## **UNBIASED PRE-AMPLIFICATION**

# High order multiplexed enrichment of specific sequences from limiting template using low-cycle PCR



## **FEATURES AND BENEFITS**

- **5X** concentrated SuperMix for lower reaction volumes / higher sample volumes
- Adding more value to your precious samples
- AccuStart II hot-start



#### **PreAmp Process Flow:**

- 1. Prepare RNA
- 2. Reverse transcribe RNA
- 3. Pool assay primers and dilute
- 4. Perform pre-amplification reaction
- 5. Dilute PreAmp reaction product
- Perform individual qPCRs for each pre-amplified gene of interest (GOI).





Fig.2A 96-gene Cq comparison for control versus 10-cycle pre-amplified cDNA. cDNA prepared from Universal Reference RNA, either 100 ng, 10 ng, or 1 ng (total RNA equivalent), were pre-amplified for 10 cycles in 20-uL reaction volumes. Following pre-amplification, product was diluted 5-fold and 0.5 uL used as template in individual qPCRs for each gene using PerfeCra SYBR Green SuperMix. Assuming 1,000-fold enrichment: S00 ng, 50 ng, or 5 ng of PreAmp product was used as template in each 10-uL qPCR. Cq for each amount of pre-amplified cDNA (Y-axis) are plotted against Cq obtained for 10 ng of control (no PreAmp) cDNA (X-axis). Idealized plots representing perfect (100%) pre-amplification efficiency for each input amount are shown in black.

Fig.2B 96-gene Cq comparison for control versus 14-cycle pre-amplified cDNA. cDNA prepared from Universal Reference RNA, either 10 ng, 1 ng, or 0.1 ng (total RNA equivalent), were pre-amplified for 14 cycles in a 20-uL reaction volume. Following pre-amplification, product was diluted 10-fold and 0.5 uL used as template in individual qPCRs for each gene using PerfeCra SYBR Green SuperMix. Assuming 16,000-fold enrichment: 400 ng, 40 ng, or 4 ng of PreAmp product, was used as template in each 10-uL qPCR. Cq for each amount of pre-amplified cDNA (Y-axis) are plotted against Cq obtained for 10 ng of control (no PreAmp) cDNA (X-axis). Idealized plots representing perfect (100%) pre-amplification efficiency for each input amount are shown in black.

PerfeCta PreAmp SuperMix is a 5X concentrated, ready-to-use reaction cocktail for unbiased, selected enrichment of target sequences from limiting amounts of starting material for downstream gene expression profiling or targeted re-sequencing. It contains all components, except primers and templates. The 5X concentrated SuperMix allows addition of higher template volumes when working with low concentration samples, and/or reduced reaction volumes. Inclusion of an inert light blue tracer dye helps visualize small reaction volumes and ensure accurate pipetting. PerfeCta PreAmp SuperMix delivers unbiased pre-amplification of up to 100 target sequences from as little as 100 pg of total cDNA. It is compatible with both TaqMan<sup>®</sup> 5'-nuclease probes or ds-DNA binding dye (i.e. SYBR<sup>®</sup> Green I) qPCR detection chemistries. A key component of PerfeCta PreAmp SuperMix is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to pre-amplification.

### **COMPONENTS**

PerfeCta PreAmp SuperMix 5X reaction buffer containing optimized concentrations of  $MgCl_2$ , dNTPs (dATP, dCTP, dGTP, dTTP), hotstart DNA polymerase, AccuVue<sup>M</sup> blue tracer dye, and stabilizers.

## **ORDERING INFORMATION**

PRODUCT	Quanta Cat. No.	Pack Size (20uL Reactions)
PerfeCta <sup>®</sup> PreAmp SuperMix	95146-040	40 x 50-µL reactions



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