# Miniaturization of a single-cell RNA-sequencing assay generates up to 4,608 transcriptome libraries from a 384-assay kit

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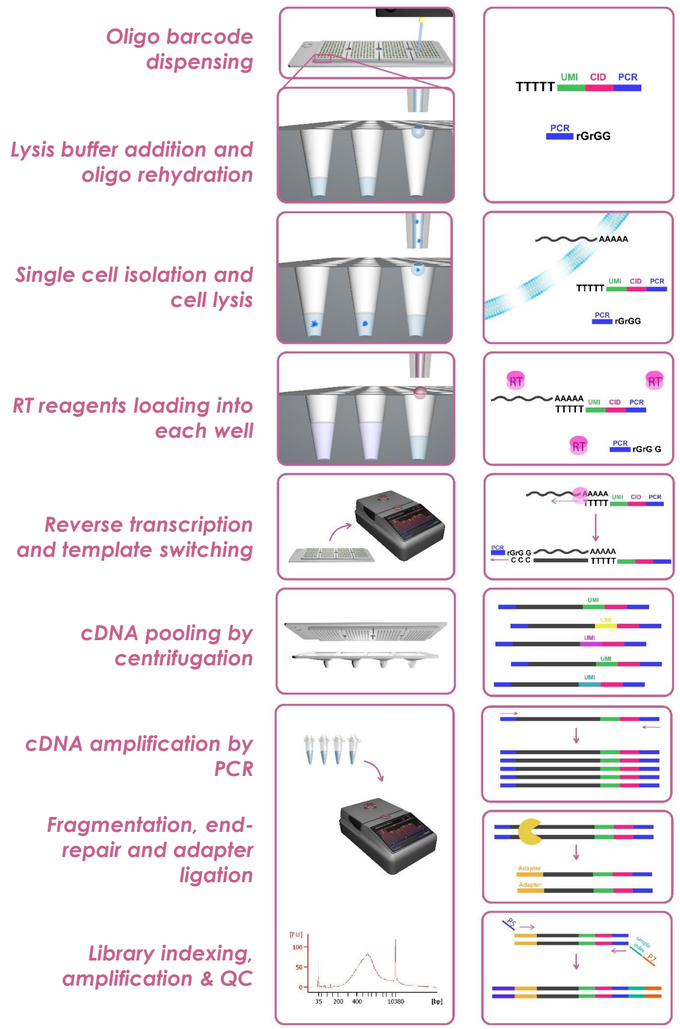
## INTRODUCTION

Single-cell RNA sequencing (scRNA-seq) methods must combine sensitivity of capturing transcript diversity within a cell with an accuracy that reflects the relative proportions of the transcripts in a cost and time-efficient manner. This preliminary work shows that the cellenONE® platform, providing both single cell isolation and automated nanoliter dispensing, drastically reduces library prep cost and handling time by miniaturizing an existing scRNA-seq commercial kit, from 5-µl → 100-nl working volume.

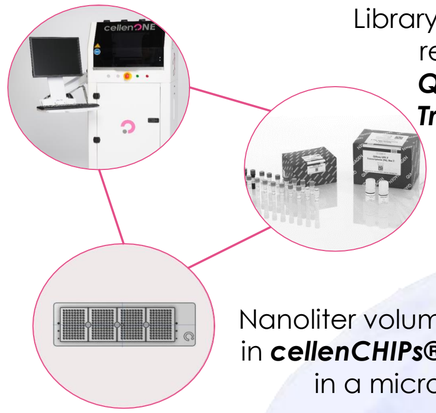
## WORKFLOW




*Working solutions (scaling quantities to 30-60 and 50-100 nl reaction volumes for cell lysis and RT reaction mixes, respectively) were prepared, and incubations were performed, according to manufacturer's instructions and data was processed using GeneGlobe, a dedicated Qiagen pipeline.*

## METHODS

Nanoliter liquid dispensing & single-cell isolation with ***cellenONE® X1***




Library preparation reagents from ***QIAseq UPX 3' Transcriptome*** kit

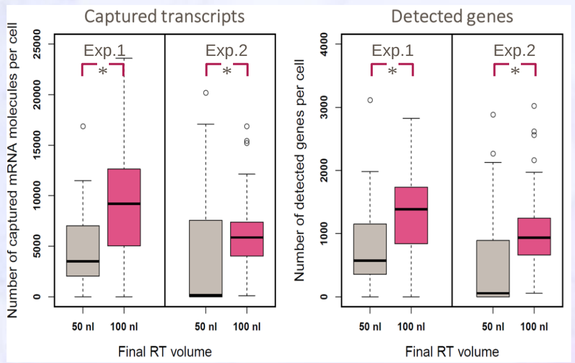
Nanoliter volume reactions in ***cellenCHIPs®*** (4x96-wells in a microscope slide footprint)

\+  Human embryonic kidney cells **= *HEK***

or  Chinese hamster ovary cells = ***CHO***

## RESULTS: Sensitivity

*Thousands of transcripts captured and genes detected per cell:*

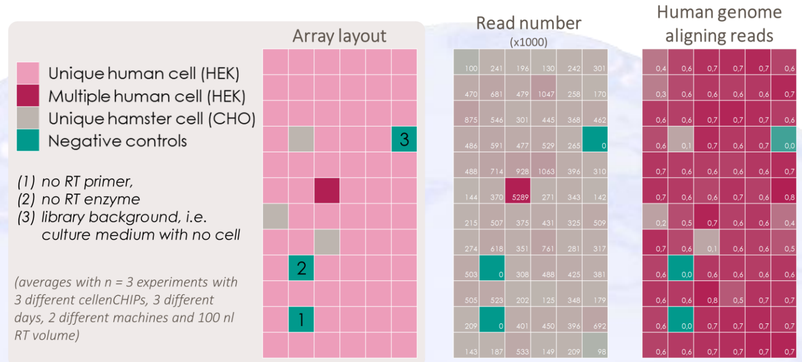



With **100 nl RT reaction**:

- ✓ Up to **23,000 captured mRNA molecules and 2,800 detected genes** per unique cell (average 8,000 and 1,200 respectively)
- ✓ **116,000 captured transcripts and 6,500 detected genes** in "Multiple" (<10) cells
- ✓ **Comparable to published QIAGEN results in original microliter-volume protocol** and to other scRNA-seq technologies (Ziegenhain, *2016, Mol Cell DOI:10.1016/j.molcel.2017.01.023!)*

## RESULTS: Contamination

*A clean signal with low background:*

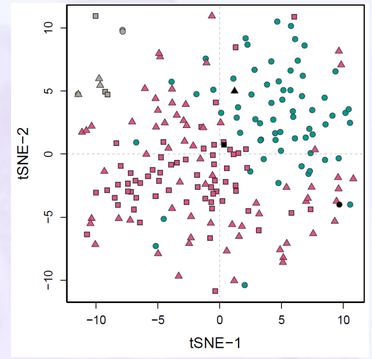



- ✓ **Low to null** cross-sample contamination
- ✓ **Low to null** library background noise

## RESULTS: Robustness

*Reproduciblility highlights transcriptome differences:*

- ✓ **Replicate** cellenCHIPs ( ■ and ▲ ) do not discriminate from one another
- ✓ Exp. 1 cell transcriptomes ( ● ) significantly discriminate From Exp. 2 transcriptomes ( ■ and ▲ )




- ✓ Hamster cells ( ■ , ▲ and ● ) always neatly discriminate from human cells ( ■ , ▲ and ● resp.)

## CONCLUSION

- ✓ Miniaturized *Qiagen's UPX 3' Transcriptome* kit for use in cellenONE® with cellenCHIP® and obtained **scRNA-seq libraries for less than 1.75 € / 2 USD per cell**
- ✓ Data quantitatively aligns with other scRNA-seq technologies
- ✓ Current work focuses on improving robustness / sensitivity:

***Watch out for an Application Note and full protocol details, to be released soon !***

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