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IMTEK

Single-cell printing for the genetic analysis of cancer cells

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Summary

We present a workflow for the isolation and genetic analysis of single cancer cells based on a Single-Cell Printer (SCP):

- Printing of single cells from the osteosarcoma cell line U2OS in wells of a 384-well microtiter plate (MTP)
- Whole genome amplification (WGA) of single-cell DNA at reduced reagent volumes
- Multiplex PCR on *LINE1* retrotransposons
- Analysis of U2OS-specific mutations

Following this workflow, a single-cell printing efficiency of 98% and uniform DNA yields after WGA were achieved. *LINE1* retrotransposons could be detected in all WGA samples, and mutations in the *TET2* and the *SLC34A2* gene, respectively.

Introduction

Cancer is based on the sequential acquisition of mutations in individual cells leading to genetically heterogeneous cell populations. There is an increasing interest in understanding this heterogeneity on a single-cell level. The isolation of single cells is still a technically challenging task in consideration of the unambiguous deposition of one single cell per well [1]. Single-cell printing allows for the gentle and proven deposition of single cells on a variety of substrates on-demand and in a non-contact manner [2], (Fig. 1).

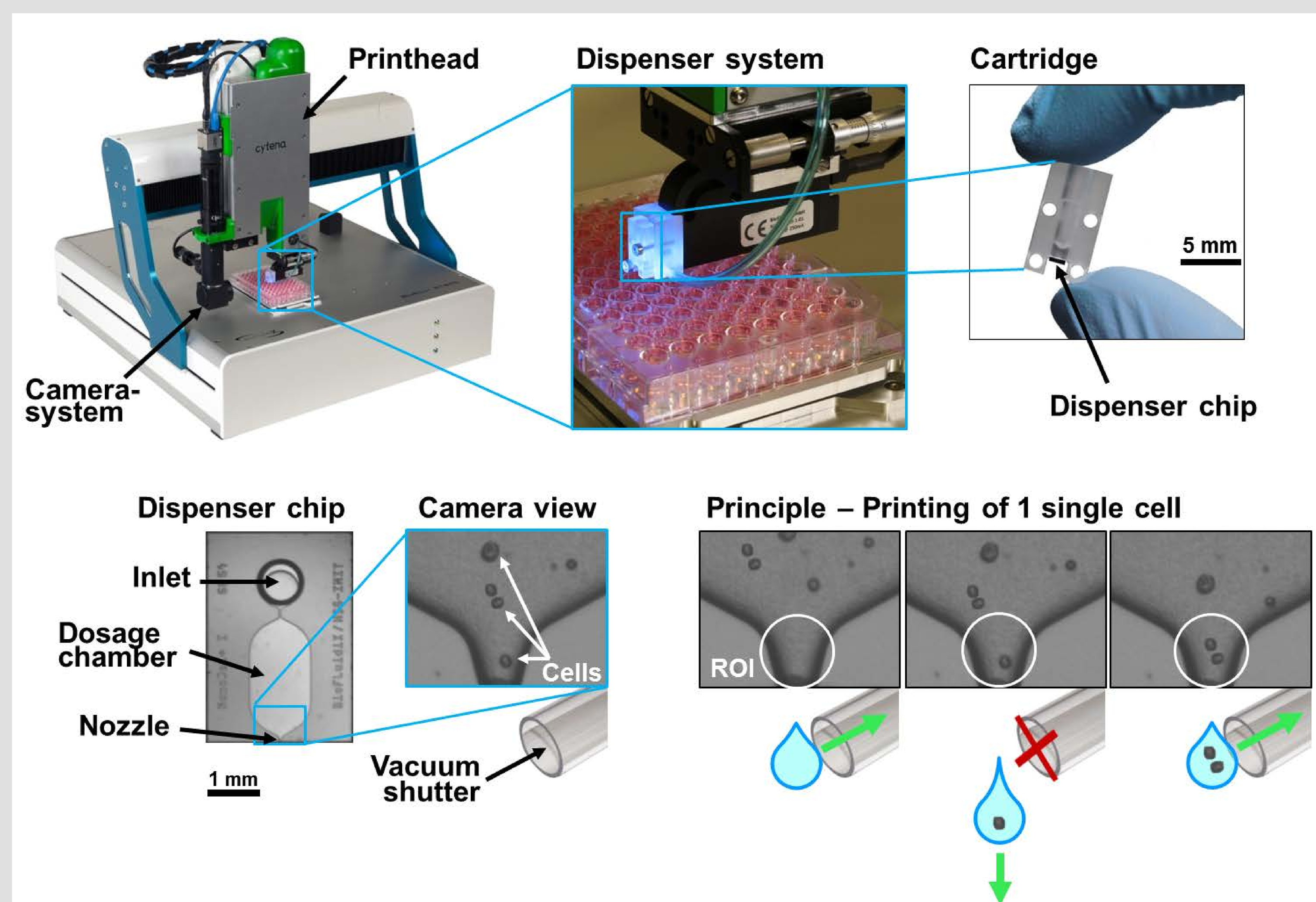


Figure 1. Single-cell printer with details of the dispensing system. The cell suspension is pipetted into the inlet of the dispenser chip. A piezo-driven actuator deflects a membrane on the back of the dosage chamber and a droplet is formed. A digital camera detects, counts and classifies the properties of cells at the nozzle before the droplet formation. Only droplets containing a single cell are delivered to the substrate. A pneumatic shutter system device removes unwanted droplets containing no or more than one cell.

Workflow

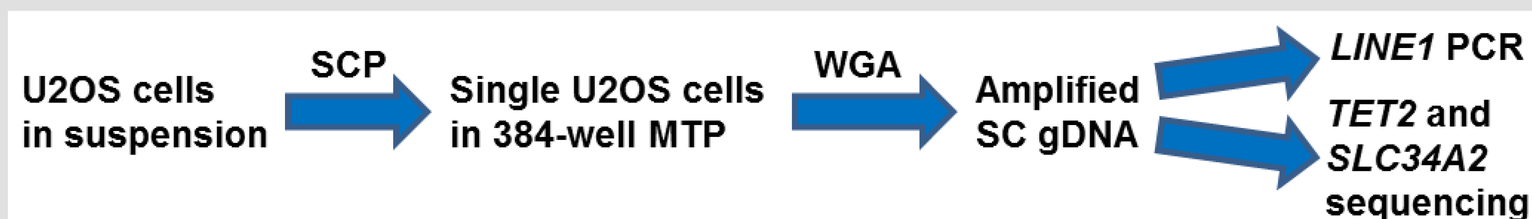


Figure 2. WGA of single-cell genomic DNA using REPLI-g™´Single Cell Kit (Qiagen) at 4-fold reduced reagent volumes compared to standard protocol for downstream single-cell genomic analyses.

Results

20 U2OS cells were printed in 20 wells, each preloaded with 1 µl PBS. Additionally 20 cells were printed in 20 dry wells resulting in a total single-cell deposition efficiency of 98% according to the SCP image series (Fig. 3).

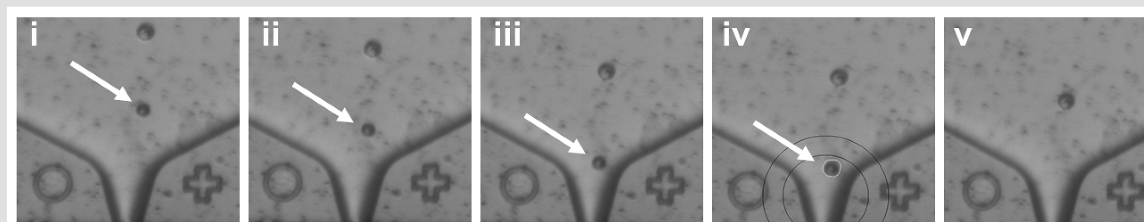


Figure 3. The SCP image series enables to track the single cell before it is ejected as a droplet.

WGA was performed on the 20 cells printed in PBS, and on four cells printed in dry wells resulting in a DNA yield of 3.9 ± 0.2 µg (PBS), and 3.8 ± 0.1 µg (dry), respectively (Fig. 4a). The WGA DNA was evaluated by a multiplex PCR on repetitive *LINE1* transposons, which revealed positive results in all WGA samples (Fig. 4b).

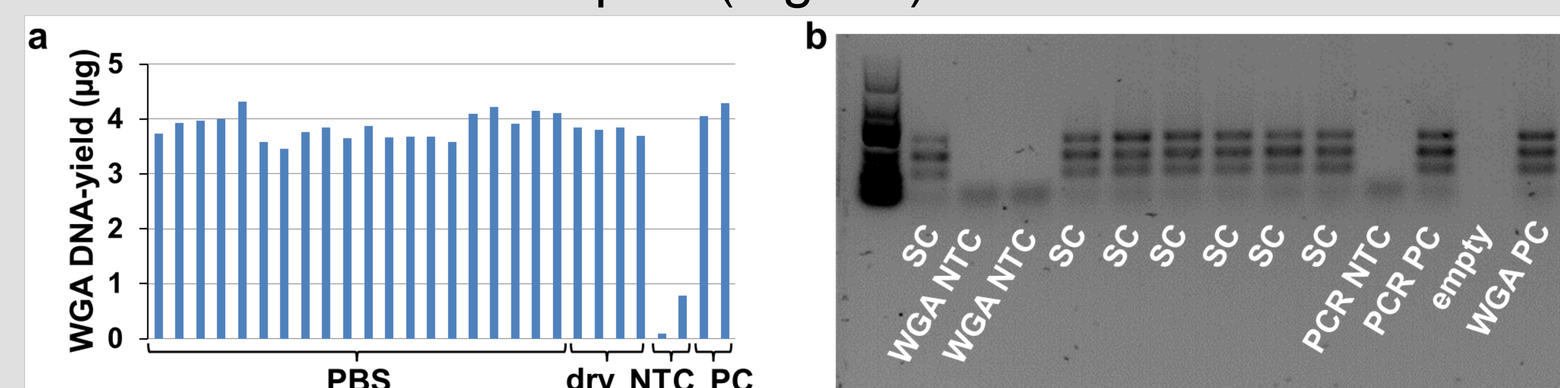
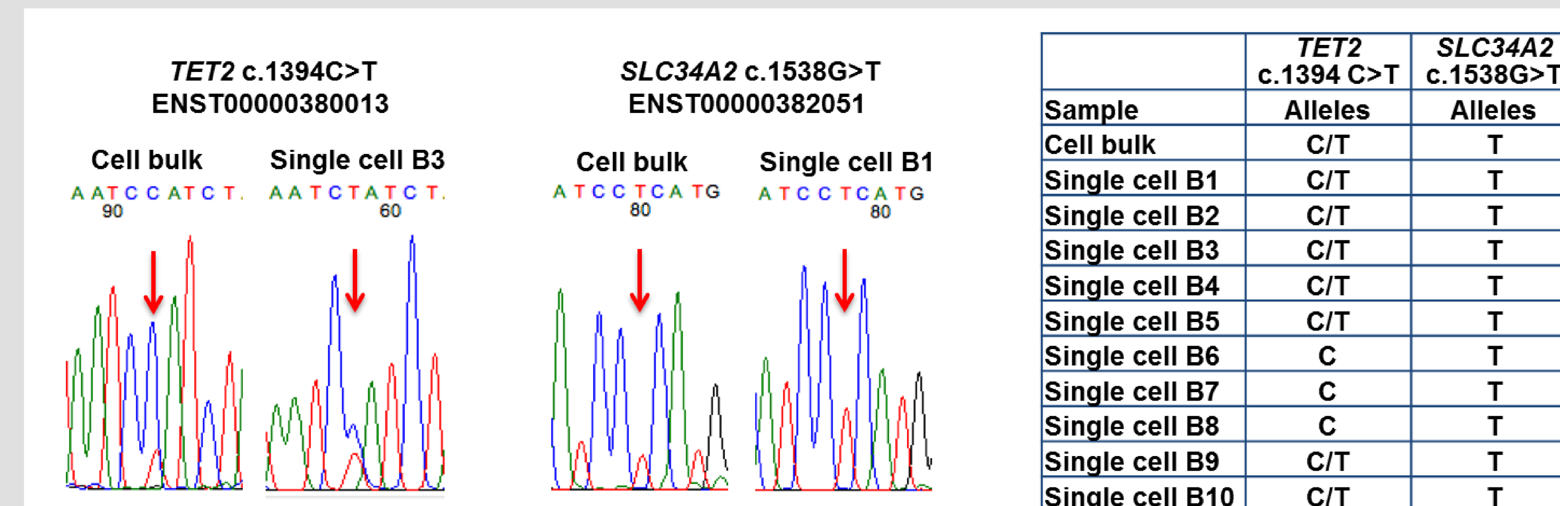


Figure 4. (a) WGA DNA yield as measured by Qubit™ quantitation. (b) Excerpt of an agarose gel shows typical triplicate bands for *LINE1* positive samples. SC: single-cell. NTC: non-template control. PC: positive control

U2OS-specific mutations in *SLC34A2* (c.1538G>T), and in *TET2* (c.1394C>T) were detected in representative WGA samples of single cells printed in PBS (Fig. 5).



	TET2 c.1394 C>T	*SLC34A2* c.1538G>T
Sample	Alleles	Alleles
Cell bulk	C/T	T
Single cell B1	C/T	T
Single cell B2	C/T	T
Single cell B3	C/T	T
Single cell B4	C/T	T
Single cell B5	C/T	T
Single cell B6	C	T
Single cell B7	C	T
Single cell B8	C	T
Single cell B9	C/T	T
Single cell B10	C/T	T

Figure 5. Analysis of U2OS-specific mutations illustrated by representative sequencing results in bulk cells and exemplary single U2OS cells. The table indicates the detected single base modifications of the respective genes.

Conclusions

We present a workflow, which exploits the cell deposition accuracy of a single-cell printer allowing for the efficient genetic analysis of single cancer cells. This workflow could be used to decipher the heterogeneity of hematological neoplasia such as acute myeloid leukemia (AML) on a genetic but also epigenetic single-cell level.

Acknowledgements

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References

[1] Gross A, *et al.* Int J Mol Sci. (2015) 16(8):16897-16919.
[2] Gross A, *et al.* J Lab Autom. (2013) 18(6):504-18.

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