBC depletion method by Stem Cell Technology (We recommend processing 0.5 mL of blood at a time and using the magnet 3 times to get the best results).

PROCESSING & LIBRARY QC

Issue: No/low bead recovery (small bead pellet after bead collection)

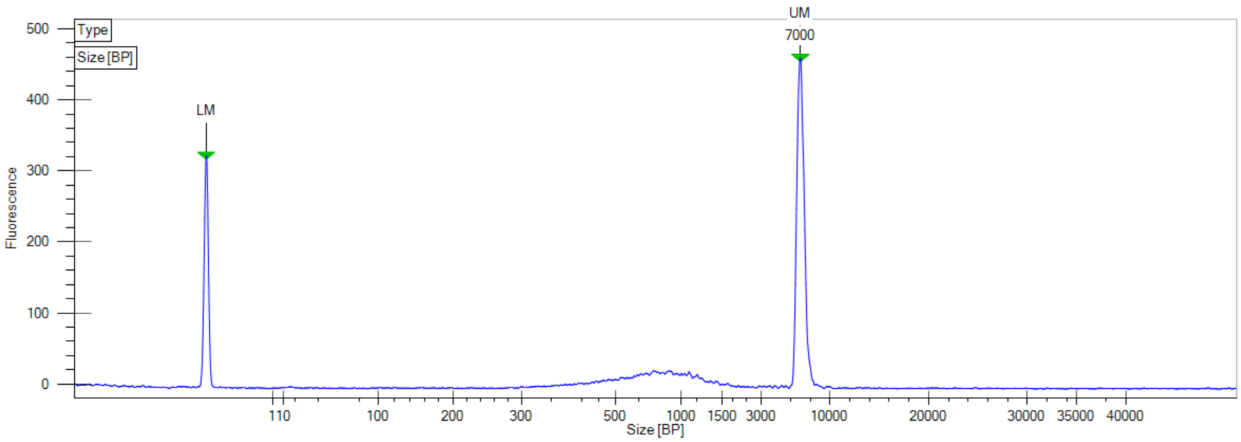
Potential cause:

- Poor transfer of the bead pellet from the Bead Collector to the filter plate
- Poor transfer of the bead pellet from the filter plate to deep well
- Insufficient centrifugation speed or time during bead collection
- The orientation of the plate adaptor in the centrifuge was not radially oriented as described in the HIVE CLX scRNAseq User Protocol (User Supplied Material section).

Recommendation:

- Confirm bead recovery is low
    1. Take 10ul from one of the 4 WTA reactions (resuspending the beads by mixing first),
    2. Load into a hemocytometer (provided in Sample Capture Reagents box).
    3. Count the number of beads that you see across all 9 quadrants (expecting 50-150 total),
    4. Divide the total count by 9 to get the average count,
    5. Multiply the average count by 4,000 to get an estimate for the total number of beads recovered.
- Check all centrifuge parameters
- Re-centrifuge the plate. If you see a bead pellet in Bead Collector, repeat transfer to filter plate
- When transferring the bead pellet to the filter plate, make sure to gently place your pipet tip at the bottom of the Bead Collector. Do not jam it down too hard or hover above the bottom. To confirm bead recovery is successful, you should see the beads in suspension in your pipet tip, and the bead pellet will be fully removed from the Bead Collector.
- Beads settle quickly, so pipet up and down rapidly before any transfer of the bead pellet
- Proceed to sequencing at risk, knowing that cell recovery is likely impacted

Issue: Low library yield (at or below 1 ng/µL) for user-loaded HIVE but CLX 1st strand control has product



DNA quantification is at or below 1 ng/uL; DNA gel electrophoresis traces appear normal with a smooth smear and the correct peak but may be hard to distinguish due to low input.

Potential cause:

- Poor sample preparation
- Sample is incompatible with HIVE scRNAseq

- Severe sample preparation, capture, or processing failure

Recommendation:

- There is enough product generated from the WTA reaction to run multiple Index PCR reactions. The Processing Kit provides enough reagents to perform three rounds of Index PCR and SPRI Clean-up for each HIVE
- Repeat the Index PCR and SPRI Clean-up steps
- If repeating the Index PCR does not fix the problem, start a new HIVE for the sample
- It may be difficult to discern low or no library yield issues due to measurement device sensitivity, non-specific signal, and how severely a step is compromised.

Issue: Low or no library yield for both user-loaded HIVE and CLX 1st strand control

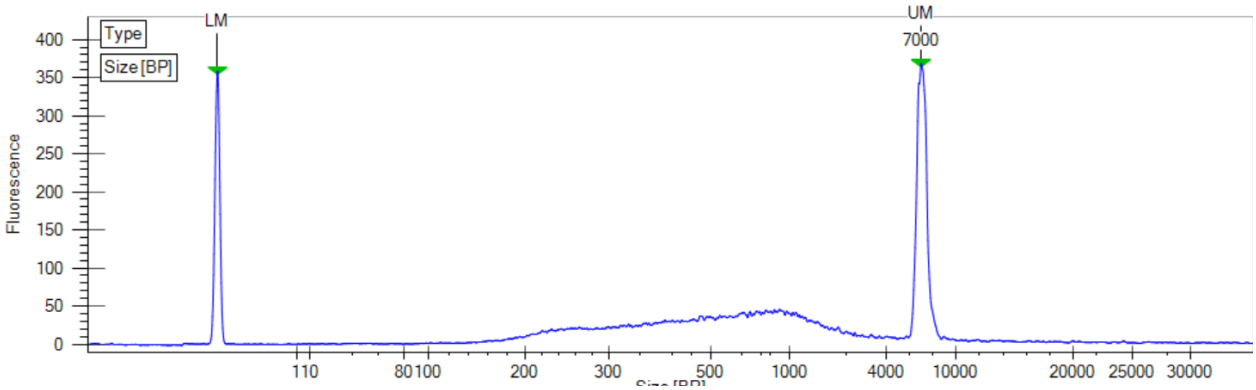
Potential cause:

- Severe library preparation failure

Recommendation:

- Repeat the WTA SPRI Clean-Up, Index PCR and Index SPRI Clean-up steps.
- If repeating the Index PCR does not fix the problem, contact support for a troubleshooting kit

Issue: Flat and broad trace with extended left slope



DNA quantification is around the threshold or at the lower end of normal range. DNA gel electrophoresis traces appear to show a smooth smear with a correct main peak, but overall show a flat pattern with the elevated left slope extending to 200 bp.

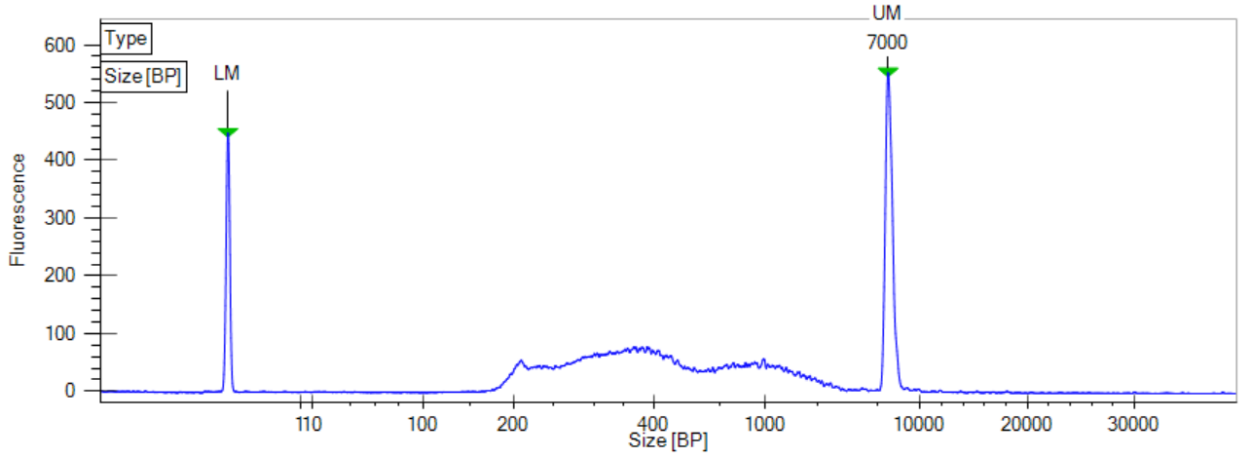
Potential cause:

- Severely compromised library preparation operations, for example:
  - Used incorrect buffer
  - Omitted NaOH denaturation
  - Missing Clean-up enzyme or disrupted incubation

Recommendation:

- Rerun the WTA SPRI Clean-Up, Index PCR and Index SPRI Clean-up
- Do not sequence if repeating Index PCR does not solve the issue. Sequencing data quality likely will be poor.

Issue: Broad trace with multiple main peaks



DNA quantification is around the threshold or at the lower end of normal range, and DNA gel electrophoresis traces appear to show a continuous smear with multiple main peaks.

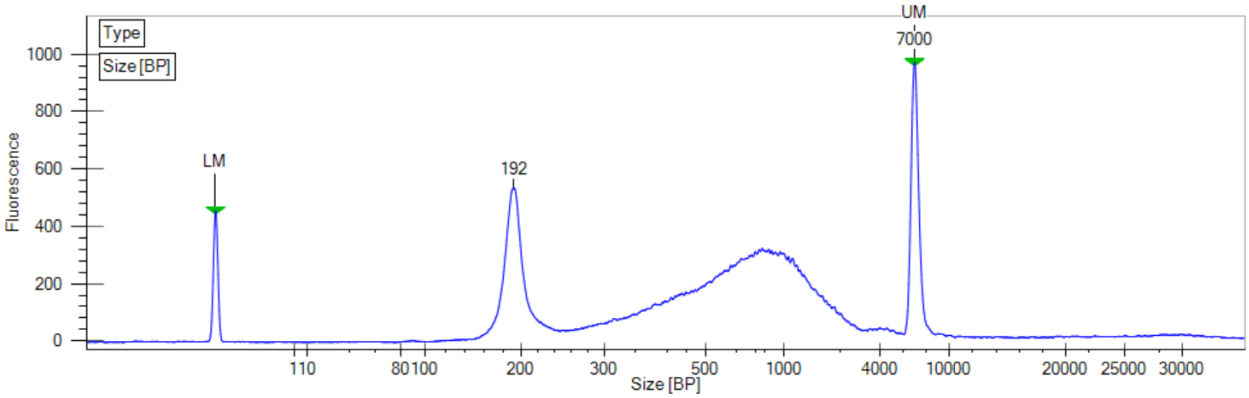
Potential cause:

- Severely degraded sample RNA
- 1st Strand synthesis reaction failure (such as missing enzyme)
- Other significant library preparation failures

Recommendation:

- Rerun the WTA SPRI Clean-Up, Index PCR and Index SPRI Clean-up
- Do not sequence if repeating Index PCR does not solve the issue. Sequencing data quality likely will be poor.

Issue: There is a spike around 200bp



DNA quantification is normal, and DNA gel electrophoresis traces appear to show a smear with the correct main peak, except that there is a sharp spike around 200bp.

Note: This spike may significantly reduce effective sequencing reads due to its small size therefore much higher molecule allocation than the gel trace signal.

Potential Cause:

- Inherent cell type trait (small peak sometimes seen with PBMCs)
- Using too much WTA product for Index PCR, for example more than 5 ng
- Failed SPRI Clean-up, such as:

- adding more SPRI Beads than recommended
- Skipping the WTA SPRI Clean-Up

Recommendation:

- Redo WTA SPRI Clean-up if skipped or correct steps were not followed
- Index PCR within recommended limits (5ng or less WTA input)
- Redo the Index SPRI Clean-up if correct Index PCR conditions were followed but:
    - correct steps were not followed -OR-
    - this spike is very large (either the spike height is higher than half of the main peak height or its area under curve (AUC) is over 10% of total)