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Product Information

U2OS GFP-HMGA1
Osteosarcoma Cell Line with GFP-tagged HMGA1

Catalog Number **CLL1036**
Storage Temperature –196 °C (liquid nitrogen)

Product Description

CompoZr® zinc finger nuclease (ZFN) technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break (www.compozrzfn.com). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The homologous recombination pathway was used to insert a transgene into a desired target location – the HMGA1 locus (NM_002131). High mobility group A1 (HMGA1, formerly known as HMG-I/Y) is a non-histone protein that preferentially binds to the minor groove of A+T-rich regions in double-stranded DNA. HMGA1 binding sites often are adjacent to or overlap with consensus binding sites for conventional transcription factors (TFs) and are thought to modulate gene expression through DNA conformation and DNA-binding recruitment.[1] A donor construct containing a fluorescent reporter gene (GFP) flanked by sequences homologous to the target site was nucleofected into U2OS cells along with ZFNs designed to cut near the genomic target site (see Figures 1a and 1b). Integration resulted in endogenous expression of a fluorescent fusion protein, GFP-High mobility group A1, with characteristic patterns of chromatin. Single cell knockin clones were isolated and followed for more than twenty passages to establish stable cell lines expressing GFP-HMGA1 from the endogenous genomic locus.

ZFN mediated gene tagging in knockin cell lines provides the basis for the development of various assays for compound screening. Here, the target gene regulation and corresponding protein function are preserved in contrast to cell lines with overexpression under an exogenous promoter.

For further information and to download sequence of modified locus, go to the website: www.wherebiobegins.com/biocells

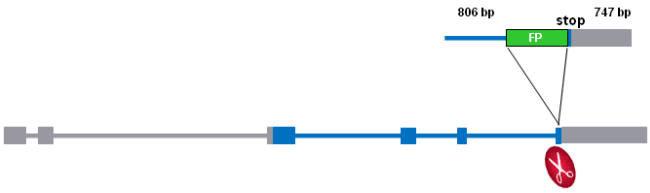
GFP and TagGFP2 are all synonymous for the fluorescent reporter gene in this document. The GFP used in this cell line originated from Evrogen, referred to as TagGFP2: http://evrogen.com/products/TagFPs.shtml

Figures 1a–c
The Design of Tag Sequence Integration at the HMGA1 Locus

1a.

Schematic of the genomic sequence at the target region for integration of the fluorescent tag GFP DNA showing ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

1b.



Schematics of HMGA1 locus showing the coding region (blue), untranslated region (gray), and the ZFN cut site (scissors). The Donor (top) has the homology arms of indicated length and the FP sequence (green) fused to the end of HMGA1 (formerly know as HMG-I/Y) coding sequence, creating a C-terminal fusion.

1c.

Schematic of HMGA1-GFP linker region. Unshaded bases represent the 3′ end of HMGA1 coding sequence for isoforms a and b. Bases added for the creation of a linker are shaded gray. Green shaded bases represent the GFP sequence. The amino acids encoded by the codons are shown in blue above the DNA sequence.

Cell Line Description
1 vial of modified U2OS cells contains ~2×10^6 cells.

Organism*: Homo sapiens* (human)
Tissue: osteosarcoma; bone

Age: 15 years
Gender: Female
Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X
CSF1PO: 13
D13S317: 13
D16S539: 11,12
D5S818: 11
D7S820: 11,12
TH01: 6,9.3
TPOX: 11,12
vWA: 14,18

Parental Cell Line: ATCC Catalog No. HTB-96™
Note: Please see HTB-96 product datasheet from ATCC for additional information about the origin of these cell lines. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Medium: Fetal bovine serum, Catalog No. F2442, at a final concentration of 10% (v/v) in McCoy's 5A Medium Modified, Catalog No. M9309. This medium is formulated for use with a 5% CO_2 in air atmosphere.

The cryoprotectant medium used is 1× Cell Freezing Medium-DMSO, Catalog No. C6164.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[2-5]

Preparation Instructions
Complete Medium: To make the complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% (v/v) in the base medium, McCoy's 5A Medium Modified, Catalog No. M9309. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Storage/Stability
Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70 °C. Storage at –70 °C will result in loss of viability.

Precaution: It is recommended that protective gloves and clothing always be used, and a full face mask always be worn when handling frozen vials. It is **important to note that some vials leak when submersed in liquid nitrogen** and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Procedure

Thawing of Frozen Cells

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (~2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml of Complete Medium and spin at ~125 × g for 5–7 minutes.
4. Resuspend cell pellet with the Complete Medium and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0–7.6) and temperature (37 °C).
5. Incubate the culture at 37 °C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended for the Complete Medium.

Subculturing Procedure

Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 ml of Trypsin-EDTA solution to flask and incubate at 37 °C for 7 minutes to detach the cells.
4. Add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels.
   Subcultivation Ratio: 1:3 to 1:6
6. Incubate cultures at 37 °C.

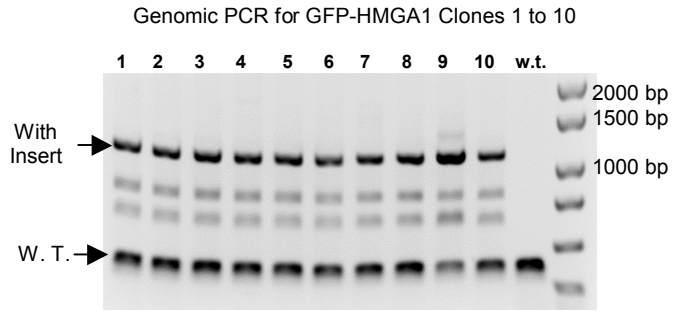
Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[6]

Results

Figures 2a–d

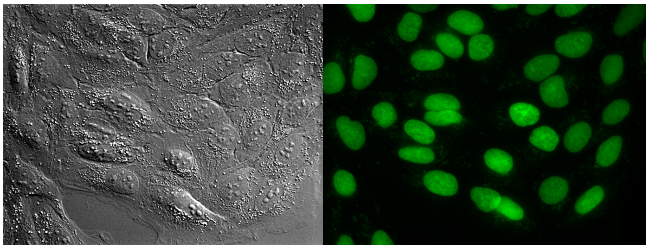
GFP- HMGA1 Single Cell Clone Verification

2a.



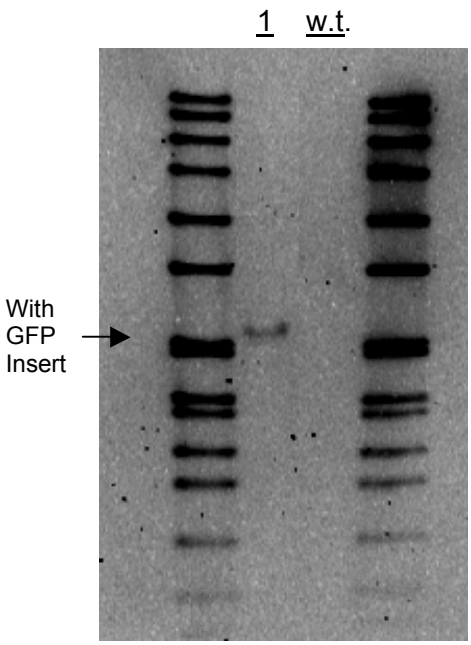
Gel electrophoresis analysis of a genomic PCR of 10 single cell clones using the forward and reverse primers specific to the HMGA1 genomic sequence. Molecular size marker and the parental line (wt) control are also shown. Clones with GFP insertion yielded a 1179 bp band.

2b.



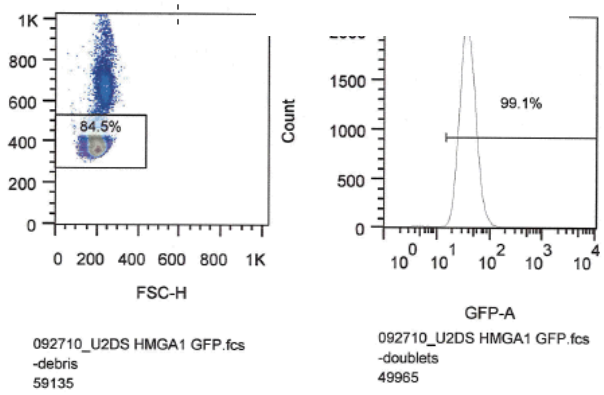
Differential interference contrast (DIC) and fluorescence microscopy images of isolated cell clone 1 expressing endogenous High mobility group A1 protein tagged with GFP (ex 450–490/em 500–550, 40×/1.4 oil)

2c.



Southern blotting for GFP-HMGA1 single cell clone 1 with DIG-labeled GFP probe. The *Kpn* I digestion yielded a 1945 bp band with GFP insertion while the W.T. was negative.

2d.



Flow cytometry analysis of GFP-HMGA1 single cell clone 1 at the end of viability test. 99.1% of the cells are GFP positive.

References

1. Reeves, R., Nuclear functions of the HMG proteins. Biochimica et Biophysica Acta, 1799, 3-14 (2010).
2. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
3. Hay, R.J. et al., eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC.
4. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1988).
5. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 4th Edition U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
6. Freshney, R.I., Chapter 10 in Culture of Animal Cells, a manual of Basic Technique by, 3rd edition, published by Alan R. Liss, (NY, NY: 1994).

Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website (www.wherebiobegins.com/biocells).

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