GENE EDITING

BIO14199 : CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

NOVEMBER 2017

InsermTransfert

BIO14199

CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

Product

- **Product:**
  - CRISPR-barcoding, a fast and highly flexible strategy which enables detection of cells containing the mutation of interest within a mass population of unmodified cells using real-time quantitative PCR or deep sequencing.
- **Application:**
  - alternative tool to the classical lentiviral DNA barcode libraries, ensuring the detection of thousands of distinct barcodes through qPCR or deep-sequencing.
  - tracing of the mutated cells immediately after DNA editing without the need to derive clones, thus providing a unique means to investigate the effects of different kinds of genomic modifications, regardless of their potential impact on cell growth, in a broad range of functional assays.
  - high-resolution tracking of single specific cancer cells allowing to identify even rare pre-existing resistant subclones potentially involved in mechanisms of acquired resistance to therapy.
- **Proof of concept:**
  - The *in vitro* results were confirmed *in vivo*, using a CRISPR-barcoding xenograft model for NSCLC. The main proofs of concept reported are related to cancer models, nevertheless this technology can be implemented in different fields of biological research.
- **Patent and publication:**
  - WO2017068120 (A1): Endonuclease-Barcoding

BIO14199

CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

Principle

- CRISPR-Barcoding principle

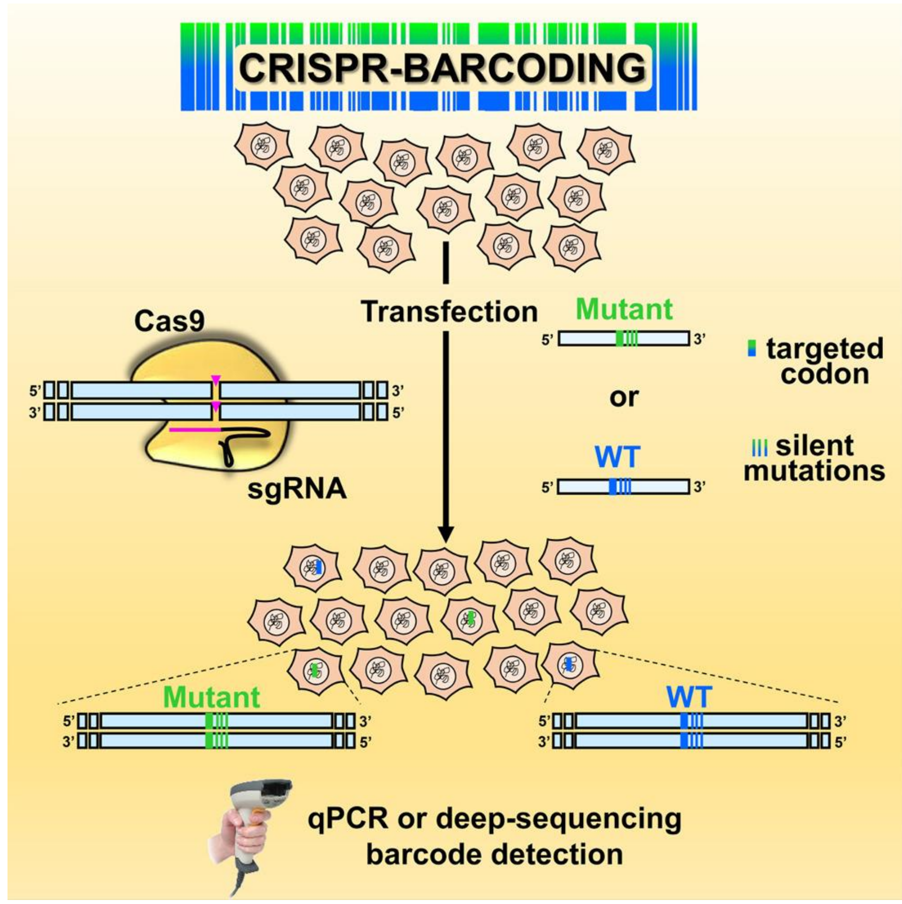

Figure 1: Schematic of the CRISPR-barcoding strategy

BIO14199

CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

Example

- CRISPR-Barcoding to Recapitulate NSCLC Resistance to EGFR Inhibitors

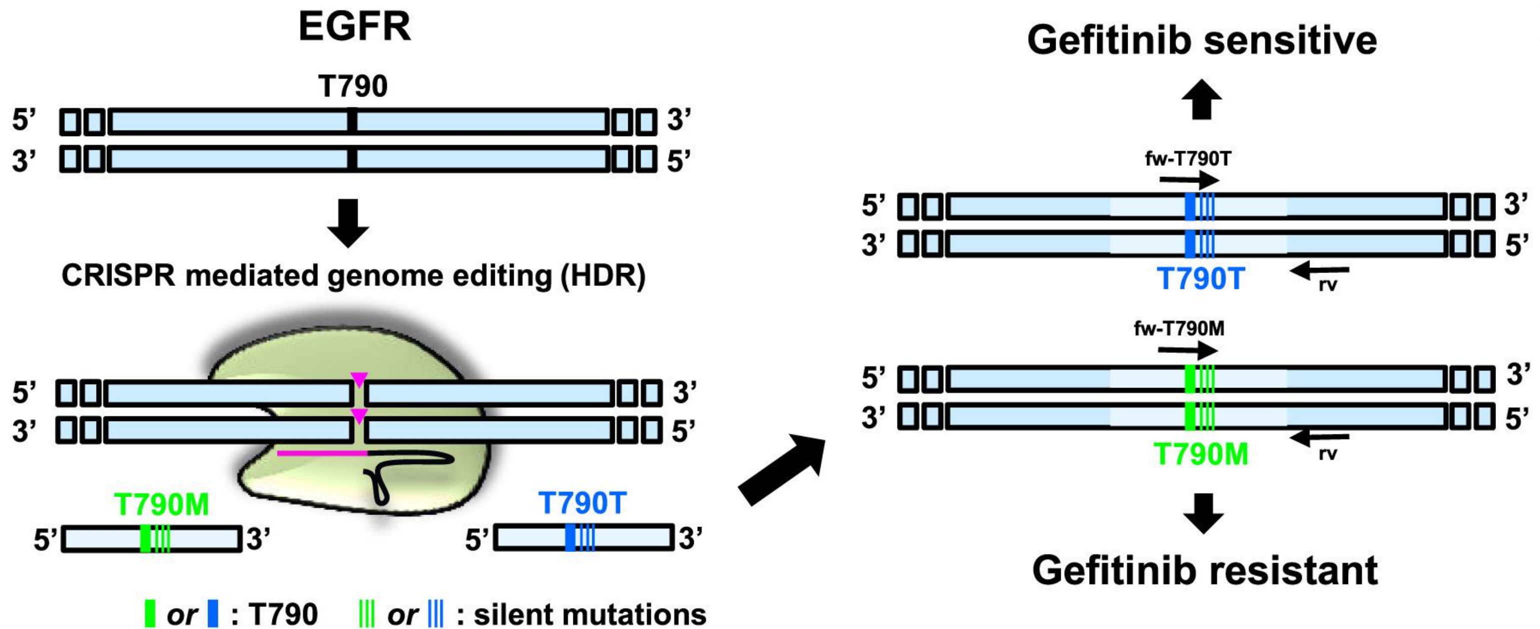

Figure 2: Schematic representation of the incorporation of EGFR-T790M and EGFR-T790T (control) barcodes in NSCLC cells.

BIO14199

CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

Example

- CRISPR-barcoding to functionally characterize oncogenic mutations in a context of intratumor heterogeneity.

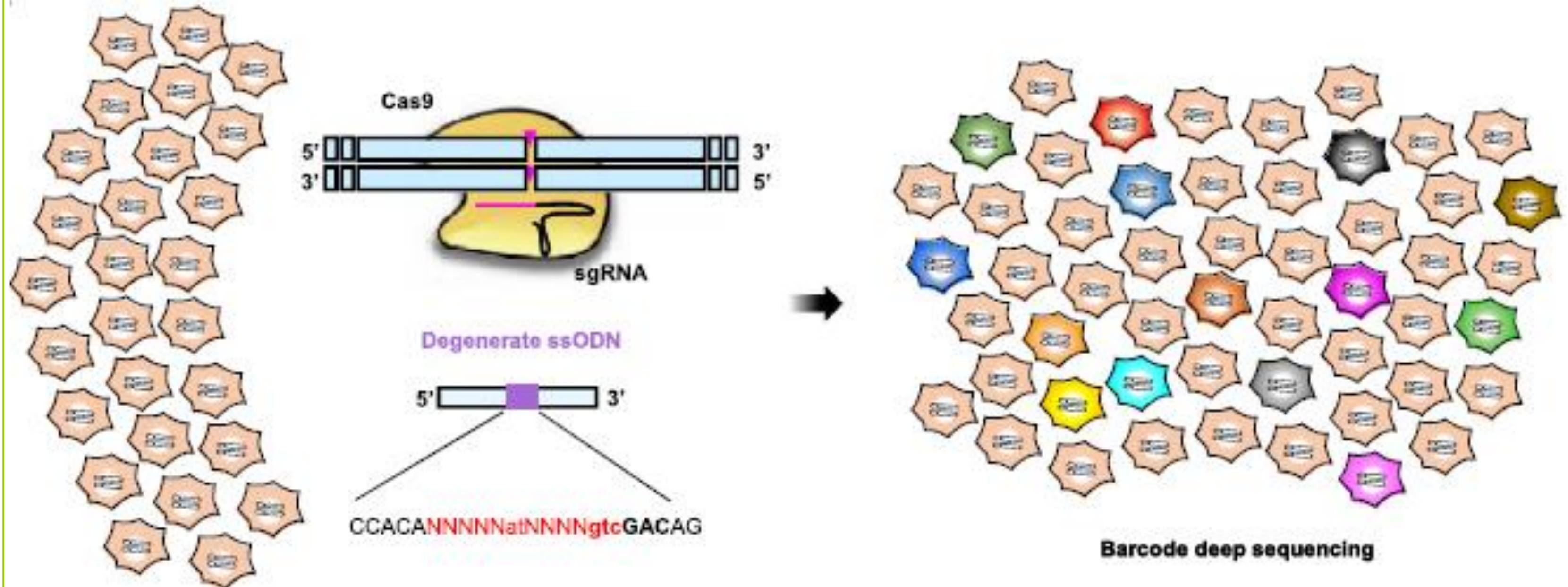

Figure 3: Through insertion of a highly complex series of degenerate sequences at a specific genomic location, CRISPR-barcoding can be used to trace several thousands of genetically labelled clones within a mass population of tumor cells.

BIO14199

CRISPR barcoding: method and kit for labeling and detecting a population of endonuclease-treated cells

Example

Inactivation of the Tumor Suppressor TP53 through CRISPR-Barcoding

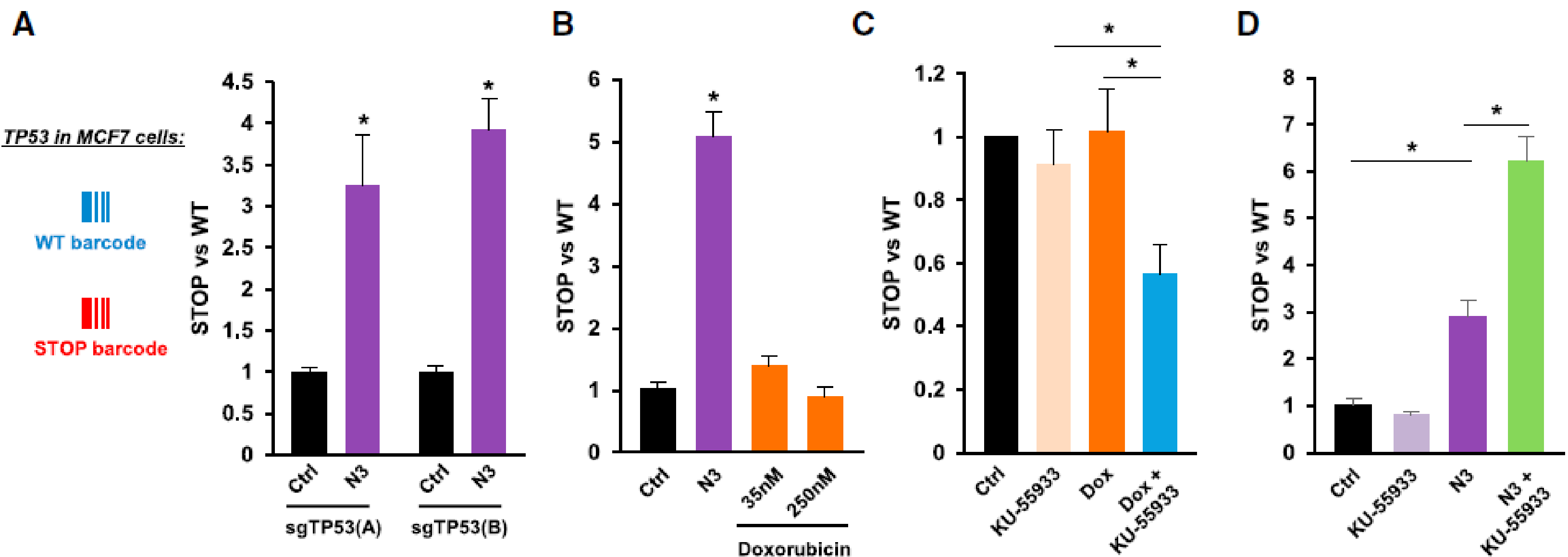

Figure 4:
(A) Effects of Nutlin-3 (N3; 10 mM, 7 days) on the TP53-STOP to TP53-WT ratio in MCF7 cells using two distinct sgRNAs (A or B). Mean ± SEM; n = 4 of one representative of three independent experiments. *p < 0.05 (Mann-Whitney test).
(B) Effects of N3 (10 mM; 7 days) or doxorubicin (Dox) on the TP53-STOP to TP53-WT ratio in HCT-116 cells. Mean ± SEM; n = 4 of one representative of three independent experiments.
(C) The cells in (B) were treated for 7 days with KU-55933 (10 mM) and/or Dox (50 nM), and the TP53-STOP to TP53-WT ratio was assessed by qPCR. Mean ± SEM of seven independent experiments.
(D) Effects of KU-55933 (10 mM) and/or N3 (10 mM, 7 days) on the TP53-STOP to TP53-WT ratio in HCT-116 cells. Mean ± SEM; n = 4 of one representative of three independent experiments.

INSERM TRANSFERT
Paris Biopark
7 rue Watt
75013 PARIS
France

Tél. +33 (0)1 55 03 01 45

stephanie.olas@inserm-transfert.fr
www.inserm-transfert.fr

InsermTransfert