

Addition of cytidine base editors to induced pluripotent stem cells for correction of p.N370S, a *GBA1* mutation responsible for type 1 Gaucher Disease

Emma Wells-Durand, under the supervision of C.L. Christensen and Dr. F.Y.M. Choy
Choy Lab, Department of Biology, University of Victoria

Emma Wells-Durand, Department of Biology
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Supervised by Dr. Francis Choy, Department of Biology

INTRODUCTION

Type 1 non-neuronopathic Gaucher Disease (GD) is a monogenic autosomal recessive disorder characterized by reduced activity of the enzyme glucocerebrosidase (GBA) in lysosomes within macrophages, which ultimately results in increased amounts of its glycolipid substrate glucocerebroside (Sidransky, 2004). The gene for GBA, *GBA1* is located on chromosome 1 (Sidransky, 2004), and contains almost 300 possible mutations responsible for GD (Hruska *et al.*, 2008). One such mutation, p.N370S (c. 1226 A>G) is an A to G point mutation (Tsuji *et al.*, 1987) located in exon 9 of *GBA1* (Koprivica *et al.*, 2000) that serves as a suitable candidate for correction using base editing, a novel gene editing technology.

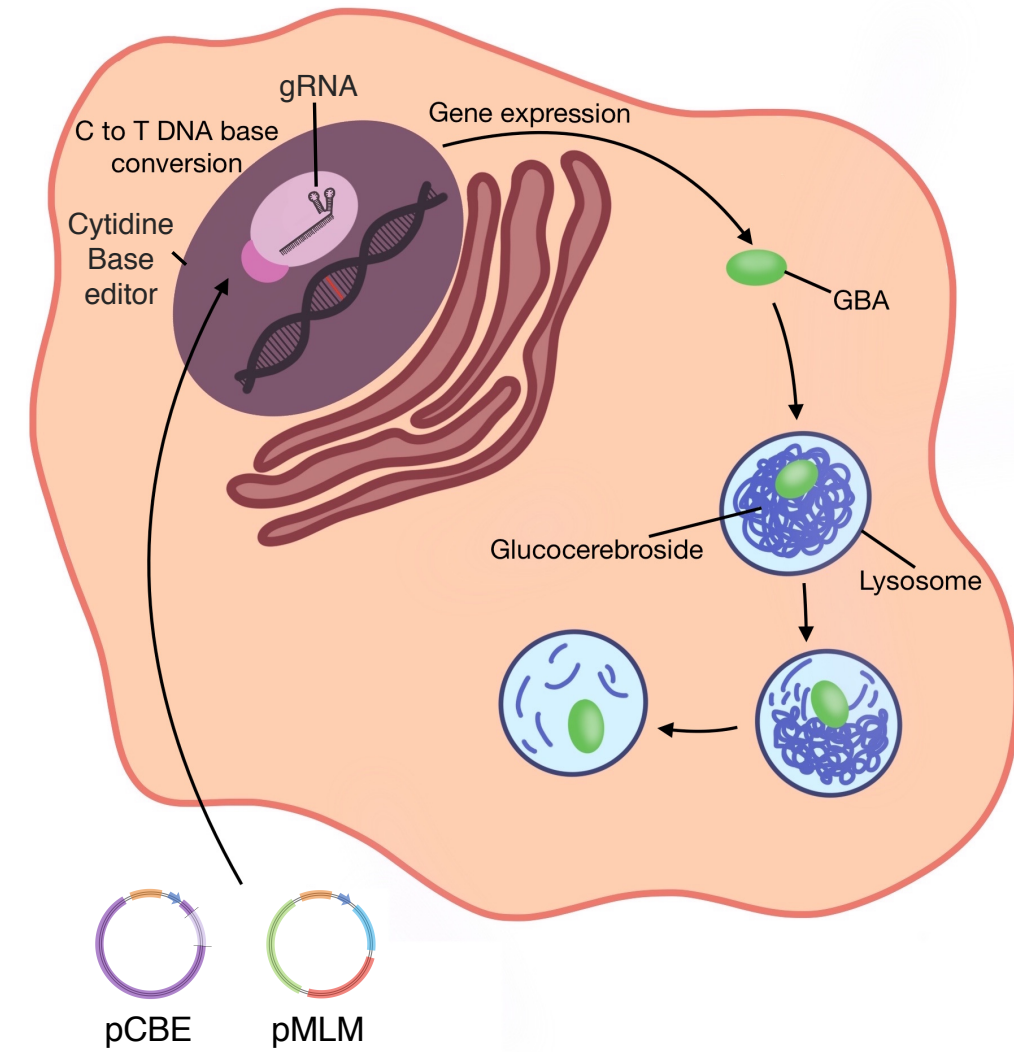


Figure 1. The transfection of a cytidine base editor and gRNA in the form of plasmids introduces a C to T base edit at N370S. The production of functional GBA results in increased metabolism of glucocerebroside in lysosomes within macrophages.

Current gene editing strategies function by making double stranded (ds) breaks at the target site as the first step towards correction (Cox *et al.*, 2015). The predominant cellular pathway that responds to dsDNA breaks, non-homologous end joining, involves the addition of unwanted insertions and deletions (indels) at the target locus, resulting in problematic and inefficient correction outcomes (Cox *et al.*, 2015). A new approach called base editing allows for the direct, programmable conversion of one DNA base to another within a pre-determined five-base-pair window, without the introduction of dsDNA breaks (Komor *et al.*, 2016). The base editor is first directed to the target locus by a single stranded guide RNA (gRNA) designed to be complementary to the target site, after which deamination and DNA base conversion occurs (Komor *et al.*, 2016). In the present study, a cytidine base editor will be used to direct a C to T conversion in the complementary strand to N370S, in patient-derived induced pluripotent stem cells (iPSCs).

REFERENCES

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RESEARCH OBJECTIVES

1. Successfully ligate a gRNA DNA sequence into a digested scaffold pMLM plasmid, and transform the gRNA and CBE plasmids into *E.coli* DH5 α cells.
2. Successfully transfect the CBE and gRNA plasmids into human N370S iPSCs, and see enough cells post-electroporation to pick colonies for monoclonal growth.
3. Establish growth of corrected monoclonal iPSC colonies.

EXPERIMENTAL PLAN

My experimental plan involves introducing two molecular components into human N370S iPSCs in the form of pMLM and pCBE plasmids: a single stranded gRNA (pMLM), and a cytidine base editor (pCBE) composed of a cytidine deaminase domain and a gRNA-binding domain. The gRNA's DNA sequence was ligated into the digested pMLM scaffold plasmid, and both plasmids were transformed into *E.coli* DH5 α cells for the generation of many plasmids.

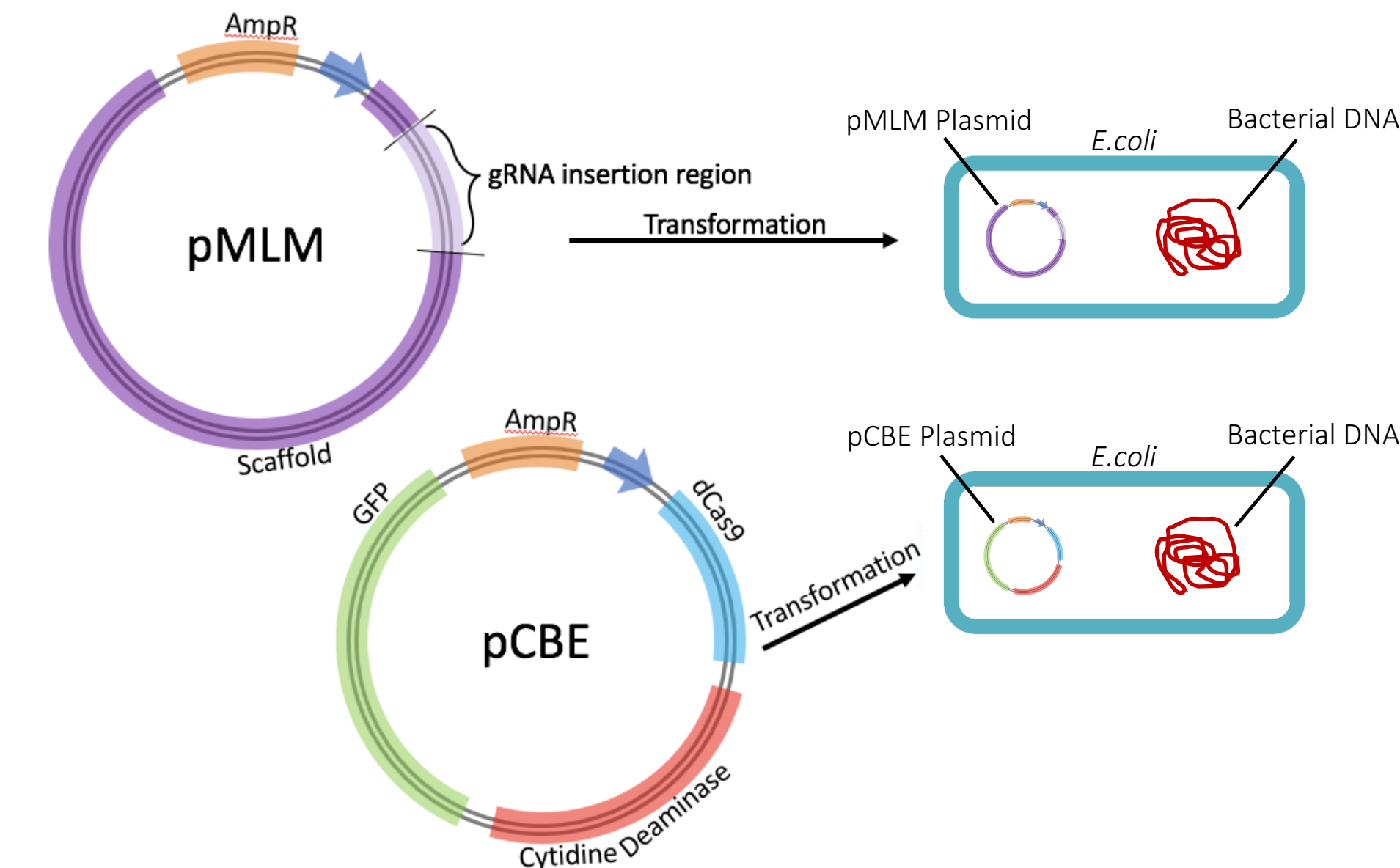


Figure 2. Schematic of the transformation of pMLM (gRNA) and pCBE (cytidine base editor) plasmids into *E.coli* DH5 α cells for the generation of many plasmids.

Plasmids were then isolated and purified from *E.coli* DH5 α cells, and subsequently transfected into human N370S iPSCs by electroporation. As the CBE plasmid expresses green fluorescent protein (GFP), viable transfected cells can be visualized under fluorescence microscopy. The aim is to see enough cells-post electroporation to pick colonies for monoclonal growth. To screen for cells that have undergone correction, mismatch primers were used to amplify the DNA sequence containing the corrected N370S mutation site, and introduce an HpyCH4III restriction endonuclease recognition sequence. Thus, only cells that have undergone correction have their amplicons digested and appear as two bands on an SDS-polyacrylamide gel. Restriction digests will be performed on polyclonal and monoclonal colonies. RNA-guided engineered nuclease (RGEN)-restriction fragment length polymorphism (RFLP) analysis was performed on N370S genomic DNA to ensure efficient targeting by the gRNA to the target locus.

RESULTS

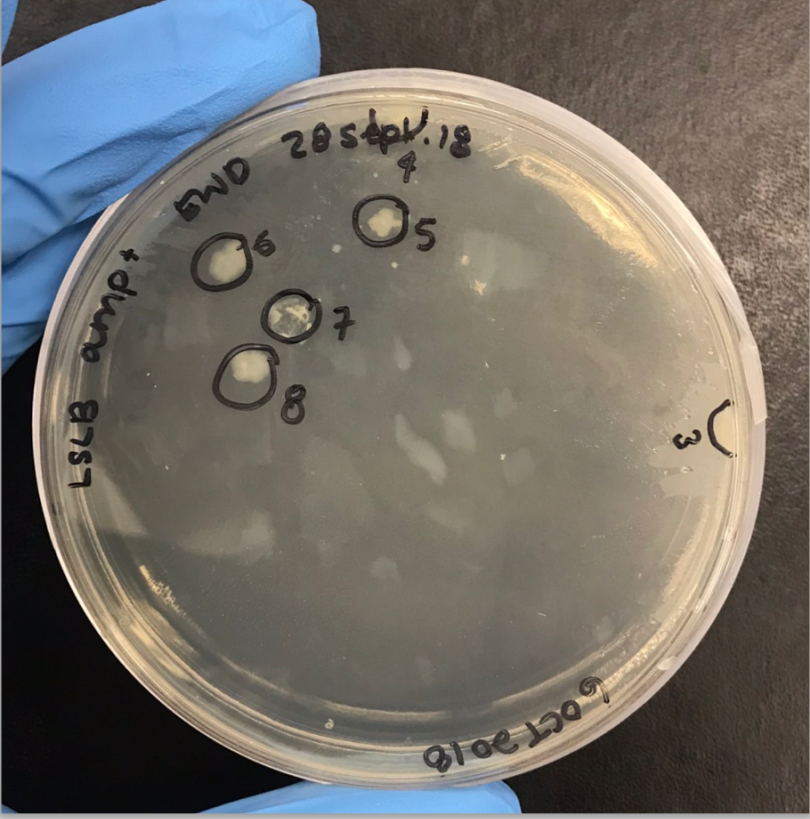


Figure 3. *E.coli* DH5 α cells were successfully transformed with CBE and gRNA plasmids. As both plasmids contain ampicillin resistance genes, successful transformation was represented by growth on ampicillin-containing media.

Figure 4. Successful transfection of plasmids into N370S iPSCs by electroporation is represented in cells fluorescing green under fluorescence microscopy. The pCBE expresses GFP protein as a selection marker.

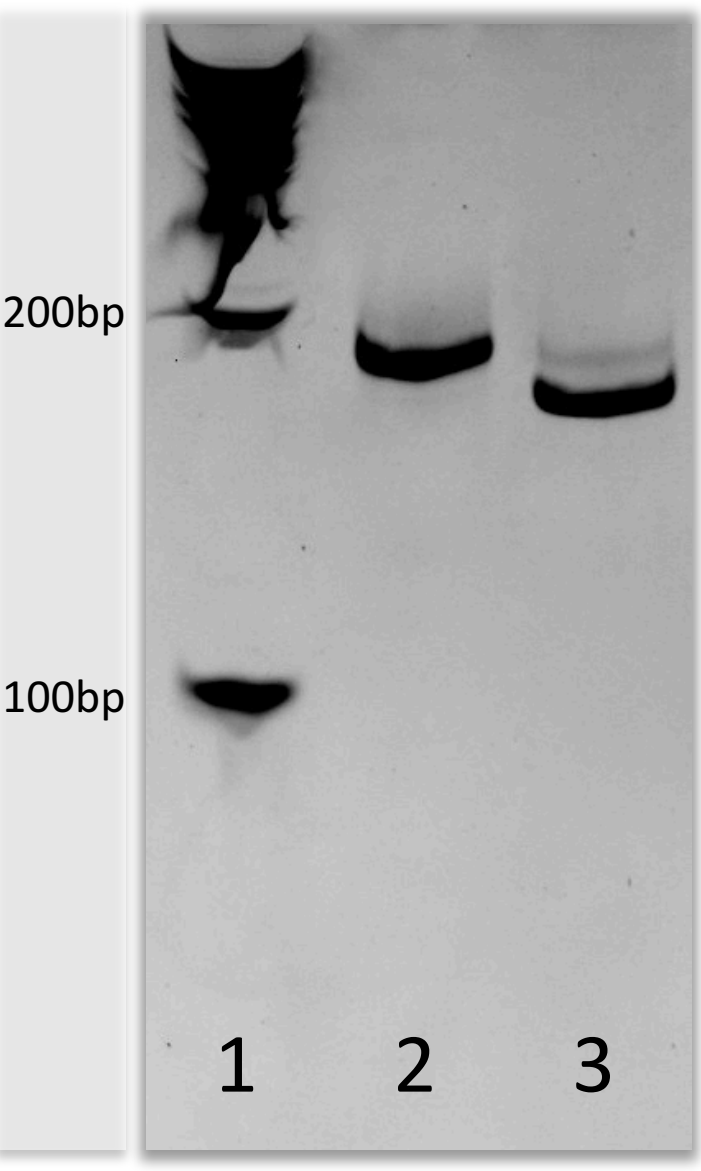
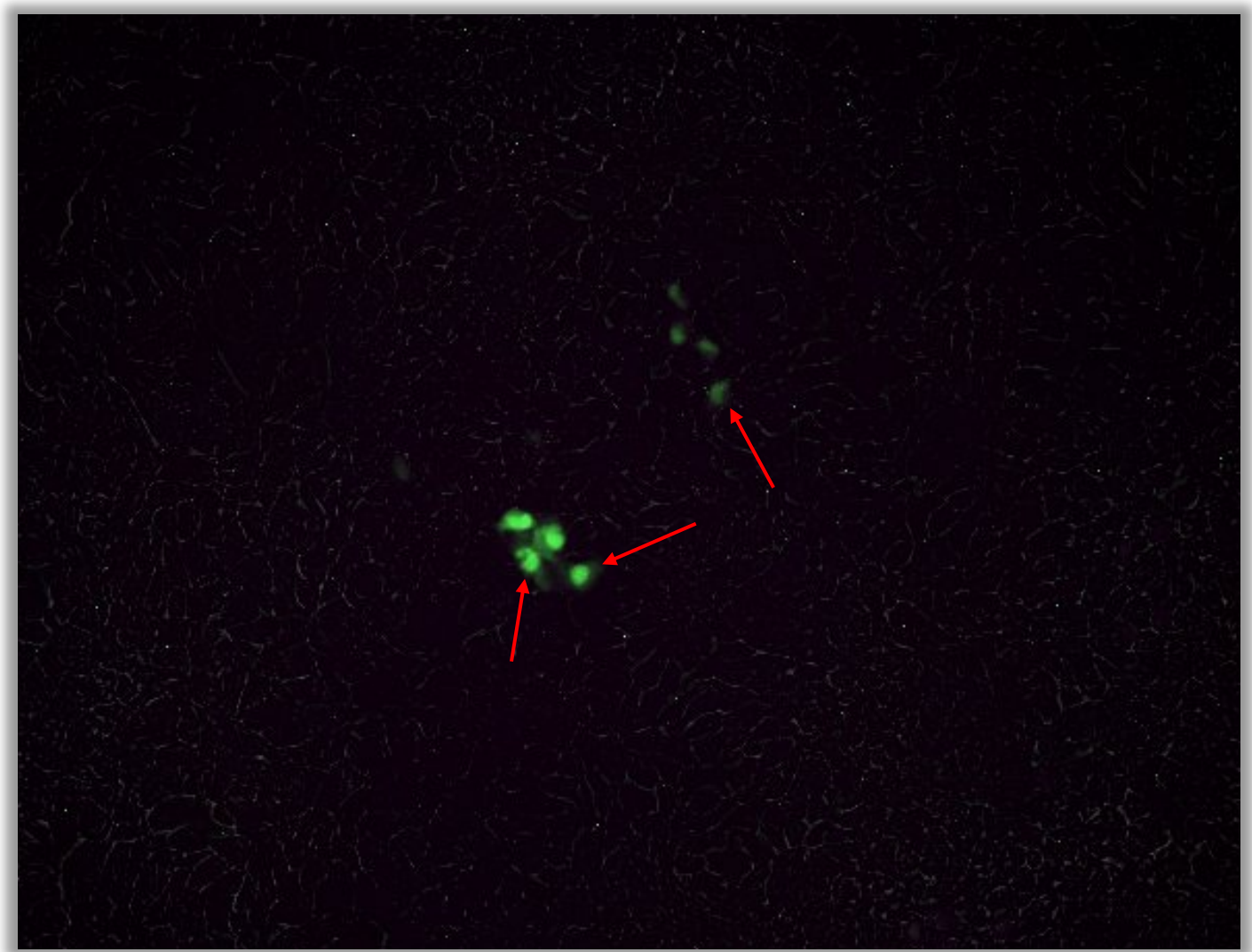


Figure 5. Proof-of-concept HpyCH4III restriction digest, run on an SDS-polyacrylamide gel. Lane 1 contains a 50bp ladder. Lane 2 contains N370S amplicons that are mutated and thus not digested, showing an expected band 190bp in length. Lane 3 contains regular genomic DNA amplicons from non-diseased iPSCs, with cleavage producing a faint undigested band 190bp in length, and a bright band of digested product 173bp in length.

Figure 6. RGEN-RFLP analysis of amplicons to test for gRNA targeting efficiency *in vitro*. Lane 1 contains a 100bp ladder. Lane 3 contains N370S amplicons digested by Cas9, confirming successful targeting of Cas9 by the gRNA to the mutation locus. Lane 2 contains a negative control, in which Cas9 was not added, and thus no cleavage is seen. Lane 4 contains a positive control, in which a known gRNA with high targeting efficiency has allowed for cleavage by Cas9.

