diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Human ChIP-seq grade b-actin promoter primer pair	
Cat. No: pp-1048-050	Format: 50 µl
Cat. No: pp-1048-500	Format: 500 µl

Product Description: This primer pair specifically amplifies a genomic region containing the human beta actin (ACTB) promoter. The 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: 150 base pairs.

Amplified region: chr7: 5536809-5536958

Specificity: Human

Format: This primer set contains both forward and reverse primers in 50 µl or 500 µl MiliQ water. The final concentration for each primer is 5 µM.

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: October 29, 2012

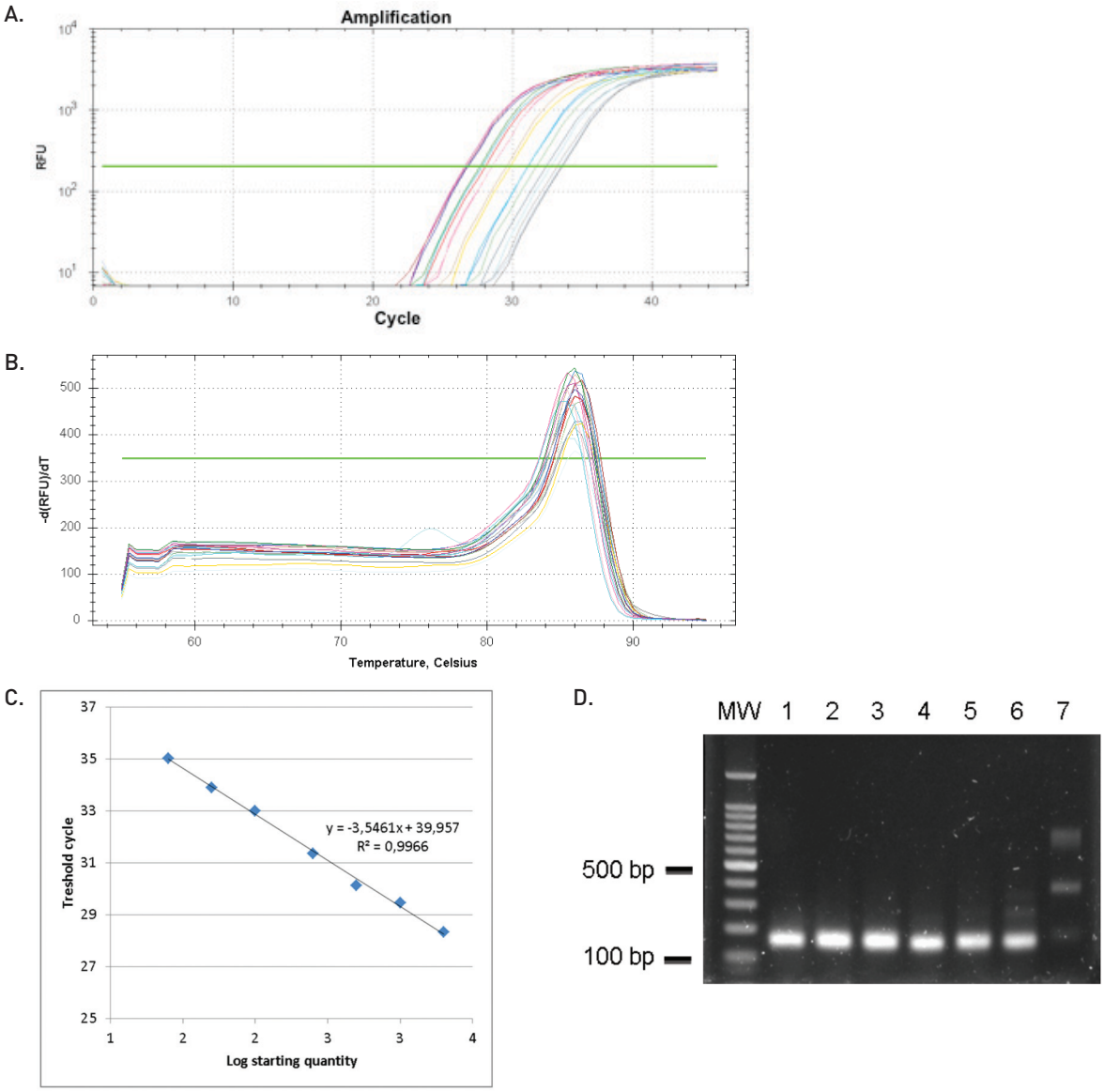

Figure 1

Sheared DNA from HeLa cells was analysed in triplicate by SYBR Green real-time PCR using the Diagenode ACTB promoter primer pair (cat. No. pp-1048-050, pp-1048-500). 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 91.4%.
Figure 1D: agarose gel electrophoresis of the PCR amplification products (2% agarose gel stained with SYBR Safe). A 100 bp molecular weight marker is shown on the left; the PCR products from 6 different dilutions (expected size 150 bp) and from the NTC are shown in lane 1-7.

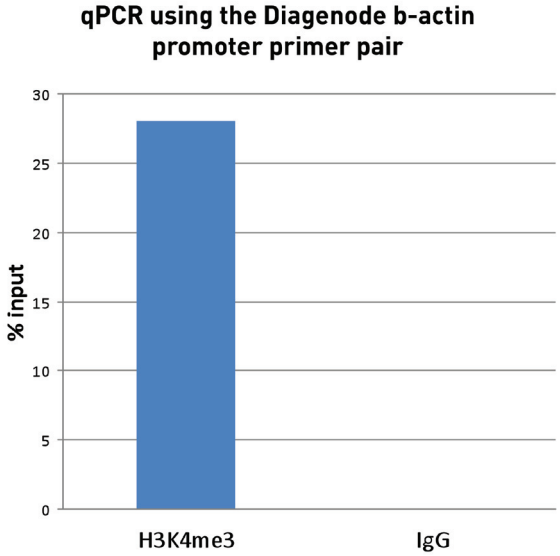

Figure 2

ChIP was perfomed on HeLa cells using an antibody against H3K4me3 (cat. No. pAb-003-050), known to be located at active promoters, and rabbit IgG used as a negative IP control. The ChIP'd samples were analysed by qPCR using the Diagenode ACTB promoter primer pair (cat. No. pp-1048-050, pp-1048-500). Figure 2 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

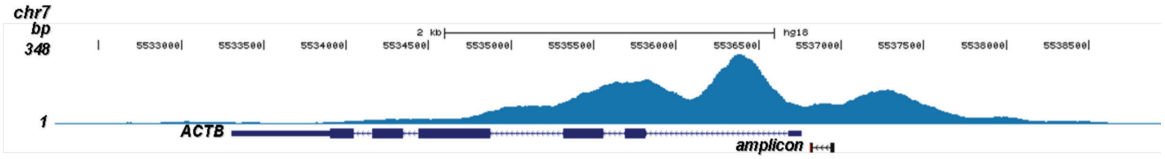

Figure 3

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 H3K4me3 profile in a region of chromosome 7 containing the ACTB gene. The position of amplicon obtained with the Diagenode ACTB promoter primer pair is indicated..