# Hot Start Amplification using OligoBeads via Gradual Release of Bound Primers

**CTGEN, US Patent Pending**
**BOOTH # 522**

## Introduction

OligoBeads provide a mean to store normalized primers used in performing enzymatic reactions including PCR. Primer bound beads eliminate the potential for pipeting errors and reduce contamination thus yielding lower repeat rates and less reagent wastage. The primers bound to the OligoBeads can be stored over a period of a few months without degradation in a nuclease free environment.

## Discussion

Primer bound OligoBeads of a same lot adsorb similar quantity of primers, ensuring that a plurality of PCR reactions can be run over a period of a few months with reproducibility. They are compatible with standard thermocyclers using 10 to 100 µL reaction volumes. A bead typically takes up a small volume, less than 2µl of the total PCR reaction volume. OligoBeads prevent the room temperature release of primers in solution. Upon the denaturation step and the first few cycles, the primers are gradually released, thus preventing mispriming and primer-dimer amplifications and ensuring low copies of template to be specifically amplified.

## Method

In the following experiments, a bead carrying two normalized primers (reverse and forward, around 15 pmol each) is transferred to a PCR tube containing DNA polymerase, PCR buffer and a 106 mer target template.

| | | |
|---|---|---|
| **Preheating** | **94ºC** | **5 min** |
| **Preheating** | **94ºC** | **30 sec** |
| **Annealing** | **75ºC** | **30 sec** |
| **Extension** | **75ºC** | **30 sec** |
| **Polymerization** | **72ºC** | **7 min** |
| **Store** | **4ºC** | **~** |

Amplifications were carried out in 50 ml reactions volumes containing 106-mer template DNA ranging from 10 to 100 copies following the PCR cycle described in the above Table. Analyses of PCR products and primer binding were conducted by Ion Exchange Liquid Chromatography.

## Results

FIG. 1 With primers pipetted and added in solution, amplification of 100 copies of template yielded two main product identified as primer dimers (peak at 8.6 min) and the target amplified 106 mer (Peak at 9.6 min). No primers at 6.4 min remained.

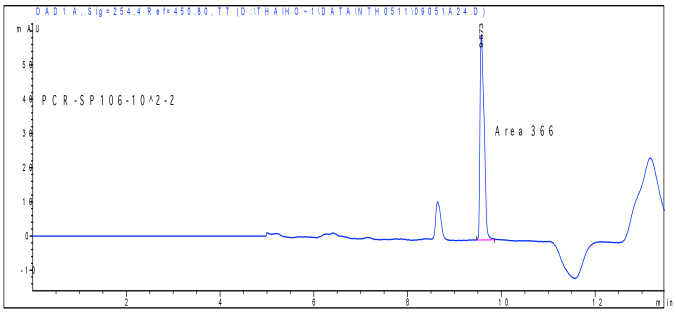


FIG. 2 and 3 When the OligoBead was used, no primer-dimer was observed even at 10 copies (left HPLC trace). Remaining primers were seen at 6.4 min and amplified product at 9.4 min. A Right HPLC trace shows the amplification of 100 copies with an oligobead of the same lot. Lower primer peaks and higher target peaks are observed.