diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Mouse Cfc1 coding primer Pairs			
Official full name: Cripto, FLR-1, cryptic family 1 **Other name:** cryptic, AV265756 **Primary source:** MGI: 109448			
Cat. No: pp-1036-050	**Size:** 50 µl	**Concentration:** 10 µM	**Lot #:** 001
Cat. No: pp-1036-500	**Size:** 500 µl	**Concentration:** 10 µM	**Lot #:** 001

10 sets of our primer pairs: 50 µl (see our list)
500 µl

Description: The primer pair cat:# pp-1036 (-050, -500) is specific to a DNA coding region in the mouse Cfc1 gene [1]. These primers can be used to amplify DNA isolated by chromatin immunoprecipitation (ChIP). Primers are optimized to be used in quantitative polymerase chain reaction (qPCR) (**Figures 1, 2 and 3**). See overview below.

Expected PCR product size: 168 base pairs (bp).

Specificity: Mouse: positive
Other species: not tested

Format: In solution in MiliQ water at the concentration of 10 µM (each oligonucleotide primer is at the final concentration of 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.

References: [1] O'Neill L.P., VerMilyea M.D. and Turner B.M. (2006) Nat. Genet. 38(7):835-41.
[2] Salomon D.S.,Bianco C., Ebert A.D., Khan N.I., De Santis M., Normanno N., Wechselberger C., Seno M., Williams K., Sanicola M., Foley S., Gullick W.J. and Persico G. (2000) Endocr. Relat. Cancer 7(4): 199-226.

Availability date: September 03, 2007

Last data sheet update: September 14, 2007

Lot #: 001/ day of the synthesis: May 25, 2007/ day of QC: August 20, 2007/ aliquoting day: August 24, 2007

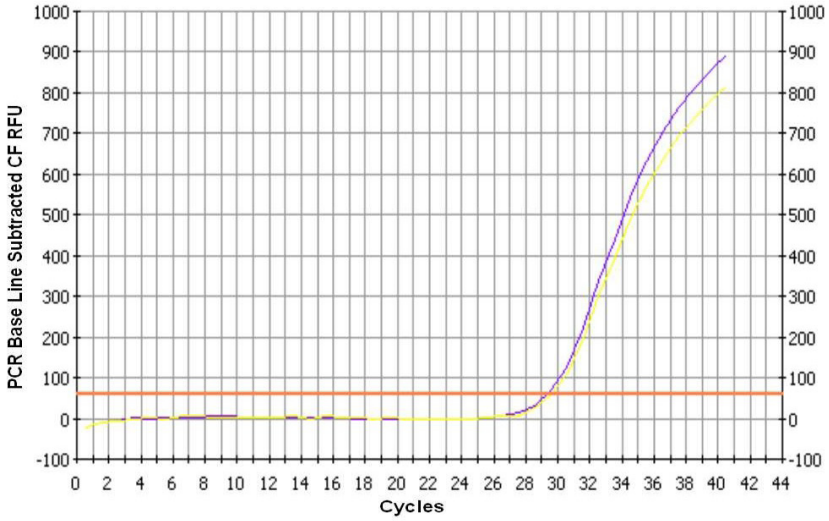

Figure 1

DNA from mouse fibroblast 3T3 cells was analyzed in duplicate by real-time PCR starting from 5 µl of DNA template (0.03 µg/ml) using the Diagenode primers to amplify a region in the mouse Cfc1 gene (cat#: pp-1036-050, -500). One µl of provided primer pairs is used by PCR of 25 µl final volume. A Real-Time PCR Detection System and iQ SYBR Green have been used. qPCR conditions used are as follows: 95°C for 3 minutes, 41 cycles of: [95°C for 60 seconds, 60°C for 60 seconds and 72°C for 90 seconds]. Duplicates are shown in yellow and purple. Threshold position is in orange.

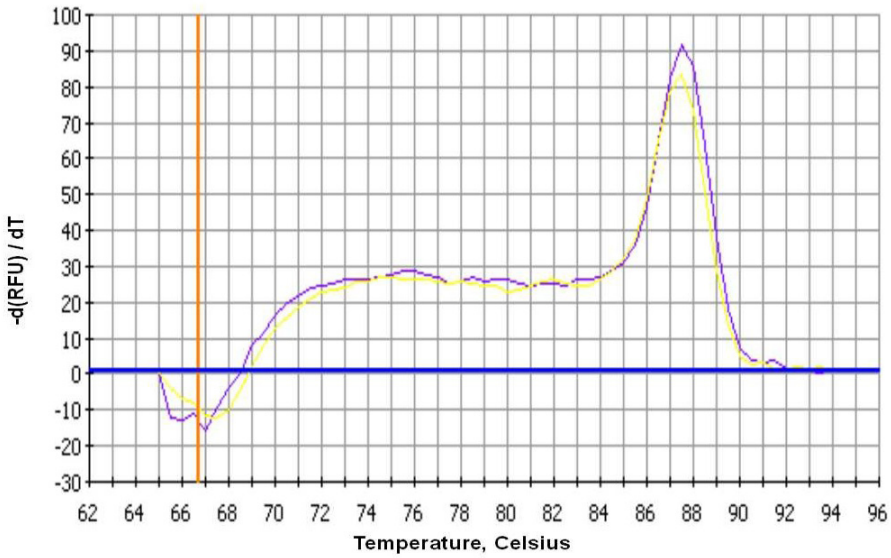

Figure 2

Melting curves obtained with the primers cat#: pp1036 (-050, -500) used in the above qPCR. Conditions were 60 cycles of 65°C for 1 minute and increment of 0.5°C per cycle. Duplicates are shown in yellow and purple.

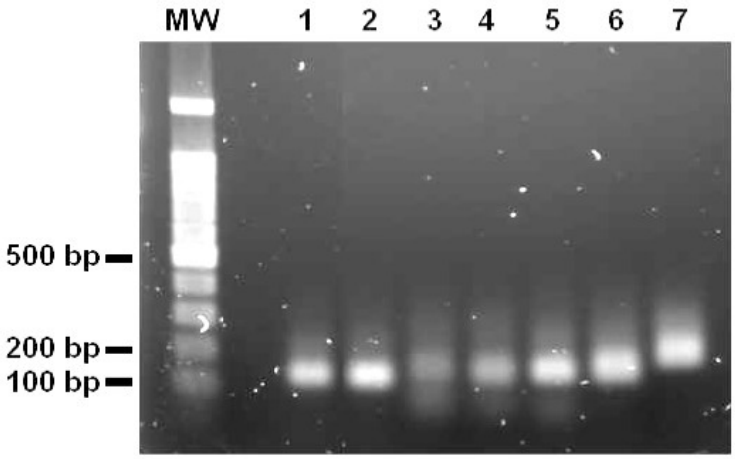

Figure 3
qPCR products were analysed by electrophoresis (1.5% agarose gel) stained with SYBR Safe and illuminated with UV light. The left lane shows molecular weight markers (MW) that decrease in size by 100 bp. Different qPCR products using different primer pairs which are available at Diagenode were tested: 1: primers for mouse Cdx2 gene promoter (pp-1025-050, -500), 2: primers for mouse Hhex gene promoter (pp-1027-050, -500), 3: primers for a coding region of the mouse Hhex gene (pp-1028-050, -500), 4: primers for a 3' region of the mouse Hhex gene (pp-1029-050, -500), 5: primers for mouse Nkx2-5 gene promoter (pp-1033-050, -500), 6: primers for mouse Cfc1 gene promoter (pp-1035-050, -500), 7: primers for a coding region of the mouse Cfc1 gene (pp-1036-050, -500). For more details about other primer pairs, see data sheet.

Overview: The EGF-CFC gene family encodes a group of structurally related proteins that serve as important competence factors during early embryogenesis in Xenopus, zebrafish, mice and humans. This multigene family consists of Xenopus FRL-1, zebrafish one-eyed-pinhead (oep), mouse cripto (Cr-1) and cryptic, and human cripto (CR-1) and criptin. FRL-1, oep and mouse cripto are essential for the formation of mesoderm and endoderm and for correct establishment of the anterior/posterior axis. In addition, oep and cryptic are important for the establishment of left-right (L/R) asymmetry. In the mouse, cryptic is not expressed in adult tissues whereas Cr-1 is expressed at a low level in several different tissues including the mammary gland [2].