Datasheet for HCT116/EGFR ΔE746-A750 cancer marker mutation cell lines (Heterozygote)

Catalog number: SL702

Product: HCT116/EGFR ΔE746-A750 cancer marker mutation cell lines(Heterozygote) with spike-in signature at 734E and 735G locus

Description: This product is a sgRNA and donor vectors (puromycin selection) for the EGFR ΔE746-A750 genome editing cell line. This cell line can be used *in vitro* as a reference for gene mutation detection or other related applications.

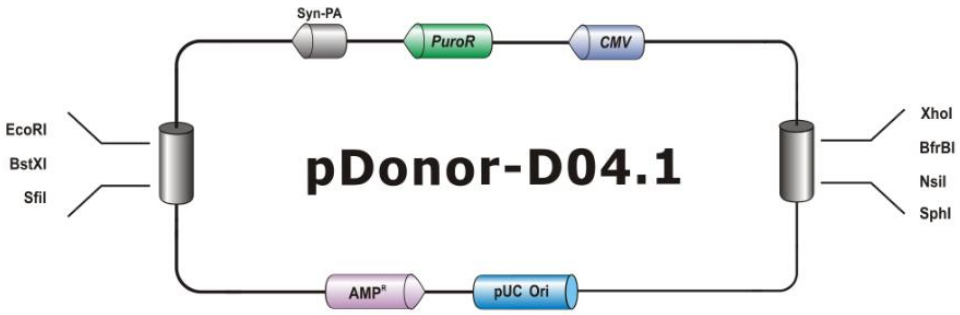
Spike-in*: with spike-in signature at 734E and 735G locus

Quantity: 1 vial of 2 x 10^6 cells; frozen

Shipping conditions: Dry ice

Storage conditions: Liquid nitrogen vapor phase. Remove the item from the dry ice packaging and check all items for damage and leakage. Place immediately into storage at or below -140 ^{0}C, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



Source of parental line: HCT116
Organism: *Homo sapiens*, human
Tissue: colon
Cell Type: epithelial

*** IP protected by 2019111467778 which was owned by GeneCopoeia, Inc.**

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins.

Safety instructions: To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur into the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in the vial exploding or losing its cap. This may cause flying debris.

Thawing procedure: The vial of cells should be thawed in a 37℃water bath with gentle agitation. For optimal performance, the vial should be thawed in under two minutes. Ensure that the cap of the vial did not loosen upon thawing, and re-tighten as needed. Spray the vial with 70% EtOH and wipe off. Repeat. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium (without selection). Centrifuge for 5 min. at 250 x g. Aspirate the medium, being careful not to disturb the pellet. Resuspend in 10 mL of complete growth medium, and place into a culture vessel of your choice. Only add selection to the medium after 24 hours in culture.

Culture conditions:

Complete Growth Medium

The base medium for this cell line is RPMI1640. For optimal growth and maintenance of selection, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Selection

puromycin to a final concentration of 0.6 μg/mL

Culture temperature:

37 ℃ with 5% CO_2

Subculture:

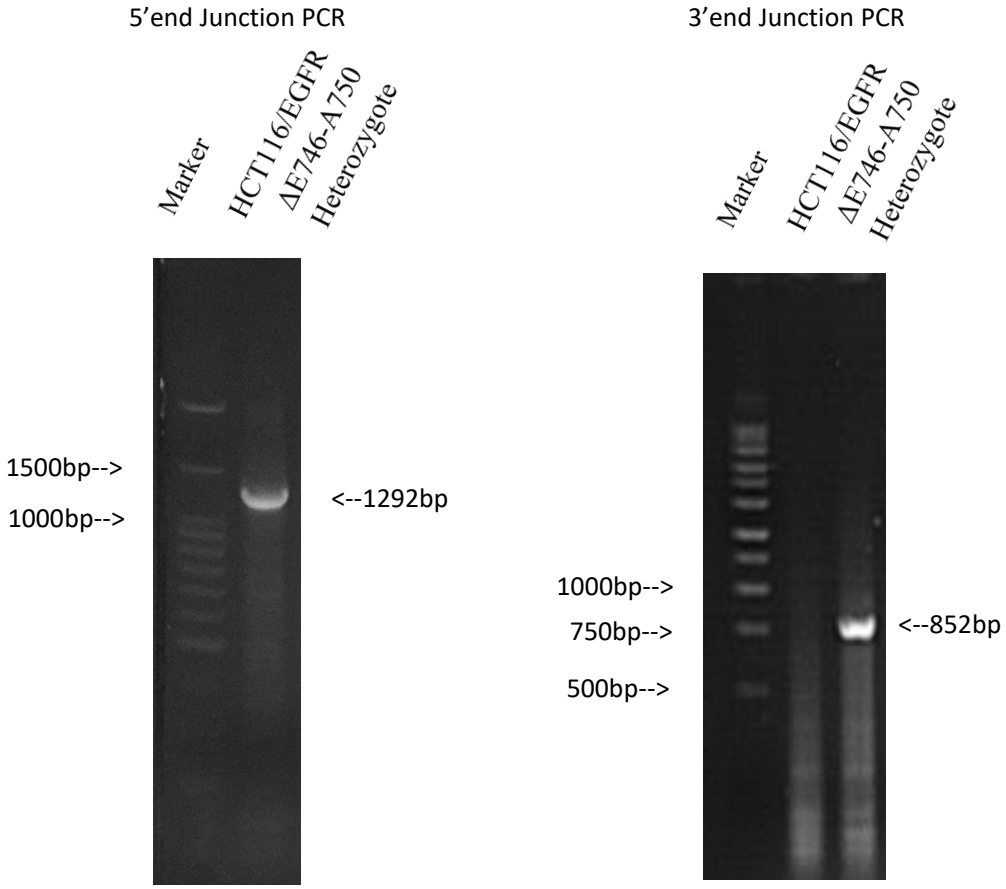
Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells and split at 1 x 10^5 viable cells/mL to 1 x 10^6 cells/mL.

Cryopreservation: Freeze slowly in complete growth medium supplemented with 5% (v/v) DMSO.

Mycoplasma: Negative
(MycoAllert Mycoplasma Detection Kit from Lonza)

Product QC:

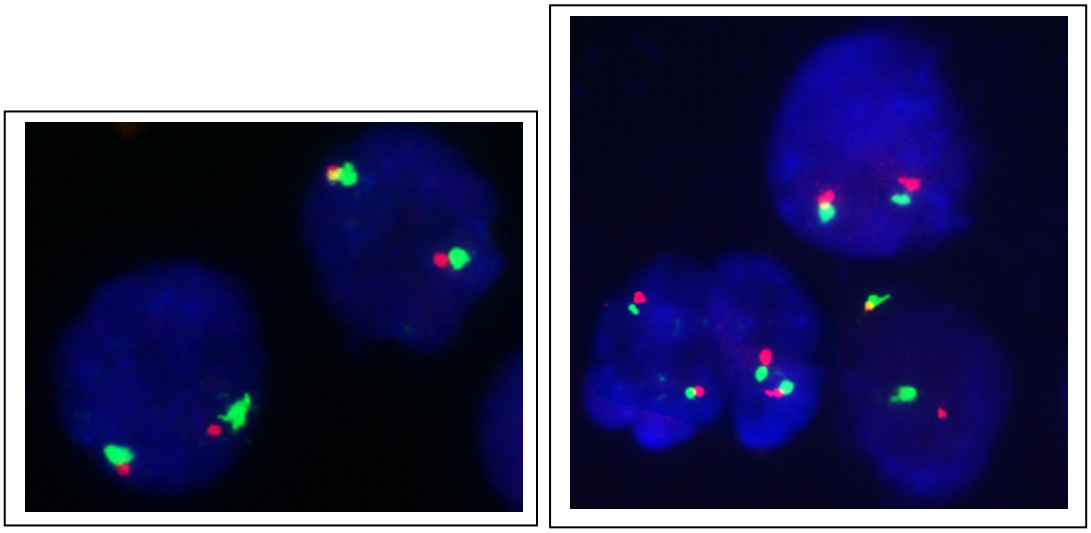
(a) Junction PCR result:



(b)Junction PCR positive statistics:

5'end Junction PCR positive amount (rate)	3'end Junction PCR positive amount (rate)	5'end and 3'end Junciton PCR double positive amount (rate)	Total sample amount
63(39.3%)	42(26.2%)	35(21.8%)	160

(c) FISH of EGFR probes on HCT cells :



HCT-116 WT

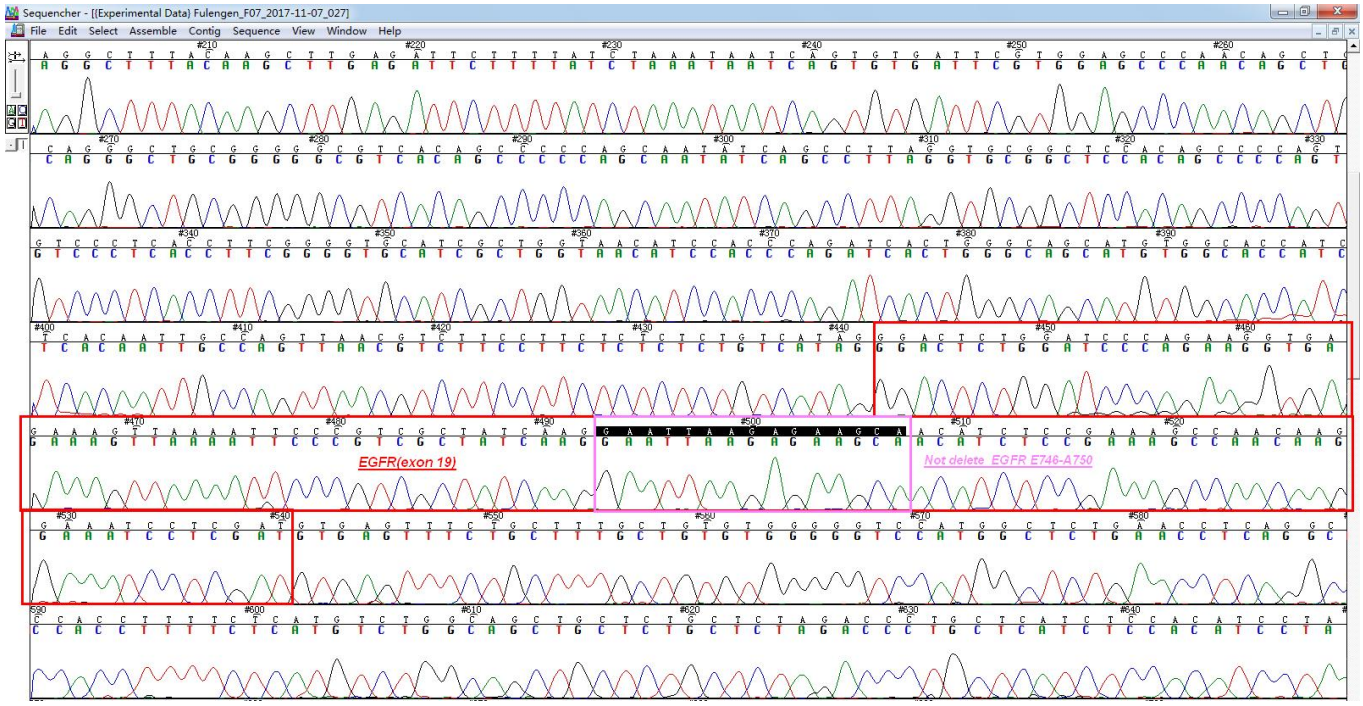
HCT-116: EGFR-Del746-750

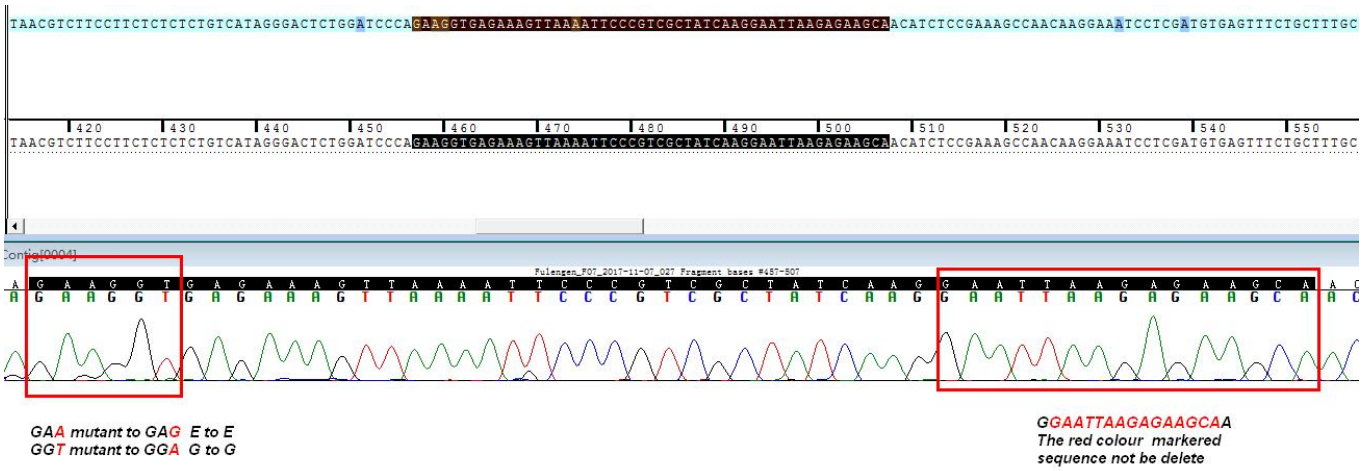
Fig.1 : **FISH of Human HCT116 cells** : GCI VividFISH™ FISH probes **CEP7/EGFR** were hybridized on **HCT-116 wild type(WT)** and **HCT-116 EGFR-Del746-750** mutated cells . The two green signals are from Chromosome 7 centromeres, while the two orange signals are representing the EGFR gene on chromosome 7. Both of them have two alleles of EGFR. The cell nuclear are counter stained with DAPI which is shown in blue.

(d)DNA Sequencing peak figure:

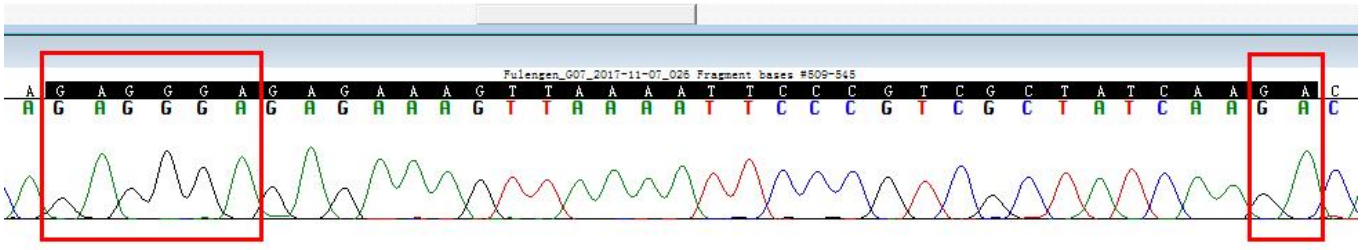
EGFR ΔE746-A750 fragment sequencing peak figure:

Subcloned sequence(show not delelte)：





Subcloned sequence(show delelte)：



GAA mutant to GAG E to E
GGT mutant to GGA G to G

GGAATTAAGAGAAGCAA
The red colour markered sequence should be delete

Citation of product: If use of this item results in a publication, please use this information: HCT116/EGFR ΔE746-A750 cancer marker mutation cell lines (SL702, GeneCopoeia, Inc., Rockville, MD).

Limited Use License

A limited use license is granted to the Buyer of the Product. The Product shall be used by the Buyer for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

C9SCL-DS-072224

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850 USA
Tel: 301-762-0888; Fax: 301-762-3888
Email: support@genecopoeia.com
Web: www.genecopoeia.com