Performing CITE-seq on the Illumina/BioRad ddSeq system

This protocol is a companion to the regular CITE-seq protocol and the Illumina/BioRad protocols. It describes how to purify and make libraries from the antibody-derived tags central to CITE-seq from samples that have been processed in the ddSeq workflow.

For CITE-seq reagent preparation, cell staining, oligo sequences, and other information please refer to the full CITE-seq protocol available at cite-seq.com.

For operating the ddSeq instrument and making subsequent libraries, please refer to the Illumina Bio-Rad SureCell WTA 3' Library Prep Reference Guide up to the "clean up first strand synthesis" step. At this step, you will need to follow the steps below to apply CITE-seq to ddSeq.

At this point, the Illumina protocol contains a purification step where the larger fraction containing the cDNA is purified away from the smaller fraction. For CITE-seq, this smaller fraction contains the Antibody-derived tags (ADTs).

Treat the sample purification beads as though they are AMPure / SPRI (they behave as though they are).

- At step 7 on page 25 of the Illumina protocol, **DO NOT DISCARD THE SUPERNATANT**. This contains the ADTs.
- Transfer the supernatant to a new 1.5 ml low bind tube.
- Continue processing the beads per the Illumina protocol to generate the transcriptome library.

- To the reserved supernatant, add an additional 120µl of AMPure/SPRI. This will bring the ratio of AMPure/SPRI to >2.0X and ensure maximum recovery of ADTs
- Mix well and incubate for 10 minutes at room temperature.
- Place tube on magnet and wait ~2 minutes until solution is clear.
- Carefully remove and discard the supernatant.
- Add 400 µl freshly prepared 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds
- Carefully remove and discard the ethanol wash.
- Add 400 µl freshly prepared 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds
- Carefully remove and discard the ethanol wash.
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol and allow the beads to air dry for 1 minute (do not over dry beads).
- Resuspend in beads in 20 µl water.

Amplify the ADT sequencing library

Set up PCR reaction:

Purified ADTs	20µl
2x Kapa Hifi	25µl
Tagment PCR adapter (TPP1) from ddSeq kit* (10µM)	1.25µl
RPI-x primer (10µM)	1.25µl
Water	2µl

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** The Illumina TPP1 primer sequence is not publicly disclosed. However, there is enough provided with the sure cell WTA kit to perform the ADT PCR.*

Cycling conditions:

95˚C 3 min
95˚C 20 sec |
60˚C 30 sec | user defined cycle number (15 is a good start - same as for cDNA)
72˚C 20 sec |
72˚C 5 min
12ºC hold

Note, if insufficient amplification achieved, additional cycles can be performed.

- Purify PCR product using 1.6X SPRI purification by adding 80 µl SPRI reagent.
- Incubate 5 minutes at room temperature.
- Place tube on magnet and wait 1 minute until solution is clear.
- Carefully remove and discard the supernatant.
- Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (first Ethanol wash).
- Carefully remove and discard the ethanol wash.
- Add 200 µl 80% Ethanol to the tube without disturbing the pellet and stand for 30 seconds (second Ethanol wash).
- Carefully remove and discard the ethanol wash.
- Centrifuge tube briefly and return it to magnet.
- Remove and discard any remaining ethanol and allow the beads to air dry for 2 minutes.
- Resuspend beads in 20 µl water.
- Pipette mix vigorously and incubate at room temperature for 5 minutes.
- Place tube on magnet and transfer clear supernatant to PCR tube.
- ADT library is now ready to be sequenced.
- Quantify library by standard methods (Qubit, BioAnalyzer, qPCR).
- ddSeq ADT library will be around 220 bp (Figure 1).

From this point, proceed with sequencing libraries as described in the Illumina / Bio-Rad literature.

ADT libraries can be pooled together with the cDNA-derived transcriptome libraries. We typically allocate 5-10% of the reads to the ADT libraries.

More information can be found in the full CITE-seq protocol.

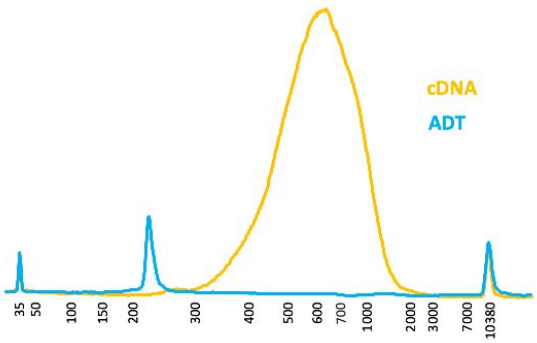

Figure 1. Example BioAnalyzer trace for ddSeq ADT library (blue) and final cDNA-derived library (orange). The ADT library should be a discreet peak at ~220 nt.

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