Accurate preamplification using dUTP and Cod UNG for integrated removal of contaminating amplicons

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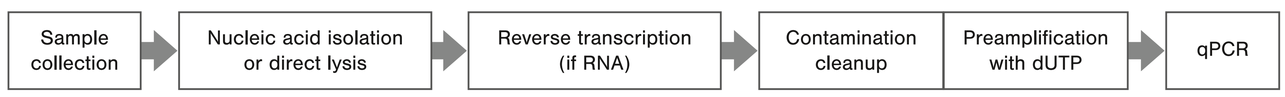
INTRODUCTION

Preamplification is often required to reliably detect and quantify rare DNA and RNA molecules in limited sample sizes. This makes downstream analyses sensitive to PCR generated amplicon contamination. The use of uracil-DNA N-glycosylase (UNG) and deoxyuridine triphosphate (dUTP) is a well known solution for carry-over contamination control in PCR. Similar approach has not been described in preamplification, where any loss of amplicons caused by re-activated UNG would be detrimental for downstream analysis. However, Cod UNG can be completely and irreversibly heat inactivated. Here we study the feasibility of performing preamplification using dUTP and Cod UNG for contamination cleanup.



Experimental workflow. Contamination cleanup and preamplification can be performed separately, or as one step.

MATERIALS AND METHODS

1. To study the effect of replacing dTTP with dUTP in target-specific preamplification, we performed multiplex PCR using 400 µM of each dNTP (dGTP, dATP, dCTP, and either dTTP or dUTP), applying optimal run conditions[1]. Amplification efficiencies of 96 individually optimized qPCR assays were estimated using DNA standard curves based on purified PCR products.

2. To investigate whether Cod UNG inhibits the amplification, we incubated 46 individual thymine-containing DNA standard curves with active or heat inactivated Cod UNG for 5 min at room temperature prior to target-specific preamplification using dUTP.

3. To assess contamination cleanup efficiency of Cod UNG, we treated 46 individual uracil-containing DNA standard curves with active or heat inactivated Cod UNG for 5 min at room temperature prior to target-specific preamplification using dUTP.

4. Finally, we tested the proposed workflow on a limited sample size by performing single-cell gene expression profiling on 92 individual human myxoid liposarcoma (MLS) 402-91 cells.

RESULTS

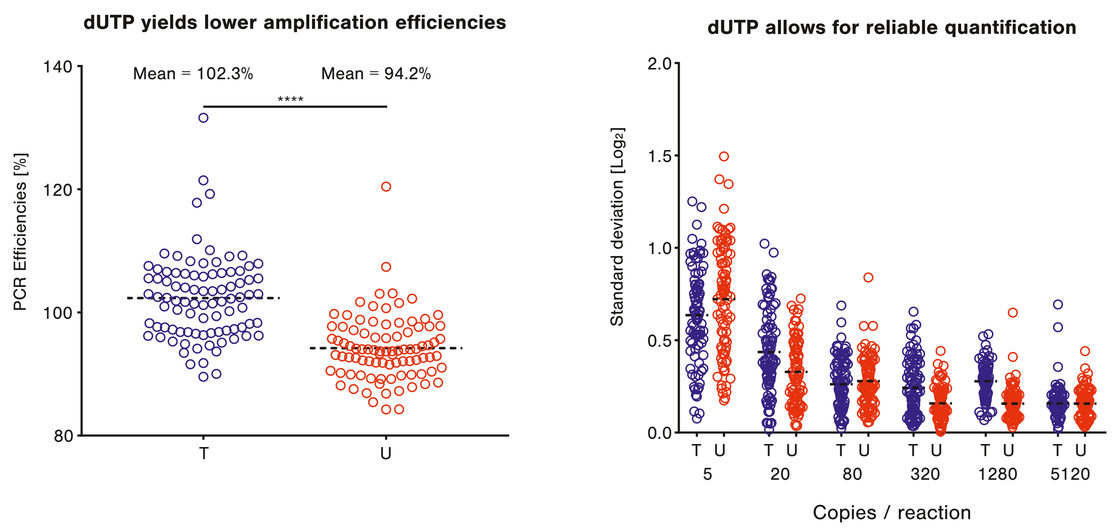


Figure 1: dUTP instead of dTTP in preamplification. PCR efficiencies were estimated using 96 individual standard curves of purified PCR products, ranging from 5 to 5120 molecules per reaction (n = 3 per dilution point). dUTP resulted in lower amplification efficiencies but not reduced reliability. **** indicate p<0.0001, using Wilcoxon matched-pairs signed rank test.

TAKE HOME MESSAGE

- Preamplification using dUTP allows for reliable quantification
- Cod UNG:
  - Does not inhibit amplification
  - Removes relevant amounts of contaminating amplicons
  - Can be totally and irreversibly inactivated

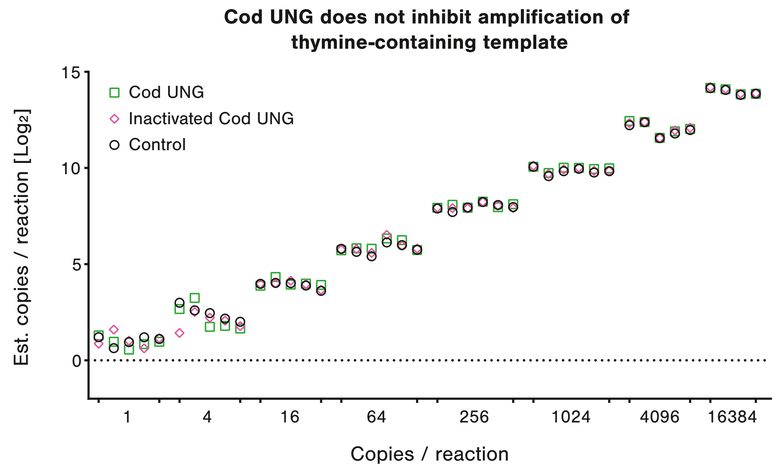


Figure 2: Cod UNG treatment of thymine-containing template. Treating 46 individual thymine-containing DNA standard curves, ranging from 1 to 16.384 molecules per reaction (n = 15 reactions per treatment), with Cod UNG prior to preamplification did not result in inhibition of amplification.

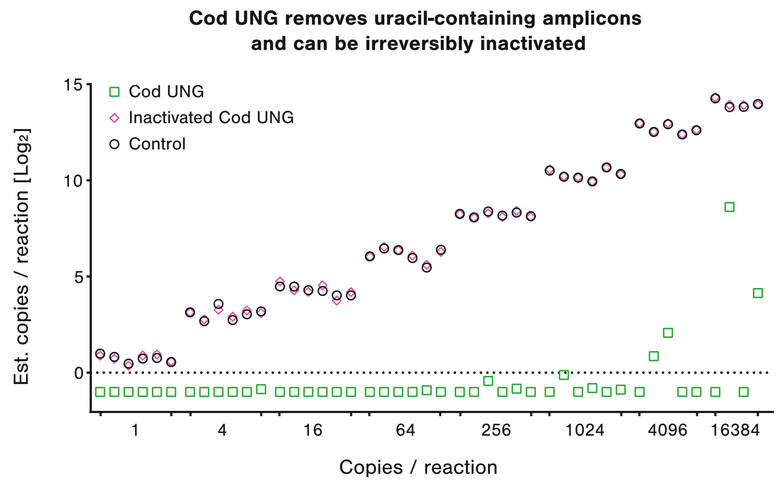


Figure 3: Cod UNG treatment of uracil-containing amplicons. Treating 46 individual uracil-containing DNA standard curves, ranging from 1 to 16.384 molecules per reaction (n = 15 reactions per treatment), with Cod UNG prior to preamplification removed all target molecules in all reactions for most assays. At the same time, heat inactivated Cod UNG (2 min at 95 C) did not differ from control.

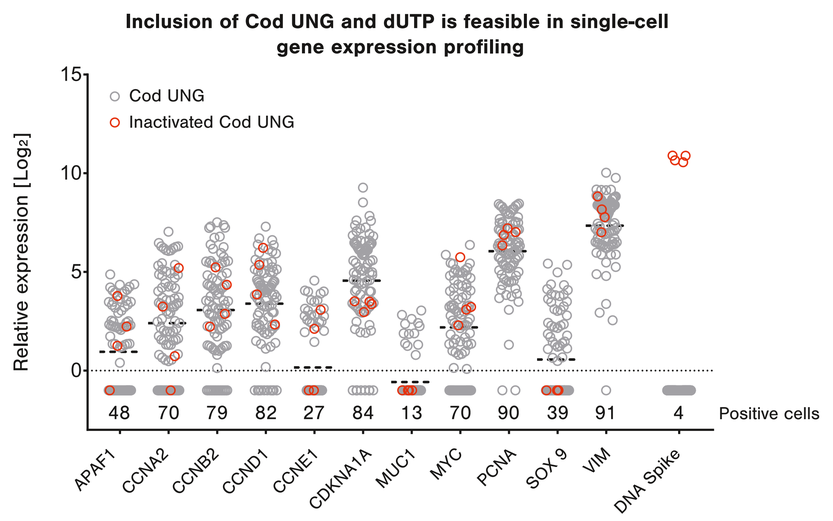


Figure 4: Single-cell gene expression. Ninety-two single cells were treated with Cod UNG in presence of 100 copies of an uracil-containing DNA Spike, followed by preamplification using dUTP. Relative expression is shown for each gene with every cell being represented by a circle. Number of cells expressing each gene is indicated. Horizontal bars indicate mean values and the dotted line indicates one molecule. Active Cod UNG removed all DNA spike (n = 88), while inactivated Cod UNG did not (n = 4).

1. Andersson *et al., Expert Rev Mol Diagn* (2015), doi: 10.1586/14737159.2015.1057124