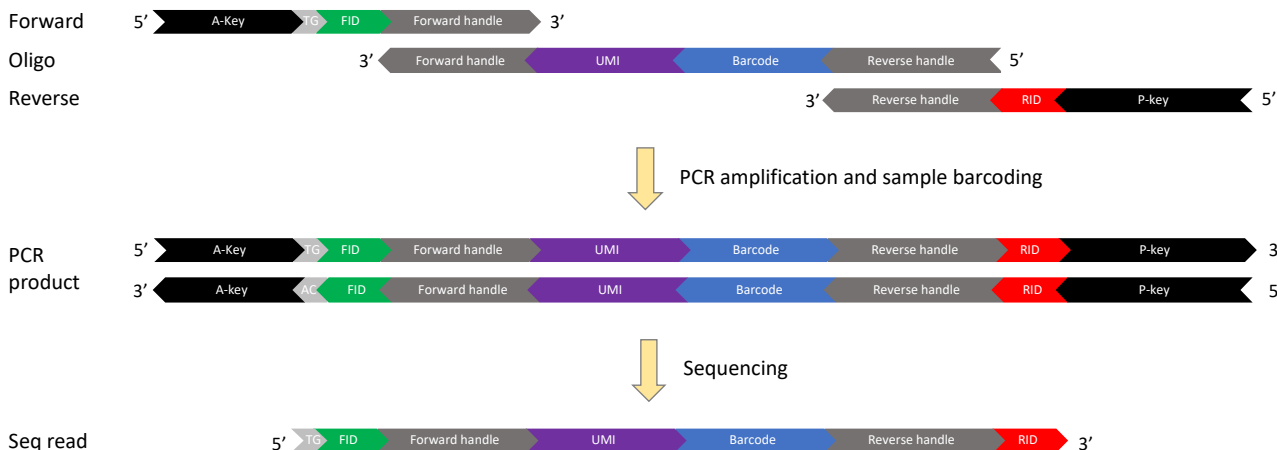


Staining Protocol

Materials required (not provided)	<p>Stain buffer: PBS, pH 7.4 containing 1-5% serum and 0.1 g/l Herring sperm DNA Wash buffer: PBS, pH 7.4 containing 1-5% serum Sorting buffer: PBS, pH 7.4 containing 50% serum 100 µM d-Biotin in PBS, pH 7.4 Antibodies identifying relevant cell surface markers (e.g. CD3, CD4, CD8) Forward and reverse amplification primers. For design of amplification primers, see Appendix A qPCR reaction components QIAquick® PCR Purification Kit</p>
Procedure	<ol style="list-style-type: none"> 1. Prepare PBMC sample and resuspend 1-3 x 10⁶ PBMC in 50-100 µl stain buffer. 2. Centrifuge dCODE Dextramer (HiT) at 10,000 x g for 1 min. 3. Preparation of dCODE Dextramer (HiT) reagent pool: <ol style="list-style-type: none"> a. Add 0.2 µl 100 µM d-Biotin per dCODE Dextramer specificity into an empty tube b. Add 2 µl of each dCODE™ Dextramer specificity and mix c. From this mixture, take 1 µl and dilute with 99 µl wash buffer. Save this for qPCR amplification as input sample (step 13). 4. Add the pool of dCODE Dextramer reagents to the cell sample and mix thoroughly. 5. Incubate at room temperature for 20 min. 6. Add relevant antibodies in the volume/concentration recommended by provider. Incubate for 20 min. 7. Washing: <ol style="list-style-type: none"> a. If staining in 4 ml tubes, add 2 ml wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing with another 2 ml wash buffer. b. If staining in 96-well microtiter plates, make 4 sequential washes using 200 µl wash buffer per well. Centrifuge at 300 x g for 5 min. between each wash and remove supernatant. 8. Resuspend cells in adequate volume of wash buffer. 9. Proceed to FACS following the guidelines and practices of your sorting facility. 10. Make a two-way sort by sorting the CD8⁺Dextramer-PE-positive population and the CD8⁺Dextramer-PE-negative population separately. 11. Collect sorted cells directly into tubes containing 500 µl sorting buffer. 12. Centrifuge the sorted cell samples at 300 x g for 10 min. Add 1 ml pure PBS and spin down for an additional 5 min at 300 x g. Discard supernatant and resuspend cell pellet in 40 µl PBS. (<i>Optional: Store at -20°C for up to 72h or proceed to the next step.</i>) 13. For each cell sample (Dextramer-PE-positive and Dextramer-PE-negative), use 20 µl cell suspension to prepare a 40 µl final PCR reaction volume. For Dextramer input mixture, use 1 µl of the diluted sample as template in a 40 µl total PCR reaction volume. 14. Purify DNA from each PCR reaction (Dextramer-PE-positive, Dextramer-PE-negative, and input mixture) using QIAquick® PCR Purification Kit following the manufacturer's instructions. Elute each sample in 20 µl water. 15. To prepare the final sample for sequencing, mix 10 µl of each of the purified Dextramer-PE-positive and Dextramer-PE-negative PCR products. Add just 1 µl PCR product from the input mixture. <i>Remaining purified PCR products can be stored at -20°C as backup.</i> 16. Send sample for sequencing.
Analysis of data	<p>Demultiplex the sequencing data according to sample (primer barcodes) and Dextramer reagents (oligo barcodes). Calculate the apparent enrichment (AE) for each reagent by dividing its read count in the Dextramer-PE-positive sample with the corresponding read count in the Dextramer-PE-negative sample. To get the specific enrichment, divide each AE with the (median of the) AE of the control Dextramer reagents.</p> <p>Sequencing reads from the Dextramer input mixture serve to validate the presence of all Dextramer reagents.</p>
Procedural notes	<p>Always keep dCODE Dextramer stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.</p>

Appendix A DNA Oligo amplification and Primer design



Note: If the amplified product is sequenced using the A-key, the sequence read corresponds to the reverse complement of the oligo.

Amplification primers:

- Amplification primers are used in the PCR reaction to amplify the target region of the DNA barcode oligo.
- Each primer consists of a region for annealing to the oligo (forward or reverse handle), a unique sample ID sequence to individual label samples for multiplex sequencing, and a sequencing handle (A-key and P-key).
- The A-key and P-key must be matched to the sequencing platform (examples below are for Ion Torrent) and can be adapted as necessary.

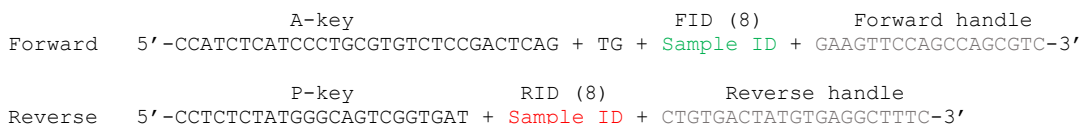


Table 1: Examples of primer sequences for Ion Torrent sequencing (color-coding as above):

Forward Primer	Reverse Primer
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGGGGTGAAGTTCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATATTGCGCCCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTCCACACGAAGTTCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATGACCCGTACTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACCTGGAAGTTCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATGGCGTACCCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGGCAGCAGAAGTTCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTTATATGTCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGAGTAACGAAGTTCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATGTCTGCTGTGACTATGTGAGGCTTTC