Single Cell in a WGA Cup: Open-well whole genome amplification

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Introduction

Genetic characterization of Circulating Tumor Cells (CTC) offers the opportunity for a “real time liquid biopsy” [1, 2]. Heterogeneity and rarity of CTC command the need for individual cell characterization. Following an enrichment procedure of CTC from blood, the identification, isolation and manipulation of single cells for further analysis without cell loss remains challenging. Here, we present a microfluidic device with open-well structures in which cells can be identified, isolated, lysed and the nucleic acids amplified following filtration. On-chip amplification will be a powerful tool to improve genetic analysis of single cells by making use of the smaller reagent volume, automation and parallel reactions of microfluidic devices [3].

Open-well Microfluidic Device

**Pneumatic Valve**

**Device Design**

Self-sorting Microwell

**Microwell Plate Design**

**Single Cell Seeding**

![Fluorescence Image of Cells in Microwells](image)

Single Cell Isolation

1. Filtrate thousands of cells through self-sorting microwell.
2. Scan self-sorting microwell plate under a fluorescence microscope.
3. Punch the bottom of microwells with cells into the reaction chamber.
4. Process isolated cell in the reaction chamber.

![Punching](image)

Peristaltic Pumping

Loading reagents to reaction chambers using peristaltic pumping

**Design**

**Sequence**

![Pumping rate: 2.5 nl/sec (10 Hz)](image)

Whole Genome Amplification on a Chip

**Isolate single cells of interest**

Load proteinase K,~150 nl

Lysis

60°C, 1 h / 95°C, 10 min

Load WGA mixture,~275 nl

Amplification

30°C, 16 h

Collect amplified-DNA out

Conclusions

We developed a novel microfluidic device for whole genome amplification of single cell isolated by self-sorting microwell plate.

References