MASSIVELY-PARALLEL DIGITAL SOLID-PHASE PCR FOR THE IN-SITU GENERATION OF A SEQUENCING-READY PICOWELL ARRAY CIRCUMVENTING emPCR

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Abstract
A novel approach to generate **sequencing-ready picowell arrays** requiring **no emPCR** and **no library beads** is presented. By **massively-parallel digital solid-phase PCR**, template DNA in length of up to 1.5 kbp is **amplified and immobilized** within 100,000 of **19 picoliter wells** of the commercial sequencing chip (PicoTiterPlate PTP™, Roche) in one single step. This approach has the great potential to circumvent the cumbersome state-of-the-art emPCR workflow in the preparation of sequencing chips.

Introduction
Many next-generation sequencing processes [1][2] comprise four steps: (1) quantification of a DNA library, (2) DNA amplification and immobilization onto beads via emPCR, (3) sorting and arranging of beads on a surface, (4) performance of a sequencing assay. Since emPCR can be error-prone, cost-intensive, and requires multiple process steps, an alternative approach to generate sequencing-ready chips is of interest [3]. We addressed this issue by a unique approach, replacing step (2) and (3) by one single step of digital solid-phase PCR [4], directly performed in a PTP used for massively-parallel pyro-sequencing.

Functional principle
PCR primers are immobilized to both, the wells of an original PicoTiterPlate, and to a planar slide that seals the PTP during PCR [5]. A PCR mix containing n template DNA molecules is distributed to m wells of the sequencing chip ($n < m$) and sealed with the slide. The sealed sequencing chip is thermocycled generating and immobilizing PCR products only in those wells that initially contained one template molecule. For analysis of the process, immobilized PCR products are detected by sequence-specific hybridization probes.

Experimental results
Functionality of massively-parallel digital solid-phase PCR in PTPs is demonstrated by simultaneously amplifying DNA in length of 346 bp and 1,513 bp. Upon sequence-specific staining, the resulting two-color image shows that amplification products of both templates are successfully immobilized to the surface of the wells and the sealing slide. The distinct signals indicate the leak-tight sealing of the wells during PCR.
When tenfold serial diluted template DNA is amplified by digital solid-phase PCR, the resulting number of positive wells scales with the number of initial template molecules. However, the number of positive wells is lower than expected, most probably due to adsorptive effects. When assuming a 250 × lower initial concentration, all results lie within the 95 % confidence interval of the expected Poisson distribution. This clearly indicates that positive wells result from amplification of a single molecule.

Conclusion and outlook
By **massively-parallel digital solid-phase PCR** performed in the **picowell sequencing chip PTP™**, **single DNA molecules** are **amplified and immobilized** within the **19 picoliter wells** and detected by hybridization. Functionality is demonstrated with template DNA as long as 1,513 bp. This is the currently **smallest on-chip solid-phase PCR** in a non-emulsion format. This approach has the great potential to **replace emPCR in the preparation of sequencing chips** saving time, costs, and material. As an outlook, our approach may enable massively-parallel single-cell analysis by isolating single cells in different wells and immobilizing specific gene products onto a substrate for further analysis.

References :
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