

Study of the impact of the R693X mutation in ASXL1 on cell lines of JAK2 V617F positive myeloid neoplasias

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INTRODUCTION

The *ASXL1* gene is the human homolog of *Drosophila melanogaster*'s additional sex comb (*Asx*) gene, located in the region of chromosome 20q11.21, formed by 13 exons and expressed in most hematopoietic cells. The *ASXL1* protein is involved in the epigenetic regulation of gene expression, interacting with histone remodeling complexes. Mutations and deletions in *ASXL1* were reported in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN), including chronic myelogenous leukemia (CML) and primary myelofibrosis (MF). MF is a hematopoietic stem cell clonal disorder characterized by gain-of-function mutations in *JAK2*, *CALR* or *MPL* genes, leading to constitutively activated JAK-STAT pathway. *ASXL1* mutations can be found in about 40% of MF patients and can occur in patients with or without *JAK2/CALR/MPL* mutations. *ASXL1* mutations were associated with an unfavorable prognosis and a higher rate of leukemic transformation. An *in vitro* study using cell lines has shown that homozygous *ASXL1* mutations lead to the absence of protein expression (loss-of-function mutations). However, the mechanisms by which *ASXL1* mutations would lead to the transformation of myeloid cells are still not fully understood. In this context, the technology of the CRISPR/Cas9 system for genetic modification of cell lines represents an interesting strategy for the study of somatic alterations of myeloid neoplasia *in vitro*. Here we intend to use the CRISPR/Cas9 system to insert the *ASXL1* R963X mutation in the NMP cell line UKE1 and study how this molecular alteration impacts cells carrying *JAK2* V617F mutation. We intend to study the methylation profile and to evaluate the protein expression of *ASXL1* in these cell lines carrying mutations.

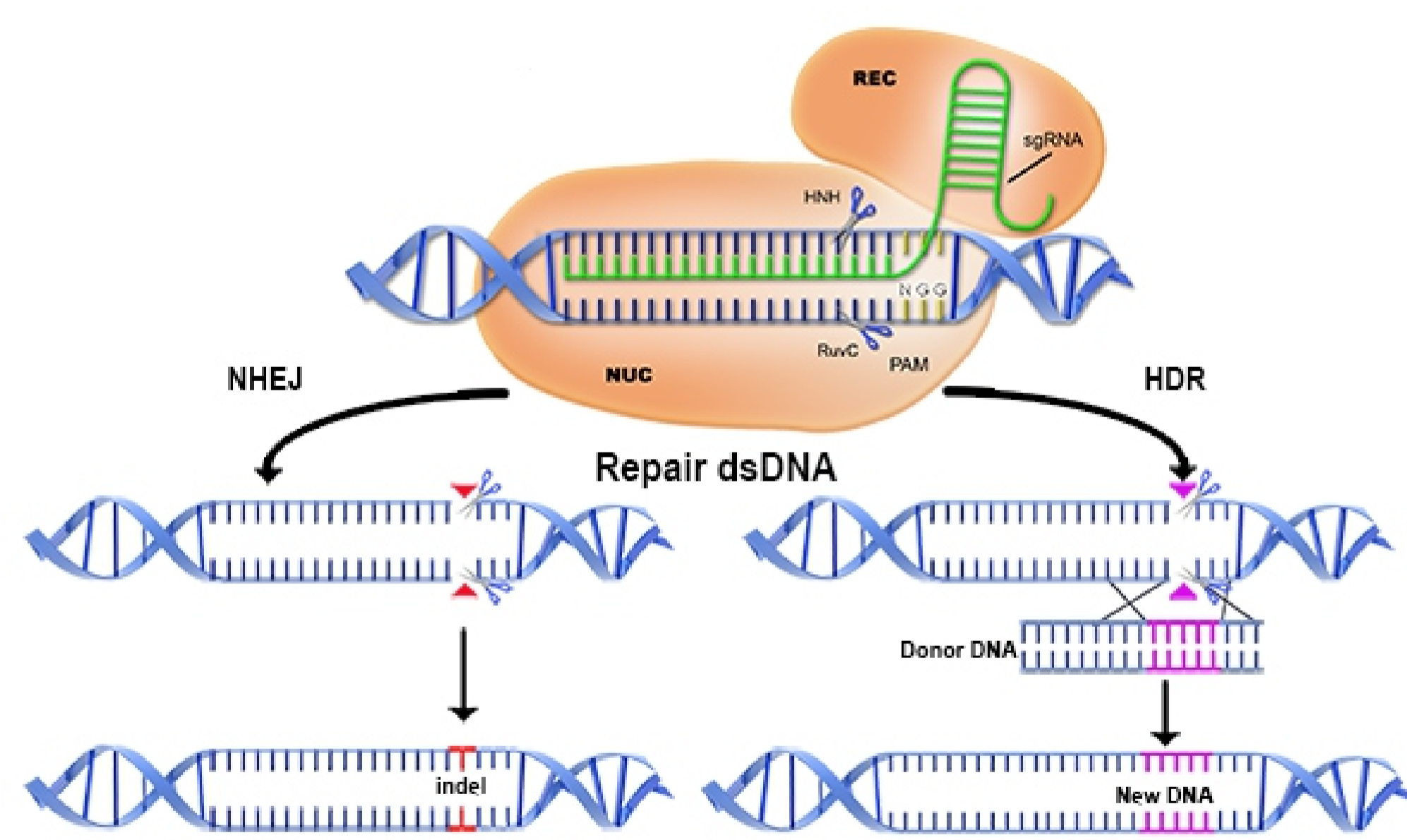


Figure 1: Representation of CRISPR/Cas9 (Savić and Schwank, 2016). In the NHEJ pathway, the ends are processed by endogenous DNA repair machinery and rejoined, which can result in random indel mutations at the site of junction and can result in gene knockout. In the HDR pathway, a repair template can be supplied which allows precise editing.

OBJECTIVES

To evaluate the impact of the R693X mutation in *ASXL1* on cell lines of JAK2 V617F positive myeloid neoplasms.

METHODS AND RESULTS

Five different specific RNAs guides (sgRNA) for the region of interest were designed using the <http://crispr.mit.edu> tool and two complementary oligos for each sgRNA were designed. The vector was digested with BbsI endonuclease linearizing vector PX458 and after we used T4 ligase to annealed oligo duplex into a specific cloning site of vector. After transformation into competent bacteria and plasmid preparation the construct was checked by sequencing using universal primer for U6 promoter region and it was possible to obtain cloned plasmids for

all five sgRNAs. First, diploid clone of the UKE-1 lineage was selected through a serial dilution of the pool and the karyotype is currently being confirmed. UKE-1 cells were currently being transfected with the plasmids obtained. After standardization we will be able to establish the study model.



Figure 2: Vector PX458 expressing Cas9 showing the cut regions of the enzyme BbsI and specific cloning site (gRNA scaffold).

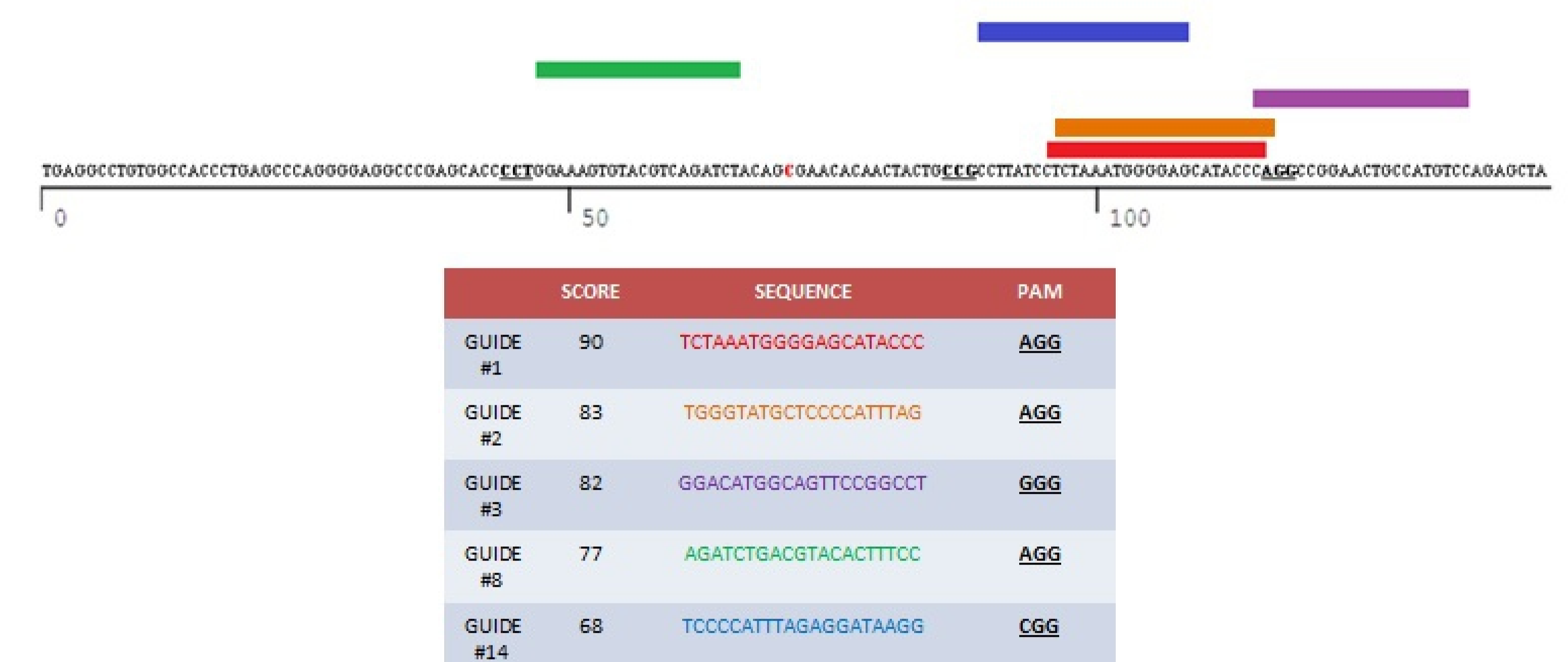


Figure 3: The five specific RNAs guides (sgRNAs) selected for the region in the *ASXL1* gene where the R693X mutation (c.2077C>T, p.R693X) is found.

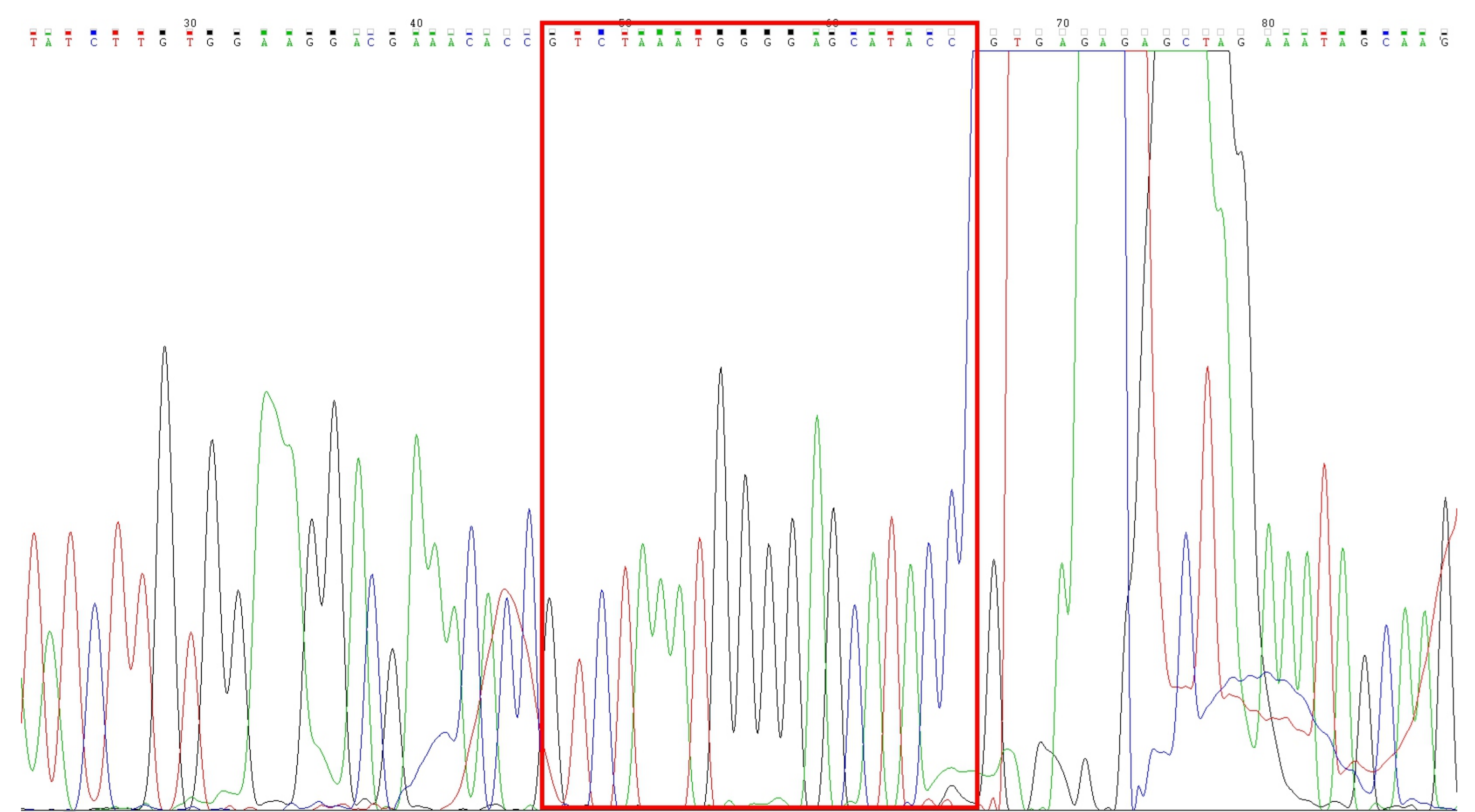


Figure 4: Electropherogram obtained after direct sequencing of plasmid PX458 using primer for U6 promoter forward region showing the guide #1_Top annealed into a specific cloning site (gRNA scaffold).