diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Human ChIP-seq grade c-fos promoter primer pair	
Cat. No: pp-1004-050	**Format:** 50 µl
Cat. No: pp-1004-500	**Format:** 500 µl

Product Description: This primer pair specifically amplifies a genomic region containing the human c-fos 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: 81 base pairs.

Amplified region: chr14:74815242-74815322

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

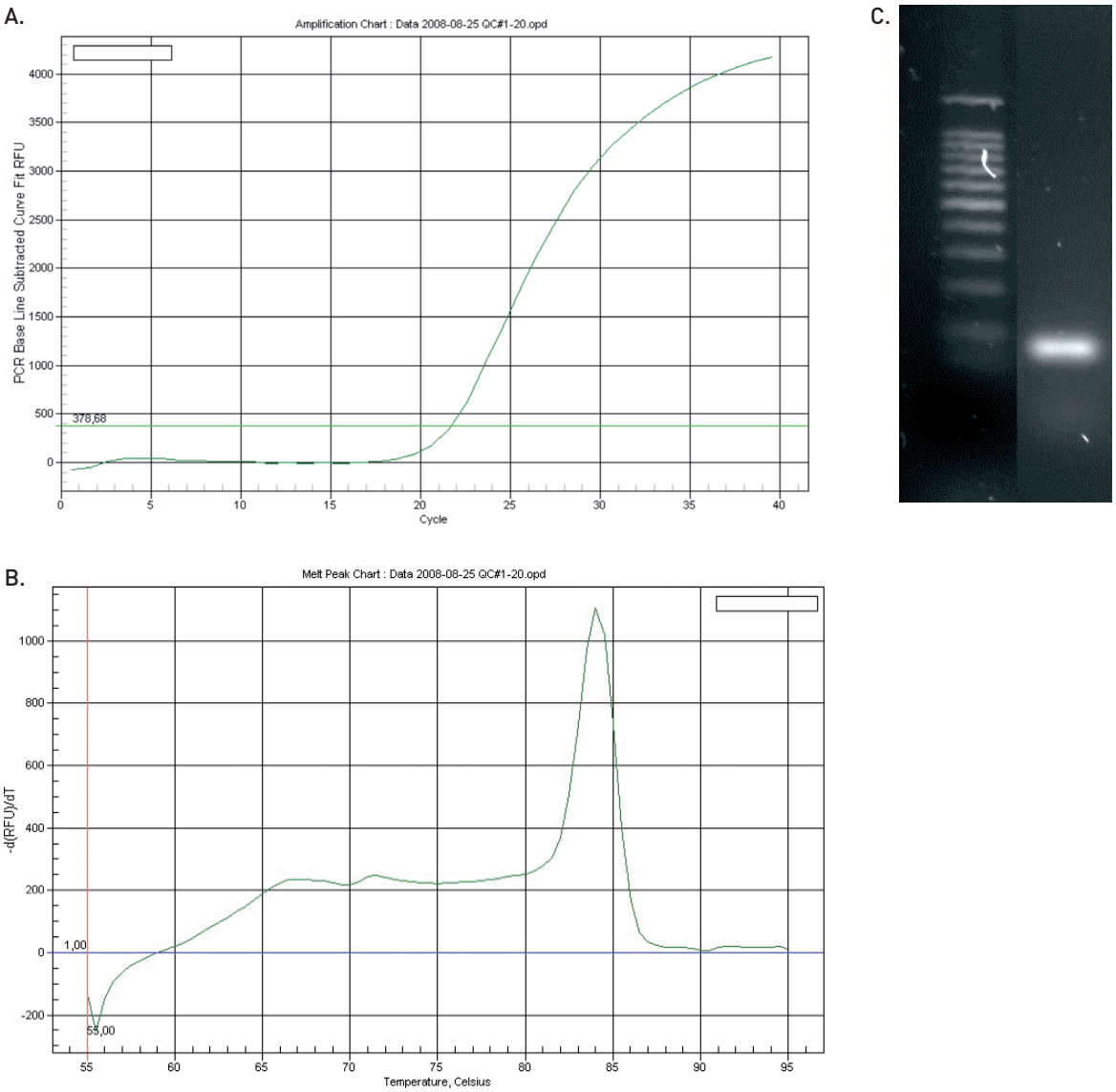

Figure 1

SYBR green real time PCR was performed on 50 ng of human genomic DNA from U2OS cells using the Diagenode c-fos promoter primer pair (cat. No. pp-1004-050, pp-1004-500). One µl of the provided primers was used in 25 µl final reaction volume. PCR conditions were as follows: incubation at 95°C for 3 min, followed by 40 cycles of 15 seconds at 95°C and 45 seconds at 60°C and a final amplification at 72°C for 2 min. Figure 1A and 1B show the amplification chart and the melting curve, respectively. Figure 1C shows the analysis of the amplification product by agarose gel electrophoresis (2% agarose gel stained with SYBR Safe). A 100 bp molecular weight marker is shown on the left, the PCR product (expected size 81 bp) is shown on the right.

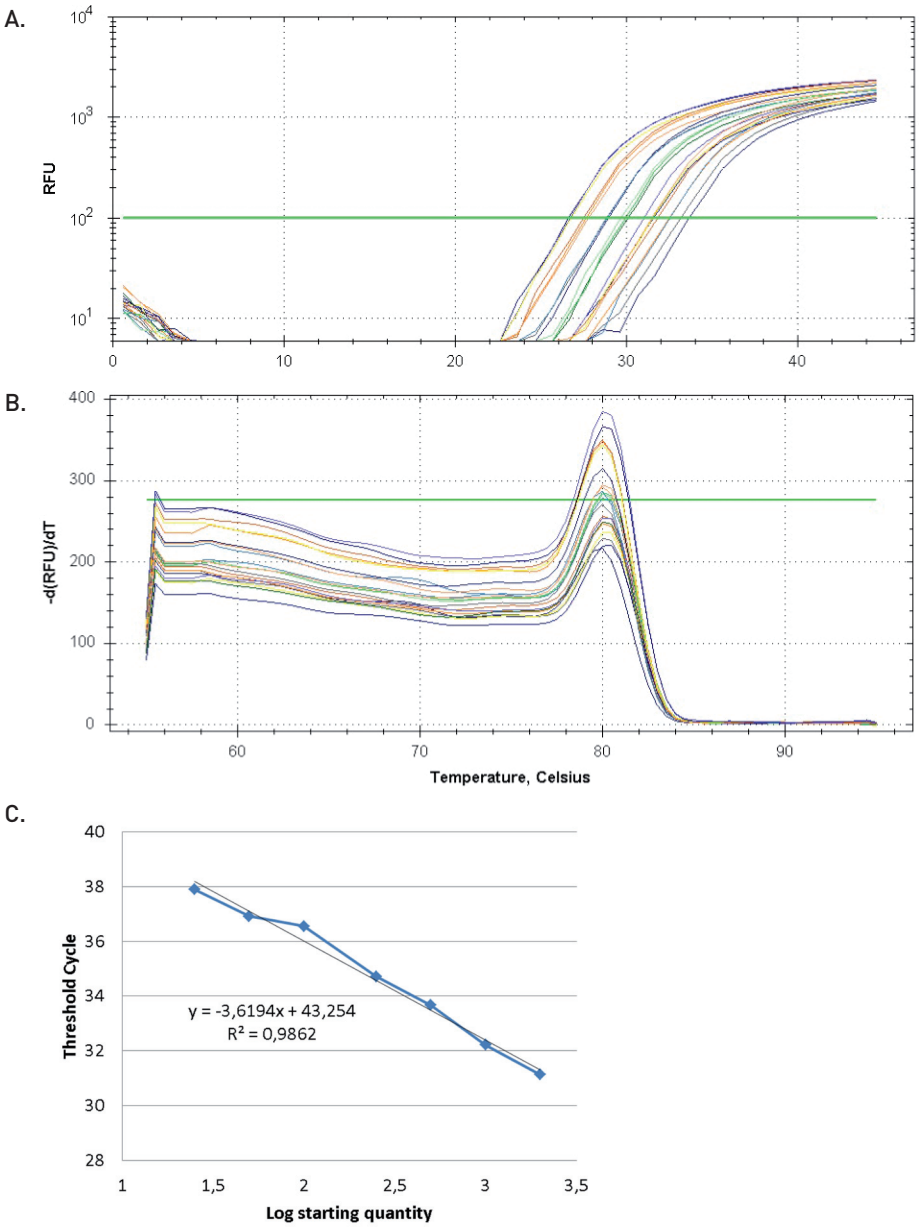

Figure 2

Sheared DNA from HeLa cells was analysed in triplicate by SYBR Green real-time PCR using the Diagenode c-fos promoter primer pair (cat. No. pp-1004-050, pp-1004-500). A dilution series starting 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 2A: amplification curves (logarithmic view). The green line represents the position of the threshold.
Figure 2B: melting curve analysis of the different amplification products.
Figure 2C: 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 89%.

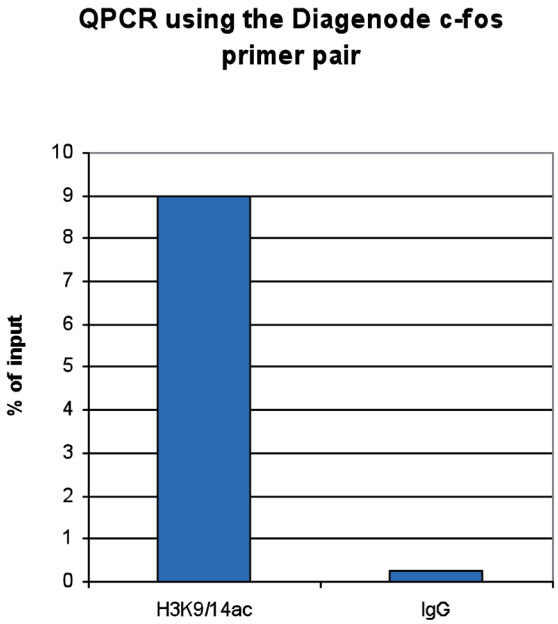

Figure 3

ChIP was perfomed on HeLa cells using an antibody against H3K9/14ac (cat. No. pAb-004-044), 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 c-fos promoter primer pair (cat. No. pp-1004-050, pp-1004-500). Figure 3 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

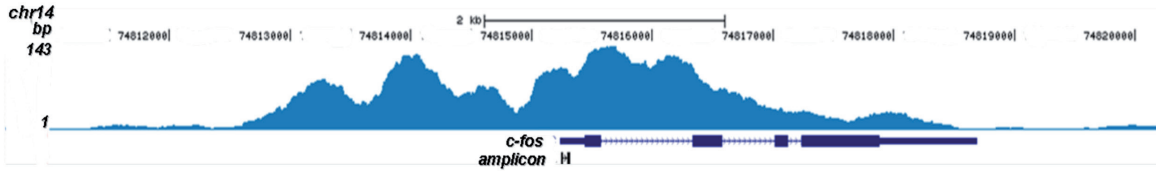

Figure 4

ChIP was performed as described above and the ChIP'd DNA was subsequently analysed by high throughput sequencing on an Illumina GAIIx. Figure 4 shows the H3K9/14ac profile in a region of chromosome 14 containing the c-fos gene. The position of amplicon obtained with the Diagenode c-fos promoter primer pair is indicated.