TALEN-induced contraction of CTG trinucleotide repeats in myotonic dystrophy type 1 cells

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Abstract

Trinucleotide repeat expansions are the cause of two dozen neurodegenerative and developmental disorders. One of these, myotonic dystrophy type 1 (Steinert disease, or DM1) is due to the expansion of a CTG triplet in the 3' UTR of the *DMPK* gene. We used highly specific DNA endonucleases to induce a double-strand break in the repeat tract to contract it below pathological length. Expression of a TALE Nuclease (TALEN) in human DM1 cells induced moderate CTG repeat contractions in only 27% of the clones analyzed. These clones exhibited large internal deletions within the TALEN, probably occurring by homologous recombination, inactivating the nuclease, and explaining its reduced efficacy. Taking advantage of the degeneracy of the genetic code, we recoded the TALEN sequence, to decrease internal redundancy and optimize codon usage. The new recoded TALEN showed increased efficacy in DM1 cells, with 68% of clones exhibiting a moderate to large contraction of the CTG repeat tract. In contract, *Staphylococcus aureus* Cas9 (*Sa*Cas9) was unable to contract the CTG repeat tract. In parallel, we completely sequenced to very high coverage the DM1 genome using the PacBio technology. It is the first DM1 genome to be totally sequenced and assembled using long reads. Long perfect and imperfect CTG trinucleotide repeats were extracted from its sequence using a dedicated homemade bioinformatics pipeline. Several clones in which the TALEN was induced were also totally sequenced. We found frequent length changes of other CTG repeats besides the one expanded at the *DMPK* locus, suggestive of off-target effects. All of them are located in introns or intergenic regions. However, improving TALEN efficacy by recoding did not increase the frequency of these off-target mutations. Therefore, TALENs could be considered as a promising gene therapy approach, not only for DM1 but also for many other microsatellite expansion disorders.

Introduction

Microsatellite expansions are responsible for over two dozen human disorders, including Huntington's disease, myotonic dystrophy type 1 (DM1 or Steinert disease), Friedreich's ataxia, fragile-X syndrome and various types of ataxias as well as severe developmental pathologies (reviewed in Orr, 2001; Orr & Zoghbi, 2007). Among these, DM1 arises from the expansion of the CTG triplet repeat within the 3' UTR of the *DMPK* gene, on chromosome 19 (Brook *et al*, 1992). DM1 is characterized as a dominant autosomal multisystemic disorder, encompassing myotonia, progressive muscle weakness and wasting, cataracts, cardiac conduction defects and cognitive dysfunction. These symptoms consistently coincide with an expansion of the *DMPK* repeat tract. In individuals without the disease, the CTG repeat length typically falls within the range of 5 to 37 triplets, whereas patients exhibit lengths ranging from 50 to 4,000 CTG. However, even among the different forms of the disease, there is substantial variability in the length of the repeat tract. For instance, the adult form of the disease can feature repeat expansions ranging from 250 to 750 CTG triplets, while the infantile form may have expansions ranging from 500 to over 1000 triplets (De Antonio *et al*, 2016). Consequently, for a given repeat length, predicting the severity of the symptoms is challenging. A patient with 500 CTG triplets could present the adult, juvenile, of infantile form of the disease, each associated with various degrees of symptom severity. This incomplete penetrance implies the involvement of additional genetic factors, beyond the *DMPK* CTG expansion, in determining DM1 severity.

At the cellular level, patient's cells exhibit abnormal nuclear aggregates of mRNA carrying expanded CUG repeats. These CUGexp-mRNA sequester the MBNL RNA binding proteins, leading to dysregulations in RNA metabolism, such as alternative splicing defects and multisystemic deficiencies that ultimately contribute to the pathology. At the molecular

level, it is the expression of pathological CUGexp-mRNA that triggers the disease. Therefore, several therapeutic approaches have been developed to target this expanded transcript, either directly or indirectly. Strategies such as inhibiting or reducing *DMPK* expression using small molecules or antisense oligonucleotides, have been explored, with several of them entering clinical trials (Pascual-Gilabert *et al*, 2021). In another approach, the expanded CUG mRNA was targeted by a mutated version of *Streptococcus pyogenes* Cas9 (*Sp*Cas9). The presence of the deficient nuclease was sufficient to decrease the amount of toxic mRNA in cell cultures and suppress RNA foci and some splicing defects (Batra *et al*, 2017).

Another therapeutic approach involved targeting the CUGexp-mRNA to prevent its interaction with the MBLN1 protein, using antisense oligonucleotides (reviewed in Izzo *et al*, 2022). More recently, a gene therapy strategy using a decoy with high affinity for CUGexp has also been developed in order to disrupt the deleterious interaction between MBNL1 protein and the expanded transcript (Arandel *et al*, 2022).

Another avenue of research focuses on the development of gene therapy approaches targeting the expanded CTG trinucleotide repeat at the DNA level, using highly specific DNA endonucleases such as Zinc-Finger nucleases (ZFN), TALE nucleases (TALEN) or the large CRISPR-Cas family (Mosbach *et al*, 2018a). Two main strategies have been pursued: one aims to contract the expanded CTG repeat by induction of a double-strand break (DSB) within the repeat tract, while the other seeks to delete it by inducing DSBs upstream and/or downstream of the repeat. The latter strategy has shown some success in DM1 cells and transgenic mice using either a modified version of the *Streptococcus pyogenes* Cas9 (e*Sp*Cas9) or the wild-type *Staphylococcus aureus* Cas9 (*Sa*Cas9) (Provenzano *et al*, 2017; Lo Scrudato *et al*, 2019; Dastidar *et al*, 2018), although it sometimes results in large deletions around the repeat tract (Agtmaal *et al*, 2017). Similar deletions have been

observed in a yeast model containing an expanded CTG repeat from a DM1 patient, where *Sp*Cas9 was used to induce a DSB within the repeat tract (Mosbach *et al*, 2020). An alternative strategy proposes to induce a DSB within the expanded CTG trinucleotide repeat in order to contract it, hopefully alleviating the symptoms of the pathology (Richard, 2015). A ZFN targeting an expanded CTG repeat was expressed in HeLa cells and triggered efficient replication-dependent shortening of the repeat tract (Liu *et al*, 2010). The *Sp*Cas9 nickase was also shown to contract the expanded CTG triplet repeat in an HEK293 cell model, although with a low but detectable efficacy (Cinesi *et al*, 2016). A TALEN was employed for the same purpose and demonstrated to induce specific contractions of an expanded CTG repeat from a DM1 patient in a *Saccharomyces cerevisiae* yeast model, achieving 99% efficacy (Richard *et al*, 2014). Efficient DSB resection by the Mre11-Rad50 complex as well as the presence of the associated Sae2 protein were necessary to shorten the repeat tract (Mosbach *et al*, 2018b).

Here, we induced both TALEN and *Sa*Cas9 in fibroblasts derived from transgenic DM1 as well as immortalized DM1 fibroblasts. Notably, *Sa*Cas9 exhibited diminished or negligible efficacy in contracting the CTG repeat tract when compared to TALEN. We observed frequent internal deletions occurring in both arms of the TALEN following transduction in human cells. To address this issue, we recoded the TALEN using the degeneracy of the genetic code, thereby reducing motif similarity and adapting it to the *Homo sapiens* codon usage preference. This newly recoded TALEN exhibited superior efficacy in contracting the CTG repeat tract compared to the wild-type nuclease. Subsequently, we conducted PacBio whole-genome sequencing and uncovered contractions in additional CTG repeats within the human genome. These findings support the presence of off-target mutations induced by the TALEN in other microsatellites.

Results

TALEN expression in mammalian cells

To ensure precise targeting of the DMPK locus, the TALEN was engineered in such a way that the left arm binds the CTG repeat tract and the right arm was designed to recognize the boundary between the repeated and non-repeated sequence (Figure 1A). In our previous work, we have reported by utilizing DSB-sequencing that breaks were induced approximately 3-4 triplets before the end of the repeat tract (Mosbach *et al*, 2018b). Both TALEN arms were cloned in a plasmid (pMOV1) flanking a GFP gene, to prevent recombination between TALEN arms following transfection (Figure 1A) (Boch *et al*, 2009). Fibroblasts derived from a hemizygous transgenic mice carrying the human *DMPK* gene with 600 CTG triplets were transfected with pMOV1. GFP-positive cells were selectively sorted, and total genomic DNA was extracted for repeat length analysis using PCR. Notably, the amplification of the repeat tract revealed that the expanded allele had contracted to less than approximately 30 CTG triplets, while control cells showed no significant length difference (Figure 1B).

Each TALEN arm was subsequently inserted into two lentivirus backbones bearing different selection markers. Immortalized human DM1 fibroblasts, referred to as ASA cells (Arandel *et al*, 2017), underwent a two-step transduction process. First, they were transduced with the lentivirus carrying the right arm and selected for neomycin resistance. Subsequently, they were transduced with the lentivirus carrying the left arm and selected for hygromycin resistance. Cells that successfully incorporated both arms were expanded clonally, and genomic DNA was extracted to assess CTG repeat length by Southern blotting. In this particular case, PCR could not be used due to the presence of an expanded *DMPK* allele with 2,000 triplets. Cells with only one TALEN arm exhibited a low level of repeat instability, with one expansion and two contractions identified among 28

alleles analyzed. Cells in which both arms were expressed showed two expansions and 10 contractions among 37 alleles analyzed. The slight increase in contractions when both arms are expressed did not reach statistical significance. Additionally, most of the observed contractions were partial, with some clones showing more than one contracted allele (Figure 1C). Note that we focused only on the expanded as detecting length changes in the smaller wild-type allele at this scale was not feasible. In conclusion, this series of experiments indicated that the wild-type TALEN demonstrated high efficiency in contracting a $(CTG)_{600}$ repeat in mice fibroblasts but displayed reduced efficacy in contracting a $(CTG)_{2000}$ expansion in human cells.

Analysis of CTG trinucleotide repeats by PacBio sequencing of ASA cells

A crucial concern with gene editing is the precision of genetic modifications. Off-target mutations are a common occurrence when utilizing *Sp*Cas9 and their prediction is not always feasible through computational tools (Tsai *et al*, 2015). In the human reference genome (GRCh38), it is estimated that there are at least 10,000 CTG trinucleotide repeats (or their complementary sequence CAG). However, this figure is likely an underestimate, particularly because sequencing very long repeats poses challenges when employing short read technologies (International Human Genome Sequencing Consortium, 2001). A more comprehensive sequencing approach, incorporating a combination of short, long and very long reads, as well as Hi-C and optical mapping, has recently led to the release of the first human genome sequence from telomere to telomere, including all centromeres that were known to contain very large repeated elements (T2T). Initial analysis of this genome sequence has suggested the presence of potentially twice as many microsatellites as previously identified, likely encompassing very long ones that were previously beyond the reach of sequencing techniques (Nurk *et al*, 2022). Consequently, we made the

decision to employ long-read sequencing as a means to evaluate possible off-target effects of the TALEN.

The ASA genome underwent sequencing using the PacBio technology, resulting in a coverage depth of 155X. HiFi reads were employed to assemble sequences, yielding 371 contigs (N50: 53 Mb), covering 99.6% of the genome. Notably, this marks the first instance, to the best of our knowledge, of a DM1 genome sequenced with long read technology. To comprehensively identify all potential CTG trinucleotide repeat, both perfect and imperfect, we developed an in-house analysis pipeline, named OSTINATO[1] (Descorps-Declère and Richard, unpublished). This program was executed on the ASA reference genome and led to the detection of 8 imperfect and two perfect CTG trinucleotide repeats spanning more than 50 triplets. This threshold was chosen as it represents the size at which the *DMPK* CTG allele becomes pathogenic.

For comparative purposes, we assessed the performance of our software against the established gold standard TRF program, which is well-recognized and widely used for tandem repeat analysis (Benson, 1999). Remarkably, TRF did not detect 2 out of the 8 imperfect CTG repeats identified by OSTINATO.

Each identified repeat displayed one or two distinct repeat lengths, which were interpreted as indicative of homozygous or heterozygous loci, although we cannot formally rule out that instability could result in repeat length alterations during cell division. It is exemplified by the *DMPK* repeat, where two distinct repeat lengths were detected for the large allele (the small allele being only 30 CTG triplets long is filtered out of our analysis). It is noteworthy that the imperfect CTG repeat lengths identified by TRF were significantly longer that those identified with OSTINATO. This discrepancy arises

[1] For: Open Solution Tallying Iterative Nucleotides Aligned in Tandem Order

from the implementation of different algorithms for imperfect repeat detection. However, both programs yielded consistent results for the two perfect CTG repeats (Table 1).

Subsequently, ten independent ASA clones were sequenced with long reads, and their genome was analyzed using the same pipeline. This set included one clone in which only the right TALEN arm was transduced, as well as 9 clones in which both arms were transduced. A comprehensive list of all sequenced clones can be found in Supplemental Table S1.

Our expectation was that the right arm alone should not induce any DSB in the ASA genome, thus resulting in CTG repeat lengths identical to the reference genome. Remarkably, this expectation held true for 6 out of 10 perfect and imperfect CTG repeats. Three contractions and one expansion were detected in four repeats, including the large pathogenic allele at the *DMPK* locus. This may be attributed to repeat instability during the growth of the culture, or to a direct effect of the right arm alone. All the other repeats did not show any significant difference with the reference genome. This outcome serves to validate the consistency of the results obtained with the OSTINATO pipeline (Table 1).

In the analysis of the nine clones in which both TALEN arms were transduced, the criterion for validating length changes was arbitrarily set at +/-10% triplets compared to the reference. The majority of these length alterations were contractions (40 out of 49 changes). Expansions were generally limited in length, except in the case of the *DMPK* locus, where two independent clones displayed significant expansion events, from 1764 to 2268 and 2260 CTG triplets (Table 1). When feasible, repeat lengths measured via Southern blots were compared with those obtained through long read sequencing (Supplemental Figure 1). This comparison revealed a strong correlation between the two methods, except in one clone (ASA30). This discrepancy may be attributed to either insufficient genome coverage in this clone, leading to a missed expanded allele, or a

contraction event occurring during the growth of this clone between the DNA extraction used for Southern blotting and the one employed for sequencing.

These findings confirmed the observation obtained by Southern blotting, indicating that TALEN expression predominantly led to contractions of the *DMPK* CTG repeat, albeit often contractions partial. Furthermore, in a minority of instances, limited expansions were also detected. Notably, this results also demonstrate that activation of this nuclease triggered frequent contractions in other long perfect and imperfect CTG repeats within the human genome. These additional contractions likely correspond to off-target DSBs induced by the nuclease.

An engineered recoded TALEN is more efficient at generating CTG repeat contractions

The utilization of lentiviral particles to deliver the TALEN allowed us to investigate their integration sites within the ASA genome. In each clone, at least one right arm and one left arm were successfully identified in the sequence, with the exception of ASA22C where only the right arm could be detected. This likely stems from incomplete genome coverage in this particular clone. To our surprise, all TALEN arms identified exhibited extensive internal deletions within the DNA-binding region. These deletions varied in size, ranging from 5 TALE motifs to encompassing the entire 15 motif DNA-binding region (Figure 2). This observation mirrored what had been previously described for TALENs expressed from lentiviruses (Holkers *et al*, 2013). These deletions strongly indicated that the TALEN activity had been inactivated by these mutations, providing a plausible explanation for the reduced effectiveness in inducing contraction. However, pinpointing the exact timing of their occurrence during the experiment was not feasible, as all clones underwent several cell divisions before being analyzed. These deletions could potentially arise through homologous recombination between repeated motifs within each DNA-binding

domain, or through template switching between internal TALE repeats during reverse transcription of the lentiviral genome, owing to the limited processivity of the reverse transcriptase (Holkers *et al*, 2013).

To address this issue and enhance TALEN efficacy, we posited that reducing nucleotide identity between TALE repeats might also decrease the frequency of template switching. Consequently, we undertook a recoding approach for both TALEN arms, ensuring that contiguous motifs shared no more than 20 identical nucleotides. In addition, the sequence was optimized using the human codon index to eliminate rare codons. A prior study from the Church lab had demonstrated that recoded TALENs maintained comparable efficacies to wild-type TALENs in human cells (Yang *et al*, 2013). Using an in-house program, we generated two DNA sequences encoding the same exact TALEN protein arms, but with reduced nucleotide identity between TALE repeats, from 94%-100% to 65%-78% (Supplemental Figure S2).

Both recoded TALEN arms (referred to as $TALEN_{RECODED}$) were cloned into the same lentiviral vectors and transduced in ASA cells, following the same procedure. These transduced cells were clonally expanded, and total genomic DNA was extracted and analyzed by Southern blotting. It is important to note that the CTG repeat length prior to transduction was higher than in the initial experiment with the wild-type TALEN, primarily due to several cells division occurring between the two experiments. It was estimated to be 2,800 triplets (± 750 triplets, given resolution), by Southern blotting.

Interestingly, it was observed that repeat contractions occurred in 50% of clones that had been transduced with only the left $TALEN_{RECODED}$ arm. This suggests that this arm, by itself, has the potential to induce CTG repeat contractions with significant frequency.

However, contractions increased to 68% when both arms were transduced. In several of these clones, contractions were nearly complete (Figure 3). In certain cases, a smear corresponding to various CTG repeat length was also observed.

Subsequently, nine clones transduced with the $TALEN_{RECODED}$ were sequenced using the PacBio technology, as previously. Among them, two contained only the left arm, while the remaining seven were transduced with both $TALEN_{RECODED}$ arms (Supplemental Table S1). We observed that the two clones transduced solely with the left arm exhibited CTG repeat contractions at the *DMPK* locus, along with several other long CTG repeats (Table 1). This observation implies that the presence of the left arm, which binds to the repeat tract, suffices to induce CTG repeat instability. This instability may arise from the generation of double-strand breaks, single-strand breaks or by interference with the replication machinery through binding to repeat tracts. Clones in which both arms were transduced displayed frequent contractions of the expanded *DMPK* allele, as well as alterations in the lengths of other CTG repeats. This suggests that the new recoded TALEN also triggers off-target mutations. In total, 29 contractions and 9 expansions were detected. It is noteworthy that the expansions observed were rather limited in size and absent in the *DMPK* gene, as opposed to the wild-type TALEN. Furthermore, it is important to highlight that the large expanded allele was undetectable in five out of nine clones, indicating that its length fell below the detection limit of OSTINATO (50 triplets). Additionally, the smears and multiple bands observed by Southern blotting (Figure 3) corresponded to multiple PacBio reads for a given repeat. Overall, the median length of CTG repeats at the *DMPK* locus exhibited a statistically significant shift towards smaller sizes with the $TALEN_{RECODED}$ in contrast to the wild-type TALEN (Figure 4, p-value= 0.0032). This shift demonstrates that the $TALEN_{RECODED}$ was considerably more effective at contracting the CTG repeat tract. Finally, in each clone, at least one intact TALEN arm was detected, except

for C9 for which genome coverage was lower and only the right arm could be detected intact. This result validated our recoding pipeline for future experiments.

Cas9 is less efficient than the TALEN to contract CTG repeats

Meanwhile, it was decided to conduct similar experiments employing the CRISPR-Cas system. Our previous work in *S. cerevisiae* demonstrated that *Streptococcus pyogenes* Cas9 (*Sp*Cas9) was markedly more proficient at inducing a DSB within a CTG repeat tract when compared to *Staphylococcus aureus* Cas9 (*Sa*Cas9) (Poggi *et al*, 2021). Fortunately, both nucleases possess a PAM 3' of the CTG repeat tract (Figure 5A). However, off-target assessments predict 175 sites (including 40 exonic sites) with 0 mismatches in the human genome for *Sp*Cas9, while *Sa*Cas is associated with only 8 sites (including 6 exonic sites). Given the importance of nuclease specificity in potential gene therapy applications, it was chosen to use *Sa*Cas9, despite its lower efficacy compared to *Sp*Cas9.

The gene encoding *Sa*Cas9 along with its associated single-guide RNA (sgRNA) was cloned in lentiviral vector containing the GFP reporter gene, and subsequently transduced into transgenic mice fibroblasts containing a $(CTG)_{250}$ expansion within the human *DMPK* gene. Cells transduced solely with the GFP gene exhibited limited contractions, whereas cells transduced with the nuclease and its corresponding sgRNA displayed substantial contractions (Figure 5B). Quantitative analysis of the signal indicated that 85% of the cells transduced with *Sa*Cas9 exhibited partial or nearly complete contraction of the repeat tract (Figure 5C).

The same lentiviral vectors were also transduced in human ASA cells. Multiple clones were expanded, and the length of the repeat tract was assessed using Southern blotting, as previously described. Although small expansions and contractions were observed in

cells transduced with the GFP alone or in combination with the nuclease, no significant distinction was discerned between the two transduction strategies (Figure 5D). Based on this series of experiments, it was concluded that *Sa*Cas9 demonstrated a much lower efficacy than the TALEN in inducing contractions within an expanded CTG trinucleotide repeat in mouse cells and proved entirely ineffective in human cells.

Materials & Methods

Plasmids

The wild-type TALEN used here is the same as the one used in *S. cerevisiae*, since the CTG trinucleotide repeat integrated in the yeast genome comes from a DM1 patient (Richard *et al*, 2014). First, the right TALEN arm from pCMha188KN16715 (Mosbach *et al*, 2018b) was cloned into pCDNA3.1 (-) (Sigma Aldrich), along with the enhanced GFP gene, to give rise to the pRight plasmid. pMOV1 was built directly in yeast, by homologous recombination between pRight, the left TALEN arm extracted from pCMha182KN9996 (Mosbach *et al*, 2018b) and an *URA3*-CEN-ARS piece of DNA from pRS416 (Sikorski & Hieter, 1989). Both TALEN arms are under the control of the CMV promoter.

For lentiviral expression, the left TALEN arm was cloned into pHR'CMVGFP_hRIF1(1924-2446)IREShygro3 (Addgene #23138) to give plasmid pTRI204. The right arm was cloned into pLVbcNEOPGK-IRES-Neo to give plasmid pTRI205. For Cas9 lentiviral expression, we used a *Sa*Cas9 and GFP-containing backbone (Addgene #118836, plasmid pTRI211) into which was cloned the *Sa*Cas9-CTG sgRNA to give plasmid pTRI 212.

The two recoded TALEN arms were ordered as synthetic pieces of DNA delivered into the pUC57-Mini vector (GenScript). The left recoded arm was cloned in pHR'CMVGFP_hRIF1(1924-2446)IREShygro3 (Addgene #23138) at BamHI and XhoI to

give plasmid pTRI213. The right recoded arm was cloned in pLVbcNEOPGK-IRES-Neo at AgeI and SalI to give plasmid pTRI214. All plasmid maps were created with SnapGene and are shown in Supplemental Figure S3.

Mice fibroblasts

Fibroblasts were isolated from transgenic mice lungs, carrying a hemizygous $(CTG)_{600}$ expansion from a human DM1 patient (Gourdon *et al*, 1997) (cell line 6423). Transfection was performed using the Viafect commercial reagent (Promega), following manufacturer conditions. Cells were transfected with the pRight plasmid, containing only the right TALEN arm, or with the pMOV1 plasmid, containing both TALEN arms. GFP-positive cells were sorted on the Institut Pasteur cytometry platform. Cells were collected 48 hours after transfection, whole genomic DNA was extracted and analyzed by PCR, using primers ST300f (GAACTGTCTTCGACTCCGGG) and ST300r (GCACTTTGCGAACCAACGAT) as previously described (Tomé *et al*, 2009).

Human ASA fibroblasts

Immortalized ASA cells (Arandel *et al*, 2017) were grown at 37°C in a 5% CO_2 humidified atmosphere, in DMEM (Gibco #61965) supplemented with 15% fetal bovine serum (FBS). Cells were passaged by briefly washing with PBS before using trypsin for 5 min at 37°C to dissociate cells, before being split in two flasks. For long-time conservation, cells were frozen in 90% FBS +10% DMSO on dry ice, before being stored in liquid nitrogen. For lentiviral transductions cells were seeded one day before at 55,000 cells/ well in 12-well plates. In the morning, media was changed for 500 µL of DMEM +5% FBS +4 µg/mL of polybrene (Merck TR-1003-G). Lentiviruses were diluted to the desired MOI in 500 µL of DMEM +5% FBS and slowly added to each well. Cells were incubated at 37°C in a 5% CO_2

humidified atmosphere overnight. The following morning, 1 mL of fresh DMEM +25%FBS was added. The following day, the medium was changed to 500 µL of DMEM +15% FBS, antibiotics selection pressure was added and maintained for 1 week.

Ring cloning

Cells were diluted to reach between 10 to 100 cells per plate. Media was changed every week until small colonies of cells were detectable under the microscope (usually around 1 month). Media was removed, plastic rings were glued around each colony using silicone. After washing with PBS, trypsin was added inside the ring to dissociate the cells. Colonies were seeded into 96-well plates. Cells were expanded reaching the desired number of cells, by passaging into 24-well plates, 6-well plates, T25 and T75 flask, for an overall culture time of 2-3 months.

DNA extraction and Southern blotting

Around $1x10^6$ cells were harvested from one T75 flask, washed in PBS and resuspended in 300 µL lysis buffer (100 mM Tris-Hcl pH8.0, 5 mM EDTA pH8.0, 0.2% SDS and 200 mM NaCl) supplemented with 600 µg of proteinase K, and incubated overnight in a water bath at 55°C. In the morning, 300 µl of phenol-CIAA were added to a Phase Lock Gel (Light) microtube (QuantaBio #2302820), following manufacturer instructions. The cell lysate is collected, added to the phenol-CIAA and manually mixed several times (no vortexing). After centrifugating at 13,000 rpm, the aqueous phase was collected, and DNA was extracted using Chloroform-IAA in a new Phase Lock Gel microtube. Collected DNA was subsequently precipitated by adding 1/10 volume of 3M NaCl and 3 volumes of absolute ethanol. Centrifugation at 13,000 rpm was carried out at 4°C for 1 hour. DNA pellet was washed in 70% ethanol and resuspended in TE.

For Southern blotting, 10 µg of DNA were digested with BamHI (60U, NEB) overnight. DNA was loaded in a 0.9% agarose +BET gel, in 1X TBE. Samples were run at 1V/cm, in 1X TBE at 4°C, overnight. A picture of the gel after migration was taken under a BioRad imaging system. The gel was quickly rinsed in distilled water and treated for one hour in 1M NaOH, followed by 2 hours in Tris 1M pH8.5, NaCl 3M. The gel was manually transferred onto a positively charged nylon Hybond-XL membrane (Cytiva #RPN203S), overnight in 6X SSC. The membrane was crosslinked under a Stratalinker at 0.120 J/cm^2 (Uvitec). Prehybridization was carried tout into Sigma PerfectHybridPlus solution at 68°C for 30 minutes. To prepare the probe, B1.4 plasmid containing a cloned 3'UTR of DMPK gene was digested with BamHI and the 1390 bp band was gel purified. The probe was labeled with alpha-32P CTP using the High Prime DNA labeling kit (Roche, #11585584001). The labelled probe was purified on a G50 sephadex column (Cytiva, #28903408) and denatured for 5 minutes at 95°C. Hybridization was carried out at 68°C overnight. Two 20 min washes were carried out in high stringency buffer (0,5% SSC + 0,1% SDS), followed by one wash in ultra high stringency buffer (0,1% SSC + 0,1% SDS). The membrane was wrapped in plastic and exposed to a phosphorimager screen for 1-2 days (or more if signal was weak).

Bioinformatics

Off-target predictions for *Sp*Cas9 and *Sa*Cas9 were evaluated using the online CRISPOR tool (Haeussler *et al*, 2016), on the human GRCh38 reference assembly. The OSTINATO pipeline is available on request to S. D.-D. or G.-F. R. and will be extensively described elsewhere.

Statistics

Length distributions at the *DMPK* locus following TALEN or $TALEN_{RECODED}$ expression were compared using the Student t-test. Off-target expansions and contractions observed with the TALEN or the $TALEN_{RECODED}$ were compared using a Fisher exact test. All Statistics were performed using the R package (Millot, 2011).

PacBio sequencing

Total genomic DNA was prepared as for Southern blotting, except that after Proteinase K treatment, the remaining pellet debris were not resuspended by pipetting but by gentle taping on the side of the tube, in order to avoid breaking high molecular weight DNA. Subsequent pipetings during phenol-CIAA treatments were performed with blue cones. PacBio libraries were prepared on the Biomics platform (Institut Pasteur), as follows. Genomic DNAs of clones in which the WT TALEN was transduced (ASA30, ASA51, ASA2, ASA53, ASA54, ASA27, ASA111E, ASA22C, ASA28 and ASA03-44, Supplemental Table S1) were sheared in g-tubes (Covaris) to an average of 50 kb length. Libraries were prepared with the SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). Sequences were performed on the Sequel PacBio machine at the Pasteur Biomics platform using the Sequel Binding Kit 3.0 and the Sequel DNA Internal Control 3.0 (Pacific Biosciences). Genomic DNAs of the reference genome (ASA3-p11) and of clones in which the $TALEN_{RECODED}$ was transduced (C3, C6, C9, C11, C12, C15, C16, C3_Left and C5_Left, Supplemental Table S1) were sheared in g-tubes to an average of 15 kb length. Libraries were prepared with a SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). Sequences were performed on a Sequel II machine, at the Institut Curie ICGex NGS platform. Loading concentration of each sample was 6 pM and run time was 20 hours for both sets of clones.

Discussion

Myotonic dystrophy type 1 is triggered by the expansion of a CTG trinucleotide repeat within the 3' UTR of the *DMPK* gene, located on the human chromosome 19. In the present study, we have demonstrated the remarkable efficacy of a TALEN in contracting the expanded repeat in transgenic mice cells and human DM1 cells. Previous therapeutic strategies for treating DM1 have primarily focused on inhibiting *DMPK* expression (Pascual-Gilabert *et al*, 2021), or targeting the CUGexp-mRNA to prevent its interaction with the MBLN1 protein (reviewed in Izzo *et al*, 2022). However, thus far, none of these approaches has advanced beyond clinical trial stages.

At the DNA level, various attempts have been made to completely remove the CTG repeat tract by inducing double-strand breaks upstream and downstream using the CRISPR-Cas9 system. Enhanced *Streptococcus pyogenes* Cas9 (e*Sp*Cas9) was transfected into immortalized myoblasts harboring 300-1,000 CTG triplets within the *DMPK* locus. Among the 85 clones examined, only 12 exhibited a pattern consistent with the complete deletion of one or two alleles. Seven out of these 12 clones exhibited a marked reduction in nuclear foci (Provenzano *et al*, 2017). PCR amplifications were conducted on seven putative off-target sites to confirm the absence of other genomic modifications.

In another study, wild-type *Sp*Cas9 was expressed in iPSC derived from DM1 patients with the same objective (Dastidar *et al*, 2018). Among 25 independent clones analyzed, 19 demonstrated deletions of the CTG repeat tract. However, nine of these exhibited deletions larger than the intended target locus, resembling observations made in *S. cerevisiae* cells using the same nuclease and a similar approach (Mosbach *et al*, 2020). *Sp*Cas9 was also expressed in DM1 patient myoblasts, which carried 2,600 CTG triplets on the expanded mutant allele and 13 triplets on the wild-type allele. Among the 103 myoblasts clones examined, 35% exhibited deletions of the repeat tract on both alleles,

while 51% showed deletions solely on the mutant allele (Agtmaal *et al*, 2017). Notably, neither of these last two reports conducted an analysis of putative off-target sites.

Lo Scrudato and colleagues utilized *Staphylococcus aureus* Cas9 (*Sa*Cas9), a smaller nuclease compared to *Sp*Cas9 and more suitable for co-packaging with the sgRNA within the same viral particle (Lo Scrudato *et al*, 2019). They expressed *Sa*Cas9 in DM1 myoblasts carrying 2,600 CTG triplets on the mutant allele. Fifty independent clones expressing the nuclease were isolated. Among these clones, five did not exhibit significant nuclear foci. Subsequently, the authors expressed the same nuclease in muscle fibers of transgenic mice harboring a large CTG expansion (1,200 CTG triplets) at the *DMPK* locus integrated in their genome (Gomes-Pereira *et al*, 2007). PCR analysis conducted on muscle fibers suggested that approximately 10% of the nuclei contained a deletion of the repeat tract. Unfortunately, no analysis of putative off-target sites was conducted.

Alternative strategies directed at modifying DNA include the insertion of a polyA signal upstream of the CTG expansion through TALEN-driven homologous recombination (Xia *et al*, 2015) and the utilization of a Zinc-Finger Nuclease (ZFN) or *Sp*Cas9 to contract CTG repeats in a HEK293 cell model containing a GFP-reporter gene that becomes active upon contraction of the CTG repeat tract (Cinesi *et al*, 2016). This latter approach closely resembles what we proposed nearly a decade ago using a TALEN (Richard *et al*, 2014). In the HEK293 experimental system, the authors observed expansions and contractions upon the introduction of a ZFN or *Sp*Cas9 targeting the CTG trinucleotide repeat. Notably, these events were more frequent when employing ZFN as compared to *Sp*Cas9. Intriguingly, when expressing a *Sp*Cas9 D10A mutant in which one of the nuclease activities was disabled, expansions occurred with a reduced frequency. Furthermore, a four-fold increase in contractions was reported when the *Sp*Cas9 nickase was co-expressed alongside its corresponding sgRNA, compared to control cells.

In the present study, we applied the previously successful approach used in budding yeast to contract an expanded CTG repeat to non-pathological length. The initial wild-type TALEN (Richard *et al*, 2014) exhibited high efficiency in murine fibroblasts carrying 600 CTG triplets. However, when the same TALEN was transduced in human cells carrying 1800 triplets, we observed less frequent contractions, of limited magnitude (Figure 1). Moreover, internal deletion occurring via homologous recombination were identified in each TALEN arm. The precise timing of these deletions remains unclear, whether they happened during reverse transcription of the lentiviral RNA genome or after integration in the ASA genome. Nevertheless, it is plausible to hypothesize that the TALEN had been actively expressed for some duration before becoming inactivated due to deletions. This hypothesis is supported by the observation of contractions occurring at the *DMPK* locus and other CTG repeats which were not evident when the same cells were transduced with the right arm alone.

By recoding the TALEN using the degeneracy of the genetic code, we were able to introduce two significant modifications. First, the recoding reduced the sequence similarity between motifs from 94%-100% to 65%-78%. This resulted in the retention of the TALEN as full-length arms in each clone analyzed, albeit some clones contained additional mutated copies of each arm. It is worth noting that recoded TALENs developed by the Church lab have previously demonstrated comparable efficacy to wild-type TALENs in inducing homologous recombination at the AAVS1 locus in hiPSCs (Yang *et al*, 2013). These recoded TALENs could also be efficiently packaged into lentiviral particles and transduced into HEK293T cells without compromising the sequence integrity of the TALE repeats. In our case, the $TALEN_{RECODED}$ was engineered to ensure that no more than 20 nucleotides of identity remained between two TALE motifs. Furthermore, rare codons were eliminated to optimize its expression in human cells. This customized $TALEN_{RECODED}$

outperformed the original TALEN in inducing CTG repeat contraction at the DMPK locus (Table 1 and Figure 5). In addition, it reduced the incidence of homologous recombination between TALE repeats, thus ensuring the preservation of at least one full-length arm in each clone. Of particular interest, the $TALEN_{RECODED}$ did not substantially elevate the occurrence of off-target contractions or expansions in other long CTG repeats (p-value=0.59 for contractions, p-value = 1.00 for expansions). This observation highlights that enhancing on-target efficacy does not necessarily lead to an increase in undesired off-target mutations. Mutations in other CTG trinucleotide repeats fall in 5 cases in genes and in 4 cases in intergenic regions. All the mutations in genes are intronic, none of them is in the coding sequence (Table 1). It is therefore reasonable to assume that their impact on gene function will be negligible.

Notably, *Sa*Cas9 displayed lower efficacy compared to the TALEN in triggering CTG repeat contractions in transgenic mice cells and was entirely ineffective in ASA cells (Figure 4). This lack of efficacy in inducing DSBs within CTG repeats mirrors observations made in *S. cerevisiae* when using the same repeats and the same nuclease. This highlights the extreme value of the yeast model in assessing the efficacy of forthcoming DNA endonucleases (Poggi *et al*, 2021).

It is noteworthy that the left arm alone triggers repeat contractions in ASA cells, a surprising finding that raises several intriguing possibilities. One hypothesis is that it may self-dimerize on the CTG repeat, potentially causing a DSB, as previously proposed by others (Liu *et al*, 2010). Alternatively, it is possible that the left arm induces a single-strand break (nick) that proves sufficient to trigger repeat contractions. Notably, one single FokI domain has been demonstrated to induce nicks on a supercoiled plasmid (Bitinaite *et al*, 1998). This latter hypothesis is compatible with findings from the Dion lab which showed that the *Sp*Cas9 nickase induces CTG repeat contractions in HEK293T cells

(Cinesi *et al*, 2016). Another possibility is that the binding of the left arm to the CTG repeat tract might trigger contractions by interfering with replication during growth of cell cultures. This effect would not be observed with the right arm alone, as it binds to the *DMPK* locus in only one copy, whereas the left arm may bind multiple times along the expanded CTG triplet repeat.

Future directions for this research encompass enhancing the TALEN specificity. This can be achieved by using different FokI domains (Miller *et al*, 2007), and examining alternative RVD for the TALE repeats. Furthermore, it is imperative to delve into the precise mechanism by which the left arm, when used alone, triggers CTG repeat contractions. Understanding this mechanism, holds the potential to provide a viable alternative to DSBs in the long-term vision of establishing an efficient gene therapy approach. Such an approach could be applied not only for DM1 but also to address all other microsatellite expansion disorders.

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Figure legends

Figure 1: TALEN design and expression in mice and human cells

A: TALEN design, showing the respective binding sites of each arm at the 3' end of the *DMPK* gene (Richard *et al*, 2014). **B:** TALEN expression in mice fibroblasts containing 600 CTG triplets. pRight: transfection with the right arm only. TALEN: transfection with both arms (pMOV1). The CTG repeat was amplified with primers ST300f and ST300r. C: TALEN expression in ASA cells. The Southern blot shows independent clones in which the right arm alone (-TALEN) or both arms (+TALEN) were transduced. Red arrows point to expansions, green arrows to contractions. Repeat lengths are shown in triplets, as determined by measurements on the blot.

Figure 2: Genomic locations and integrity of TALEN arms after transduction in ASA cells.

For each clone, the chromosome number (Chrom.) as well as the nucleotide after which the arm was integrated is shown (Insertion). Empty boxes show the extent of internal deletions in each arm. ND: the insertion point could not be precisely determined due to low sequence quality.

Figure 3: $TALEN_{RECODED}$ transduction in ASA cells.

A: The Southern blot shows independent clones in which the left arm alone (Left) or both arms ($TALEN_{RECODED}$) were transduced. GM09197: Huntington's disease patient fibroblasts used as a control. WT TALEN: ASA cells before transduction. GReen arrows point to contractions, green brackets to smears. **B:** length distributions of CTG repeats at the *DMPK* locus following transductions with the wild-type TALE (top) or the $TALEN_{RECODED}$ (bottom).

Figure 4: Length distributions of CTG repeats at the DMPK locus, following TALEN or TALEN$_{RECODED}$ expression.

Only clones in which the large allele could be detected are shown (see Table 1). The X axis shows the length categories (in triplets), the Y axis shows the percentage of HiFi reads in each category. **A:** Length distribution following transduction with the TALEN. **B:** Length distribution following transduction with the TALEN$_{RECODED}$. Black bars: ASA3-p11 reference genome, dark grey bars: cells transduced with one arm alone, light grey bars: cells transduced with both arms.

Figure 5: *Staphylococcus aureus* Cas9 expression in mice and human cells.

A: Design of the sgRNA and location of the PAM on the 3' sequence of the *DMPK* gene. **B:** Transduction of GFP alone or *Sa*Cas9, its sgRNA and GFP in heterozygous transgenic mice fibroblasts carrying 250 CTG triplets. The CTG repeat was amplified with primers ST300f and ST300r. **C:** Quantifications of PCR signals from B. **D:** The Southern blot shows independent ASA clones in which the GFP alone or *Sa*Cas9, its sgRNA and GFP were transduced. Although small contractions or expansions could be detected in some clones, no significant differences were found between both experiments.

Supplemental Figure S1

Correlation between CTG repeat length measured on Southern blot (X axis) and PacBio sequencing (Y axis).

Supplemental Figure S2

Top: Sequence comparison of TALE repeats between the original wild-type TALEN and the TALEN$_{RECODED}$. Red rectangles show sequence stretches at least 3 nucleotide long that

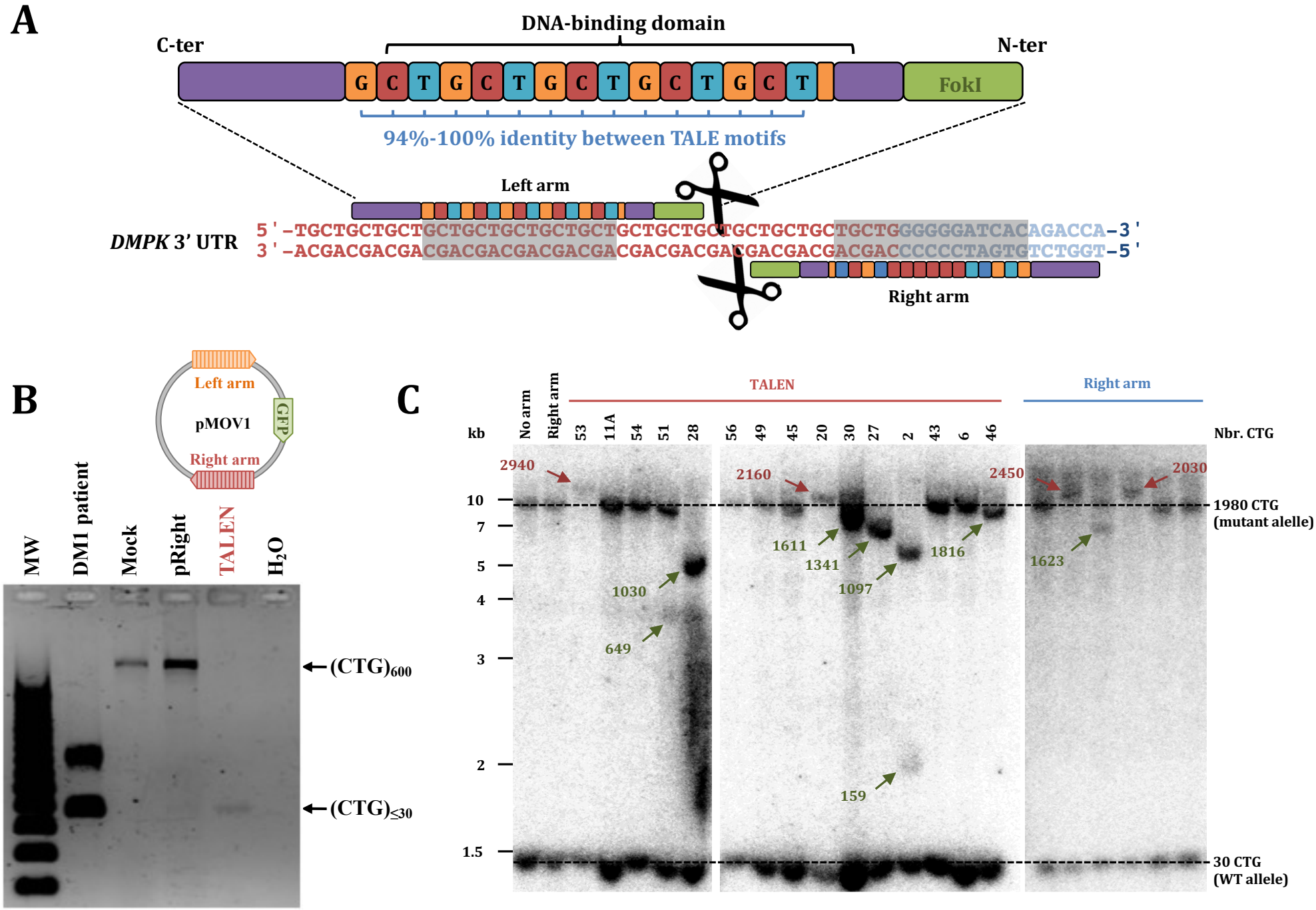
were conserved between the original and the recoded motif. The two large black rectangles underline the six nucleotides encoding the RVD. **Bottom**: Multiple alignments of the 15 TALE motifs of each arm, for the TALEN and the $TALEN_{RECODED}$. Alignments were performed using Clustal Omega, minimum and maximum identity percentages between two motifs were calculated by MView. Both programs were run on the NCBI-EBI multiple alignment portal (https://www.ebi.ac.uk/Tools/msa/).

Supplemental Figure S3

Plasmids used in this study. They are all available on request to G.-F. R.

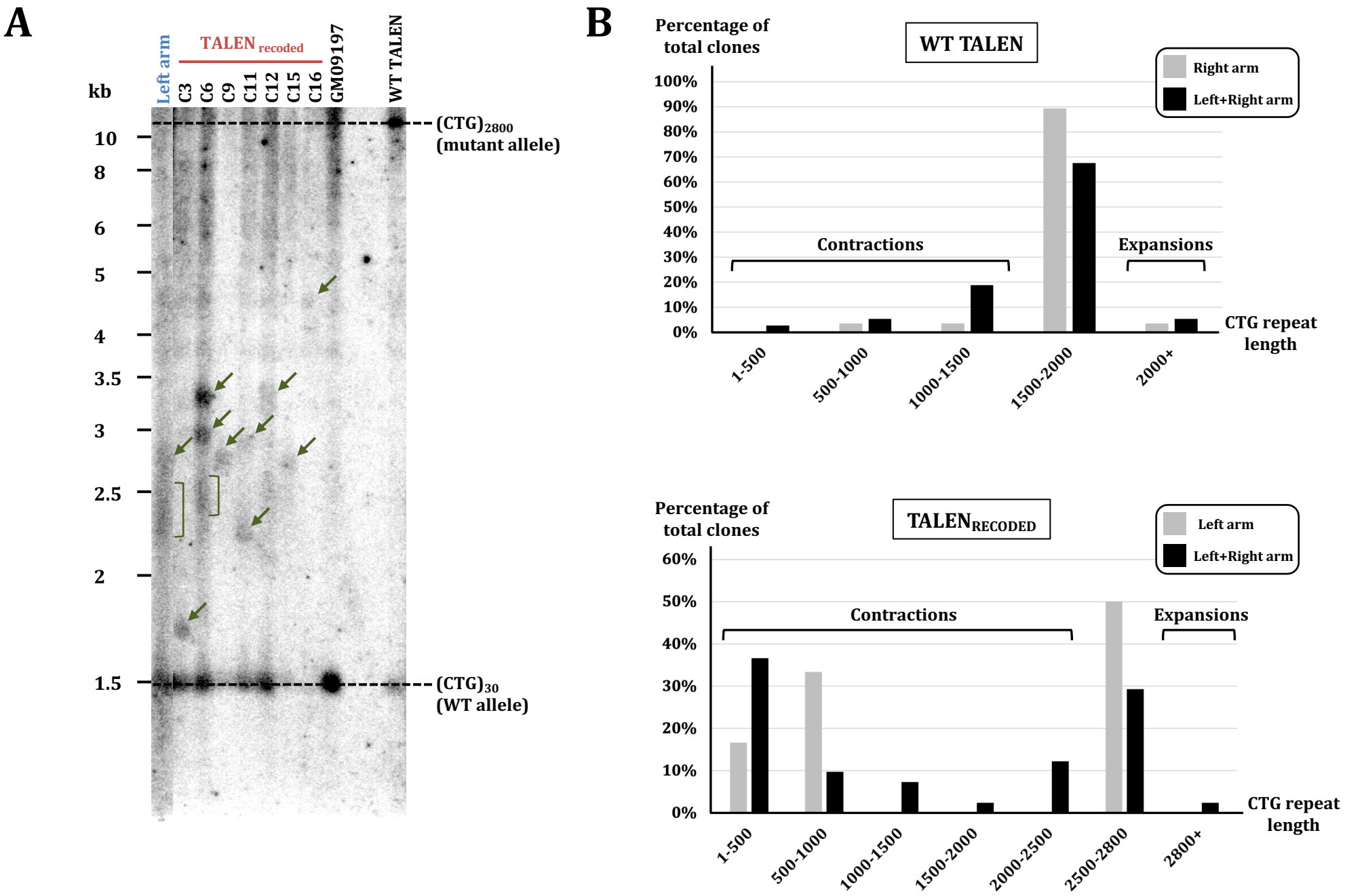
Supplemental Table S1

Summary of PacBio sequences. For each clone, TALEN arms, the number of SMRTCells and genome coverage obtained by sequencing are shown. Note that TALEN clones were sequenced on a Sequel machine, whereas $TALEN_{RECODED}$ clones were sequenced on a Sequel II.

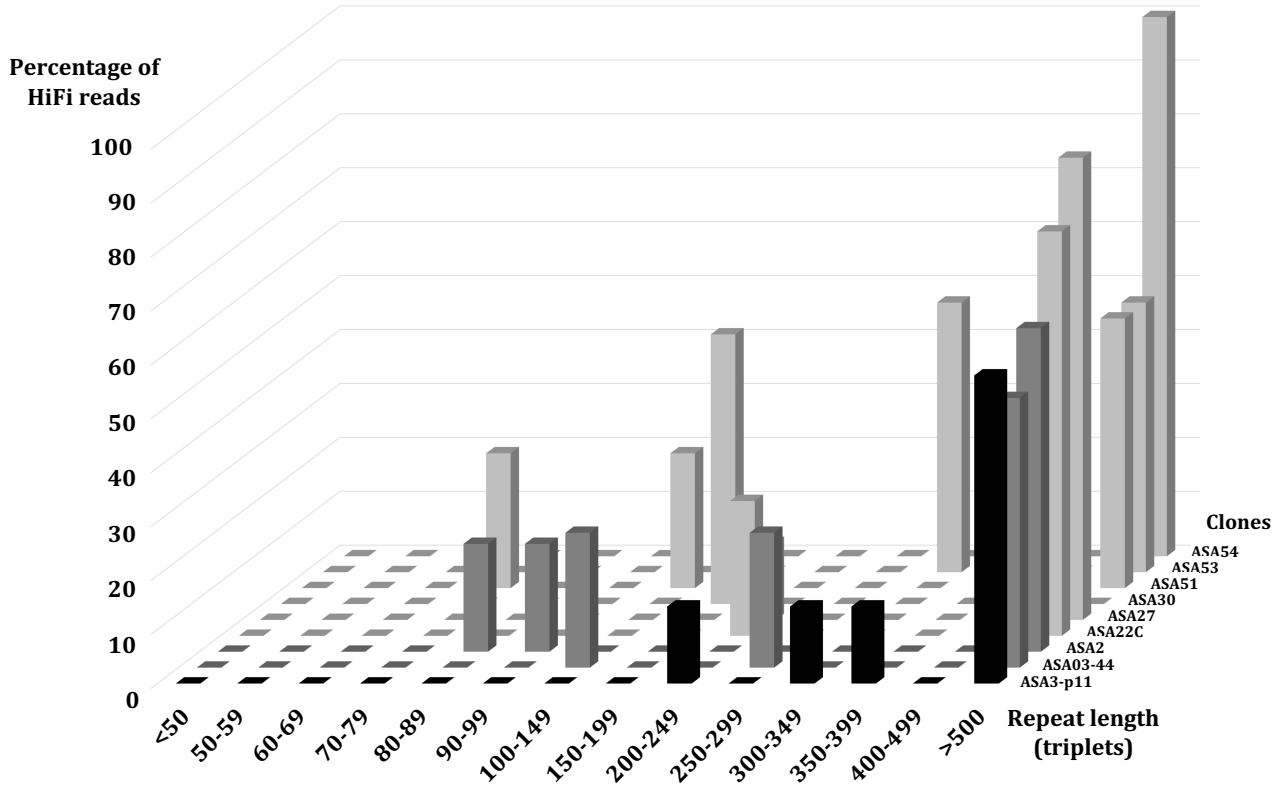

A
DNA-binding domain
C-ter
N-ter
G C T G C T G C T G C T G C T
FokI
94%-100% identity between TALE motifs
Left arm
DMPK 3' UTR
5'-TGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGGGGGGATCACAGACCA-3'
3'-ACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACCCCCCTAGTGTCTGGT-5'
Right arm
B
Left arm
pMOV1
GFP
Right arm
MW
DM1 patient
Mock
pRight
TALEN
H2O
(CTG)600
(CTG)≤30
C
kb
No arm
Right arm
TALEN
Right arm
53
11A
54
51
28
56
49
45
20
30
27
2
43
6
46
Nbr. CTG
2940
2160
2450
2030
10
7
5
4
3
2
1.5
1980 CTG
(mutant alelle)
1611
1341
1097
1816
1623
1030
649
159
30 CTG
(WT allele)

WT TALEN

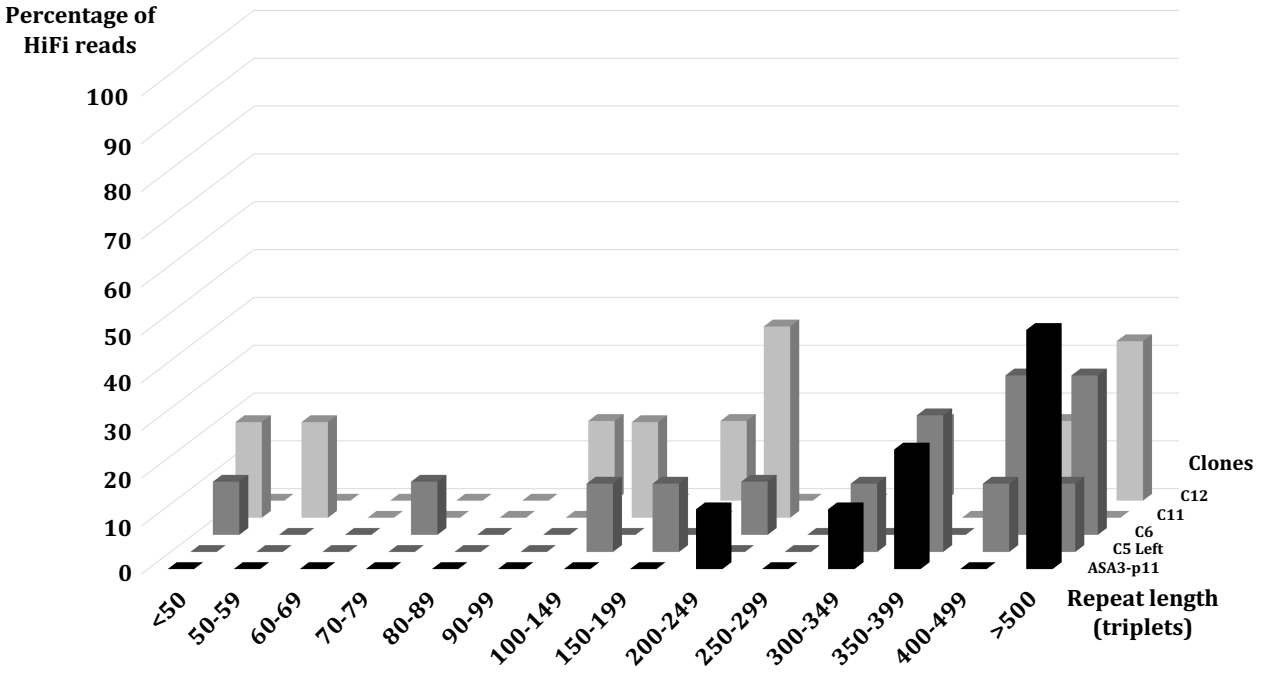
Clone	Chrom.	Insertion	TALEN arms	
ASA30	16	53,203,762		L
	17	40,248,469		R
ASA51	3	43,705,128		L
	12	104,116,196		L
	X	120,541,797		R
	11	65,075,578		ND
ASA2	1	226,251,110		L
	12	35,994,594		R
ASA53	1	226,243,903		L
	15	40,369,424		R
ASA54	1	226,247,770		L
	15	40,382,620		R
ASA27	1	226,248,197		L
	X	78,103,806		R
ASA111E	21	6,722,176		L
	4	142,097,315		R
	7	49,511,359		R
ASA22C	4	142,102,943		R
ASA28	20	25,597,534		L
	12	54,013,787		R
ASA03-44*	ND	ND		L


A
kb
Left arm
TALEN recoded
C3
C6
C9
C11
C12
C15
C16
GM09197
WT TALEN
10
8
6
5
4
3.5
3
2.5
2
1.5
(CTG)2800
(mutant allele)
(CTG)30
(WT allele)
B
Percentage of total clones
WT TALEN
Right arm
Left+Right arm
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Contractions
Expansions
CTG repeat length
1-500
500-1000
1000-1500
1500-2000
2000+
Percentage of total clones
TALENRECODED
Left arm
Left+Right arm
60%
50%
40%
30%
20%
10%
0%
Contractions
Expansions
CTG repeat length
1-500
500-1000
1000-1500
1500-2000
2000-2500
2500-2800
2800+

A


Percentage of HiFi reads
100
90
80
70
60
50
40
30
20
10
0
<50
50-59
60-69
70-79
80-89
90-99
100-149
150-199
200-249
250-299
300-349
350-399
400-499
>500
Repeat length (triplets)
Clones
ASA54
ASA53
ASA51
ASA30
ASA27
ASA22C
ASA2
ASA03-44
ASA3-p11

B


Percentage of HiFi reads
100
90
80
70
60
50
40
30
20
10
0
<50
50-59
60-69
70-79
80-89
90-99
100-149
150-199
200-249
250-299
300-349
350-399
400-499
>500
Repeat length (triplets)
Clones
C12
C11
C6
C5 Left
ASA3-p11

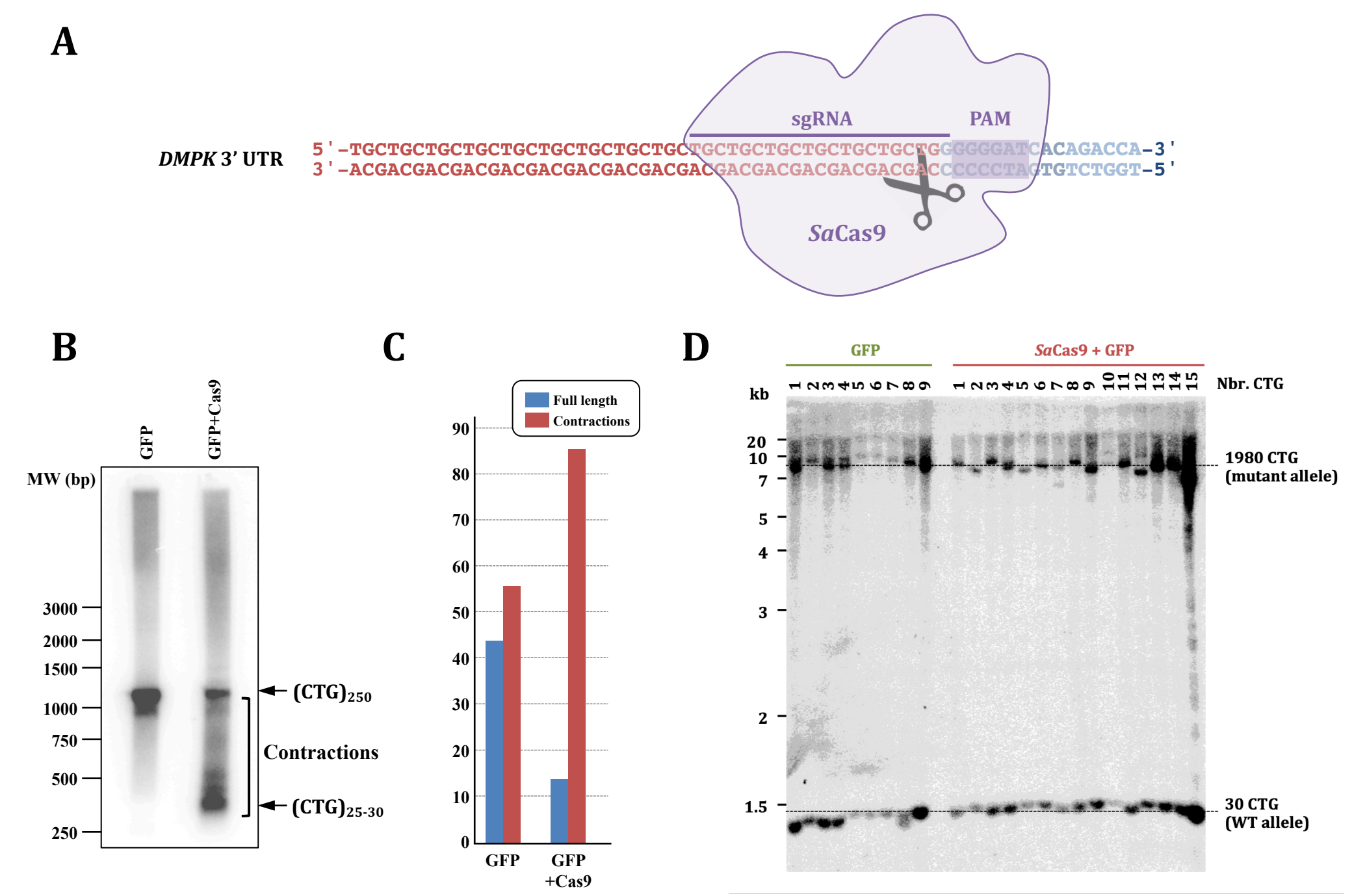

A
DMPK 3' UTR
5'-TGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGGGGGATCACAGACCA-3'
3'-ACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACCCCCCTAGTGTCTGGT-5'
sgRNA
PAM
SaCas9
B
GFP
GFP+Cas9
MW (bp)
3000
2000
1500
1000
750
500
250
(CTG)250
Contractions
(CTG)25-30
C
Full length
Contractions
90
80
70
60
50
40
30
20
10
0
GFP
GFP +Cas9
D
GFP
SaCas9 + GFP
kb
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Nbr. CTG
20
10
7
5
4
3
2
1.5
1980 CTG (mutant allele)
30 CTG (WT allele)

Table 1: Median length of perfect and imperfect CTG repeats as determined by PacBio sequencing

Grey numbers identify length differences of +/- 10% relative to the ASA3-p11 reference

Repeat consensus	Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Perfect (CTG)61		DMPK CTG expansion		
Chromosome	**2**		**3**		**4**		**6**		**8**		**10**		**15**		**15**		**17**		**19**		
Repeat ID#	2p13_1		3p25		4q35		6q25		8q24		10q24		15q11		15q23_2		17q21_2		19q13		
Gene	*SNRNP27*		*TPRXL*		-		*SLC22A22*		-		-		-		*MYO9A*		*CA10*		*DMPK*		
Location	Intron		Intron		Intergenic		Intron		Intergenic		Intergenic		Intergenic		Intron		Intron		3' UTR		
ASA3_p11 (reference)	84	86		125	78	110	101		62		52		42		86	86	61			311	1764
ASA03-44 (right arm)	84	86	71	124	78	109	100		62		51		42	66	75	86	64			194	1872
ASA2	82		81	121	71	105			61						56	88	60		93	632#	1094
ASA22C	69	78	108	121	67	79			37	55				54	57		57		238	559#	1638
ASA27	84	86		125	79	110	103	185	62		51		57	69	75	87	61		202		1264
ASA28	75		72	124	80	101				54					79	86					
ASA30					62	77									62		51			417#	
ASA51	68	81		122	66	102			61						59	83	57		89	574#	2260
ASA53	67	83	69	126	78	109			62		53		52	69	59	90	57			338	>1053*
ASA54	76	86		123	78	110	101		62		52		45		78	91	61		1154	1775	2268
ASA111E	56	77			62				51					60	61		61				
Found by TRF	148		200		116	158	109	592	No		147	163	No		157		64			316	1766
Contractions (40)	6	1	5		4	2			2	2					8		1		5	1	3
Expansions (10)								1					2	5							2

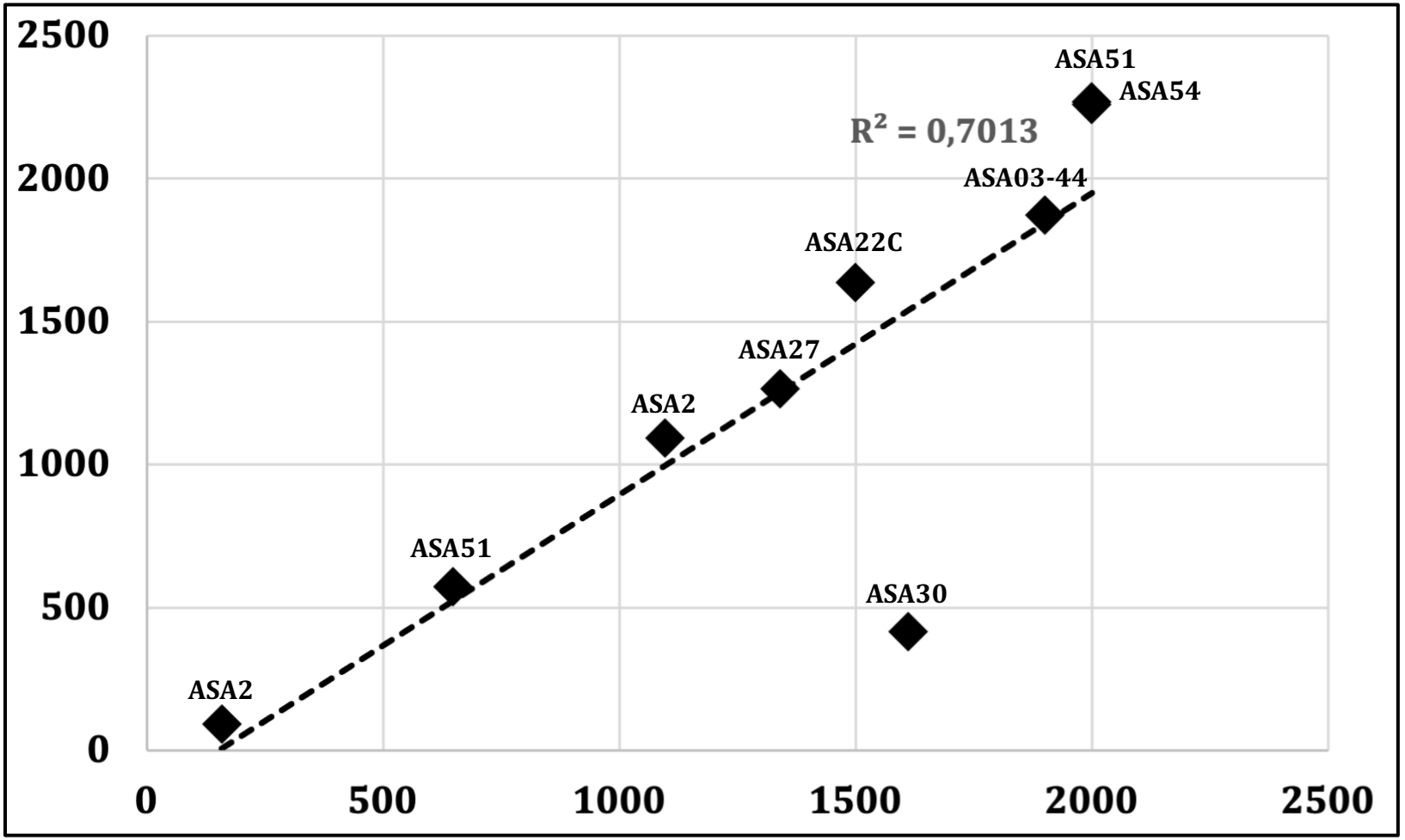
Repeat consensus	Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Imperfect CTG		Perfect (CTG)61		DMPK CTG expansion		
Chromosome	**2**		**3**		**4**		**6**		**8**		**10**		**15**		**15**		**17**		**19**		
Repeat ID#	2p13_1		3p25		4q35		6q25		8q24		10q24		15q11		15q23_2		17q21_2		19q13		
Gene	*SNRNP27*		*TPRXL*		-		*SLC22A22*		-		-		-		*MYO9A*		*CA10*		*DMPK*		
Location	Intron		Intron		Intergenic		Intron		Intergenic		Intergenic		Intergenic		Intron		Intron		3' UTR		
ASA3_p11 (reference)	84	86		125	78	110	101		62		52		42			86	61			311	1764
C3_Left (left arm)	79	84	111	124	79	110	100	178	61	51	50		59		77	87	61				
C5_Left (left arm)		85		125	67	110	100	186	60		51				65	87	63	55	143	389#	
C3	77	84		125	73	110	100		61	54	54		49	63	81	87	60				
C6	61	85	86	125	78	109			62	55	52		52		57	86	62	54	57	231	510#
C9				123	78	109			61		51				62						
C11	78	85	97	124	67	109			62		51		66		60	86	60	55	50	149	227
C12	63	84	111	125	78	110	100		61		51		51	70	52	84	61		99	199	475#
C15																					
C16																					
Contractions (29)	2		4		2					3					7			3	4	3	1
Expansions (9)								2					5	2							

+/- 10% difference

(Lengths in triplets)

* the repeat is not entirely covered by an HiFi read, so precise length cannot be determined

\# it is not possible to determine whether these length changes correspond to contraction of the large allele or expansion of the smaller one


2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
ASA51
ASA54
R² = 0,7013
ASA03-44
ASA22C
ASA27
ASA2
ASA51
ASA30
ASA2

Left TALEN arm Binding domain: $G_1C_1T_1G_2C_2T_2G_3C_3T_3G_4C_4T_4G_5C_5T_5$

```
>G1 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G1 (recoded)    TTGACACCTCAACAAGTTGTTGCTATTGCATCAAATAACGGAGGTAAGCAGGCACTGGAGACCGTTCAGCGGCTGCTCCCTGTGCTTTGTCAAGCTCATGGT
>C1 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C1 (recoded)    CTTACTCCAGAACAAGTCGTCGCAATCGCTTCCCATGACGGGGGCAAACAGGCCCTTGAGACTGTCCAACGCTTGTTGCCAGTTTTGTGCCAGGCACACGGA
>T1 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T1 (recoded)    CTGACTCCCCAACAGGTGGTAGCTATCGCCTCTAACGGTGGCGGAAAGCAAGCTTTGGAGACAGTCCAAAGGCTCCTGCCCGTTCTCTGCCAGGCCCACGGC
>G2 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G2 (recoded)    CTTACACCACAACAAGTTGTTGCTATTGCTAGTAACAATGGCGGAAAACAAGCCCTGGAGACTGTGCAGCGGCTCCTTCCTGTCCTTTGTCAGGCTCATGGG
>C2 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C2 (recoded)    CTCACTCCTGAGCAGGTCGTCGCAATCGCATCACATGACGGCGGGAAGCAAGCATTGGAGACCGTTCAACGCCTGCTGCCAGTGTTGTGTCAAGCACACGGA
>T2 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T2 (recoded)    CTGACCCCCCAGCAAGTCGTCGCAATCGCTAGCAATGGTGGAGGTAAACAGGCTCTGGAAACAGTGCAAAGACTTCTCCCCGTCCTCTGCCAAGCCCACGGT
>G3 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G3 (recoded)    TTGACACCTCAACAAGTTGTTGCAATTGCAAGCAATAACGGCGGCAAACAGGCCCTGGAAACTGTGCAGCGCCTTCTCCCAGTTTTGTGTCAGGCACATGGA
>C3 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C3 (recoded)    CTTACTCCAGAACAGGTGGTCGCTATTGCTAGTCATGACGGAGGAAAGCAAGCTCTTGAAACAGTTCAACGGCTCCTTCCTGTTCTTTGCCAAGCTCATGGG
>T3 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T3 (recoded)    CTCACGCCTCAGCAAGTGGTCGCCATAGCCTCAAATGGAGGAGGTAAGCAAGCATTGGAAACCGTCCAAAGATTGTTGCCCGTGCTCTGCCAGGCCCATGGT
>G4 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G4 (recoded)    CTGACACCTCAACAAGTCGTCGCAATTGCTTCCAACAATGGAGGCAAGCAGGCTCTGGAAACTGTCCAGAGGCTGCTCCCCGTCTTGTGTCAAGCTCATGGT
>C4 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C4 (recoded)    TTGACTCCAGAGCAGGTTGTTGCTATAGCATCACATGACGGCGGAAAACAGGCCCTGGAGACCGTGCAGAGACTTCTTCCTGTGCTTTGCCAAGCACACGGC
>T4 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T4 (recoded)    CTCACCCCTCAACAAGTAGTGGCCATTGCCTCTAACGGGGGCGGTAAGCAAGCATTGGAAACAGTTCAACGCTTGCTGCCAGTCCTCTGTCAGGCCCATGGG
>G5 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G5 (recoded)    CTCACTCCTCAACAAGTGGTTGCTATTGCTTCTAATAATGGAGGGAAACAAGCTCTTGAAACCGTTCAGCGGCTCCTTCCAGTGCTCTGTCAAGCACATGGA
>C5 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C5 (recoded)    CTTACACCAGAACAGGTTGTCGCAATAGCAAGTCATGACGGGGGCAAGCAGGCCCTTGAGACAGTCCAAAGACTTTTGCCTGTTCTTTGCCAAGCTCACGGC
>T5 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T5 (recoded)    TTGACCCCCAACAGGTTGTGGCGATTGCCAGCAATGGGGGGGAAAACAGGCACTGGAAACTGTGCAGAGGTTGCTGCCCGTCTTGTGCCAGGCACACGGG
>half-T (original)    TTGACCCCTCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGCGGCAGGCCGGCGCTGGAG
>half-T (recoded)     TTAACGCCCCAACAGGTCGTTGCGATCGCGTCTAATGGAGGGGGCAGACCAGCGTTGGAG
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Right TALEN arm Binding domain: $G_1T_1G_2A_1T_2C_1C_2C_3C_4C_5C_6A_2G_3C_7A_3$

```
>G1 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G1 (recoded)    CTCACACCTCAACAGGTTGTTGCCATTGCATCAAACAATGGAGGTAAACAAGCACTGGAAACCGTTCAGAGACTTCTCCCTGTCCTTTGTCAGGCTCATGGT
>T1 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T1 (recoded)    CTGACTCCCCAGCAGGTCGTGGCTATCGCCTCTAATGGGGGCGGAAAGCAGGCTTTGGAGACAGTGCAAAGGCTGCTGCCCGTTCTCTGCCAGGCCCACGGG
>G2 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G2 (recoded)    CTTACACCACAACAGGTGGTTGCTATTGCTAGTAACAACGGCGGAAAACAAGCCCTCGAAACTGTCCAGAGGCTCTTGCCTGTCCTTTGTCAAGCCCATGGT
>A1 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>A1 (recoded)    TTGACCCCTGAACAAGTGGTAGCGATTGCGAGCAACATCGGGGGGAAGCAAGCACTGGAGACTGTGCAAGCCCTGCTCCCAGTGCTGTGTCAGGCTCATGGC
>T2 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>T2 (recoded)    CTGACCCCCCAGCAAGTCGTGGCAATCGCCTCCAATGGCGGAGGTAAACAGGCTCTTGAAACAGTCCAGAGACTTCTGCCCGTCCTCTGCCAAGCCCACGGT
>C1 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C1 (recoded)    CTTACTCCAGAACAAGTCGTCGCCATCGCTAGCCACGACGGGGGCAAACAGGCCCTGGAGACTGTGCAACGCTTGCTTCCAGTTTTGTGCCAGGCACATGGA
>C2 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C2 (recoded)    CTCACTCCTGAGCAGGTCGTGGCAATCGCATCACATGATGGTGGGAAGCAAGCATTGGAGACCGTTCAGCGCTTGCTGCCAGTGCTGTGTCAAGCACACGGA
>C3 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C3 (recoded)    TTGACCCCCGAACAGGTCGTCGCCATTGCTAGTCACGACGGAGGAAAGCAGGCTCTTGAAACAGTTCAAAGGCTCCTTCCTGTCCTTTGCCAGGCTCATGGG
>C4 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C4 (recoded)    CTGACTCCAGAGCAAGTTGTTGCTATCGCCTCACATGATGGGGGAAAACAGGCCCTCGAGACCGTGCAGAGACTGTTGCCTGTTCTCTGCCAAGCACACGGA
>C5 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C5 (recoded)    CTTACACCCGAACAGGTGGTCGCAATAGCAAGCCACGACGGTGGTAAGCAGGCACTTGAGACAGTCCAAAGACTTCTCCCTGTGCTTTGCCAGGCCCACGGT
>C6 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C6 (recoded)    CTCACTCCAGAGCAGGTTGTTGCCATTGCCTCCCATGATGGAGGGAAACAAGCTCTCGAAACTGTTCAGAGGTTGCTTCCCGTCCTCTGTCAAGCTCACGGG
>A2 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>A2 (recoded)    CTGACGCCAGAGCAAGTGGTGGCCATCGCTTCTAATATCGGCGGCAAGCAGGCCCTGGAAACAGTTCAAGCTTTGCTACCCGTGTTATGCCAGGCCCACGGC
>G3 (original)   TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>G3 (recoded)    CTGACCCCTCAGCAGGTTGTGGCAATTGCATCCAACAACGGTGGGAAACAAGCACTCGAAACTGTGCAGCGCCTTCTCCCAGTTTTGTGTCAAGCACATGGA
>C7 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>C7 (recoded)    TTGACACCCGAACAGGTTGTCGCTATCGCCAGTCATGACGGCGGTAAGCAGGCCTTGGAGACCGTCCAGAGGCTCCTTCCCGTGCTGTGCCAGGCACATGGG
>A3 (original)   TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
>A3 (recoded)    CTAACCCCGGAGCAGGTAGTGGCCATTGCTTCTAATATTGGGGGCAAACAAGCTCTCGAGACGGTACAGGCCCTCTTGCCAGTTTTGTGTCAAGCGCACGGC
>half-T (original)    TTGACCCCTCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGCGGCAGGCCGGCGCTGGAG
>half-T (recoded)     TTAACCCCCCAACAGGTCGTTGCGATCGCGTCTAATGGAGGGGGCAGACCAGCGTTGGAG
```

Left TALEN arm (Identity between motifs: 94.1%-100%)

```
                 10        20        30        40        50        60        70        80        90       100
C1/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C2/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C3/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C4/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C5/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G1/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G2/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G3/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G4/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G5/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T1/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T2/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T3/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T4/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T5/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
```

Right TALEN arm (Identity between motifs: 94.1%-100%)

```
                 10        20        30        40        50        60        70        80        90       100
C1/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C2/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C3/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C4/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C5/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C6/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
C7/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
A1/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
A2/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
A3/1-102         TTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G1/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G2/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
G3/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATAATGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T1/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
T2/1-102         TTGACCCCCCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTCCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCACGGC
```

Left TALEN$_{RECODED}$ arm (Identity between motifs: 66.7%-78.4%)

```
                 10        20        30        40        50        60        70        80        90       100
T5/1-102         TTGACCCCCCAACAGGTTGTGGCGATTGCCAGCAATGGGGGGGAAACAGGCACTGGAAACTGTGCAGAGGTTGCTGCCCGTCTTGTGCCAGGCACACGGG
T3/1-102         CTCACGCCTCAGCAAGTGGTCGCCATAGCCTCAAATGGAGGAGGTAAGCAAGCATTGGAAACCGTCCAAAGATTGTTGCCCGTGCTCTGCCAGGCCCATGGT
T4/1-102         CTCACCCCTCAACAAGTAGTGGCCATTGCCTCTAACGGGGGCGGTAAGCAAGCATTGGAAACAGTTCAACGCTTGCTGCCAGTCCTCTGTCAGGCCCATGGG
T1/1-102         CTGACTCCCCAACAGGTGGTAGCTATCGCCTCTAACGGTGGCGGAAAGCAAGCTTTGGAGACAGTCCAAAGGCTCCTGCCCGTTCTCTGCCAGGCCCACGGC
T2/1-102         CTGACCCCCCAGCAAGTCGTCGCAATCGCTAGCAATGGTGGAGGTAAACAGGCTCTGGAAACAGTGCAAAGACTTCTCCCCGTCCTCTGCCAAGCCCACGGT
G1/1-102         TTGACACCTCAACAAGTTGTTGCTATTGCATCAAATAACGGAGGTAAGCAGGCACTGGAGACCGTTCAGCGGCTGCTCCCTGTGCTTTGTCAAGCTCATGGT
G4/1-102         CTGACACCTCAACAAGTCGTCGCAATTGCTTCCAACAATGGAGGCAAGCAGGCTCTGGAAACTGTCCAGAGGCTGCTCCCGTCTTGTGTCAAGCTCATGGT
G3/1-102         TTGACACCTCAACAAGTTGTTGCAATTGCAAGCAATAACGGCGGCAAACAGGCCCTGGAAACTGTGCAGCGCCTTCTCCCAGTTTTGTGTCAGGCACATGGA
C1/1-102         CTTACTCCAGAACAAGTCGTCGCAATCGCTTCCCATGACGGGGGCAAACAGGCCCTTGAGACTGTCCAACGCTTGTTGCCAGTTTTGTGCCAGGCACACGGA
C2/1-102         CTCACTCCTGAGCAGGTCGTCGCAATCGCATCACATGACGGCGGGAAGCAAGCATTGGAGACCGTTCAACGCCTGCTGCCAGTGTTGTGTCAAGCACACGGA
C4/1-102         TTGACTCCAGAGCAGGTTGTTGCTATAGCATCACATGACGGCGGAAAACAGGCCCTGGAGACCGTGCAGAGACTTCTTCCTGTGCTTTGCCAAGCACACGGC
G2/1-102         CTTACACCACAACAAGTTGTTGCTATTGCTAGTAACAATGGCGGAAAACAAGCCCTGGAGACTGTGCAGCGGCTCCTTCCTGTCCTTTGTCAGGCTCATGGG
G5/1-102         CTCACTCCTCAACAAGTGGTTGCTATTGCTTCTAATAATGGAGGGAAACAAGCTCTTGAAACCGTTCAGCGGCTCCTTCCAGTGCTCTGTCAAGCACATGGA
C3/1-102         CTTACTCCAGAACAGGTGGTCGCTATTGCTAGTCATGACGGAGGAAAGCAAGCTCTTGAAACAGTTCAACGGCTCCTTCCTGTTCTTTGCCAAGCTCATGGG
C5/1-102         CTTACACCAGAACAGGTTGTCGCAATAGCAAGTCATGACGGGGGCAAGCAGGCCCTTGAGACAGTCCAAAGACTTTTGCCTGTTCTTTGCCAAGCTCACGGC
```

Right TALEN$_{RECODED}$ arm (Identity between motifs: 65.7%-78.4%)

```
                 10        20        30        40        50        60        70        80        90       100
T1/1-102         CTGACTCCCCAACAGGTCGTAGCTATCGCCTCTAACGGTGGCGGAAAGCAAGCTTTGGAGACAGTGCAAAGGCTCCTGCCCGTTCTCTGCCAGGCCCACGGG
T2/1-102         CTGACCCCCCAGCAAGTCGTCGCAATCGCTTCCAATGGTGGAGGTAAACAGGCTCTTGAAACAGTGCAAAGACTTCTCCCCGTCCTCTGCCAAGCCCACGGT
A2/1-102         CTAACGCCAGAGCAAGTTGTAGCCATCGCTTCTAATATCGGCGGGAAACAAGCCCTGGAAACAGTTCAAGCTTTGCTACCCGTGTTATGCCAAGCCCACGGC
A3/1-102         CTAACACCGGAGCAGGTAGTTGCGATTGCTTCTAATATCGGGGGTAAACAAGCCCTCGAGACGGTACAAGCCCTCCTTCCCGTTTTGTGTCAAGCGCACGGC
C1/1-102         CTTACTCCAGAACAAGTCGTCGCAATCGCTTCCCATGACGGGGGGAAACAGGCCCTTGAGACTGTGCAACGCTTGCTTCCAGTTTTGTGCCAGGCACACGGA
C2/1-102         CTCACTCCTGAACAGGTCGTCGCAATCGCATCACATGACGGGGGGAAGCAAGCATTGGAGACCGTTCAACGCTTGCTGCCAGTTTTGTGTCAAGCACACGGA
A1/1-102         TTGACCCCTGAACAAGTGGTAGCGATTGCGAGTAACATCGGGGGGAAACAAGCACTGGAGACTGTGCAAGCTTTACTCCCAGTGTTGTGTCAAGCTCATGGG
C4/1-102         CTTACTCCAGAACAGGTTGTTGCTATAGCATCACATGACGGGGGAAAACAGGCCCTCGAGACCGTGCAGAGACTTCTTCCTGTTCTTTGCCAAGCACACGGA
C5/1-102         CTTACACCAGAACAGGTTGTCGCAATAGCAAGTCATGACGGGGGTAAGCAGGCCCTTGAGACAGTGCAAAGACTTCTCCCTGTTCTTTGCCAAGCTCACGGT
C7/1-102         CTCACACCAGAACAGGTTGTCGCTATAGCAAGTCATGACGGAGGTAAACAAGCCCTCGAGACCGTGCAGAGGCTCCTTCCCGTCTTGTGCCAAGCACATGGG
G3/1-102         CTGACACCTCAACAAGTTGTTGCAATTGCATCCAACAACGGGGGGAAACAAGCCCTCGAAACTGTGCAACGCCTTCTCCCAGTTTTGTGTCAAGCACATGGA
C6/1-102         CTCACTCCAGAACAGGTTGTTGCAATTGCTTCCCATGACGGAGGGAAACAAGCTCTCGAAACTGTTCAAAGGTTGCTTCCCGTCCTTTGTCAAGCTCACGGG
C3/1-102         CTTACTCCAGAACAGGTCGTCGCTATTGCTAGTCATGACGGAGGAAAGCAAGCTCTTGAAACAGTTCAAAGGCTCCTTCCTGTCCTTTGCCAAGCTCATGGG
G1/1-102         CTCACACCTCAACAAGTTGTTGCTATTGCATCAAACAACGGAGGTAAACAAGCACTCGAAACCGTTCAAAGACTTCTCCCTGTCCTTTGTCAAGCTCATGGT
G2/1-102         CTTACACCACAACAAGTTGTTGCTATTGCTAGTAACAACGGCGGAAAACAAGCCCTCGAAACTGTGCAAAGGCTCCTTCCTGTCCTTTGTCAAGCTCATGGG
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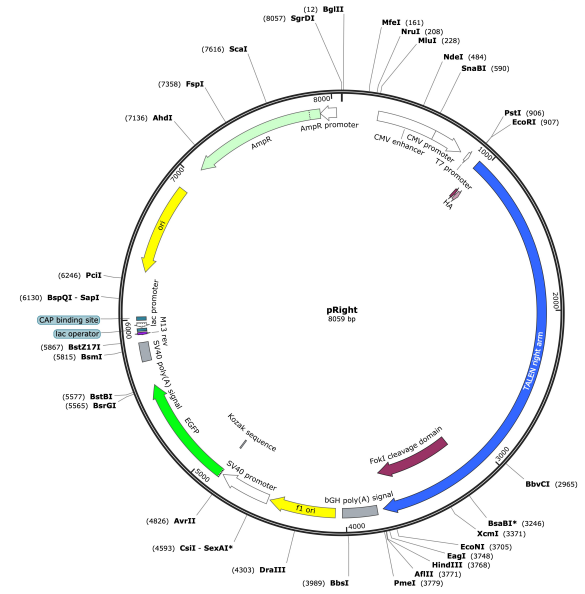
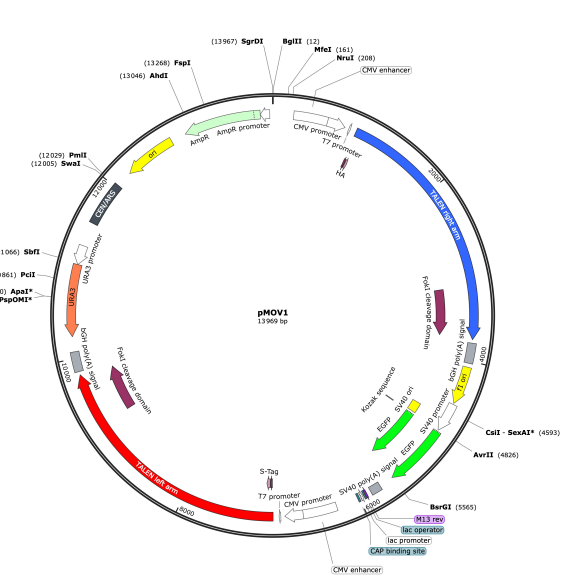
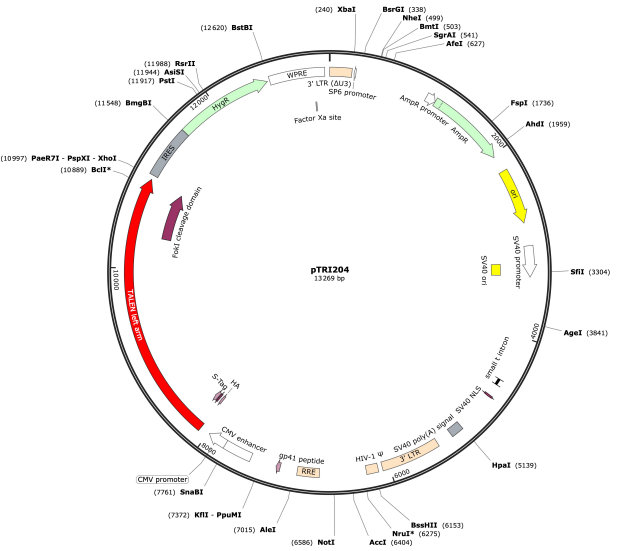
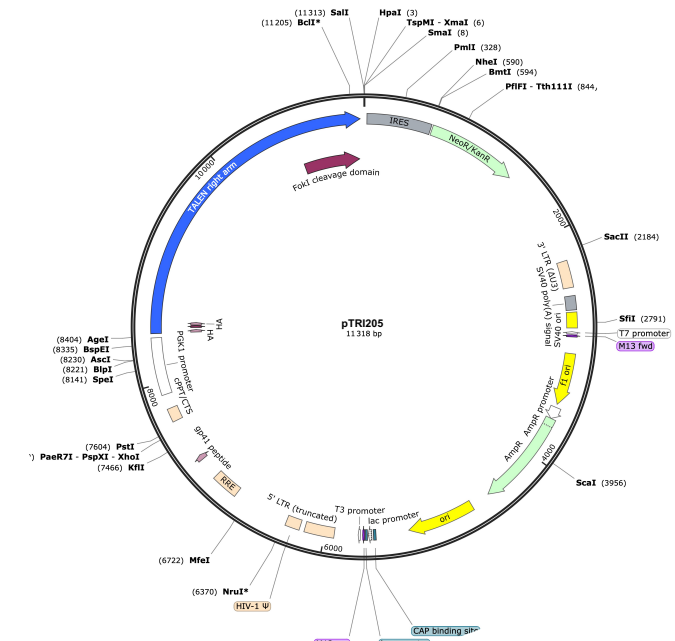
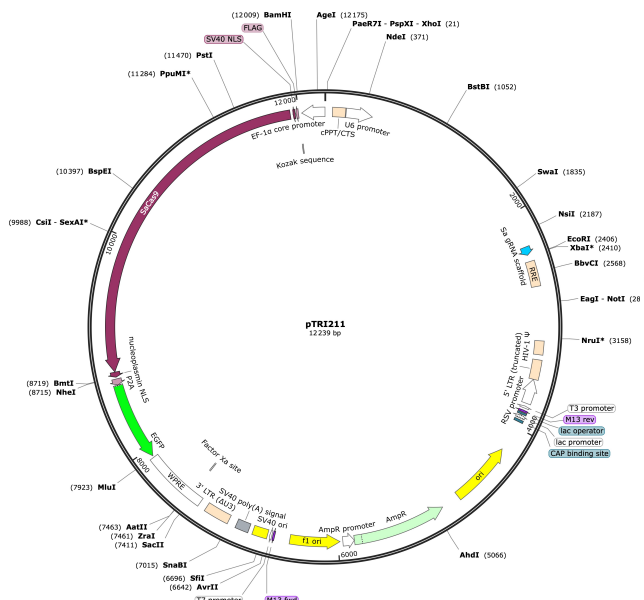
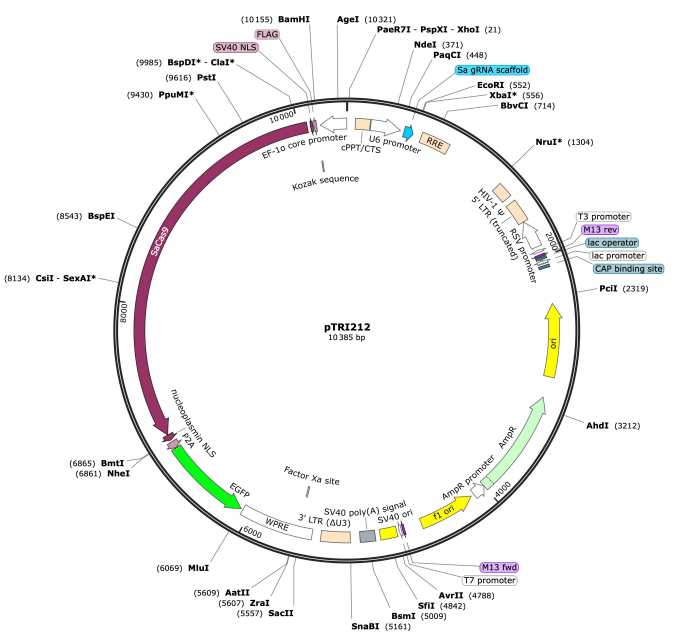

pRight
8059 bp
(12) BglII
(8057) SgrDI
MfeI (161)
NruI (208)
MluI (228)
NdeI (484)
SnaBI (590)
PstI (906)
EcoRI (907)
(7616) ScaI
(7358) FspI
(7136) AhdI
(6246) PciI
(6130) BspQI - SapI
CAP binding site
lac operator
(5867) BstZ17I
(5815) BsmI
(5577) BstBI
(5565) BsrGI
(4826) AvrII
(4593) CsiI - SexAI*
(4303) DraIII
(3989) BbsI
PmeI (3779)
AflII (3771)
HindIII (3768)
EagI (3748)
EcoNI (3705)
XcmI (3371)
BsaBI* (3246)
BbvCI (2965)
AmpR
AmpR promoter
CMV enhancer
CMV promoter
T7 promoter
HA
TALEN right arm
FokI cleavage domain
Kozak sequence
SV40 promoter
f1 ori
bGH poly(A) signal
EGFP
SV40 poly(A) signal
M13 rev
lac promoter
ori

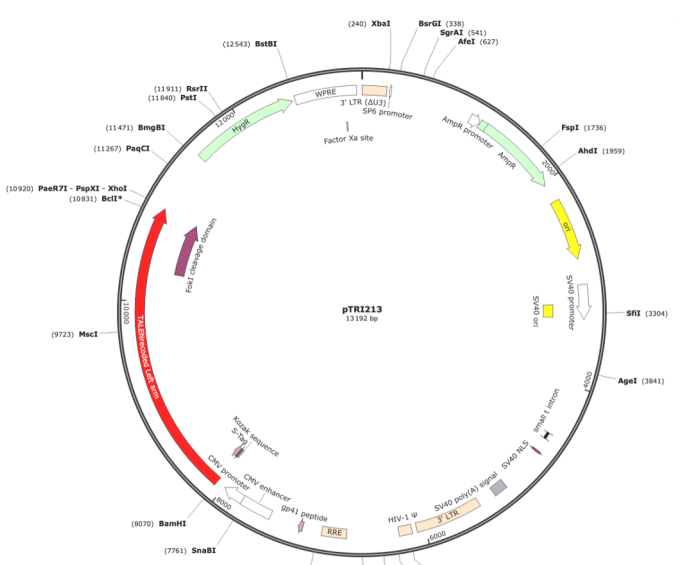

pMOV1
13969 bp
(13967) SgrDI
BglII (12)
MfeI (161)
NruI (208)
CMV enhancer
(13268) FspI
(13046) AhdI
(12029) PmlI
(12005) SwaI
(11066) SbfI
(10861) PciI
(10670) ApaI*
(10666) PspOMI*
(4593) CsiI - SexAI*
AvrII (4826)
BsrGI (5565)
M13 rev
lac operator
CAP binding site
AmpR
AmpR promoter
CMV promoter
T7 promoter
HA
TALEN left arm
FokI cleavage domain
SV40 poly(A) signal
Kozak sequence
SV40 ori
EGFP
S-Tag
TALEN right arm
bGH poly(A) signal
ori


pTRI204
13269 bp
(240) XbaI
BsrGI (338)
NheI (499)
BmtI (503)
SgrAI (541)
AfeI (627)
(12620) BstBI
(11988) RsrII
(11944) AsiSI
(11917) PstI
(11548) BmgBI
(10997) PaeR7I - PspXI - XhoI
(10889) BclI*
FspI (1736)
AhdI (1959)
SfiI (3304)
AgeI (3841)
HpaI (5139)
(7761) SnaBI
(7372) KflI - PpuMI
(7015) AleI
(6586) NotI
AccI (6404)
NruI* (6275)
BssHII (6153)
WPRE
3' LTR (ΔU3)
SP6 promoter
Factor Xa site
AmpR promoter
AmpR
HygR
IRES
FokI cleavage domain
TALEN left arm
SV40 ori
SV40 promoter
f1 ori
ori
SV40 NLS
smaI intron
SV40 poly(A) signal
3' LTR
HIV-1 ψ
RRE
gp41 peptide
CMV enhancer
CMV promoter
S-Tag
HA


pTRI205
11318 bp
(11313) SalI
HpaI (3)
(11205) BclI*
TspMI - XmaI (6)
SmaI (8)
PmlI (328)
NheI (590)
BmtI (594)
PflFI - Tth111I (844,
SacII (2184)
SfiI (2791)
T7 promoter
M13 fwd
ScaI (3956)
(8404) AgeI
(8335) BspEI
(8230) AscI
(8221) BlpI
(8141) SpeI
(7604) PstI
(7466) KflI
(6722) MfeI
(6370) NruI*
M13 rev
lac operator
CAP binding site
IRES
NeoR/KanR
FokI cleavage domain
TALEN right arm
3' LTR (ΔU3)
SV40 poly(A) signal
SV40 ori
SV40 promoter
f1 ori
AmpR
AmpR promoter
ori
lac promoter
T3 promoter
5' LTR (truncated)
RRE
gp41 peptide
HIV-1 ψ
PGK1 promoter
cPPT/CTS
HA


pTRI211
12239 bp
(12009) BamHI
AgeI (12175)
PaeR7I - PspXI - XhoI (21)
NdeI (371)
FLAG
SV40 NLS
(11470) PstI
(11284) PpuMI*
BstBI (1052)
SwaI (1835)
NsiI (2187)
EcoRI (2406)
XbaI* (2410)
BbvCI (2568)
EagI - NotI (28
NruI* (3158)
AhdI (5066)
(10397) BspEI
(9988) CsiI - SexAI*
(8719) BmtI
(8715) NheI
(7923) MluI
(7463) AatII
(7461) ZraI
(7411) SacII
(7015) SnaBI
(6696) SfiI
(6642) AvrII
EF-1α core promoter
cPPT/CTS
U6 promoter
Kozak sequence
SeCas9
Sa gRNA scaffold
RRE
RSV promoter
5' LTR (truncated)
T3 promoter
M13 rev
lac operator
lac promoter
CAP binding site
nucleoplasmin NLS
P2A
EGFP
Factor Xa site
WPRE
3' LTR (ΔU3)
SV40 poly(A) signal
SV40 ori
f1 ori
AmpR promoter
AmpR
ori
T7 promoter
M13 fwd


pTRI212
10385 bp
(10155) BamHI
AgeI (10321)
PaeR7I - PspXI - XhoI (21)
NdeI (371)
PaqCI (448)
FLAG
SV40 NLS
(9985) BspDI* - ClaI*
(9616) PstI
(9430) PpuMI*
Sa gRNA scaffold
EcoRI (552)
XbaI* (556)
BbvCI (714)
NruI* (1304)
PciI (2319)
AhdI (3212)
(8543) BspEI
(8134) CsiI - SexAI*
(6865) BmtI
(6861) NheI
(6069) MluI
(5609) AatII
(5607) ZraI
(5557) SacII
SnaBI (5161)
BsmI (5009)
SfiI (4842)
AvrII (4788)
EF-1α core promoter
cPPT/CTS
U6 promoter
Kozak sequence
SeCas9
RRE
HIV-1 ψ
5' LTR (truncated)
RSV promoter
T3 promoter
M13 rev
lac operator
lac promoter
CAP binding site
ori
nucleoplasmin NLS
P2A
EGFP
Factor Xa site
WPRE
3' LTR (ΔU3)
SV40 poly(A) signal
SV40 ori
f1 ori
AmpR promoter
AmpR
M13 fwd
T7 promoter


pTRI213
13192 bp
(240) XbaI
BsrGI (338)
SgrAI (541)
AfeI (627)
(12543) BstBI
(11911) RsrII
(11840) PstI
(11471) BmgBI
(11267) PaqCI
(10920) PaeR7I - PspXI - XhoI
(10831) BclI*
FspI (1736)
AhdI (1959)
SfiI (3304)
AgeI (3841)
(9723) MscI
(8070) BamHI
(7761) SnaBI
(7015) AleI
(6586) NotI
AccI (6404)
NruI* (6275)
BssHII (6153)
WPRE
3' LTR (ΔU3)
SP6 promoter
Factor Xa site
AmpR promoter
AmpR
HygR
FokI cleavage domain
TALEN Bleo/cocktail right arm
SV40 ori
SV40 promoter
ori
SV40 NLS
smaI intron
SV40 poly(A) signal
3' LTR
HIV-1 ψ
RRE
gp41 peptide
CMV enhancer
CMV promoter
S-Tag
Kozak sequence

pTRI214
11241 bp
(10840) AatII
(10838) ZraI
SacII (22)
(10476) NdeI
SfiI (629)
T7 promoter
M13 fwd
(9923) PflFI - Tth111I
(9430) PaqCI
(9407) PmlI
(9087) SmaI
(9085) TspMI - XmaI
(9082) HpaI
(9074) SalI - SgrDI
(8985) BclI*
ScaI (1794)
(8289) AsiSI
(7415) AfeI
NruI* (4208)
(6242) AgeI - SgrAI
(6173) BspEI
(6068) AscI
(6059) BlpI
(5979) SpeI
PstI (5442)
PspXI (5410)
KflI (5304)
3' LTR (ΔU3)
SV40 poly(A) signal
SV40 ori
f1 ori
AmpR promoter
AmpR
ori
M13 rev
T3 promoter
lac promoter
CAP binding site
lac operator
HIV-1 ψ
5' LTR (truncated)
RRE
gp41 peptide
cPPT/CTS
PGK1 promoter
HA
Kozak sequence
FokI cleavage domain
TALEN Bleo/cocktail left arm
NeoR/KanR

ASA clone ID#	TALEN arms	Nbr. SMRTCells	Genome coverage
ASA3-p11	None	4	155
ASA30	Right+Left	4	13
ASA51	Right+Left	4	15
ASA2	Right+Left	4	14
ASA53	Right+Left	7	68
ASA54	Right+Left	3	12
ASA27	Right+Left	7	83
ASA111E	Right+Left	4	16
ASA22C	Right+Left	4	19
ASA28	Right+Left	4	15
ASA03-44	Right	7	31
C3_Left	Left recoded	2	69
C5_Left	Left recoded	2	27
C3	(Right+Left)recoded	2	49
C6	(Right+Left)recoded	2	46
C9	(Right+Left)recoded	2	7
C11	(Right+Left)recoded	2	28
C12	(Right+Left)recoded	2	40
C15	(Right+Left)recoded	2	33
C16	(Right+Left)recoded	2	5
Total		**70**	