



# Generation of *TP53*-Deficient Cell Lines Using the CRISPR/Cas9 Genomic Engineering System to Investigate the Role of p53 in the Regulation of the Antiviral and Cancer Genomic Deaminase, APOBEC3B

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## Background

The human *TP53* gene encodes the tumor suppressor, p53. In response to DNA damage, p53 activates repair machinery and promotes cell cycle arrest. If the extent of genomic mutation is beyond repair, p53 induces apoptosis. P53 inactivation is a hallmark of cancer cells. Loss of functional p53 enables cells to bypass cell cycle checkpoints and proliferate with damaged genomes, thus contributing to the process of transformation.<sup>1</sup>

In addition to p53, altered expression of other proteins has been implicated in tumor progression. The cytosine deaminase, APOBEC3B (A3B), normally functions as part of the innate and adaptive immune responses. A3B is found at low levels in the nucleus of normal tissue where it catalyzes the deamination of cytosine to uracil in single stranded DNA. In contrast, A3B is over-expressed in at least 19 different cancers, including breast, cervical and head/neck. Additionally, the A3B signature mutation, cytosine-to-thymine, makes up the majority of the mutations observed in these tumors. Based on these data, A3B has been hypothesized as a major source of genetic mutations in transformed cells.<sup>2,3,4,5</sup>

Cervical and head/neck cancers are driven by HPV infection, and recent evidence from the Harris lab has indicated that high-risk HPV strains cause the up-regulation of A3B. It has been shown that this virus-mediated up-regulation is dependent on the viral protein, E6. E6 is a ubiquitin ligase that causes p53 inactivation by inducing its degradation. Inactivation of p53 is found in A3B overexpressing breast cancers,<sup>6</sup> and in estrogen receptor-positive breast cancer specifically, high A3B expression correlates with reduced disease-free and overall survival.<sup>7</sup> Developing strategies to target A3B would prove useful in a clinical setting.

## Hypothesis & Rationale

Based on these data, we hypothesize that p53 is a transcriptional repressor of A3B, and that loss of functional p53 by E6 enables A3B gene transcription to progress unimpeded. The resultant high levels of A3B activity promote large-scale genomic DNA mutation and provide an environment suitable for cellular transformation. To test this hypothesis, we will use the CRISPR/Cas9 Genomic Engineering System<sup>8</sup> to create both *TP53* knockout and knockdown cell lines.

Using this model system, we expect to see no difference between the levels of A3B expression induced by E6-expressing versus non-expressing HPV in *TP53*<sup>-/-</sup> cells. This result would suggest that HPV-induced A3B up-regulation necessitates inactivation of *TP53* by E6. Additionally, overexpression of A3B by *TP53*-deficient cells may induce a state of hypermutation not suitable for cell survival, and thus, may promote death by synthetic lethality<sup>3,5</sup>. If this is the case, we expect to see an increase in apoptotic gene expression and decreased cell viability.

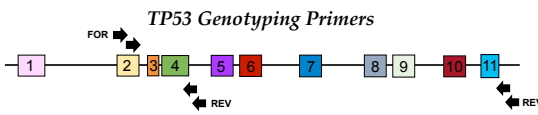
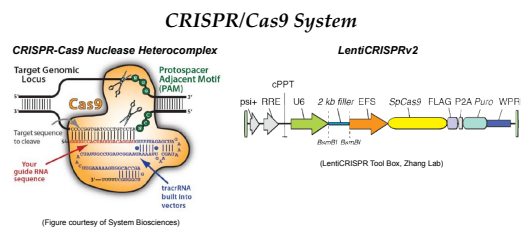
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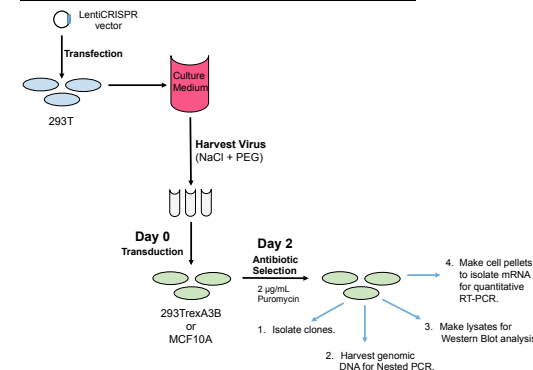
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## Methods

### Construction of Lenti/CRISPR vectors targeting *TP53*

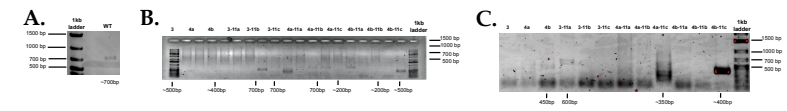


### Generation of *TP53*-deficient cell lines

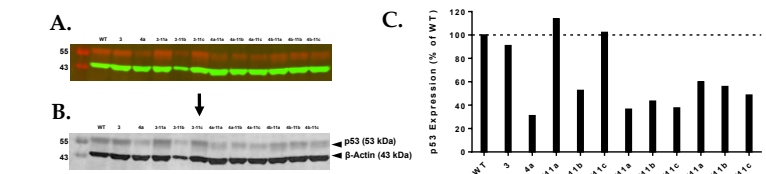


## Results

**Figure 1. Nested PCR on genomic DNA isolated from puromycin-resistant 293TrexA3B mixed populations transduced with *TP53*-targeted vectors.** *TP53*-specific primers flanking exons 3 and 4 (A & B) or 3 and 11 (C) were used to perform nested PCR on genomic DNA isolated from WT (A) or pools of puromycin-selected 293TrexA3B cells transduced with LentiCRISPR vectors specific for *TP53* (B & C). Reactions were run on 1% agarose gels to identify bands suitable for sequencing.



**Figure 2. P53 expression in WT versus puromycin-resistant 293TrexA3B mixed populations transduced with *TP53*-targeted vectors.** Immunoblots were imaged with the Odyssey (LICOR) (A), converted to grayscale (B), and band intensity was quantified using ImageJ. Each p53 band was normalized to its respective  $\beta$ -actin control. The level of WT expression was set to 100%, and the relative level of p53 expression as a percent of WT was depicted in a bar graph (C) made using Prism 4.



## Summary

The mechanism of E6-dependent A3B up-regulation in the context of virus infection has not yet been fully elucidated, and may involve transcriptional repression by p53. *TP53*-deficient cell lines represent a novel system to determine whether or not p53 normally represses A3B transcription in this setting. Using the CRISPR/Cas9 Genomic Engineering System, we have demonstrated decreased *TP53* expression in the model cell line 293TrexA3B as proof of concept, with the expectation of reproducibility in clonal populations and more relevant cell lines (e.g., tumor-derived). Once *TP53*-null 293TrexA3B clones are obtained, A3B can be induced using doxycycline, and the level of A3B expression in a *TP53*-competent versus a *TP53*-deficient environment will be assessed. An increase in A3B signature mutations (C-G to T-A within TCA motifs<sup>9</sup>) will be identified using sequencing. The possibility that A3B overexpression promotes death by synthetic lethality will be examined by assessing cell viability, and by looking for increased pro-apoptotic protein expression. P53 is impaired in the majority of cancers, and A3B has been implicated in at least 19, thus, identifying the mechanisms of viral-mediated A3B induction has a global impact not only for viral pathogenesis, but also for cancer progression. The potential of A3B as a candidate for targeted therapies is high, and warrants further investigation.