A fast and reliable method for detecting base editing in clonal cell lines

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Abstract

One of the most powerful applications of genome editing is the introduction of base changes in specific genomic sites that mimic single-nucleotide polymorphisms (SNPs) related to human diseases or the introduction of stop codons to generate gene knockouts. However, screening a large number of clones to identify edited clonal cell lines containing the engineered base of interest is still a bottleneck, especially in the absence of a phenotypic readout. A couple of potential approaches for identifying edited cells are Sanger sequencing and next-generation sequencing (NGS). However, Sanger sequencing is not easy to apply in a high-throughput manner, and although NGS allows screening of 96-well plates, it is very costly.

To address this need, we developed a SNP-detection method that allows quick screening of clones from a 96-well plate. The method is based on PCR amplification of the genomic target site followed by an enzymatic assay with a fluorescence readout. The overall workflow takes approximately four hours to complete and the final fluorescent signal indicating the presence of the introduced SNP can be detected using a standard plate reader, without requiring any special instrumentation.

As a proof-of-concept, we applied this method to successfully detect all the possible transitions in several human loci using genomic DNA as template or directly in cultured human fibroblasts. As a final test, this method was used to screen for clonal human iPS cell (hiPSC) lines carrying tyrosinemia-related SNPs introduced via genome editing.

Conclusions:

- The Guide-it™ SNP Screening Kit provides a quick, high-throughput method to identify edited clonal cell lines containing the engineered base of interest, regardless of their zygosity (homozygous or heterozygous).
- SNP detection using this method is independent of the engineered nucleotide substitution and the surrounding targeted genomic loci.

1 Workflow for detection of base editing in clonal cell lines

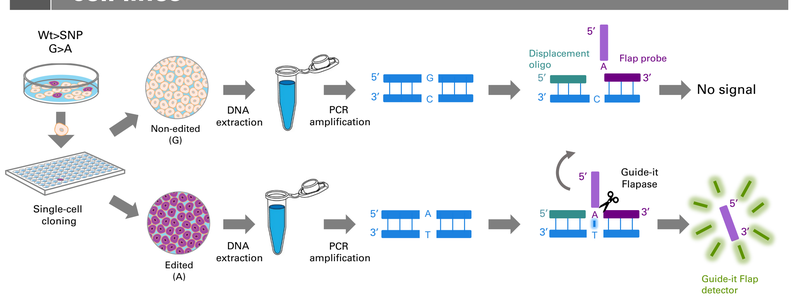

Figure 1. The Guide-it SNP Screening Kit provides a method for detecting single base editing. In this example workflow, the detection of a G>A transition (where G is the wild-type base edited to an A) is shown. After the genome editing event, single cells are isolated via FACS or limiting dilution and expanded to clonal cell lines that can be wild-type (G) or successfully edited (A). After DNA extraction and subsequent PCR amplification of the target site, the PCR product is annealed with two different complementary oligo probes—the Displacement oligo and the Flap probe—that hybridize with the DNA sample in the region adjacent to the interrogated base (defined as the actual SNP base for which you are screening). After the annealing of the two DNA oligos with the PCR product, Guide-it Flapase recognizes a complete base-pairing between the Flap probe and the PCR product in the resulting three-dimensional structure and subsequently cleaves the Flap. This cleavage event causes the release of the Flap oligo, which is detected downstream by the Guide-it Flap detector, generating a fluorescence signal that can be measured using a plate reader. Therefore, the detection of a fluorescence signal is equivalent to an exact base pairing between your Flap probe and the DNA sample.

2 Detection of all possible base transitions

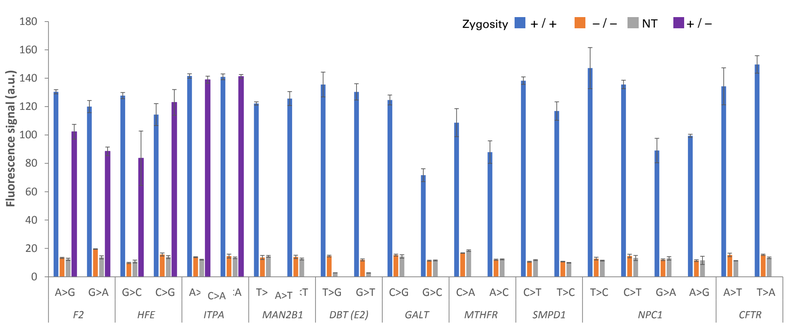

Figure 2. Detection of all of the different base transitions from genomic DNA. The performance of the assay was assessed for homozygous and heterozygous samples using various SNPs in human genes as models for single nucleotide edits. Ten nanograms of genomic DNA from the different samples (obtained from the Coriell Institute) was used as a template for PCR amplification and the generated PCR products were used for the detection of SNPs using the Guide-it SNP Screening Kit. All transitions were successfully detected, even in the cases where the sample was heterozygous for the SNP. This demonstrates the effectiveness of the assay to detect a desired edit, regardless of the nature of the base transition.

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3 Detection of SNPs in human fibroblasts

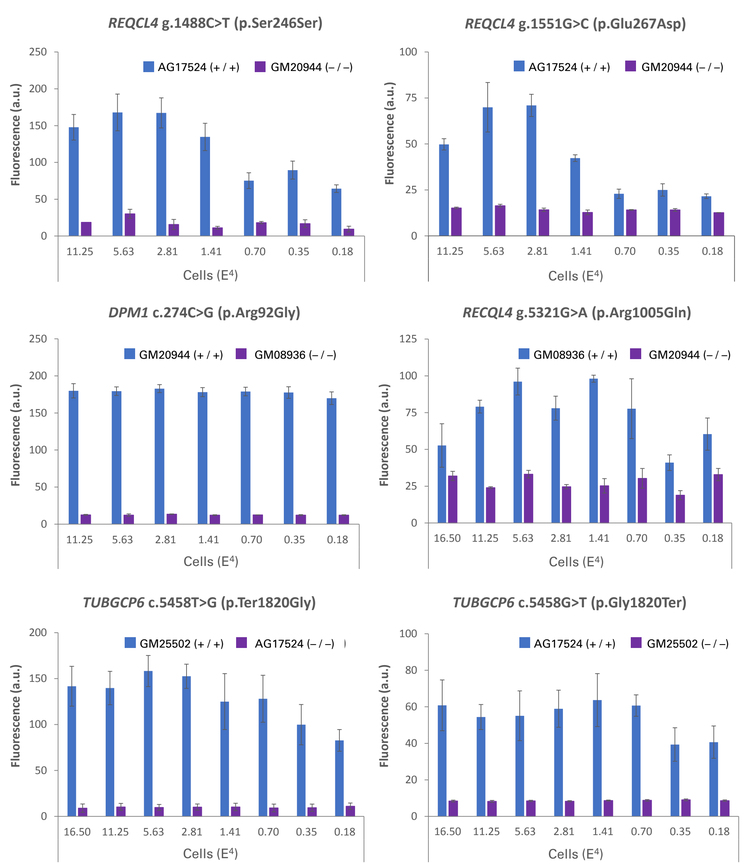

Figure 3: Detection of various SNPs in cells. Fibroblasts that carried different SNPs in various genes (*RECQL4, DPM1* and *TUBGCP6*) were obtained from the Coriell Institute (the details of the position and nature of the base transitions are denoted above each graph). For each base transition, two cells lines were analyzed: one carrying the desired SNP and the other one being wild-type for that site (denoted by the blue and purple bars respectively). Cells were plated at different densities ranging from 1.6 x 10^5 to 1.8 x 10^3 cells per well in 96-well plates. After 48 hours, genomic DNA was extracted using MightyPrep DNA Reagent and used as a template in a PCR reaction in order to amplify the region encoding the SNP. PCR products were interrogated for the different SNPs using the Guide-it SNP Screening Kit. In all the cases, a considerably higher fluorescent signal was detected in the cell lines encoding the SNP. These examples demonstrate the successful application of the detection assay in human cells cultured at various cell densities.

4 Application in sample genotyping

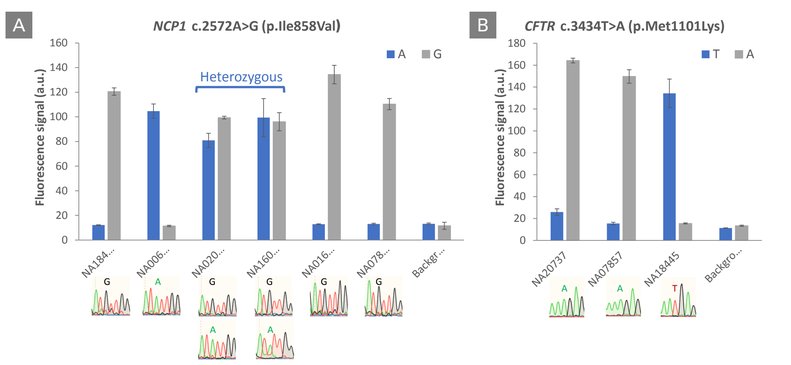

Figure 4. Use of the Guide-it SNP Screening Kit for genotyping. Panel A. In case of the *NPC1* gene, the assay was designed to detect the A to G transition at nucleotide 2572 (c.2572A>G) which results in a missense mutation at codon 858 (p.Ile858Val). Only the sample NA18445 from Coriell Institute was reported in the database to carry this mutation (positive control). The assay was able to determine which samples were homozygous or heterozygous for that specific mutation using two independent experimental Flap probes designed to detect G or A. Samples NA02051 and NA16000 were heterozygous, while all other samples (NA01619 or NA07857) were homozygous. **Panel B.** In the case of *CFTR* mutation, the Guide-it SNP Screening kit was able to detect that sample NA20737 had a T to A transition at nucleotide 3434 (c.3434T>A p.Met1101Lys) resulting in a missense mutation in exon 17b. The fluorescence levels, in this case, were similar to the ones obtained using sample NA07857, which is reported to have the same T>A transition. The assay also detected that sample NA18445 was wild-type (T) in that position. All results were confirmed by Sanger sequencing.

5 Screening for SNPs related to tyrosinemia in hiPSCs

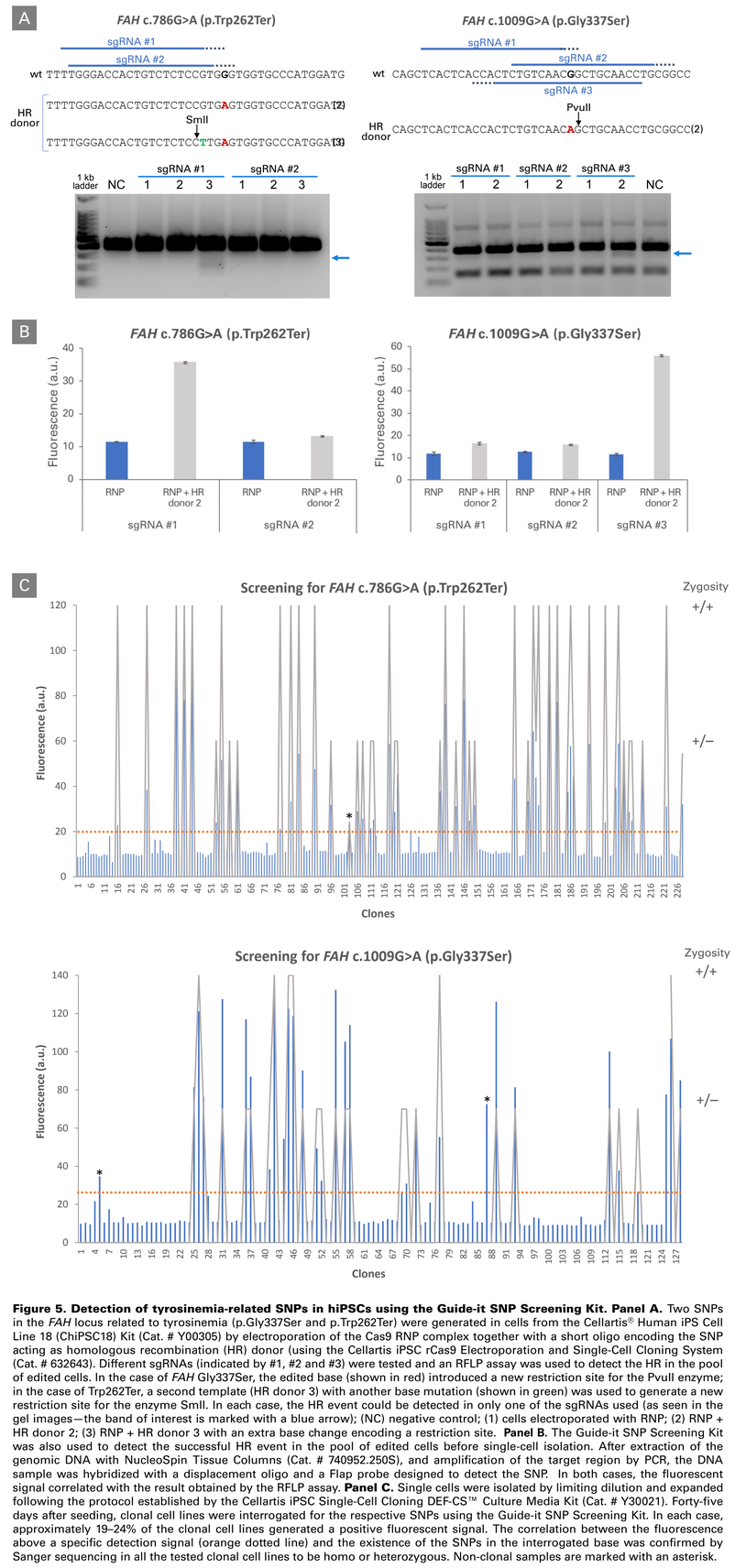

Figure 5. Detection of tyrosinemia-related SNPs in hiPSCs using the Guide-it SNP Screening Kit. Panel A. Two SNPs in the *FAH* locus related to tyrosinemia (p.Gly337Ser and p.Trp262Ter) were generated in cells from the Cellartis® Human iPS Cell Line 18 (ChiPSC18) Kit (Cat. # Y00305) by electroporation of the Cas9 RNP complex together with a short oligo encoding the SNP acting as homologous recombination (HR) donor (using the Cellartis iPSC rCas9 Electroporation and Single-Cell Cloning System (Cat. # 632643). Different sgRNAs (indicated by #1, #2 and #3) were tested and an RFLP assay was used to detect the HR in the pool of edited cells. In the case of *FAH* Gly337Ser, the edited base (shown in red) introduced a new restriction site for the PvuII enzyme; in the case of Trp262Ter, a second template (HR donor 3) with another base mutation (shown in green) was used to generate a new restriction site for the enzyme SmlI. In each case, the HR event could be detected in only one of the sgRNAs used (as seen in the gel images—the band of interest is marked with a blue arrow); (NC) negative control; (1) cells electroporated with RNP; (2) RNP + HR donor 2; (3) RNP + HR donor 3 with an extra base change encoding a restriction site. **Panel B.** The Guide-it SNP Screening Kit was also used to detect the successful HR event in the pool of edited cells before single-cell isolation. After extraction of the genomic DNA with NucleoSpin Tissue Columns (Cat. # 740952.250S), and amplification of the target region by PCR, the DNA sample was hybridized with a displacement oligo and a Flap probe designed to detect the SNP. In both cases, the fluorescent signal correlated with the result obtained by the RFLP assay. **Panel C.** Single cells were isolated by limiting dilution and expanded following the protocol established by the Cellartis iPSC Single-Cell Cloning DEF-CS™ Culture Media Kit (Cat. # Y30021). Forty-five days after seeding, clonal cell lines were interrogated for the respective SNPs using the Guide-it SNP Screening Kit. In each case, approximately 19–24% of the clonal cell lines generated a positive fluorescent signal. The correlation between the fluorescence above a specific detection signal (orange dotted line) and the existence of the SNPs in the interrogated base was confirmed by Sanger sequencing in all the tested clonal cell lines to be homo or heterozygous. Non-clonal samples are marked with an asterisk.