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Product Information

U2OS BFP-LMNB1 RFP-ACTB
Osteosarcoma Cell Line with BFP-tagged LMNB1 and RFP-tagged ACTB

Catalog Number **CLL1038**
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 transgenes into two desired target locations – the ACTB locus (NM_001101, β-actin) and the LMNB1 locus (NM_005573, Lamin B1). Donor constructs containing fluorescent reporter genes (RFP for ACTB and BFP for LMNB1) flanked by sequences homologous to the target site were nucleofected sequentially into U2OS cells along with ZFNs designed to cut near the genomic target site (see Figures 1a, 1b, 2a, and 2b). Integration resulted in endogenous expression of both fluorescent fusion proteins, RFP–β-Actin and BFP–Lamin B1, that polymerize to form characteristic patterns of actin fibers and are easily seen in the nuclear envelope, respectively. Single cell knockin clones were isolated and followed for more than twenty passages to establish stable cell lines expressing RFP-ACTB BFP-LMNB1 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.

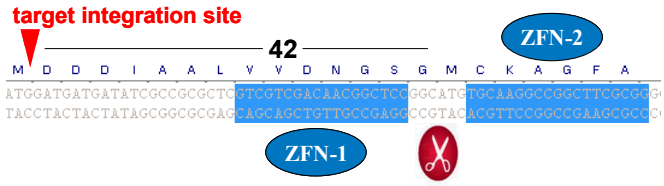
For further information and to download sequence of modified locus, go to the website: www.wherebiobegins.com/biocells

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

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

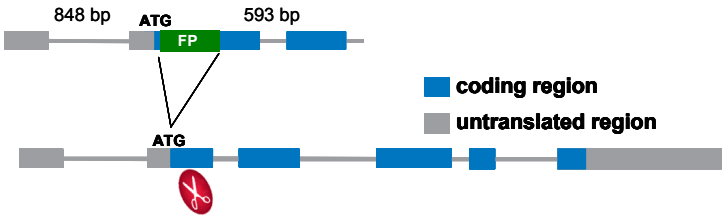
Figures 1a and 1b.
The Design of Tag Sequence Integration at the ACTB Locus

1a.



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

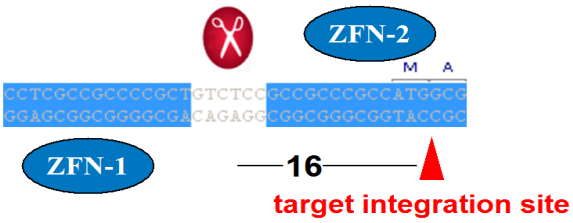
1b.



Schematics of ACTB 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 beginning of ACTB coding sequence (the N-terminal fusion).

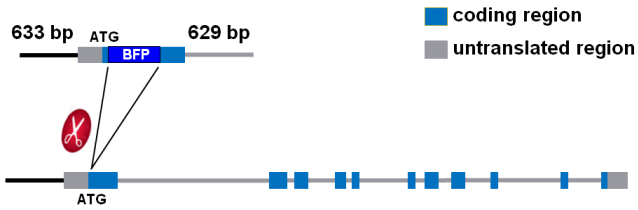
Figures 2a and 2b.
The Design of Tag Sequence Integration at the LMNB1 Locus

2a.



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

2b.



Schematics of LMNB1 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 (dark blue) fused to the beginning of LMNB1 coding sequence (the N-terminal fusion).

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.[1-4]

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.

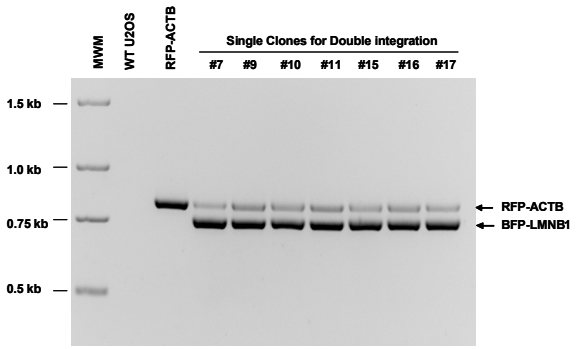
Subculturing Procedure
Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 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.[5]

Results
Figures 3a–3e
RFP-ACTB BFP-LMNB1 Single Cell Clone Verification

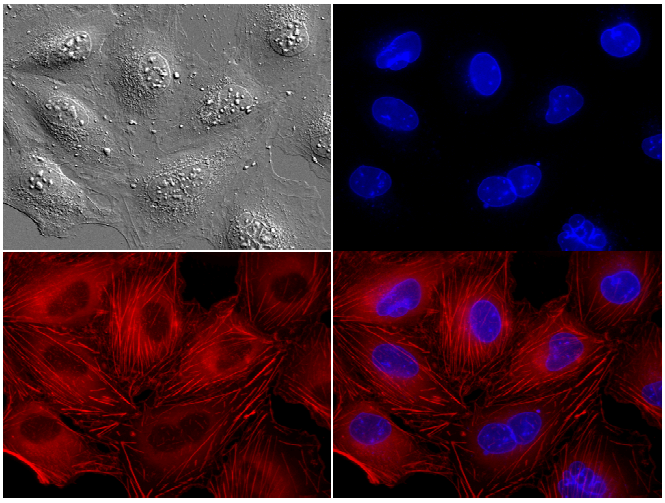
3a.

Junction PCR to Detect Double Integration of RFP-ACTB and BFP-LMNB1 In Selected Single Clones



Gel electrophoresis analysis of a junction multiplex PCR of 7 single cell clones using the forward primer specific to the targeted RFP/BFP sequence and the reverse primer specific to the ACTB and LMNB1 genomic sequences, respectively. Molecular size markers and the parental line (U2OS) control are also shown.

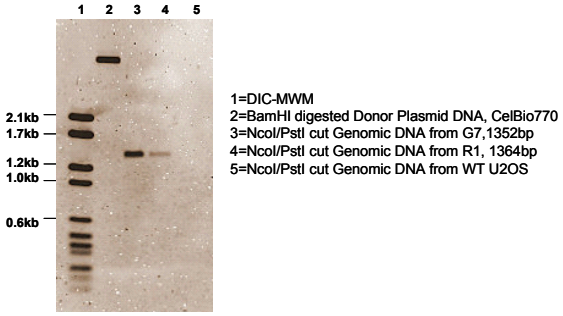
3b.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone simultaneously expressing endogenous ACTB and LaminB1 protein tagged with RFP and BFP, respectively. (ex 450–490/em 500–550, 40×/1.4 oil)

3c.

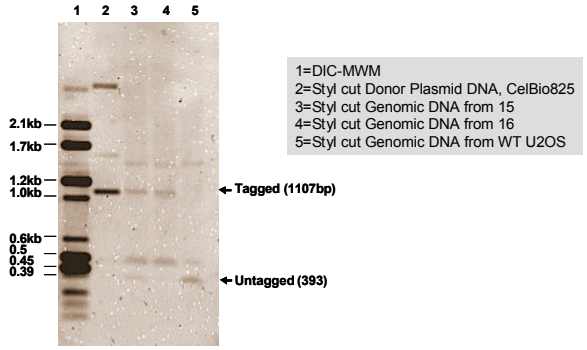
Southern Analysis of GFP and RFP Tagged ACTB in U2OS Single Clone #G7 and R1



Southern blotting for ACTB final single cell clones with DIG-labeled RFP probe showing 1364 bp band. Positive (donor plasmid) and negative (parental line) controls are shown as well.

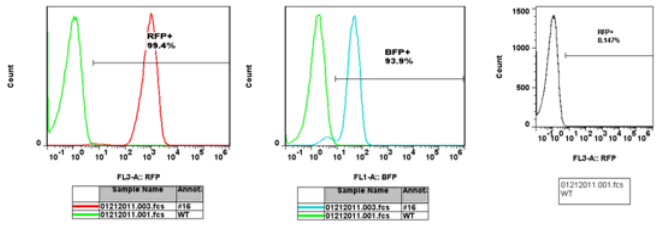
3d.

Southern Analysis of BFP Tagged LmnB in r-ACTB U2OS Clone 15 and 16 by DIG-LmnB Probe



Southern blotting for LMNB1 final single cell clones with DIG-labeled LMBNB1 probe showing 1107 bp band. Positive (donor plasmid) and negative (parental line) controls are shown as well.

3e.



Flow cytometry analysis of RFP-ACTB BFP-LMNB1 single cell clone 16 at the end of viability test. 99% of the cells are RFP positive and 94% of the cells are BFP positive.

References

1. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
2. Hay, R.J. et al., eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC.
3. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1988).
4. 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
5. 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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