diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Human ChIP-seq grade Myoglobin Exon 2 primer pair		
Cat. No: pp-1006-050	**Format:** 50 µl	**Concentration:** 10 µM
Cat. No: pp-1006-500	**Format:** 500 µl	**Concentration:** 10 µM

Product Description: This primer pair specifically amplifies a genomic region from exon 2 of the human myoglobin gene. These Primers are thoroughly tested and optimized for routine SYBR® Green Real-Time qPCR assay following ChIP and for ChIP-sequencing library validation (e.g. before and after ChIP-seq library preparation).

Amplicon length: 76 base pairs.

Amplified region: chr22:34,336,993-34,337,068

Specificity: Human

Format: 10 µM solution in MilliQ water (5 µM of each primer)

Storage: For long storage, store at -20°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Last data sheet update: April 18, 2013

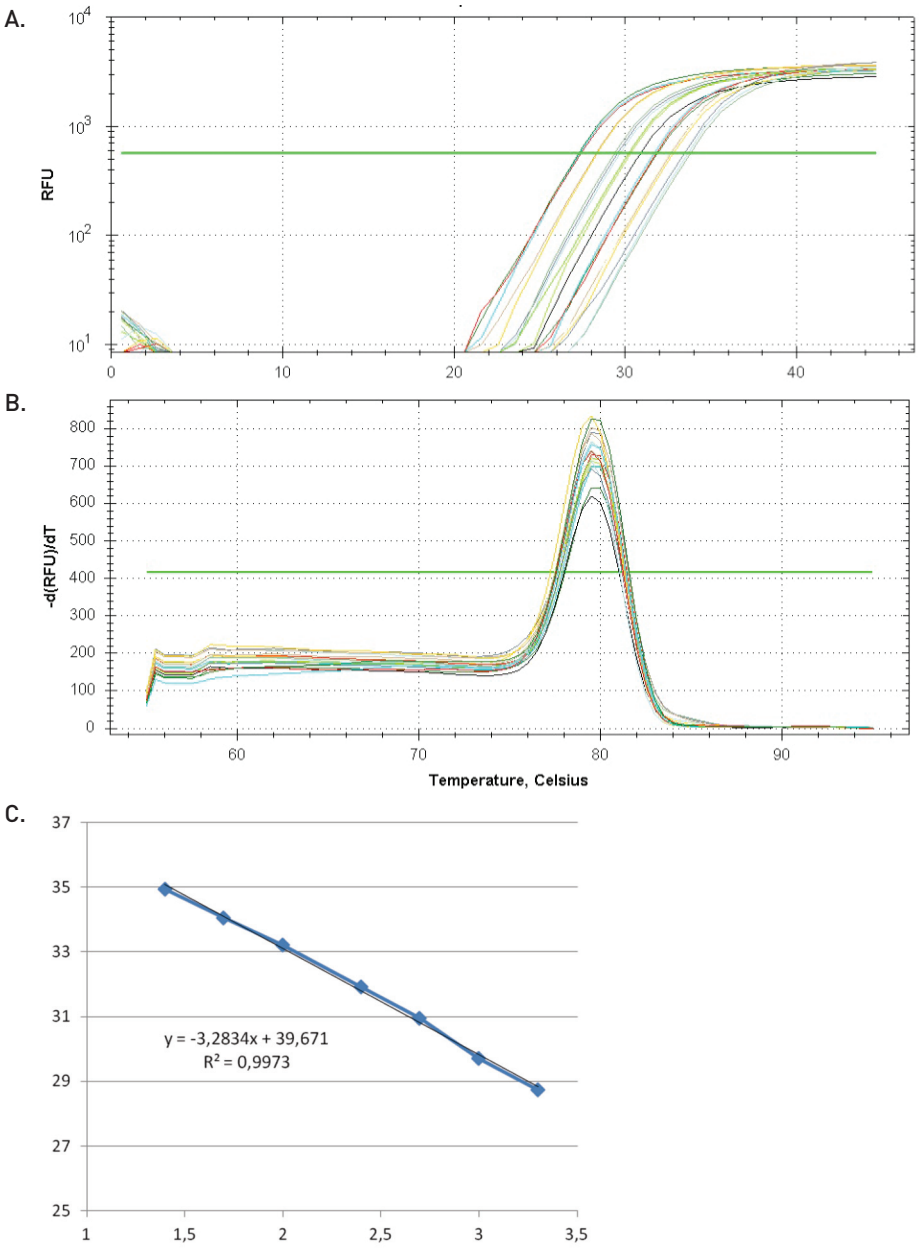

Figure 1

Sheared DNA from HeLa cells was analysed in triplicate by SYBR® Green real-time PCR using the Diagenode MB ex2 Primer Pair (cat. No. pp-1006-050, pp-1006-500). A dilution series ranging from 2 ng to 25 pg of DNA template was amplified with 1 µl of the provided primers in 25 µl total reaction volume on an iQ5 thermocycler (Biorad). qPCR conditions were as follows: incubation at 95°C for 10 minutes, followed by 45 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C and a final extension for 10 minutes at 72°C.

Figure 1A: amplification curves (logarithmic view). The green line represents the position of the threshold.

Figure 1B: melting curve analysis of the different amplification products.

Figure 1C: plot of the Ct value (mean of 3 replicates) against the log of the initial DNA amount. The reaction efficiency, as calculated from the slope of this curve, is 101.6%.

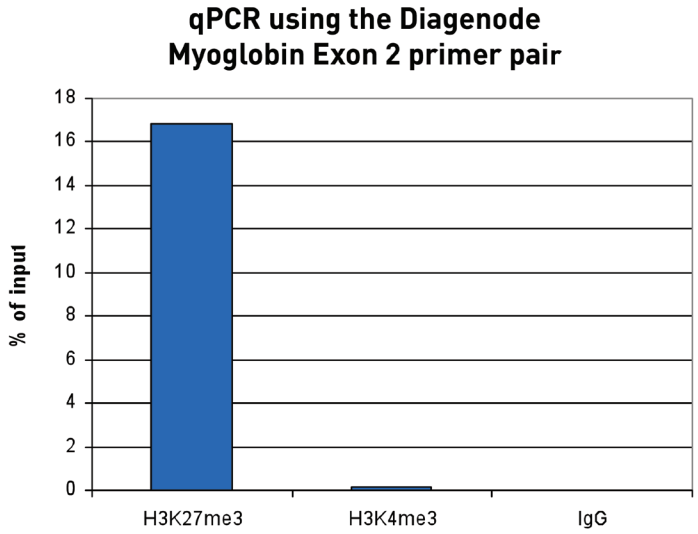

Figure 2

ChIP was perfomed on HeLa cells using antibodies against H3K27me3 (cat. No. pAb-069-050), located at inactive genomic regions, and H3K4me3 (cat. No. pAb-003-050), known to be located at active promoters. Rabbit IgG was used as a negative IP control. The ChIP'd samples were analysed by qPCR using the MB ex2 Primer Pair (cat. No. pp-1006-050, pp-1006-500). Figure 2 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

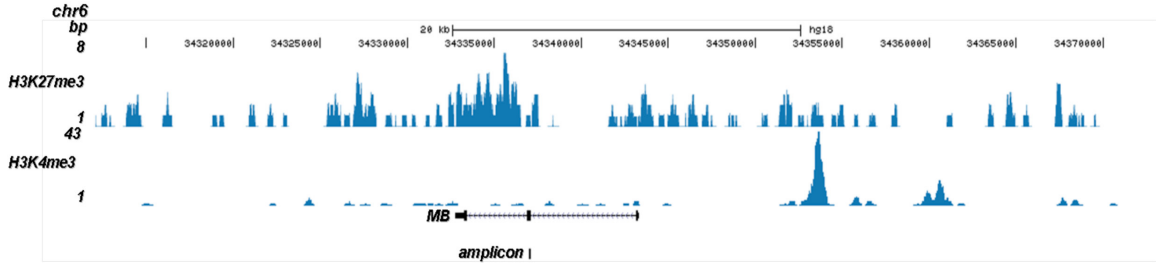

Figure 3

ChIP was performed as described above and the ChIP'd DNA was subsequently analysed by high throughput sequencing on an Illumina GAIIx. Figure 3 shows the H3K27me3 and H3K4me3 profiles in a region of chromosome 6 containing the MB gene. The position of amplicon obtained with the Diagenode MB ex2 Primer Pair (cat. No. pp-1006-050, pp-1006-500). is indicated.