

# CLindex®

## Increase sample throughput and reduce cost

This technical note describes the CLindex® sample multiplexing workflow and evaluates its performance metrics including labeling efficiency, effects on the transcriptome, cell clustering, and viability.

### Overview

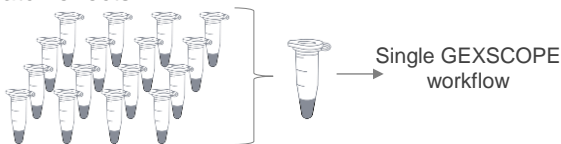
High-throughput single-cell sequencing technologies enable researchers and clinicians to assess how tissues or cell populations evolve in different diseases at single-cell level. During the past ten years, throughput and sensitivity of single-cell sequencing have advanced constantly. Nowadays, these improvements allow researchers to investigate samples from different origins and analyze thousands of cells of each sample. To meet the increasing need for higher sample throughput in translational research and precision medicine, we developed CLindex®, a novel multiplexing method using click chemistry to add a sample-tag to cells.

### Highlights

- **Unbiased** – works with all species and tissues types
- **Gentle** – maintains high cell viability rates
- **Compatible** – supports usage of high-density chip to capture over 120,000 cells on one chip
- **Fast** – finishes sample labeling in <30 min
- **Reliable** – achieves multiplexing of up to 16 samples

## Introduction

A hallmark of high-throughput single-cell sequencing technology is the molecular tagging of cells with barcoding sequences. Each mRNA molecule is tagged with both, a common cell specific barcode and a unique molecular identifier (UMI). The information from both tags enable the analysis of the transcriptome at single-cell resolution. To increase sample throughput, we developed CLindex®, a flexible sample multiplexing solution for single cell RNA-sequencing studies that is compatible with samples of different species. CLindex® adds a sample-specific index to the surface of each cell that is contained in a defined sample. The CLindex® Sample Multiplexing Kit enables pooling up to 16 samples and capturing genetic information of up to 120,000 cells on one chip, when combined with our high-density microwell chip (Figure 1). The possibility to tag and pool multiple samples into a single experiment increases sample throughput and reduces batch effects.



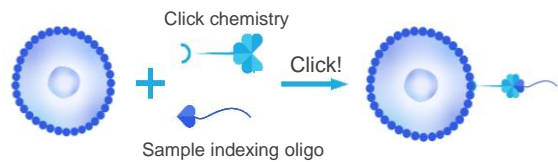
**Figure 1.** The CLindex® Sample Multiplexing Kit enables pooling of up to 16 samples to analyze their transcriptome at single-cell resolution with one microfluidic chip.

## Workflow

CLindex®, together with our single-cell sequencing library prep kit and CeleScope data analysis pipeline, covers the complete process from sample preparation to data analysis. The CLindex® sample indexing oligos have unique sample indexing sequences, a poly-A stretch, and a PCR handle sequence (Figure 3). After labeling, the cells from each sample with a unique CLindex® tag are pooled. This pooled and tagged cell suspension is loaded onto a microfluidic chip to partition single cells into microwells. Following

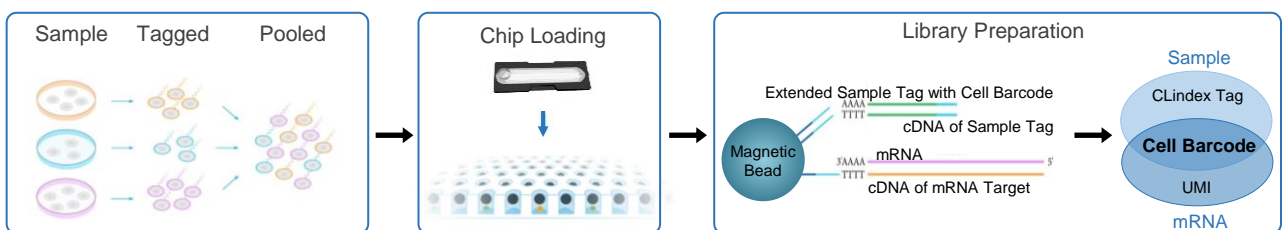
## CLindex® Click-Chemistry

Our CLindex® multiplexing technology employs click-chemistry for efficient, unbiased sample tagging. A sample indexing oligo contains a chemical group with high affinity to cell surface proteins and therefore labels the cells of each sample with a click chemistry reaction (Figure 2). The tagging is efficient and robust, since CLindex® sample indexing oligos are covalently bound to the cells. A major advantage of the click chemistry-based labeling compared to antibody-based labeling is that the cell surface proteins can be crosslinked by click chemistry, making the method suitable for samples from different species. Furthermore, the entire process of sample tagging takes only 30 min.



**Figure 2.** Click chemistry-based sample tagging. A chemical group binds to specific, yet highly abundant, cell surface proteins, and is labeled with one of the 16 sample indexing oligos.

cell lysis, barcoding beads in each microwell capture and barcode the CLindex® sample indexing sequences and cellular mRNAs, simultaneously. During the reverse transcription, the sample indexing oligos can be converted to cDNA. A sample tag library and a separate RNAseq library from the same single cells are subsequently generated and sequenced. Since both libraries contain a common denominator, the cell barcode, sample and cellular origin of each transcript can be determined during bioinformatic analysis.



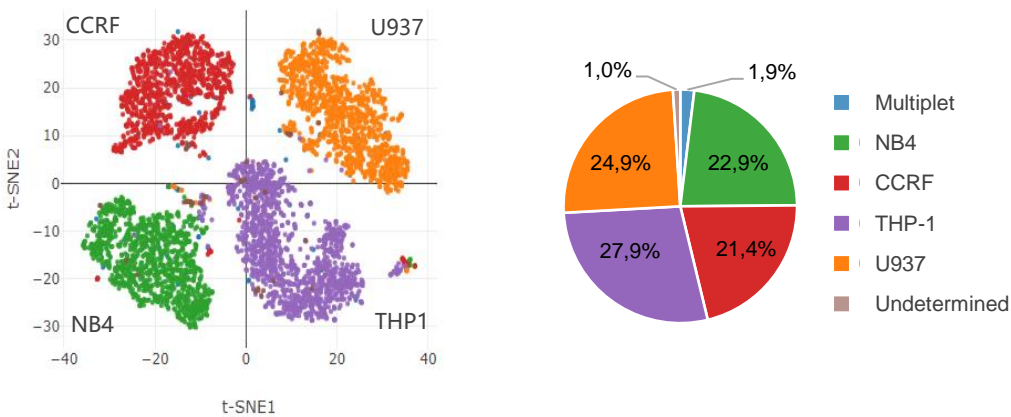
**Figure 3.** Workflow of CLindex sample multiplexing, chip loading, and library preparation.

## High Labeling Efficiency

Four cell lines (NB4, THP1, U937 and CCRF) were labeled using CLindex® and pooled in equal parts prior to cell parsing on the SCOPE-chip™, followed by cDNA library construction, and sequencing (Figure 4). A total of 4965 cells was captured. Cell clustering and annotation confirmed that sample indices were distributed evenly and correlated well with their labeled cell line samples. The percentage of correctly labeled cells was as high as 97%, with only 3% being either multiplets or undetermined. Furthermore, CLindex® sample tagging does not impair capturing,

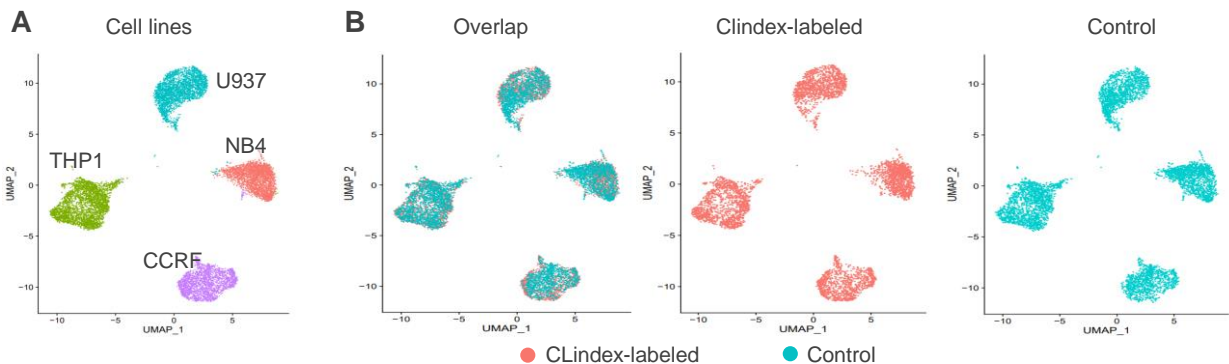
**Table 1.** CLindex® sample multiplexing does not interfere sample processing and sequencing.

	Control (no labeling)	CLindex®
Estimated No. of Cells	5 698	4 965
Mean Reads per Cell	17 776	19 406
Median Genes per Cell	2 428	2 873
Median UMI per Cell	6 073	8 309
Total Genes	25 289	25 308



**Figure 4.** Four different cell lines were labeled using CLindex® for multiplexing. CLindex® sample labeling is highly efficient (97%) and unbiased. NB4: Human Acute Promyelocytic Leukemia Cell Line; U937: Human Histiocytic Lymphoma Cell Line; THP1: Human Peripheral blood Monocyte Cell Line; CCRF: Human Acute Lymphoblastic Leukemia Cell Line.

sequencing, or mapping as shown by the performance metrics in Table 1. Compared to the same four cell lines pooled without labeling, CLindex® sample tagging does not alter overall gene expression patterns (Figure 5). Thus, CLindex® sample tagging is unbiased, efficient, and shows no biological impact.

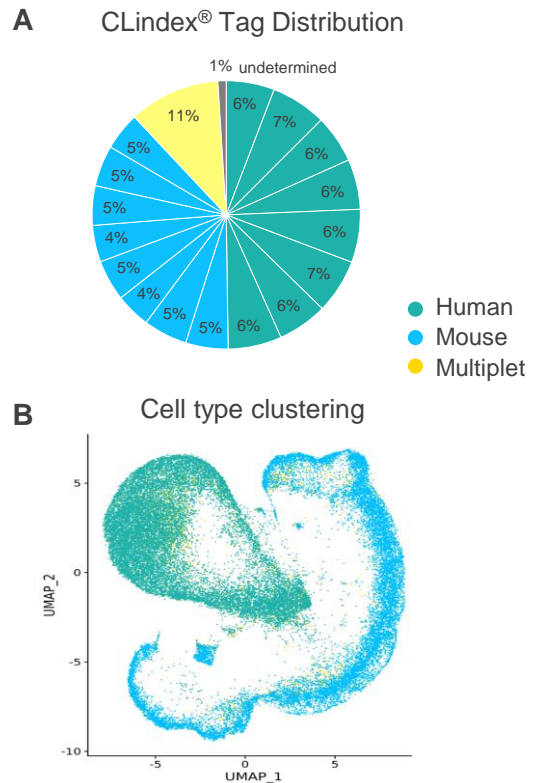


**Figure 5.** CLindex® sample tagging has no impact on overall gene expression patterns. (A) Cells colored by their CLindex® sample tag. (B) Cells colored by presence or absence of CLindex® sample tag.

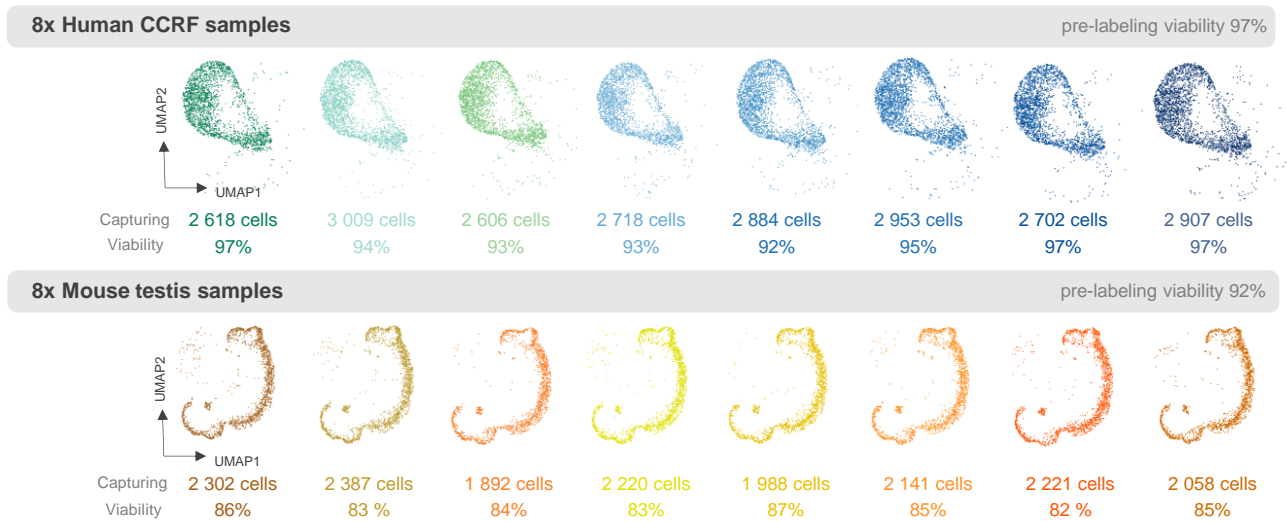
# Sample Labeling Across Different Species

Despite the diversity of nature, there is a surprising number of features, which are highly conserved among different organisms. CLindex® takes advantage of this by efficiently labeling cell surface proteins that are highly conserved across different species. Sixteen individual samples, of which eight were mouse testis and eight were human CCRF cell line samples, were labeled with CLindex® sample tags and pooled to be processed using the high-density (HD) SCOPE-chip (250,000 microwells). More than 40,000 cells were captured and sequenced. The results showed an equal distribution of captured cells between species and samples (Figure 6). As low as 11% of the sample tags corresponded to multiplets and only 1% could not be determined. Consequently, 88% of all cells were efficiently labeled and used for subsequent analysis. The UMAP overlap plot of all 16 samples distinctly separates samples according to the two species. Thus, CLindex® sample multiplexing combined with the HD SCOPE-chip allows high sample throughput at low multiplet occurrence.

Deconvoluted multiplexed samples reflect inter-species differences and intra-species consistency regarding presence of cellular subtypes (Figure 7). Multiplexing showed little effect on the cell viability, which was maintained at >92% in human CCRF samples and >82% in mouse testis samples. This proves high adaptability of CLindex® to different species and validates cross-species multiplexing.



**Figure 6.** A total of 16 samples with 8 mouse testis samples and 8 human CCRF cell line samples were multiplexed using CLindex® sample tagging. Pooled samples were represented at equal frequencies, while a low multiplet rate occurred (A). Also, the UMAP overlap plot of all 16 samples distinctly separates samples according to the two species (B). Thus, CLindex® sample tagging had no impact on cell clustering.

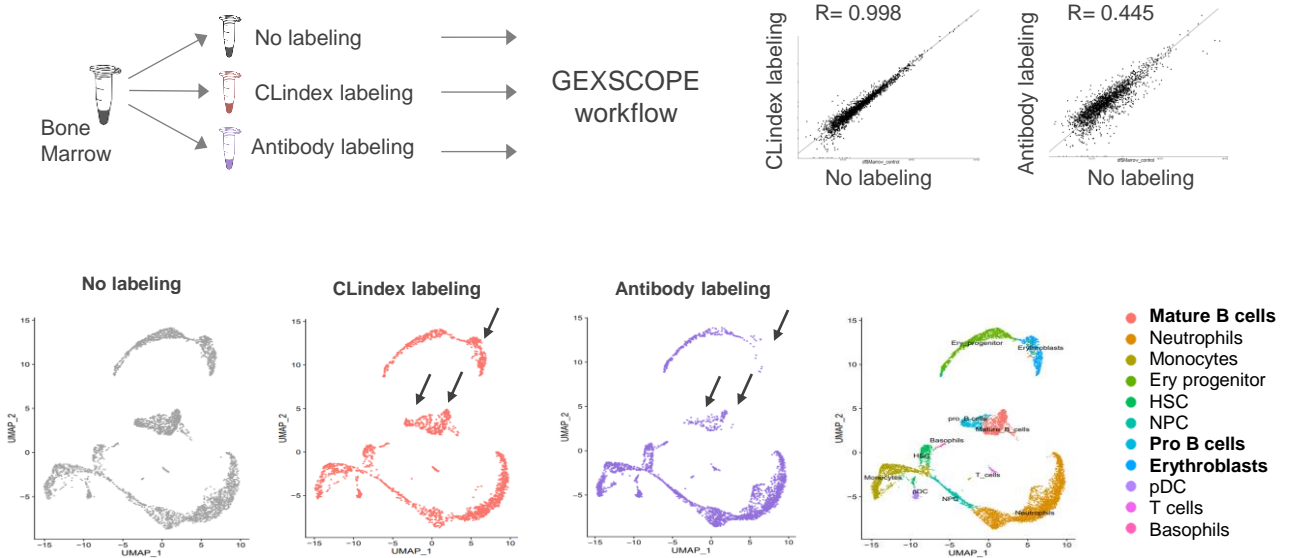


**Figure 7.** CLindex allows efficient and unbiased cross-species samples multiplexing, while maintaining cell viability at high levels. Species-specific cellular subtypes are labeled and captured free of bias.

## CLindex® Sample Labeling Includes all Cell Subtypes

To evaluate the performance of CLindex® sample tagging, mouse bone marrow samples were labeled with CLindex® and with an antibody-based labeling method from a different provider in parallel (**Figure 8**). In contrast to the antibody-based labeling method, the transcriptome of cells labeled with CLindex®

correlated well with the transcriptome of non-labeled cells. Superior to antibody-based labeling, some cell types, such as mature B cells, pro-B cells, and erythroblasts were present with in the CLindex® analysis, while largely lost during the antibody-based labeling process.



**Figure 8.** CLindex® allows unbiased sample labeling of a population of different cell types found in mouse bone marrow. A high correlation was observed between the transcripts identified independent of the labeling with CLindex sample tags ( $R=0.998$ ).

## Conclusion

In summary, the CLindex® Multiplexing Kit is a complete solution for high sample throughput and allows fast processing at low cost. All cell subtypes are evenly labeled, and the method is applicable to different species. The labeling has no impact on gene expression or cell clustering and only a minor impact on cell viability.



### Product described in this technical note

Catalog	Product	Size
1050064	CLindex® Sample Multiplexing Kit 16-Plex S	1 RXN
1050065	CLindex® Sample Multiplexing Kit 16-Plex L	4 RXN *

\*Note that the 4RXNs kit doesn't contain 64 different labels, but 4 times 16 labels

### Related products

- High-density (HD) microwell chip
- GEXSCOPE® Single Cell Kits

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