diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Human NANOG promoter Primer Pairs			
Official full name: Nanog homeobox **Primary source:** HGNC: 20857			
Cat. No: pp-1012-050	**Size:** 50 µl	**Concentration:** 10 µM	**Lot #:** 001
Cat. No: pp-1012-500	**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-1012 (-050, -500) is specific to a DNA region in the human NANOG gene promoter as shown below (**Figure 1**) [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 2, 3 and 4**). See overview below.

Expected PCR product size: 95 base pairs (bp).

Specificity: Human: 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] Dahl J. A. and Collas P. (2007) Stem Cells 25 (4):1037-46.
[2] Pan G. and Thomson J.A. (2007) Cell Res. 17 (1): 42-9.

Availability date: July 16, 2007

Last data sheet update: August 03, 2007

Lot #: 001/ day of the synthesis: May 25, 2007/ day of QC: June 11, 2007/ aliquoting day: July 23, 2007

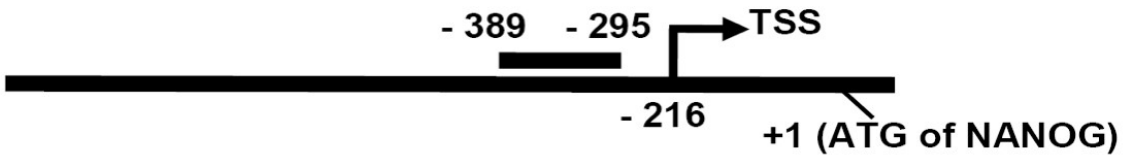

Figure 1
Region amplified in the promoter of the NANOG gene. Region amplified by polymerase chain reaction (PCR) using the primer pair cat#: pp-1012-050, -500 is indicated by a bar and the nucleotide number relative to the ATG translation initiation site is given. Position of the transcriptional start site (TSS) is indicated.

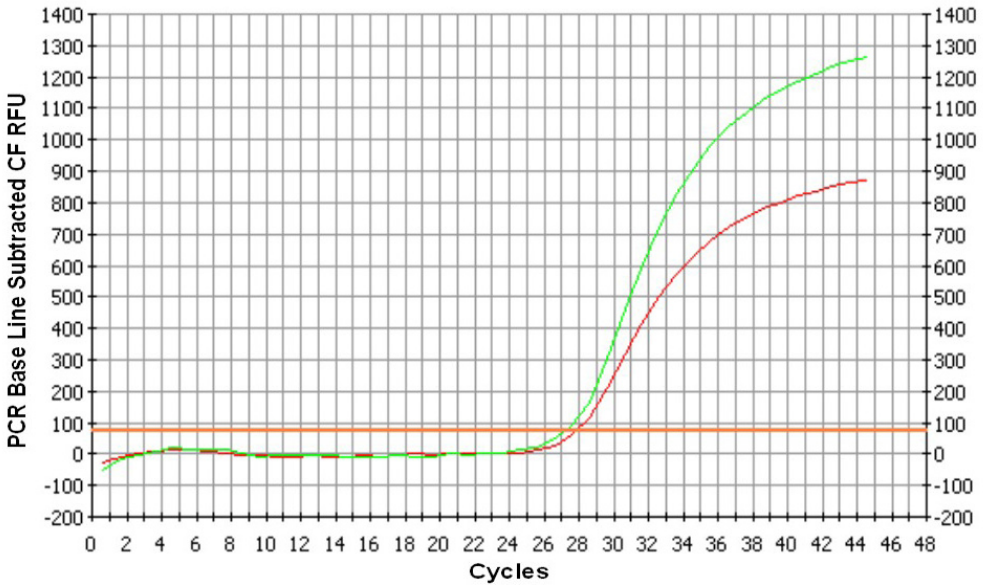

Figure 2.
DNA from undifferentiated human teratocarcinoma NCCIT cells was analyzed in duplicate by real-time PCR starting from 5 µl of DNA template (0.01 µg/ml) using the Diagenode primers to amplify a region of the NANOG gene promoter (cat#: pp-1012-050, -500). One µl of provided primer pairs is used 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, 40 cycles of: [95°C for 30 seconds, 60°C for 30 seconds] and 1 cycle of 72°C for 30 seconds. Duplicates are shown in red and green. Threshold position is in orange.

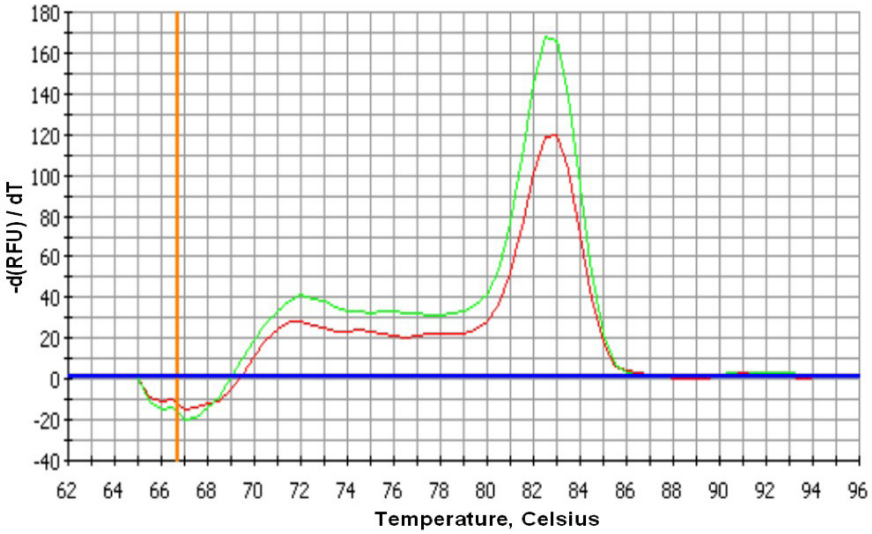

Figure 3
Melting curves obtained with primers cat#: pp-1012 (-050, -500) used in the above qPCR. Conditions were 60 cycles of 65°C for 1 minute and increment of 0.5°C per cycle. Duplicates are shown in red and green.

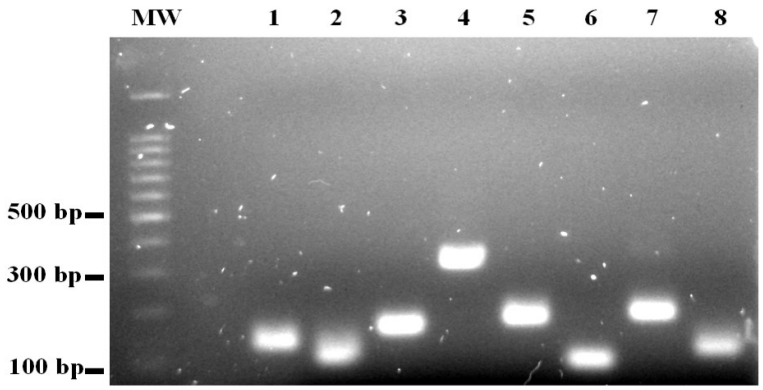

Figure 4.
qPCR products were analysed by electrophoresis (1.5% agarose gel) stained with SYBR Safe and illuminated with UV light. The left lane shows molecular weight markers (MW) that decrease in size by 100 bp. Different qPCR products using different primer pairs which are available at Diagenode were tested: 1: primers for human LMNA gene promoter (cat#: pp-1011-050, -500), 2: primers for human NANOG gene promoter (cat#: pp-1012-050, -500), 3: primers for human OCT4/A (-2,602/-2,435) (cat#: pp-1013-050, -500), 4: primers for OCT4/B (-2,340/-2,042) (cat#: pp-1014-050, -500), 5: primers for OCT4/C (-1,725/-1,564) (cat#: pp-1015-050, -500), 6: primers for OCT4/D (-1,491/-1,409) (cat#: pp-1016-050, -500), 7: primers for OCT4/E (-318/-150) (cat#: pp-1017-050, -500), 8: primers for human PAX 6 gene promoter (cat#: pp-1018-050, -500). For more details about other primer pairs, see data sheet.

Overview: Nanog homeobox is a transcription regulator involved in inner cell mass (ICM) and embryonic stem (ES) cells proliferation and self-renewal. Nanog imposes pluripotency on ES cells and prevents their differentiation towards extraembryonic endoderm and trophectoderm lineages [2].