## Precision genome editing using synthesis-dependent repair of Cas9-induced DNA breaks

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#### **Abstract**

The RNA-guided DNA endonuclease Cas9 has emerged as a powerful new tool for genome engineering. Cas9 creates targeted double-strand breaks (DSBs) in the genome. Knock-in of specific mutations (precision genome editing) requires homology-directed repair (HDR) of the DSB by synthetic donor DNAs containing the desired edits, but HDR has been reported to be variably efficient. Here, we report that linear DNAs (single and double-stranded) engage in a high-efficiency HDR mechanism that requires only ~35 nucleotides of homology with the targeted locus to introduce edits ranging from 1 to 1000 nucleotides. We demonstrate the utility of linear donors by introducing fluorescent protein tags in human cells and mouse embryos using PCR fragments. We find that repair is local, polarity-sensitive, and prone to template switching, characteristics that are consistent with gene conversion by synthesis-dependent strand-annealing (SDSA). Our findings enable rational design of synthetic donor DNAs for efficient genome editing.

#### Significance

Genome editing, the introduction of precise changes in the genome, is revolutionizing our ability to decode the genome. Here we describe a simple method for genome editing that takes advantage of an efficient mechanism for DNA repair called synthesis-dependent strand annealing. We demonstrate that synthetic linear DNAs (ssODNs and PCR fragments) with ~35bp homology arms function as efficient donors for SDSA repair of Cas9-induced double-strand breaks. Edits from 1 to 1000 base pairs can be introduced in the genome without cloning or selection.

#### Introduction

Precision genome editing begins with the creation of a double-strand break (DSB) in the genome near the site of the desired DNA sequence change ("edit") (1). Generation of targeted DSBs has been greatly accelerated in recent years by the discovery of CRISPR-Cas9, a programmable DNA endonuclease that can be targeted to a specific DNA sequence by a small "guide" RNA (crRNA) (2). DSBs are lethal events that must be repaired by the cell's DNA repair machinery. DSBs can be repaired via imprecise, non-homology-based repair mechanisms, such as non-homologous end-joining (NHEJ), or by precise, homologydependent repair (HDR) (3). HDR utilizes DNAs that contain homology to sequences flanking the DSB (termed homology arms) to template the repair. If a synthetic "donor" DNA containing the desired edit is available when the DSB is generated, the cellular HDR machinery will use the donor DNA to repair the DSB and the edit will be incorporated at the targeted locus (1). Several studies have reported that single-stranded oligonucleotides (ssODNs) can be used to introduce short edits (<50 bases) ((4) and references therein). ssODNs that target the DNA strand that is first released by Cas9 after DSB generation have been reported to perform best (5). This strand preference, however, has only been tested for small edits near the DSB and has not been noticed at all loci (4). Edits at a distance from the DSB (>10 bp) are recovered at lower frequencies (4, 6). Recovery of large edits (such as GFP knock-ins) has also been reported to be inefficient, requiring large plasmid donors with long (>500nt) homology arms or selection markers to recover the rare edits (3). Large insertions have been obtained through non-homologous or micro-homology-mediated end joining reactions (NHEJ and MMEJ), but these approaches require simultaneous Cas9induced cleavage of donor and target DNAs (7-13).

We documented previously that, in *C. elegans*, HDR can be very efficient provided that the donor DNAs are linear (14). Linear donors do not appear to integrate at the DSB, but instead are used as templates for DNA synthesis, as in the synthesis-dependent strand annealing (SDSA) model for gene conversion (Figure 1) (15). In SDSA, the DSB is first resected to yield 3' overhangs on both

sides of the DSB (Figure 1A). The 3' overhangs invade the donor and are extended by DNA synthesis copying donor sequences (Figure 1B). Bridging of the DSB is completed when the newly synthesized strands withdraw from the donor and anneal back to each other at the locus (Figure 1C). In *C. elegans*, donors for SDSA can be single (ssODNs) or double-stranded (PCR fragments), and require only short homology arms (~35 bases) to engage the DSB. The repair process is sensitive to insert size and prone to template switching, where synthesis can "jump" between two overlapping donors (14). In human cells, SDSA has been proposed as a repair mechanism for ssODNs (4, 16), but not for double-stranded donors, which are thought to participate in a different HDR pathway (16, 17). Here, we investigate the sequence requirements for linear donors to engage the DSB repair machinery in human cells. Our findings suggest that ssODNs and PCR fragments both engage in a SDSA-like type of gene conversion, and we demonstrate the utility of PCR fragments to create fluorescent protein knock-ins in human cells and mouse embryos. Our findings suggest simple donor DNA design principles to maximize editing efficiency.

#### **Materials and Methods**

#### Detailed results, sequences and solutions

Table S1 lists all experiments, including detailed conditions and results of experimental replicates. Table S2 to S5 lists sequences of linear donors, plasmids, PCR primers and cr/sgRNAs, respectively. Position of the cr/sgRNAs on the loci targeted in this study can be found in Figure S1.

Repair templates, Cas9, cr/tracrRNAs and plasmids for cell culture ssODNs (ultramers) and PCR primers where ordered from IDT and reconstituted at 50uM and 100uM respectively in water. For the Illumina sequencing experiment shown in Figure 7B, ssODNs and primers were ordered PAGE purified. PCR fragment donors were synthesized as described in (18).

Cas9 protein was purified as described in (19). crRNAs and tracrRNA were ordered from IDT and reconstituted in 5mM Tris-HCl pH7.5 at 130uM. *PYM1* sgRNA cloning was cloned as described in (20). Plasmids containing repair templates were made using gBlock gene fragments (IDT) and InFusion cloning kit (Clontech), and purified using Qiagen mini-prep kit and eluted in H2O.

#### Cas9 RNP nucleofection:

With the exception of experiments at the *PYM1* locus (see below), all experiments in this study used Cas9 RNP delivery (21). Nucleofections using Cas9 RNP were performed as described (22). HEK293T cells or HEK293T cells expressing a truncated GFP (GFP1-10) (23) were grown to 50-75% confluency, trypsinized, pelleted and resuspended at 800000 cells / 80ul of PBS. Just before nucleofection, PBS was replaced with 80ul of Nucleofection kit V (Lonza). 40ul of Cas9 RNP mix (see below) was added to the cells in suspension in Nucleofector kit V and processed using an Amaxa Nucleofector 2b machine (Lonza) using the A023 program. Cells were transferred to culture media and analyzed for fluorescence 3 days after.

The Cas9 RNP mix contains: 6.5uM of crRNA and tracrRNA, 1.6ug/ul of Cas9, a variable concentration of repair templates (see Table S1 for details), 10.4% Glycerol, 131mM KCl, 5.2mM Hepes, 1mM MgCl2, 0.5mM Tris-HCl, pH7.5. For sequencing of GFP edits at the *Lamin A/C* locus, cells were sorted (at the JHU Ross Flow Cytometry Core Facility) for GFP signal and cloned in 96 wells plates for genotyping or pooled in a 6-well plate for microscopy analysis. Single cell clones were lysed using QuickExtract DNA Extraction Solution (Epicentre) and genotyped by PCR using Phusion taq (NEB) with genomic primers outside of the HDR fragment. PCR products were analyzed on agarose gel and sequenced (see Figures S6 and S7).

#### Cas9 plasmid transfections

For experiments at the *PYM1* locus, Cas9 and the sgRNA were delivered on plasmids. HEK293T cells were grown to 50-75% confluency in 6 wells plate (with

2ml of culture media per wells). 10.8ul of Cas9 plasmid mix (containing 3.6ul of X-tremeGENE 9 DNA Transfection Reagent from Roche, 892ng of plasmid pX458 containing *PYM1* sgRNA and 3.24pmol of repair template) was added to 120ul of optiMEM glutaMAX media (ThermoFisher), incubated for 15min at room temperature, and next added to the cells. 48h after transfection, cells were sorted for GFP signal (to select for cells that received pX458) and grown out as single cell clones. The single cell clones were lysed and genotyped by PCR. PCR products were directly analyzed on agarose gel or mix with *EcoR1* (NEB) and the corresponding Restriction Enzyme (RE) buffer, digested over-night and analyzed on agarose gel.

#### Cytometer analysis

For each experiment, 5,000 to 10,000 cells were analyzed using a Guava EasyCyte 6/2L (Millipore) cytometer. Cells were scored as GFP+ if they exhibited a higher signal than 99.5% of non-transfected control cells.

HEK293T (GFP1-10) cells exhibit a higher basal green fluorescence than wild-type HEK293T cells. Cytometer analysis could not be performed on these cells for GFP11-tagged Lamin A/C and SMC3. For those experiments, as well as for RFP tagging, cells were analyzed by fluorescence microscopy and scored manually (see below).

#### Microscopy

Cells were fixed in 4% PFA and mounted with DAPI. Cells were imaged using a confocal microscope with a 63X objective. > 50 fields of cells (>1000 cells) were selected in the DAPI channel, photographed, and analyzed for GFP or RFP expression manually.

#### PCR amplicons for Illumina sequencing

HEK293T (GFP1-10) were nucleofected with different combinations of repair ssODNs (Fig 7B, Table S1). To control for possible template-switching during PCR amplification, we also introduced single donors (wild-type or mutant) in two

separate cell populations and combined the cells during PCR amplification. 60h after nucleofection, cells were trypsinized, washed in PBS, and 500000 cells were lysed in 40ul of QuickExtract DNA Extraction Solution. 40ul of H2O was added to each lysis. A total of 6ul of DNA from each experiments were PCR amplified using Phusion Taq and the primer 390 (Forward, in the left end of the insert) and the primer 1849 (Reverse, in the Lamin A/C locus downstream of the right HA of the ssODN used for repair) for 10 cycles at 68.5C (see Table S4 for primer sequences). After 10 PCR cycles, no band could be detected on agarose gel and ethidium bromide staining. Each PCR reaction was purified using Qiagen Minelute columns and eluted in 10ul of H2O. 2ul of each PCR were amplified using Phusion tag at 65C for 20 cycles. PCR reactions did not reach an amplification plateau with this number of cycles. The PCR reactions were performed using primers 1928 (Forward, containing the Illumina sequence and annealing in the same region than primer 390) and Reverse primers containing the Illumina sequence and a specific barcode. The Illumina reverse primers anneal with the Lamin A/C locus just upstream of primer 1849 and downstream the right HA of the ssODN used for repair.

PCR amplicons were purified on a 10% non-denaturing TBE/PAGE gel and the band corresponding to the PCR product was cut from the gel, eluted over-night, and precipitated with isopropanol. After resuspension, sample concentrations were quantified on a bioanalyzer, and the barcoded samples were pooled to a concentration of 0.4uM per sample in 10 ul. This sample was submitted to the Johns Hopkins School of Medicine Genetics Resources Core Facility for 250 cycle paired-end sequencing on an Illumina MiSeq instrument.

#### Illumina Sequencing analysis

After de-multiplexing of barcoded samples, the 3' adaptor and all downstream nucleotides were trimmed from the forward reads using Cutadapt (<a href="http://journal.embnet.org/index.php/embnetjournal/article/view/200">http://journal.embnet.org/index.php/embnetjournal/article/view/200</a>), and the resulting sequences were mapped to the insert + Lamin A/C locus using Bowtie 2 (24). After removing reads that did not fully map to the template and low-quality

reads (Q score less than 35; error probability of 0.00032), sequences were parsed for template switching. To score template switches, we evaluated sequencing reads at diagnostic positions and determined whether each position matched the sequence of the wild-type or mutated template. Reads with a diagnostic nucleotide that did not match either the wild-type or mutated template were discarded. Because the PCR control sample contained a mixture of the fully wild-type and fully mutated templates, we used the first diagnostic position (from the right side of the insert) only as an "anchor" to determine the initial identity of the template; this position was not used to score switching. Thereafter, whenever two or more contiguous diagnostic nucleotides indicated a switch in template identity, we scored this as a switch. For the control sample in which both templates were wild-type, we used the "1/6" mutated template for comparison, to determine the rate of false-positive switches in the assay. Because the PCR control experiment was performed with the wild-type and "1/6" mutated template (Table S1), we also used the "1/6" mutated template for scoring switches in this sample. See Table S6 for details.

#### Cas9 RNP injection in mouse zygotes

All mouse experiments were carried out under protocols approved by the JHU animal care and use committee.

The PCR fragment donor was synthesized as described in (18). The plasmid donor was generated using a gBlock and restriction enzyme cloning, and purified by Qiagen midi-prep kit and eluted in injection buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA). Pronuclear injections of zygotes (from B6SJLF1/J parents (Jackson labs)) was performed by the JHU Transgenic facility at a final concentration: 30ng/ul Cas9 protein (PNABio), 0.6uM each of crRNA/TracrRNA (Dharmacon) and PCR donor (3ng/ul or 5ng/ul) or plasmid donor (10ng/ul). The Cas9 protein, crRNA, tracrRNA were combined from stocks at 1000ng/ul, 20uM, 20uM respectively and incubated at 4C for 10 minutes. Then injection buffer was added to dilute to the final working concentrations above (Table S1) along with repair vector or fragment. The solution was microcentrifuged 5 min at 13000xg

and the solution used for injection. Pups were genotyped using genomic primers immediately outside of the PCR donor sequence, or using one primer in mCherry and one upstream of the 483 bp homology arms in the case of the plasmid donor. Genomic DNA from all pups was also subjected to PCR amplification with internal mCherry specific primers to identify random insertions of the donor template (locus-specific mCherry negative/internal mCherry product positive). We identified 7 pups (11%, out of 60 pups without mCherry insertion at the *Adcy3* locus) with potential transgenic insertions of the PCR fragment at other undetermined loci. In contrast, we identified no transgenics (0%, out of 20 pups without mCherry insertion at the *Adcy3* locus) when using the plasmid donor.

#### Results

## PCR fragments with short homology arms are efficient donors for genome editing in HEK293T cells

In human cells, ssODNs and plasmids are most commonly used as donors for genome editing (3). To determine whether PCR fragments can also function as donors, we attempted to knock-in GFP at three loci using PCR fragments amplified from a GFP-containing plasmid. The GFP-coding insert (714 bp) was amplified using hybrid primers containing sequences to target GFP and ~35 bp locus-specific homology arms (HAs). The HAs were designed to insert GFP in frame with the target open-reading frame 0, 11 and 5 bp away from a Cas9 cleavage site in the *Lamin A/C*, *RAB11A*, and *SMC3* ORFs, respectively (Figures 2 and S2). The PCR fragments (0.33-0.21uM) and *in vitro*-assembled Cas9-guide RNA complexes were introduced by nucleofection into HEK293T cells without selection as in (22). The efficiency of GFP integration was examined 3 days later by cytometer or fluorescence microscopy (see Material and Methods). We obtained 14.9%, 17.5% and 13.9% GFP+ positive cells for the *Lamin A/C*, *RAB11A* and *SMC3* loci, respectively (Figure 2B and S2B). In each

case, the cells expressed GFP in a pattern consistent for the targeted ORF (Figures 2D and S2C).

Reducing the molarity of the PCR fragments by 10-fold reduced efficiency by ~1/2 (Compare Figures 2B and 2C). Increasing the length of the homology arms to 500 bp did not increase editing efficiency significantly, even when controlling for the reduced molarity of the longer PCR fragments (Figure 2C). Reducing the length of the homology arms to ~15 bp, however, decreased efficiency (Figure 2B). PCR fragments with no homology arms or homology arms for a locus not targeted by Cas9 yielded GFP+ edits near background levels (Figures 2, S2, S3 and Table S1). Plasmid donors with ~500 bp homology arms also performed poorly (Figure 2C) as reported previously (7). We conclude that PCR fragments function as efficient donors in HEK293T cells, performing similarly to ssODNs, and better than plasmids with much longer homology arms. Because ~35 bp homology arms are convenient to introduce by PCR amplification, we used that length for subsequent experiments. 30-40 nt homology arms have also been reported to be optimal for ssODNs (4).

#### Editing efficiency is sensitive to insert size

To test the effect of insert size on editing efficiency, we added varied sizes of DNA sequence to the GFP insert. For ease of synthesis and to maintain equimolar amounts of donor DNAs, we introduced donor fragments at the same low molarity (0.12uM). We found that inserts beyond 1kb performed very poorly, yielding less than 0.5% edits (Figure 3A). By varying the size of the homology arms, we found that the size of the insert, and not the overall size of the donor DNA, determines editing efficiency. An 1188 bp donor (714 bp insert with two 237 bp HAs) performed as well as a 780 bp donor with the same size insert and 33 bp HAs (10% versus 11% edits, Figure 3A). The 1188 bp donor, however, performed much better than a 1188 bp donor with a longer insert (1122 bp) and 33 bp HAs (10% versus 0.5% edits, Figure 3A).

To test whether decreasing insert size below the size of GFP would increase editing efficiency, we took advantage of the split-GFP system (22, 23).

In this system, the 11<sup>th</sup> beta-strand of GFP (57 bp, GFP11) is knocked-in in cells expressing a complementary GFP fragment (GFP1-10). We generated PCR products containing the GFP11 insert and ~35 bp HAs and introduced these at 0.33uM. We obtain 44.9% edits at the *Lamin A/C* locus (Figure 3B) and 37.4% at the *RAB11A* locus (Figure 3C). A donor with no homology arms yielded only 1.3% edits (Figure S3B). Again, we found that increasing insert size reduced efficiency, down to 20% for a 993 bp insert (Figure 3B). We conclude that dsDNAs engage in an efficient repair process that requires only 35 bp homology arms, but favors relatively short inserts (<1kb at the molarities tested here).

#### Repair is a polarity-sensitive process.

In the SDSA repair model, when using a double-stranded donor, strand invasion can be initiated on either side of the DSB, since both strands in the donor are available for pairing (Figure 1) (15). In the case of single-stranded donors (ssODNs), however, the model predicts that repair will initiate only on the right or left side of the DSB depending on the polarity of the ssODN. To test this prediction, we designed ssODNs with a GFP11 insert and only one HA targeting either the left or right side of the Cas9-induced DSB in *Lamin A/C* and *RAB11A* (Figure 4). ssODNs with only one homology arm were able to function as donors, presumably because NHEJ can repair the gap on the side with no HA (see discussion). As predicted by the model, the polarity of the ssODN had a profound effect on editing efficiency. Editing efficiency was highest with ssODNs that could anneal to a complementary 3' end at the DSB (Figure 4). These observations are consistent with a replicative repair process initiated by pairing between 3' overhangs at the DSB and the HAs on the donor.

## Recoding of sequences between the DSB and the edit increases recovery of distal edits.

Editing efficiency is known to decrease with increasing distance between the edit and the DSB (6). This observation is also consistent with replicative repair, which predicts that synthesis that generates sequence complementary to

the other side of the DSB will promote "premature annealing" back to the locus before synthesis of the edit (Figure 5). To test this prediction directly, we designed an ssODN donor with two inserts: a proximal insert (restriction enzyme site) at the DSB in the PYM1 locus and a distal insert (3xFlag) 23 bases away from the DSB. Each insert was flanked by an HA targeting the PYM1 locus (Figure 5A). We generated 63 single cell clones and genotyped the PYM1 locus by PCR and Sanger sequencing (see Material and Methods). 46% of the clones contained only the proximal edit and 12.6% contained both the proximal and distal edits. The finding that ~80% of the edits contained only the proximal edit is consistent with "premature" annealing using sequence between the two edits. To test this hypothesis, we mutated 7 bases in the 23 bases region separating the proximal and distal edit. The mutations were designed to reduce homology with the locus while preserving coding potential (Figure 5A). This partial recoding reduced the frequency of proximal edit-only clones to 10.3% and increased the frequency of proximal+distal edits to 25.8%. We conclude that sequences on the donor that span the DSB can trigger premature annealing and prevent the incorporation of distal edits.

To test whether internal homologies can also participate in the repair process in double-stranded donors, we performed a similar experiment with a PCR fragment designed to incorporate GFP11 at the DSB, and tagRFP 33 bases from the DSB in the *Lamin A/C* locus (Figure 5B). We recovered 10.8% GFP-only edits and 8.6% GFP-RFP double positives. Partial recoding of the sequence between GFP11 and tagRFP (by introducing 10 silent mutations) reduced the percent of GFP-only edits to 4.4% and raised the percent of GFP-RFP double positives to 17.6%. We conclude that internal homologies on double-stranded templates can also interact with the targeted locus. Since both polarities are present in double-stranded templates, internal sequences could participate in principle in both the initial invasion step and the annealing step back to the locus.

Polarity of single-stranded donors affects incorporation of distal edits

Donors that contain edits designed to be inserted at a distance from the DSB have one HA that matches sequences immediately next to the DSB (proximal HA) and one HA at a distance from the DSB (recessed arm) (Figure 6A). We tested whether proximal and recessed arms initiate repair with similar efficiencies using a series of 22 ssODNs with inserts ranging from 0 to 41 nucleotides from the DSB at four loci (Figure 6). In all ssODNs, the sequence between the DSB and edit was partially recoded to increase the frequency of edit incorporation as described in the previous section. Strikingly, we observed an increasing bias for a particular polarity with increasing edit-to-DSB distance (Figure 6B). The favored polarity changed whether the edit (and recessed arm) was positioned to the left or right of the DSB (sense polarity when the edit is on the left side of the DSB, and antisense when the edit is on the right side). ssODNs with inserts close to the DSB did not show much polarity bias (Figure 6B). These findings are consistent with a replicative model for repair where proximal arms directly abutting the DSB are favored to initiate a round of DNA synthesis that will copy the edit. Recessed arms can be used for annealing back to the locus, although that process also appears to favor proximal arms. Even when using ssODNs with the correct polarity and recoding between the DSB and the edit, we still observed an inverse correlation between editing efficiency and edit-to-DSB distance (Figure S4). Finally, we note, that, unlike ssODN polarity, the polarity of the guide RNA used to create the DSB had only a minor effect, if any, on efficiency (Figure 6B).

#### Repair is prone to template switching between donors

In *C. elegans*, we observed that sequential rounds of invasion and synthesis ("template switching") can create edits that combine sequences from overlapping donors (14). To test whether template switching also occurs in human cells, we combined two donors in a single editing experiment. One donor was an ssODN with two HAs and an insert containing GFP11 with a STOP codon that prevents translation of the full-length fusion (Figure 7A). The second donor was an ssODN of the same polarity but with no HAs and no STOP codon (wild-

type GFP11 insert). Consistent with template switching, we obtained 4% GFP+ edits when using both donors, compared to 0.3% and 0.6% GFP+ edits when using only the first or second ssODN, respectively (Figure 7A).

To visualize template switching more directly, we combined wild-type donors with recoded donors where the GFP11 insert contained several silent mutations and used Illumina sequencing to sequence the insertion en masse (Figure 7B). Using recoded donors with silent mutations every 12 bases in the GFP11 insert, we identified evidence of template switching in 1.4% of edits ("chimeric edits", see Materials and Methods). Interestingly, the same experiment performed with donors that contained silent mutations every 6 or every 3 nucleotides resulted in only 0.5% and 0% chimeric edits, respectively (Figure 7B, Figure S5 and Table S6). The chimeric edits could not have resulted from sequential rounds of Cas9 cleavage and repair, since the edit destroyed the crRNA pairing sequence. The chimeric edits also could not have arisen during PCR amplification, since we observed no chimeric edits in a control experiment mixing two different cell populations (Figure S5). We conclude that template switching occurs between donors *in vivo* and is sensitive to the degree of homeology between donors.

#### Accuracy of repair is asymmetric

To investigate the accuracy of repair with linear donors, we isolated GFP+ and GFP- cells by fluorescence-activated cell sorting from a single editing experiment targeting the *Lamin A/C* locus with a GFP-containing PCR fragment (Figure S6). Each cell was grown out as a clone and the *Lamin A/C* locus was amplified using two primers flanking the insertion site. As expected, all 48 GFP+ clones contained at least one *Lamin A/C* allele with a full-size insert (4 were homozygous with two edited alleles). We sequenced the GFP insert in 23 of the 48 GFP+ clones and identified 20 precise insertions and 3 imprecise insertions containing small in-frame indels at the left or right junction (Figures S6/S7). We also sequenced the wild-type-sized allele in 11 of the 44 heterozygous GFP+ clones, and identified 2 with wild-type sequence, 6 with indels at the DSB, and 3

with small inserts (<100 bp) corresponding to either the N-terminus or C-terminus of GFP (Figure S7). We also screened 37 GFP- clones by PCR and, surprisingly, identified 10 that contained inserts at the *Lamin A/C* locus. We sequenced 7 of the 10 inserts and identified 3 with a full-size GFP insert with out-of-frame indels at one junction and 4 with smaller GFP inserts (Figure S7).

In total, we sequenced 13 imprecise GFP edits and found only one internal deletion and one insertion in the wrong orientation (Figure S7). All other imprecise edits were full-size or truncated GFP fragments inserted in the correct orientation. All had one precise junction on the non-truncated terminus of GFP. The other junction was imprecise and contained indels (Figure S7). These observations are consistent with an asymmetric repair process that uses different mechanisms to initiate and resolve repair (see Discussion).

## mCherry-tagging of a mouse locus using a PCR donor with short homology arms

To test whether linear DNAs with short homology arms could also be used in a mammalian animal model, we designed a PCR fragment to insert mCherry in the mouse adenylyl cyclase 3 (Adcy3) locus. The PCR fragment contained a 739 bp insert flanked by two 36 bp homology arms designed to insert mCherry in frame near the C-terminus of Adcy3. The PCR fragment and in vitro assembled Cas9 complexes were co-injected into mouse zygotes, and the resulting pups were genotyped by PCR and Sanger sequencing (Figure S8). We identified 27/87 pups with a correct size insertion at the Adcy3 locus (31% edit efficiency). Sequencing of 10 full-size mCherry edits revealed them all to be precise (no indels). A parallel editing experiment using an mCherry supercoiled plasmid with 500 bp HAs yielded 5 edits from 25 pups (20% edit efficiency). Similar knock-in efficiencies were also reported recently using long single-stranded donors (25). We conclude that PCR fragments with short HAs function as efficient donors in mouse embryos. PCR fragments yield edits at frequencies similar to plasmids and ssDNA donors, but with the added convenience of ease of synthesis especially for long inserts.

#### **Discussion**

In this report, we demonstrate that PCR fragments are efficient donors for genome editing in human cells and mouse embryos. PCR fragments require only short homology arms (HAs ~35 bp) and can be used to integrate edits up to 1kb. PCR fragments (and ssODNs) appear to participate in a replicative repair mechanism that broadly conforms to the SDSA model for gene conversion. Our findings suggest simple guidelines to streamline donor design and maximize editing efficiency (Figure S9).

#### Linear DNAs repair Cas9-induced DSBs by templating repair synthesis

In principle, linear donors could repair Cas9-induced breaks by integrating directly at the DSB. For example, microhomology-mediated end-joining (MMEJ) could cause donor ends to become ligated to each side of the DSB (8). Alternatively, HAs on the donor could form holiday junctions with sequences on each side of the DSB. Cross-over resolution of the two holiday junctions could cause donor sequences to become integrated at the DSB. This type of HDR has been proposed to underlie genome editing with plasmid and viral donors (16). In these models, repair is symmetric: the same mechanism (MMEJ or recombination) is used to ligate donor sequences to each side of the break. In contrast, our observations suggest that repair with linear donors proceeds by an asymmetric, likely replicative, process. First, ssODNs with only one HA show strong polarity specificity (Figure 4), consistent with a specific requirement for pairing with 3' ends at the DSB (Figure 1). Second, recessed HAs (HA at a distance from the DSB) are rarely used to initiate a repair event, but can be used to resolve a repair event (Figure 6). Third, internal homologies on the donor can bypass integration of distal edits (Figure 5). Fourth, most imprecise edits have asymmetric junctional signatures (Figure S7). These observations suggest that

the repair process is polar like DNA synthesis and has different requirements to initiate and resolve repair. These findings are consistent with the SDSA model for gene conversion (15) (Figure 1). SDSA initiates with DNA synthesis templated by the donor to extend 3' ends at the DSB, and resolves by annealing of the newly replicated strand(s) back to the locus. Our observations suggest that initiation of DNA synthesis is the most homology-stringent step, requiring a ~35 base HA on the donor complementary to sequences directly adjacent to one side of the DSB. The observations that HAs longer than 35 bases do not perform significantly better, and that distal HAs perform more poorly, suggests that resection exposes only short regions of ssDNA on either side of the DSB. In contrast to the initiation step, the resolution step has more relaxed homology requirements. Recessed arms can be used for that step, and in fact repair can proceed with no HA on the "annealing side" (Figure 4). In that case, NHEJ (or MHEJ) is used to fuse the newly replicated strand to the other side of the DSB. One possibility is that NHEJ or MHEJ competes with annealing during resolution, especially in the case of long edits where synthesis has a higher chance of stalling before reaching the distal HA or before synthesis of a complementary strand primed from the other side of the DSB (Figure 1). Consistent with this view, we recovered several partial GFP insertions that were integrated in the correct orientation but contained one imprecise junction on the truncated side of GFP.

Partial edits due to premature withdrawal of the newly replicated strand from the donor should be less frequent with shorter inserts. Consistent with this prediction, we found that editing efficiency is inversely proportional to insert size. At the *Lamin A/C* locus, we obtained 45% edits for a 57 bp insert, 26% edits for 714 bp insert (GFP) and 20% edits for a 993 bp insert. The size of the insert, and not the overall size of the donor, correlated with efficiency (Figure 3). A likely possibility is that the low processivity of repair polymerases (26) increases the chances of aberrant dissociation/annealing events on long inserts.

We also obtained evidence for dissociation and invasion events between donors. Such "template switching" causes sequences from overlapping donors to become incorporated in the same edit. We found that template switching is

sensitive to the degree of homology between donors and is reduced significantly by mutations every 3 or 6 bases. Similarly, recoding of sequences between the DSB and the edit can reduce the occurrence of premature annealing events that terminate synthesis on the donor before copying of the edit. Template switching may also explain why editing efficiency is sensitive to donor molarity, since high donor molarity is predicted to lower the frequency of aberrant dissociation/annealing events during synthesis. It will be interesting to determine which repair polymerases are responsible for synthesis templated by linear donors and whether their processivity characteristics account for our observations of template switching. In this regard, it is interesting to note that we identified a higher frequency of full-length edits (and lower frequency of partial edits) in mice compared to HEK293T cells. This difference could reflect differences in the properties of the enzymes that mediate SDSA in the two systems. Alternatively, the higher precision in mice could be due to a more efficient method for delivering donors at high molarity (pronuclear injection in mouse zygotes versus nucleofection in HEK293T cells).

## SDSA as a repair mechanism for Cas9-induced DSBs: implications for genome editing

The demonstration that both ssODNs and PCR fragments engage in SDSA to repair Cas9-induced DSBs in human cells has two important implications for genome editing. First, the SDSA model makes simple predictions for optimal donor design (Figure S9). These predictions improve editing efficiencies for edits at distance from the DSB, and eliminate the effort and expense used in creating donor DNAs with unnecessarily long homology arms. Linear donors with short homology arms can be chemically synthesized as single-stranded or double-stranded DNA fragments without any cloning. In this manner, tagging of genes with GFP can be achieved readily, without resourcing to split-GFP approaches that also require expression of a complementary GFP1-10 fragment (22). Second, because SDSA is thought to be a widespread mechanism for DSB repair among eukaryotes (27), it is likely that the

approaches outlined here will be applicable to other cell types and organisms. We documented previously that PCR fragments with short HAs perform well in *C. elegans* (14), and we demonstrate here the same for HEK293T cells and mouse embryos. It will be interesting to investigate whether linear donors with short HAs can also be used for genome editing in pluripotent cells and post-mitotic cells.

#### **Acknowledgments**

We thank the JHU GRCF sequencing facility, the JHU Transgenic facility and the JHU Ross Flow Cytometry Core Facility for expert support. We also thank Andrew Holland and Tyler Moyer for discussions and help with tissue culture and Boris Zinshteyn for discussions about Illumina sequencing and data analysis. This work was supported by National Institutes of Health (NIH) [grant number R01HD37047 to G.S., R01DC004553 to R.R., F32GM117814 to A.F.]. G.S. and R.G. are investigators of the Howard Hughes Medical Institute. D.H.G. is a Damon Runyon Fellow supported by the Damon Runyon Cancer Research Foundation (DRG-2280-16).

#### Figure legends

### Figure 1: Synthesis-dependent strand annealing (SDSA) model for gene conversion

Diagrams showing SDSA model for gene conversion (after (15)). Each line corresponds to a DNA strand: locus - grey, donor - blue, edit - green. Dotted lines represented newly synthesized DNA.

- A. DSB is resected creating two 3'overhangs on each side of the DSB.
- **B.** Strand invasion / DNA synthesis: The overhangs pair with complementary strands in the donor and are extended by DNA synthesis.
- **C.** Annealing: The newly synthesized strands withdraw from the donor and anneal back at the locus. Ligation seals the break.

## Figure 2: PCR fragments with short homology arms are efficient donors for GFP insertion

**A.** Diagrams showing PCR donors for GFP insertion at the *Lamin A/C* and *RAB11A* loci. Locus - grey, GFP - green, HA (Homology Arm) - blue, DSB - vertical line. GFP was inserted at the DSB in *Lamin A/C* and 11 bp upstream of the DSB in *RAB11A* locus.

- **B.** Graphs showing % of GFP+ cells obtained with PCR donors with HAs of the indicated lengths (33/33 refers to a right HA and a left HA, each 33 bp long). Insert size in all cases was 714 bp. PCR fragments were nucleofected in HEK293T cells at the concentration indicated and cells were counted by flow cytometer 3 days later. For this and all other figures, see Table S1 for details.
- **C.** Graphs showing % of GFP+ cells obtained with PCR or plasmid donors with HAs of the indicated lengths. Insert size in all cases was 714 bp. PCR fragments were nucleofected in HEK293T cells at the concentration indicated and cells were counted by flow cytometer 3 days later.
- **D.** Confocal images of cells 3 days after nucleofection. GFP: green, DNA: blue. The GFP subcellular localizations are as expected for in frame translational fusions.

#### Figure 3: Editing efficiency increases with decreasing insert size

- **A.** Knock-in of donors containing full-length GFP at the *Lamin A/C* locus. PCR fragments were nucleofected in HEK293T cells at the concentration indicated and cells were counted by microscopy 3 days later. HAs were 33/33, except for the last column where HAs were 237/237.
- B. Knock-in of donors containing full-length GFP or GFP11 at the *Lamin A/C* locus. PCR fragments were nucleofected at the concentration indicated in HEK293T (expressing GFP1-10) and cells were counted by microscopy 3 days later. HAs were 33/32 (57 and 336 bp inserts) or 33/33 (714 and 993 bp inserts).
  C. Knock-in of donors containing full-length GFP or GFP11 at the *RAB11A* locus (11 bp upstream of DSB). PCR fragments were nucleofected at the concentration

indicated in HEK293T (expressing GFP1-10) and cells were counted by flow cytometer 3 days letter. HAs were 33/33.

#### Figure 4: Repair is a polarity-sensitive process

- **A.** Diagrams showing pairing of ssODNs (antisense: dark blue, and sense: light blue) with resected ends of the locus (grey). Dotted lines denote DNA synthesis as in Figure 1. The ssODNs have only one 32-33 bp HA and a 126 bp insert (containing a combination of 3xFlag and GFP11 green).
- **B.** Normalized efficiency of sense *vs* antisense ssODNs containing only one HA corresponding to sequence on left or right side relative to DSB. The polarity that allows pairing between the ssODN and resected ends (as shown in diagram in A) is favored. Numbers on top of each column indicate the % of GFP+ cells determined by microscopy (*Lamin A/C*) or flow cytometer (*RAB11A*).

## Figure 5: Recoding of sequences between the DSB and the edit increases recovery of distal edits.

**A.** Schematics showing possible interactions between resected locus (grey, *PYM1* locus) and ssODNs coding for proximal and distal edits. Dotted lines denote DNA synthesis. Proximal edit (green, Restriction Enzyme RE site) is 1 bp to the right of the DSB and distal edit (red, 3xFlag) is 23 bp to the left of the DSB. Asterisks denote silent mutations in the region between the distal and proximal edits. Circles show the relative frequency of cells containing both edits (purple), only the proximal edit (green), or only the distal edit (red). % refers to the percent of each edit types among all the cell clones analyzed analyzed by PCR genotyping (size shift) and RE digestion. N refers to the total number of edited cell clones.

**B.** Schematics showing possible interactions between resected locus (grey, *Lamin A/C* locus) and PCR fragments coding for proximal and distal edits. Dotted lines denote DNA synthesis. Proximal edit (green, GFP11) is at the DSB and distal edit (red, tagRFP) is 33 bp to the right of the DSB. Asterisks denote silent mutations in the region between the distal and proximal edits. Circles show the

relative frequency of cells containing both edits (purple), only the proximal edit (green), or only the distal edit (red). Edits were determined by microscopy. % refers to the percent of each edit type among all cells analyzed by microscopy. N refers to the total number of edited cells.

#### Figure 6: Polarity of ssODNs affects incorporation of distal edits

**A.** Schematics showing possible interactions between resected locus (grey) and ssODNs (light or dark blue depending on polarity) coding for a distal edit (green). Dotted lines denote DNA synthesis. Asterisks denote silent mutations in the region between the DSB and the distal edit.

**B.** Normalized efficiency of sense vs antisense ssODNs with edits inserted to the left of the DSB, near the DSB, or to the right of the DSB. Distance from the DSB is indicated under each experiment. The locus and guide RNA and polarity are also indicated below. ssODN polarity has little effect on editing efficiency for proximal edits. In contrast, ssODN polarity has a large effect for distal edits. The favored polarity changes depending on whether the distal edit is positioned to the left or right of the DSB. Note that the favored ssODN polarity does not correlate with crRNA polarity (for example, first two columns in the graph show crRNAs 1776 and 1777 which cut at the same position but have opposite polarity). Experiments involving the *PYM1* locus were done on HEK293T that were cloned out and genotyped by PCR genotyping (size shift) for 3xFlag insertion (see Figure 5). All other experiments were performed on HEK233T (GFP1-10) cells that were directly scored for GFP+ by flow cytometer or microscopy 3 days after nucleofection. Numbers on top of each column indicate the overall % of edits. Note that overall frequency decreases with increasing distance from the DSB (see Figure S4).

#### Figure 7: Repair is prone to template switching between donors

**A.** Schematics showing repair of a DSB at the *RAB11A* locus with two donors. Donor 1 contains GFP11 with a STOP codon and two HAs. Donor 2 contains

GFP11 with no stop codon and no HA. Double arrows indicate identical sequence shared between the donors.

Graphs showing the percent of GFP+ cells (Y axis, as determined by flow cytometer) for each donor combination (X axis). Each donor used separately gives % of GFP+ close to background whereas donor 1 + donor 2 gives GFP positives cells (4%, GFP expression confirmed by microscopy). As a reference, an ssODN identical to donor 1 but without the STOP codon gives 19.6% edits (discontinuous right column).

**B.** Schematics showing repair of a DSB at the *Lamin A/C* locus with two donors. Donor 1 contains GFP11 and two HAs. Donor 2 contains a recoded GFP11 (stars) with no HA. Double arrows indicate identical sequence shared between the donors. In this experiment, the edits were amplified en masse by PCR using a locus-specific primer and an insert-specific primer and sequenced by Illumina sequencing (see Material and Methods).

Graph showing the % of reads with evidence of template switching (Y axis) for each donor combination (X axis). Donor 1 + donor 2 without mutations and donor 1 + donor 2 with 1 mutations every 3 nucleotides (1/3) show no evidence of template switching (0%), whereas donor 1 + donor 2 (1/6) and donor 1 + donor 2 (1/12) show evidence of template switching (0.5% and 1.4% respectively). See Figure S5 and Table S6 for details.

#### Figure S1: crRNAs used in this study

Schematics showing guide RNAs (arrows) used in this study mapped on *Lamin A/C*, *RAB11A*, *SMC3*, *PYM1* (human) and *Adcy3* (mouse) loci. Grey boxes indicate coding exons, only the first and last exons are shown for *Lamin A/C*, *RAB11A*, *SMC3*, and mouse *Adcy3*. For each guide, arrows indicate the 3' end. Numbers indicate position of the DSB relative to the ATG or STOP codon. Chemically synthesized crRNAs were used at all loci, except for *PYM1* where we used a plasmid-encoded sgRNA. Guide RNA sequences are in Table S4.

## Figure S2: tagging with GFP of the *SMC3* locus using PCR repair template with short homology arms

**A.** Diagram showing PCR donor for GFP insertion at the *SMC3* locus. Locus - grey, GFP - green, HA (Homology Arm) - blue. GFP was inserted 5 bp to the right of the DSB.

**B.** Graphs showing % of GFP+ cells obtained with PCR fragments with HAs of the indicated lengths. Insert size in all cases was 714 bp. PCR fragments were nucleofected in HEK293T cells at the concentration indicated and cells were counted by flow cytometer 3 days letter.

**C.** Confocal images of cells 3 days after nucleofection. GFP: green, DNA: blue. The GFP subcellular localization is as expected for in-frame translational fusion to *SMC3*, a nuclear protein.

## Figure S3: Flow cytometer plots of cells tagged with PCR repair templates Flow cytometer plots showing the number of cells (Y axis) and their GFP intensity (X axis).

**A.** Lamin A/C, RAB11A and SMC3 were targeted in HEK293T cells with an eGFP containing PCR fragment with or without ~35 bp Homology Arms (HAs). Green double arrows indicate the % of GFP+ cells. For every experiment, non-nucleofected cells were also run through the flow cytometer to determine background fluorescence (<0.5% cells). Note that donors without HAs yield GFP+ values slightly above background, consistent with a low level of integration by NHEJ or MMEJ.

**B.** RAB11A was targeted in HEK293T (GFP1-10) cells using a GFP11-containing repair template with or without ~35 bp Homology Arms (HAs). Green double arrows indicate the % of GFP+ cells. Non-nucleofected cells were also run through the flow cytometer to determine background fluorescence (<0.5% cells). Note that HEK293T cells that express GFP1-10 cells have a higher intrinsic fluorescence than HEK293T cells.

#### Figure S4: Insertion efficiency relative to distance from the DSB

Graph showing the efficiency % of editing (Y axis) vs distance from the DSB (X axis) (data from Figure 6). Each line links editing experiments performed with the same guide RNA. ssODNs (optimal polarity, Figure 6) were designed to insert the edit at varying distances from the DSB as indicated. For all ssODNs, the sequence between the edit and the DSB was partially recoded to minimize premature annealing and Cas9 re-cutting of the edited locus while preserving coding potential.

#### Figure S5: Illumina sequencing to monitor template switching

**A.** Schematic representation of the experimental design (see Figure 7). Stars in color represent silent mutations used to monitor template switching.

**B.** The probability of a mutation (relative to the "No mutation" template) at each nucleotide position in the region of the repair template, after removal of incompletely mapped and low-quality reads. Bars are color-coded by identity of the incorporated nucleotide. Green: A, blue: C, black: G, red:T. PCR control: Two cell populations that received separately a wild-type ssODN or a mutant ssODN (1/6 mutations) were combined for PCR amplification. This control was used to determine basal levels of template switching that might occur during PCR amplification. These levels are 25-fold lower than observed in cells cotransfected with the same two ssODNs (0.02% versus 0.50%).

## Figure S6: Derivation of GFP+ and GFP- clones from a single editing experiment targeting the *Lamin A/C* locus with a GFP-containing PCR fragment

**A.** Schematic showing the donor (green with blue Homology Arms - HAs) and targeted locus (grey). HEK293T cells were edited at the *Lamin A/C* locus with an eGFP PCR donor with 33/33 HAs, and FACS-sorted as GFP+ and GFP- cells. The clones were amplified and examined by confocal microscopy. All GFP+ cells exhibit the expected nuclear membrane localization expected from a GFP translation fusion with *Lamin A/C*.

**B.** Statistics of genotyping results for GFP+ and GFP- single clones. See text and Figure S7 for details.

#### Figure S7: Structure of imprecise GFP knock-in edits

Schematics showing the GFP inserts obtained in the experiment described in Figure S6.

Lamin A/C locus (grey line), Full-length left HA (L, 33 bp) and right HA (R, 33 bp) (blue), GFP (green, with length of GFP sequence indicated), Indel (red). GFP+ indicates cells with Lamin A/C GFP signal.

- A. Precise edit for reference
- B. Edits with imprecise right junctions
- (b1) Contain an 11 bp duplication of the *Lamin A/C* locus sequence just downstream the right HA.
- (b2) Contain a 6 bp deletion of the *Lamin A/C* locus sequence just downstream the right HA.
- (b3) Contain a deletion of the last 19 bp of the right HA and of the 8 bp just downstream the right HA sequence.
- (b4) Contain an 11 bp deletion inside the right HA.
- (b5) Contain only the 363 first bp of GFP sequence.
- (b6) Contain only the 70 first bp of GFP sequence followed by a 4 bp insertion and a full deletion of the right HA together with a 4 bp deletion of the *Lamin A/C* locus sequence just downstream the right HA sequence. Sequencing from wild-type size allele from Het GFP+ cell.
- (b7) Contain only the 22 first bp of GFP sequence followed by a 5 bp insertion and a deletion of the first 13 bp of the right HA. Sequencing from wild-type size allele from Het GFP+ cell.
- **C.** Edits with imprecise left junctions.
- (c1) Contain a 23 bp duplication of the left HA just upstream the GFP sequence.
- (c2) Contain on the left side the 8 first bp of GFP, followed by the 25 bp of the left HA sequence upstream of GFP, and followed by full-length GFP sequence.
- (c3) Contain a 52 bp insertion followed by the last 469 bp of GFP sequence.

(c4) Contain a deletion of the last 7 bp of the left HA followed by the last 68 bp of

GFP sequence. Sequencing from wild-type size allele from Het GFP+ cell.

D. Edit with internal deletion

(d1) Contain the 556 first bp of GFP sequence followed by a 12 bp insertion and

the last 13 bp of GFP sequence.

E. Edit with inverted insertion

(e1) Contain the left HA and first 501 bp of GFP sequence inverted.

Figure S8: mouse Adcy3 locus tagging with mCherry using PCR donor with

short homology arms

**A.** Schematic representation of the mouse *Adcy3* locus repair strategy: mCherry

(red), Homology Arms (HA, blue), locus (grey lines), DSB (blue line).

**B.** Example of genotyping PCRs using primers flanking the DSB (outside the

HAs) and run on agarose gel. The upper bands ('insert' arrow) correspond to the

mCherry insertion. Details can be found in Materials and Methods and Table S1.

Figure S9: Guidelines for donor design

A. Schematic showing typical editing experiment to introduce an edit (green box)

at a distance from the DSB (stippled line).

**B.** Recommendations based on results presented in this study. We refer readers

to (5, 21) for additional recommendations for ssODNs designed to insert edits at

27

the DSB.

Table S1: Detailed experimental conditions and results

Table S2: Repair templates used in this study

Table S3: Plasmids used in this study

Table S4: Primers used in this study

#### Table S5: crRNA/sgRNA used in this study

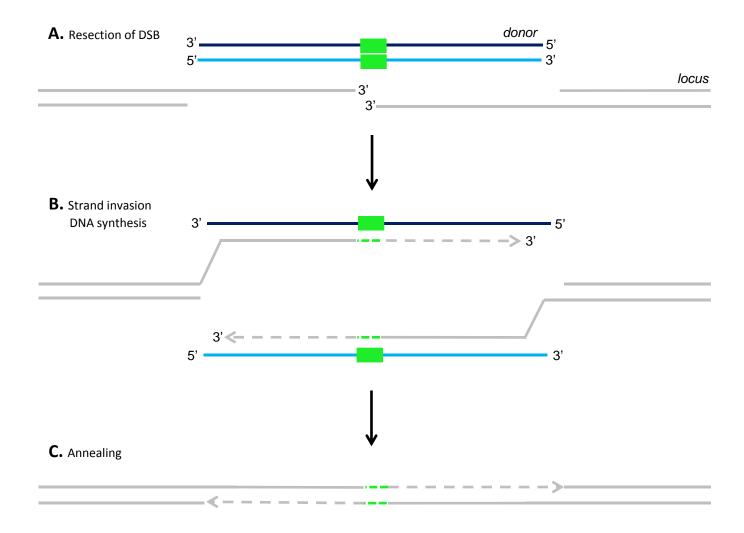
#### Table S6: Classification of reads from Illumina sequencing

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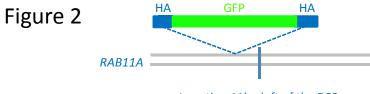
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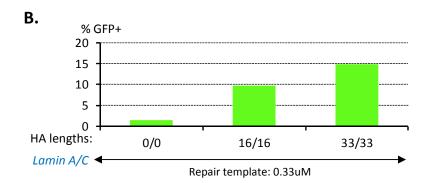
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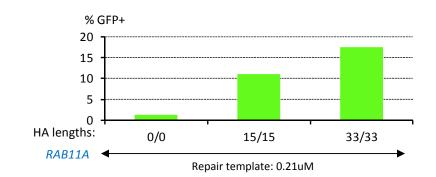
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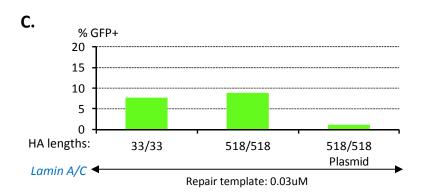
Insertion at the DSB

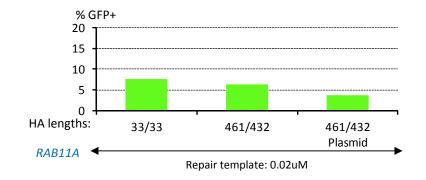


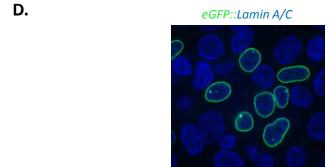
Insertion 11bp left of the DSB











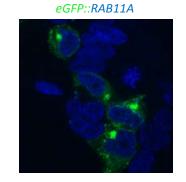
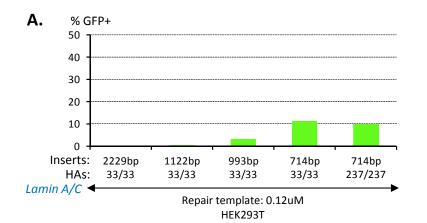
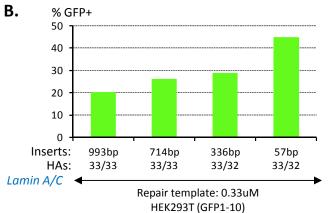


Figure 3





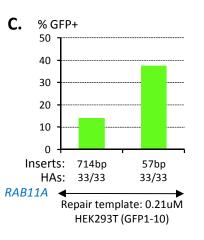


Figure 4

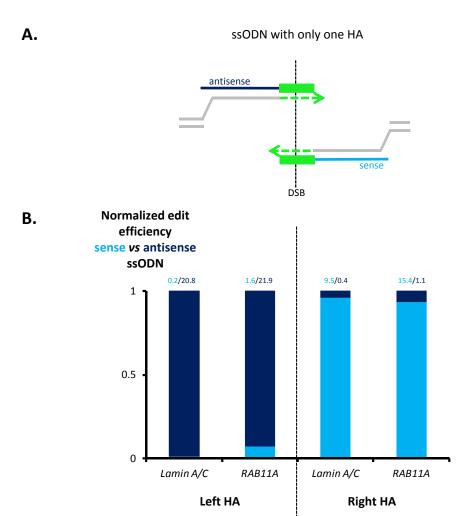
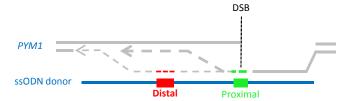


Figure 5





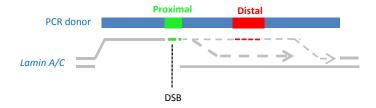


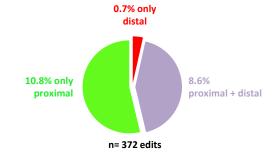
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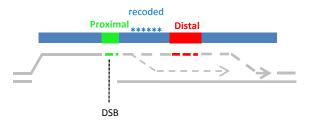
DSB



#### В.







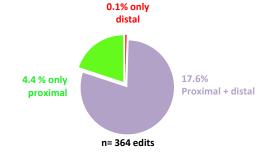


Figure 6

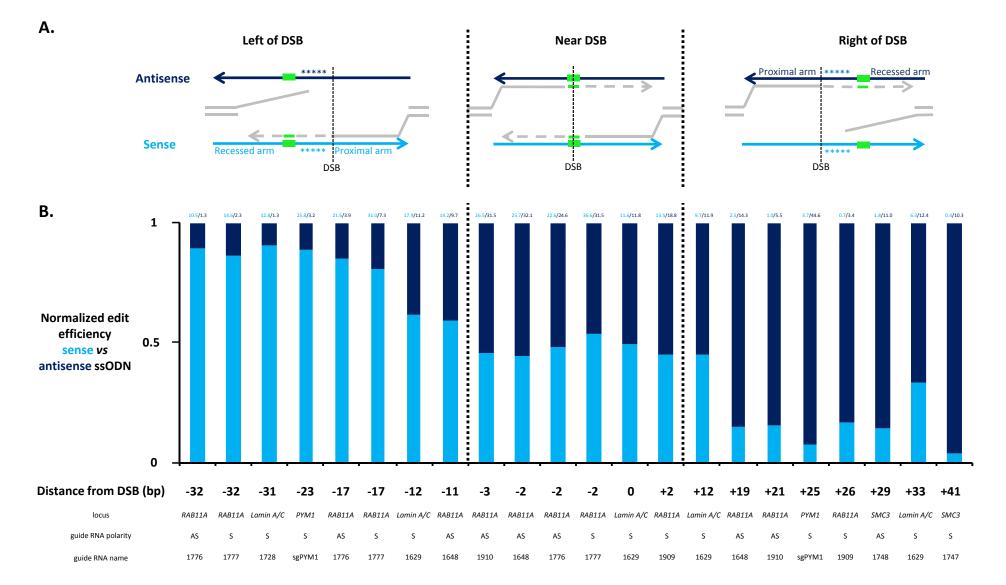
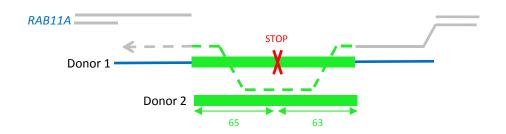
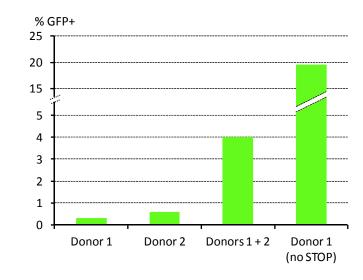


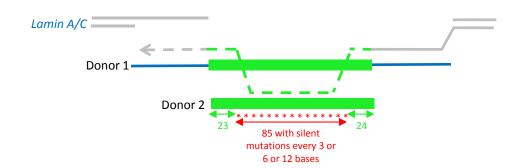
Figure 7

A.





В.



% of reads with template switching

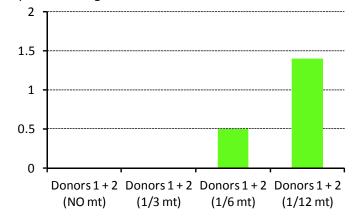
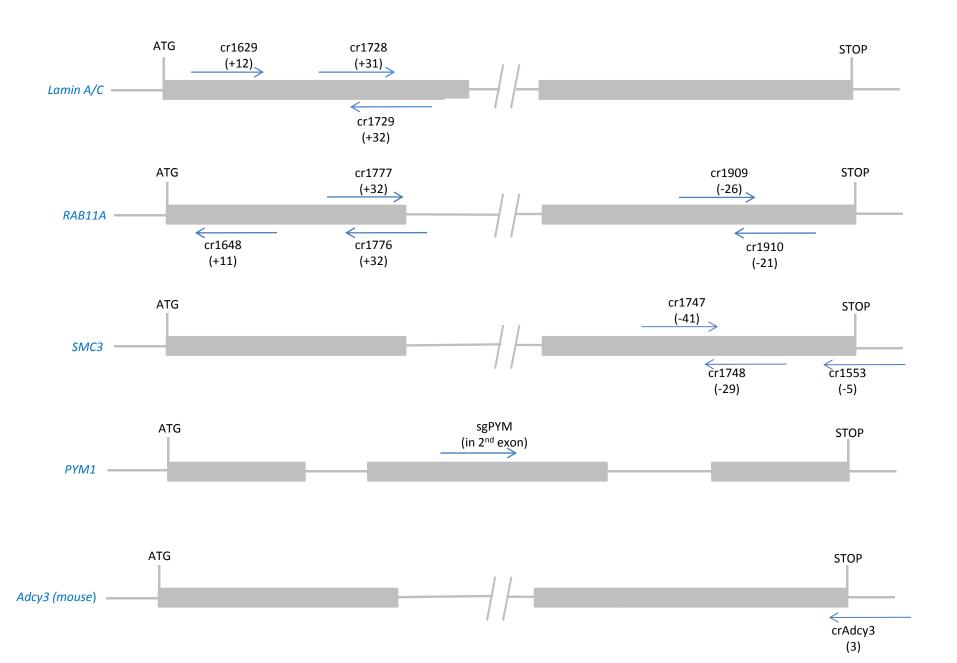


Figure S1



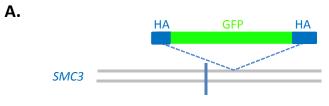
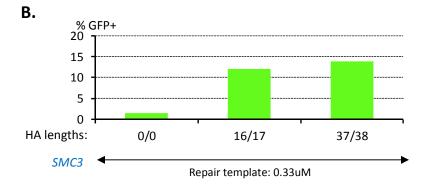


Figure S2

Insertion 5bp to the right of the DSB



C.

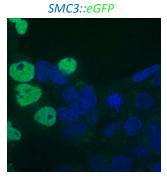


Figure S3

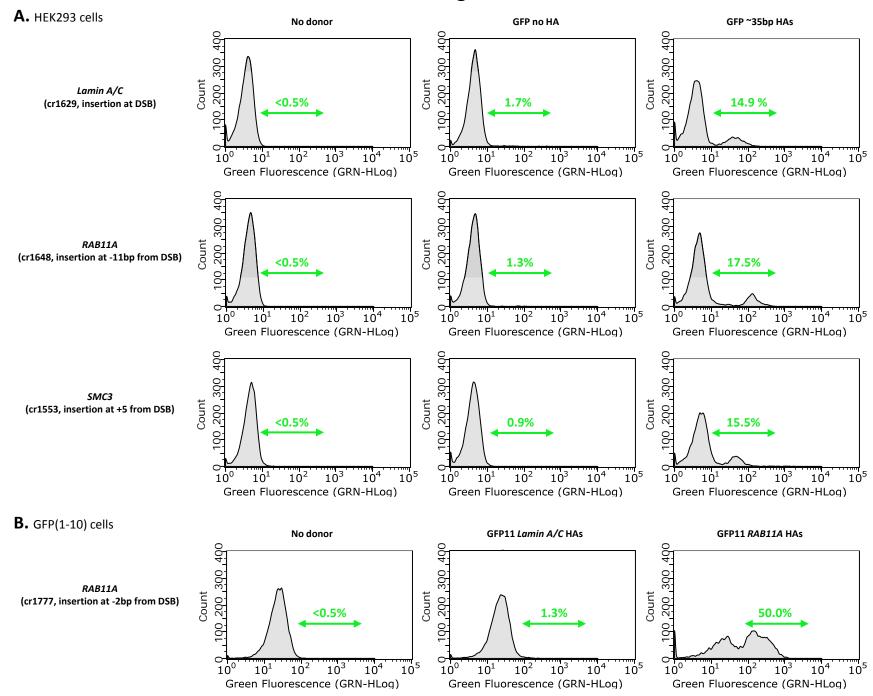


Figure S4

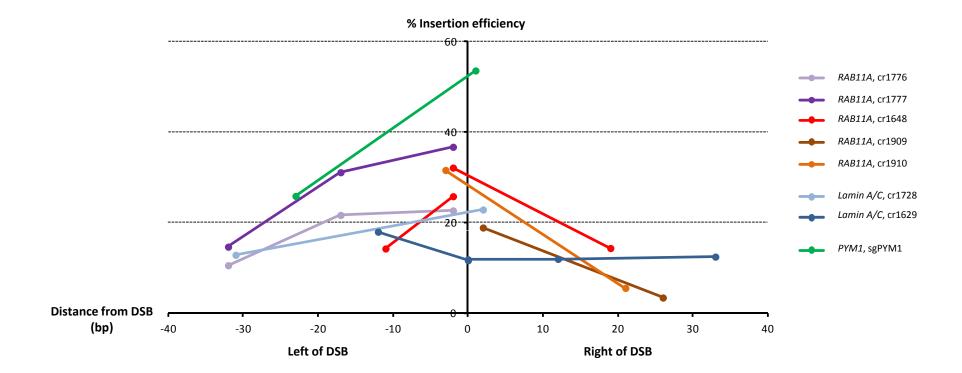
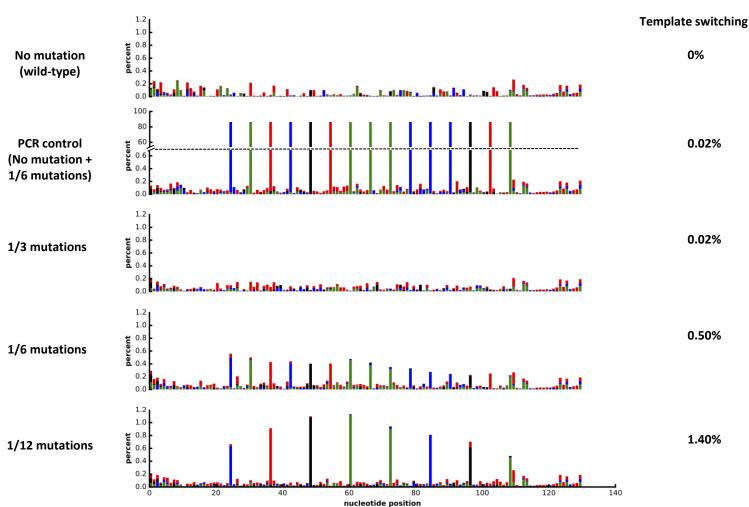


Figure S5





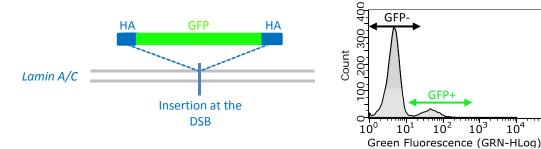


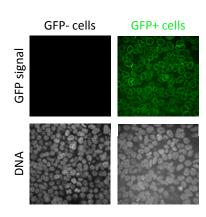


## Figure S6

10<sup>5</sup>







п.
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_				
	GFP- fraction	GFP+ fraction		
% cells GFP+ (microscopy)	0% (0/1884)	98% (1744/1778)		
% of cell with insertion (PCR genotyping)	27% (10/37)	91.7% Het (44/48) 8.3% Homo (4/48)		
Sequencing results	6/7 with GFP Indel 1/7 inverted GFP insertion	20/23 with correct insertion 3/23 with GFP in frame Indel		

Figure S7

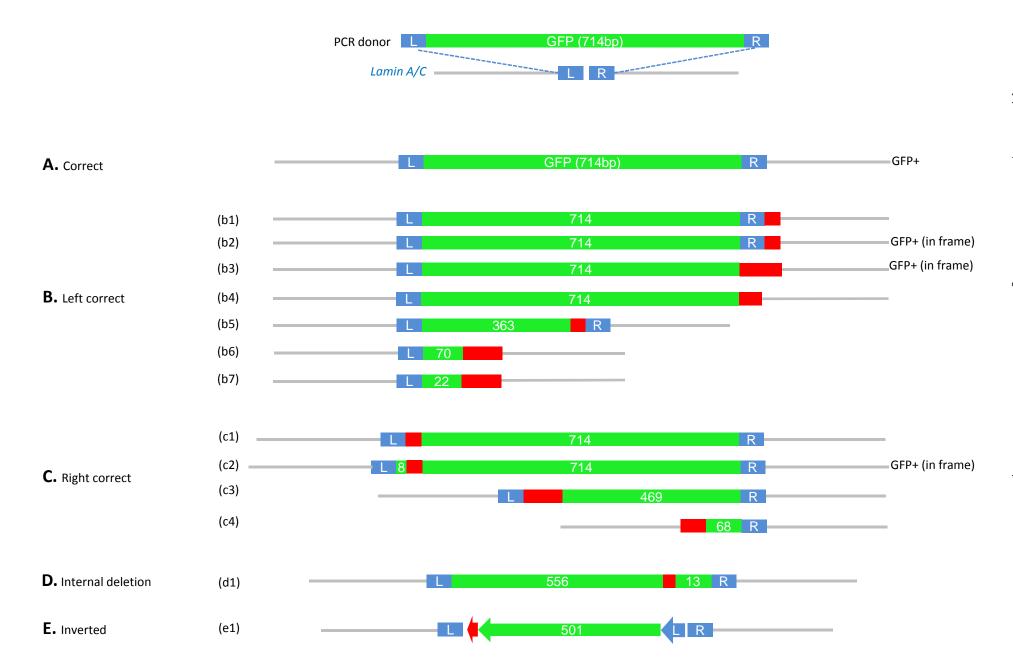


Figure S8

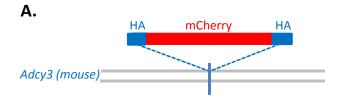
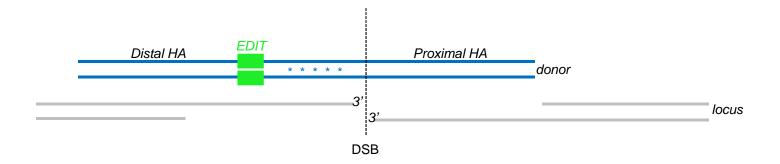




Figure S9

A.



В.

- 1. Edit: less than 30 bases from DSB (and less than 1kb in length if insertion).
- 2. HAs: ~35 bases.
- 3. At least one proximal HA (directly abutting DSB) with no/few mutations. If using an ssODN, make sure proximal HA is at 3' end of the ssODN.
- 4. Recode sequence between edit and DSB to prevent premature annealing (asterisks). Also include here any mutations needed to prevent recutting by Cas9.
- 5. For small edits that cannot be identified by size change, also add a restriction site in edit (or distal to edit) to facilitate detection.

				college/college colleges		TABLE SS: Detailed experimental con	ditions and results				
sat	Cell type	Cach delivery	came	orkna/ugkna polarity (relative to gene coding sequence)	Regair template type/same	Repair template left, fright Hamallagy Arms (nucleorides/basepains) FRIUMSS 2 / S2 / S	Repair template palarity (relative to gene cading sequence)	Distance between Diff and ed (bg, relative to Diff)	Regair template concentration (uM for KNP/Nucleofection)	(nucleotides/kasepairs)	Efficiency (N) (for cytometer analysis non-nucleofested cells 45.5%)
eCFP: Lamin A/C eCFP: Lamin A/C	HEIZ937 HEIZ937	RM/Nucleofection RM/Nucleofection	09NA 1629 09NA 1629	s s	PCR 1650/932 PCR 1858/1859	0/0 36/56	dichia.	0	0.88	714 714	Cytometer: 1.5 (average of independent experiments: 1.8 = 1.7)  Cytometer: 9.7 (sechnical replicates: 9.4 and 50.5)
eGFP: Lamin A/C	HEK2937	#M/Nucleofection	ORNA 1629	s	PCR 1618/1618 or PCR 1763/1764	11/11	didNA		0.88	714	Cytometer: 14.9 (average of independent experiments: 19.2 = 14.9 = 13.2 = 15.1 = 16.2 = 12.5 = 16.9 = 12.7 = 11.8 = 18.1). Sequencing results can be found in Figure 56 and 57
eGFP: Lamin A/C	H942937	#MP/Nucleofection	C/RNA 1629	s	PCR 1763/1766	31/33	dichia		0.09	714	Cytometer: 7.7 (average of independent experiments: 7.5 + 2)
eGFP: Lamin A/C eGFP: Lamin A/C	1612107 1612107	RM/Nucleofection RM/Nucleofection	ORNA 1629 ORNA 1629	s s	PCR 1761/1762 Placed 1716	518/518 518/518	diDNA diDNA	0	0.09	754 754	Cytometer: 9.9 (average of independent experiments: 9.4 + 9.4)  Cytometer: 1.1 (average of independent experiments: 1.1 + 1.2)
egip: rarita egip: rarita	HEK2937 HEK2937	RM/Nucleofection RM/Nucleofection	09NA 1648 09NA 1648	AS AS	PCR 1610/932 PCR 1818/1819	0/0 15/15	diDNA diDNA	-11 (recoded) -11 (recoded)	021 021	714 714	Cytometer: 1.8 Cytometer: 11.0
egep-rabila egep-rabila	HEK2937 HEK2937	#MP/Nucleofection #MP/Nucleofection	09NA 1648 09NA 1648	AS AS	PCR 1880/1881 or PCR 1652/1668 PCR 1880/1881	11/11	dichia.	-11 (recoded) -11 (recoded)	021	714 714	Cytometer: 17.5 (average of independent experiment: 17.5 + 22.5 + 12.6)  Cytometer: 7.6
eGFP-RARITA	H642937	#MP/Nucleofection	ORNA 1648	AS	PCR 1806/1867	461/432	dichia	-53 (recoded)	0.02	714	Cytometer: 6.4
ecop: Louis AC or ecop: Resile	HER2927 HER2927	RMP/Nucleofection RMP/Nucleofection	09NA 1648 09NA 1629	AS S	Place of 1791 PCR 1653/1653	661/632 No HA, PCR containing eGFP with RABITA HAS used with Lamin A/C	didNA dicular didNA	-11 (recoded)	0.02	714	Cysometer: 3.7 Microscope: no Lamin A/C or RARITA eGFP signal
SMC3: +GFF	H6K293T	#MP/Nucleofection	ORNA 1558	AS	POR 1690/WHZ	0/0	diDNA	+5 (recoded)	0.88	714	Cytometer: 1.4 (average of independent superiments: $1.9 \circ 0.9$ )
SMCX: vGFP SMCX: vGFP	HER293T HER293T	RM/Nucleofection RM/Nucleofection	ORNA 1553 ORNA 1553	AS AS	PCR 1604/1605 PCR 1554/1555	16/17 27/28	diDNA diDNA	+5 (recoded) +5 (recoded)	0.89	714 714	Cytometer: 12.0 (average of independent experiments: 10.1 = 1.k8)  Cytometer: 13.9 (average of independent experiments: 16.4 + 15.5 + 11.2 + 9.0 + 13.9 + 13.4 + 15.4 + 13.1)
TDV: eGFP: extra-sequence: tageFP (2229 bp insert): Lamin A/C TSV: eGFP: extra-sequence (1122 bp	H6K293T	#MP/Nucleofection	09NA 1629	s	PCR 2005/2006	11/11	diDNA		0.12	2229	Microscope: 0.0 (0)'s406, but few positives can be found when the entire microscope (lide was examined)
intert): Lamin A/C extra-sequence: eGPP (RM) bp intert): Lamin A/C	H612931 H612931	RNP/Nucleofection RNP/Nucleofection	C9NA 1629 C9NA 1629	s	PCR 2005/2005 PCR 2005/1609	11/11 11/11	dicha.		0.12 0.12	1122 983	Microscope: 0.5 Microscope: 3.2
eGFP: Samin A/C eGFP: Samin A/C	HEK293T HEK293T	RM/Nucleofection RM/Nucleofection	C9NA 1629 C9NA 1629	s	PCR 1618/1619 PCR 2018/2019	217/217	diDNA diDNA		032	714 714	Microscope: 11.4 Microscope: 10.0
extra sequence: eGPP (MRS by least): Lamin A/C	HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	ORNA 1629 ORNA 1629	s s	PCR 2009/1609 PCR 1618/1609	11/11	dichia.		0.89	993 754	Microscope: 26.4 (average of independent experiments: 31.2 + 25.7 + 22.3)
GPTI: extra-sequence (Biblip inset): comin A/C	HEX2907 (GFP1-10)	#MP/Nucleofection	ORNA 1629	5	PCR 2051/2052	31/12	didNA.		0.88	336	Microscope: 29.0
GPII:Lamin A/C eGP:RABIIA	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	ORNA 1629 ORNA 1648	S AS	PCR 2003/2004 PCR 1653/1653	11/12	dichia dichia	-11 (recoded)	039	57 714	Microscope: 44.9 Cysameter: 54.1
GP11:RAB11A GP11:RAB11A	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1648 09NA 1777	AS S	PCR 2008/2009 PCR 2014/2015	21/23	didha didha	-11 (recoded)	021	\$7 \$7	Cytometer: 37.4 Cytometer: 50.0
GFF1:RAB11A or GFF11:Lonin A/C	HEX2937 (GFP1-10)	#M/Nucleofection	C/RNA 1777	5	PCR 2003/2006	No HA, PCR containing GFPS1 with Lomin A/C HALused with RABISA CHNA	diDNA		0.88	57	Cysometer: 1.8
GP11: Arling: Lonio A/C	HEX293T (GFP1-10)	#MP/Nucleofection	CRNA 1629		6100N 1798	CHNA FIGURE 4 38/0			5	126	Microsope: 0.2
GP11: Mrlag: Lorein A/C	HEX293T (GFP1-10)	#MP/Nucleofection	09NA 1629	5	6600N 1789	20/0	AS.		6	126	Microscope: 20.8
Juliag: GP11: Lamin A/C Juliag: GP11: Lamin A/C	HEX2907 (GFP1-10) HEX2907 (GFP1-10)	RM/Nucleafection RM/Nucleafection	09NA 1629 09NA 1629	5 5	600N 1706 600N 1706	6/32 6/32	s As		5	126 126	Microcope: 8.5 Microcope: 0.4
GP11: Juliag: Anklist GP11: Juliag: Anklist	HEX293T (GFP1-10) HEX293T (GFP1-10)	859/Nucleofection 859/Nucleofection	09NA 1648 09NA 1648	AS AS	6000N 1816 6000N 1817	niez aiez	s as	*1	s s	126 126	Cytometer: 1.6 Cytometer: 21.9
Auflag: GIP11:ANR11A	HEX293T (GFP1-10) HEX293T (GFP1-10)	RM/Nucleofection RM/Nucleofection	09NA 1648 09NA 1648	AS AS	6000N SR29 6000N SR20	9703	s as	4	s .	126	Optioneter: 15.4
Juring: GP911:Ank15A  PM811:All reserver and Juring Zillip opernoon		RM/Nucleofection  Plannid (Carls:T2A:SEP) SEP				PSURSS / 6 / 50		4	S 3.34pmail of oxbbN and 993ng of Carth plannid in a mix of		
(non-recoded)	H812937	satingl/frandector	qPM1	ś	1100N 1582	36/17	ś	+1 and -23	of Cach placed in a mix of 10 Med containing 8 Med of X- termed SNR 9 8 2-8 pensi of outbox and 842 ng of Cach placed in a mix of 10 Med containing 8 Med of X-	6 and 66	PCR cingle cell colonies (predicted size shift and Restriction Sizyme digest): 12.6 (B/KK) for KK and hafing invertion, 0 (B/KK) for hafing invertion alone, di. 0 (29/KK) for KK invertion alone
PMET: All near cut and Aurilag 200p upstream (recoded)	H\$12931	Plaunid (Cach:T2A:GFP; GFP sarting)/Transfection	sgPM1	s	HDDN 1580	36/17	s	+1 and -29 (recoded)	of Card plannid in a mix of 10 Rul containing 8 feet of X- tremedélNE 9 3 34pmei of 0000N and 892ng	6 and 66	PCK single cell colonies (predicted size shift and Restriction Extyree digest): 25.8 (15)/58, including 3 homozygous) for 86 and Asiling insertion, 1.7 (1/58) for 8xFlag insertion alone, 10.3 (4/58) for 86 in sertion alone
PMET: No near cut and Juliag 280p upstream (recoded)	H942937	Plaunid (Cach:T2A:GFP; GFP sarting)/Transfection	sgPIM1	s	6100N 1581	36/17	AS	+1 and -29 (recoded)	it 24pmoil of ox20N and 992ng of Cardyplasmid in a mix of 10 Rul containing it fiul of X- tremediately	6 and 66	PCR single cell colonies (predicted size shift and Restriction Snayme digest): k.2 (2,6/1) for RS and ReFilip Insertion, 0 (5,6/1) for ReFilip Insertion alone, 0 (5,6/1) for RS insertion alone
GFP11 at our and togistP 22tip downstream (non-recoded):Lamin A/C	HEX293T (GFP1-10)	#MP/Nucleofection	c/9NA 1629	s	PCR 1969/1989 (on plasmid 1892)	31/33	diche.	0 and +88	023	798 (57 + 33 + 708 )	Microscope: 8.6 (160/1802) for GPP11 and tag8FP insertion, 0.7(18/1802) for tag8FP insertion alone, 10.8 (199/1802) for GPP11 insertion alone
GFF11 or cut and togRFF 23tg downtream (recoded: Lamin A/C	HEK293T (GFP1-10)	#MP/Nucleofection	C9NA 1629	s	PCR 1969/1989 (on plasmid 1899)	31/23	dichia	0 and +33 (recoded)	028	798 (57 + 33 + 708 )	Microscope: 17.6 (288/1629) for GPT1 and tag8FP insertion, 0.1 (A/1629) for tag8FP insertion alone, 6.4 (73/1629) for GPP1 insertion alone
(WOODS) Linux A/C	HEX2937 (GPP1-10)	RM/Nucleofection	ORNA 1629	s	1890) HODN 1620	PIGURES 6 / 54 31/12	ś			57	tagror science acces, 6.6 (7.6) tazisi tor GPP11 science acces  Microscope: 11.6
6911: Lamin A/C	HEX293T (GFP1-10)	RM/Nucleofection	C/9NA 1629	s	HOON 1792	31/12	AS		s s	57	Microscope: 11.8
GP11:Lamin A/C GP11:Lamin A/C	HEX2937 (GFP1-10) HEX2937 (GFP1-10)	RM/Nucleofection RM/Nucleofection	09NA 1629 09NA 1629	5	HDDN 1679	20/25 20/25	s as	-12 (recoded) -12 (recoded)	5	57 57	Microscope: 17.9 Microscope: 11.2
GP11:Lamin A/C GP11:Lamin A/C	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1629 09NA 1629	s s	MOON 1798 MOON 1796	11/11	S AS	+12 (recoded) +12 (recoded)	5	57 57	Microscope: 8.7 Microscope: 11.9
GP11: Lamin A/C	HEX293T (GFP1-10)	#MP/Nucleofection	C/9NA 1629	5	HOON 1795	33/33	ś	+38 (recoded)	5	57	Microscope: 6.3
GP11:Lamin A/C GP11:Lamin A/C	HEX290T (GFP1-10) HEX290T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1629 09NA 1729	S AS	HDDN 1796	10/11	AS S	+ili (recoded) -il2 (recoded)	s s	57 57	Microscope: 12.4 Microscope: 1.5
GP11:Lamin A/C GP11:Lamin A/C	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	ORNA 1729 ORNA 1728	AS S	6000N 1797 6000N 1796	38/33	AS S	-RI (recoded)	s s	\$7 \$7	Microscope: 0.2 Microscope: 13.8
GP11: Lamin A/C	HEX2937 (GFP1-10)	RM/Nucleofection	ORNA 1728 ORNA 1648	s As	HOON 1777	20/34	AS .	-it (recoded)	\$	57 57	Microsope: 1.8 Opposite: 142
GPII:RABIIA	HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	C/RNA 1648	AS AS	HDDN 1779	20/23	s Aš	-11 (recoded)	5	57	Cysometer: 9.7
GP11:RAB1IA GP11:RAB1IA	HEX293T (GFP1-10) HEX293T (GFP1-10)	RM/Nucleofection RM/Nucleofection	CRNA 1648 CRNA 1648	AS AS	6000 1866 6000 1866	11/11	s as	4	s s	57 57	Cytometer: 25.7 Cytometer: 32.1
GPP11:RAB1IA GPP11:RAB1IA	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	ORNA 1648 ORNA 1648	AS AS	000N 1881 000N 1882	33/34 33/34	S AS	+18 (recoded) +18 (recoded)	5	57 57	Cytometer: 2.5 Cytometer: 14.k
GFP11:RAW11A	HEX293T (GFP1-10)	RNP/Nucleofection	C/RNA 1777	s	6100N 1792	33/34	ś	-32 (recoded)		57	Cytometer: 14.6 (superimental replicate: 14.4)
GPP11: RABILA GPP11: RABILA	HEX293T (GFP1-10) HEX293T (GFP1-10)	RM/Nucleofection RM/Nucleofection	C/RNA 1777	s s	600N 1768 600N 1888	31/34	AS S	-82 (recoded) -97 (recoded)	5	\$7 \$7	Cycometer: 2.3 (experimental replicate: 1.3) Cycometer: 31.0
GP11:RAB11A GP11:RAB11A	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1777	s s	6000N 1886 6000N 1827	21/34	AS S	-17 (recoded)	s 5	\$7 \$7	Cysometer: 7.8 Octometer: 36.6
GFP11:RAB11A	HEX2937 (GFP1-10)	#MP/Nucleofection	09NA 1777	s	6000N 1828	33/34	AS	4	6	57	Cytometer: 31.5
GP11: AND11A GP11: AND11A	HEX2007 (GFP1-10) HEX2007 (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	08NA 1776 08NA 1776	AS AS	HDDN 1782 HDDN 1788	23/34 23/34	š Aš	-12 (recoded) -12 (recoded)	s s	57 57	Cytometer: 10.5 (experimental replicator: 10.1) Cytometer: 1.8 (experimental replicator: 1.4)
GPP11:RAB11A GPP11:RAB11A	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 17% 09NA 17%	AS AS	6000 1838 6000 1836	33/34 33/34	S AS	-17 (recoded)	s s	57 57	Cytometer: 21.6 Cytometer: 3.9
GP11.AAR1A GP11.AAR1A	HEX293T (GFP1-10) HEX293T (GFP1-10)	#M/Nucleofection	ORNA 1776 ORNA 1776	A6	HDDN 1827 HDDN 1828	33/34 33/34	S AS	4	5	87	Cytometer: 22.6 Cytometer: 26.6
A48134: 6F911	HEX293T (GFP1-10)	RNP/Nucleofection	C/RNA 1810	AS	HOON 1911	35/12	ś	4		57	Cytometer: 26.5
ANDA: GPEI	HEX293T (GFP1-10) HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1910 09NA 1910	AS AS	600W 1912 600W 1928	16/12 16/15	AS S	-8 +25 (recoded)	5 5	\$7 \$7	Cytometer: 31.5 Cytometer: 1.0
AMELA: GP11 AMELA: GP11	HEX293T (GFP1-10)	RMP/Nucleofection RMP/Nucleofection	09NA 1910 09NA 1909	AS S	6000N 1925 6600N 1911	16/15 28/25	AS S	+21 (recoded) +2	5	57 57	Cysometer: 5.5 Cytometer: 15.5
A48134: 6FF11	HEX2907 (GFP1-10)	RM/Nucleofection	c/8NA 1909		HOON 1912	20/25	AS	+2		57	Cytometer: SRR
AMELIA: 6991 AMELIA: 6991	HEX2907 (GPP1-10)	RM/Nucleofection RM/Nucleofection	CRNA 1909 CRNA 1909	5 5	HDDN 1922 HDDN 1923	16/15 16/15	s as	+26 (recoded) +26 (recoded)	5 5	57 57	Cysometer: 0.7 Cysometer: 3.4
SMC2: GFF11 SMC3: GFF11	HEX293T (GFP1-10) HEX293T (GFP1-10)	RM/Nucleofection RM/Nucleofection	09NA 1768 09NA 1768	AS AS	HOON 1751	34/28 34/28	s as	+29 (recoded) +29 (recoded)	s s	57 57	Microscope: 1.8 Microscope: 11.0
SMCR: 68911 SMCR: 68911	HEX2907 (GPP1-10)	RM/Nucleofection RM/Nucleofection	09NA 1767 09NA 1767	s s	600N 1758 600N 1754	34/28	s as	+65 (recoded) +65 (recoded)	s s	57 57	Microscope: 0.4 Microscope: 20.3
SMEE: GFF21  PFM2: All ever cut and lafting 25thp  dissertment incoded.	HEK293T (GPP1-10) HEK293T	RMP/Nucleofection  Plannid (Cach: T2A::GFP; GFP  sortion) (Counterfaction	opper star	s s	600N 1764 600N 1988	34/68	AS S	+61 (recoded) +1 and +25 (recoded)	3.24pmoi of ox20N and 892ng of Cardyplasmid in a mix of	57 6 and 66	Microscope: 50.k  PCR single cell colonies (predicted das delth and Restriction Snoyme diget): 1.7  (2)/64) for Hi and NeTag insertion, 0 (5/Hi) for heling insertion alone, 0 (5/Hi) for Hi intertion alone.
downstram (recoded)  PYME: All near cut and heliop 25top downstram (recoded)	H92937	sating)/Transfection  Plaunid (Carls:T2A:16FF; GFP sating)/Transfection	gPMs		sicon 158s	30/40	Ali	+1 and +25 (recoded)	10.8ul containing 8.6ul of X- temed&NE 9 8.24pmol of oxDDN and 892ng of Card plasmid in a mix of 10.8ul containing 8.6ul of X-	6 and 66	
									TremeGENE 9		PCR cirgle cell colonies (predicted size shift and Restriction Enzyme digest): 64.6 (21/62, including 7 homosygous) for RI and Jahlag Intertion, 0 (5)(47) for 3xFlag Intertion alone, 10.6 (5)(47) for RI is sertion alone
PMI: bring	HEX2007 HEX2007 (0095-10)	Plannid (Cadr.T3A::GFP; GFP sarting)/frandlection RMP/Nucleofection	uphM1	S AS	H00N 1518	6/43 33/33	6	-1	of Cach planned in a mix of 10 Rul containing 8 feet of X- tremedible 9	66	PCK single cell colonies (pre-dicted size shift): Sil.5 (Rb/S6, induding 7 homozygow)  Microscope 2.0
69711:12466 A/C	HEX2007 (GP1-10) HEX2007 (GP1-10)	EMPRECEDIATION EMPRECEDIATION	09NA 1729 09NA 1728 09NA 1648	š	MOON 1786 MOON 1786 MOON 1997 and MOON 1879	33/63 32/63 9959857/55 33/63 and non-applicable	i	3	\$	57 57	Microscope: 2.0 Microscope: 22.8 Consener: 0.1
STOP Agence: GP11 with STOP framestift: some expense: RABIIA same expense: GP11 with STOP only: same sequence: RABIIA	HEX293T (GFP1-10)	RMP/Nucleofection	CRNA 1648	AS AS	(unrelated) siction 1956 and siction 1979 (unrelated)	XX/XX and non-applicable	s and na		S and S	133	Cysometer: 0.8 Cysometer: 0.6
eutro-sequence: GFP12 (vestur schilds): eutro- sequence: ANR11A	HEX293T (GFP1-10)	#MP/Nucleofection	ORNA 1648	Aú	sicon 1955 and sicon 1979 (unreliced)	no HA but 65/Nik homologous sequences flanking the STOP (/frameshift) in GPP11 and non- applicable	S and na		5 and 5	129	Cynometer: 0.6
extra-sequence: GFFII with STOP/flumeshift: retra-sequence: ARRITA + extra-sequence: GFFII (retra-seQUM); extra- sequence: GFFII (retra-seQUM); extra- sequence:	HEX293T (GFP1-10)	RNP/Nucleofection	ORNA 1648	AS	6000N 1967 and 6000N 1965	33/33 and no HA but 65/63	S and S		S and S	133 and 129	Cynometer: 4.0
inquence extra sequence: GPE1 with SRSP only: extra sequence: RABLIA + extra-sequence: GPE1 (recow coDDM): extra-sequence	HEX293T (GFP1-10)	#MP/Nucleofection	09NA 1648	AS	1000N 1956 and 1100N 1955	33/33 and no HA but 65/63	Sand S		S and S	132 and 129	Cysometer: 4.1
(VECUM CODA): AUTO-INQUARCE AUTO-INQUARCE: ANITO-INQUARCE: ANI	HEX2937 (GFP1-10)	#M/Nucleofection	CRNA 1648	Aú.	sicon 1964 and sicon 1879 (unristed)	13/33 and non-applicable	S and ra		S and S	132	Cycometer: 19.6
extro-sequence: GFF11-Myc without mutation: extro-sequence: Lamin A/C	HEX293T (GFP1-10)	#M/Nucleofection	09NA 1629	ś	HOON 1799	11/12	ś	0	6	132	DNA was extracted from cells edited with scDDN 1799 or coDDN 1885. Next the DNA from these experiment was mixed and the insertion was PCR amplified
extro-sequence: GP11-Myc with 1/6 mutation: extro-sequence: Lonio A/C	HEX293T (GFP1-10)	#MP/Nucleofection	ORNA 1629	s	600W 1885	31/12	6		6	132	and sequenced by Bluminian technology (Earcode 25). This control was performed to ensure that template oxiding do not occur during PCR amplication (PCR control in Figure SS and Table S6)
musabanc: ratio sequence: Lamin A/C  extra sequence: GP11 Myc; extra sequence: Lamin A/C + extra sequence: GP11- Myc (eithout musaban); extra sequence	HEK2987 (GFP1-10)	and the second	ORNA 1629						5 and 5	132	
		mm/mm/8086000				33/32 and oxbbN without HA and no mutation					PCR amplicon sequencing using Blumina technology (Baroode S, No mutation). See Figure SS and Table SS
extra-sequence: GP11-Myc; extra- sequence: Samin A/C + extra-sequence: GP11- Myc (1/8 mutations); extra-sequence	HEX293T (GFP1-10)	#MP/Nucleofection	ORNA 1629	ś		33/32 and sciON without HA and 1 mutation every list	ś	۰	5 and 5	132	PCR amplicon sequencing using Illumina technology (Barcode 6, 1/8 mutation s). See Figure SS and Table S6
extra-orqueces: GP114Ays; extra- orquence; Lamin AyC + extra-orquence; GP11- Myc (L/G mutations); extra-orquence	HEX293T (GFP1-10)	#MP/Nucleofection	ORNA 1629	ś	1100N 1799 and 1100N 1805	30/92 and socion without HA and 1 mutation every list	ś		5 and 5	132	PCR amplicon sequencing using Illumina technology (Rarcode 7, 1/6 mutations). See Figure SS and Table S6
extra-sequence: GFP11-Myc: extra- sequence: Lamin A/C + extra-sequence: GFP11- Myc (1/12 mutations): extra-sequence	HEX293T (GFP1-10)	#MP/Nucleofection	ORNA 1629	ś	1100N 1799 and 1100N 1806	reaction every tare.	ś		5 and 5	132	PCR amplican sequencing using Ellumina technology (Barcode R, 1/12 mutations), See Figure SS and Table S6
exto-enquence: GFP11-Myc without mustabol: exto-enquence: RABILLA or exto- enquence: GFF11-Myc without mustabol: exto- sequence: Lamin A/C	HEX2907 (GFP1-10)	RM/Nucleofection	09NA 1668	AS	HDDN 1799	No HA, colbin containing extra- cequence. SPF11-Myc without mutation: with sequence with Lamin A/C HAL used with ANRIZA critica.		۰		132	No PCR positive signal was detected (Marcode II)
organice: GPE1-Myc without mutation: with organice: Lomin A/C	Personal (GPT-10)	mary man tenter to on	LIMMA 1848	Ab	100AN 1788	Lamin A/C HALused with RARIZA CIRNA	•	0		.62	nor run puncuer signar wild directed (Bascade II)
mouse Adryl: mCheny	Pronuclear injection of mouse embryos	RNP/Injection	or Adapit	AS	Raunid plt6-ACirCtermGeromi mCheny	- 480/621	diDNA dicular	between -4/+2	3. Som of plasmid regain template and abeglut Case protein and Oslam of ordes/Trachfolk is injection buffer Som or Stand of PCR repair template and abeglut Case protein and Oslam of ordess/Trachfolk is injection	799	PCR or mouse tail DNA jaredicted size shift; 20.0 (5/25), ON (5/20) of the negative for Aday's incherry insertion are positive by PCR using micherry.
									orRNA/TracrRNA in injection buffer SetM or 30 MM of POX regain has obtain		internal primers.  PCR or mouse tail DNA (predicted size shift): 31.0 (27)97, including 3
mouse Adityl: mCherry	Pronuclear injection of mouse embryos	RNP/Injection	crAdcyll	AS	PCR 1596/1587	36/86	diche	between -4/+2	protein and B31g/ul Cadl protein and CuluM of orRMA/TracHMA in injection	799	PCR on mouse tail DNN (predicted size shift): \$1.0 (27/87, including it homozygoud). \$0 out of \$5 Her clones for incherry have perfect insertion. \$1.6% (7/NS) of the negative for Actlys incherry insertion are positive by PCR using incherry interests primers.

					USE \$2: Repair templates used in this stud		
Gene targeted	LINE	Repair template coding for		Distance from DSB	Type and Name P	Polarity (S=Sense; AS=Ant/Sense	) Sequence
	non-applicable	unrelated siOON (142nt)	non-applicable	non-applicable	HDDN 1379 HDDN 1620	non-applicable	Ecolocomentile collectus filmina conficer citative constituente de la matematica de la colocita de la colocita de la filmina constituente de la colocita del la colocita de la colocita de la colocita de la colocita de la colocita del la colocita de la colocita del la colocita de la colocita de la colocita de la colocita del la colocita d
Lamin A/C	1629 1629	GFP11 GFP11	13/32 13/32		HOON 1620 HOON 1732	S AS	accegnage-genage- as sidon) 1620 but AS
Lamin A/C	1629	GFP11	33/32		PCR 2003/2004 (on ssDDN 1620/1772)	diDNA	as siDDN 1620 but diDNA
Lamin A/C	1629	eGEP	0/0		PCR 1630/832 (on plasmid 1698)	diDNA	—eGFP—
Lamin A/C	1629	eGEP	16/16	0	PCR 1858/1859 (on plasmid 1716)	diDNA	catgogoccopics—eGFP—cagogogococco
Lamin A/C	1629	eGEP	13/21		PCR 1618/1619 (on plasmid 1698) or PCR 1743/1744 (on plasmid 1716)	diDNA	grigoca волідо одде сліддаці вососей се — «GFP— следоду редосласо фольтурация од сладо
					PCX 1743/1744 (on politica 1719)		ggcgloggigactcagtgttogcgggagcgccgccacctacaccagccaacccagatccgaggtcgacagcgcccagatcccaagcctgccag
Lorento AAT	1629	wCC0	217/217		PCR 2058/2059 (on plasmid 1716)	diDNA	cologicistic construiti de la construit construiti de la construit de la cologici del cologici de la cologici de la cologici della cologici d
Lamin A/C	1629	-	24//24/		PCX 2058/2059 (on positing 1739)	DILBA	enjoggegege.co.cog.njogggggggggggggggggggtatter.co.cog/digingco.cocg.go/disco.gggggggggggggggggggggggggggggggggggg
							chricesterstigentelecertrapercapateurgapsterapstagene majertapateurgische einzelegene percenten ge- gestigenten ein mit mageste gestigen weste gegenze der der der seine einzelegen einzeren der
Lamin A/C	1629	eCEP	518/518	0	PCR 1741/1742 (on plasmid 1716)	diDNA	streccetice—ages—
							anguir gett gjerggeta en neg med gjerge gengete gjeggetage er gengete gengete gengete en neg mejet gjengstigte oper gre mgjigt oggeste magjeog och er gage opprint gjeggetage og magjeocht genere en neg mejet gjengstigte oper greg
							Generate statements of the properties of the pro
							ARTE AND A STATE OF THE ARTE A
Lamin A/C	1629	eCEP	518/518	0	Plasmid 1716	dsQNA circular	see Table SI grigoraactigorggoniggagaccongor —GFP11:extra-sequence (A36 bp insert)—
Lamin A/C	1629 1629	GFP11:extra-sequence (236 bp insert)	13/32 23/33		PCR 2051/2052 (on plaumid 2050) PCR 2049/1619 (on plaumid 2042)	diDNA diDNA	cageggegeacceggageggggegragge entrecasetreconcentramanaccontec—entramanacon-eGP 1993 bo inserti—
Lamin A/C	1629	TEV:=GFP::extra-sequence (1122 bp insert) TEV:=GFP::extra-sequence::tag6FP	11/21		PCR 2005/2015 (on plasmid 1894)	DIDNA	cape garder control of the control o
Lamin A/C	1629	insert) TEV::eGFP::estra-sequence::tagRFP (2229 bp insert)	23/23		PCR 2005/2006 (on plaumid 1894)	diDNA.	Explicement@collicesplikealmencelitergra, egsb-matra-medmence:rmlegab (5550 pb jumest)
Lamin A/C	1629	GFP11:3xFlag	33/0	0	HOON 1788	s	Frienreikeikernthiliterrandursthippistarendurbeiteithipistarenduktioner.  Erfermerikeikernthiliterreikernthiliterrikeithil
Lamin A/C	1629	GFP11:3xFlag	33/0	0	HODN 1789	AS	as siOON 1788 but AG
Lamin A/C	1629	3xFlag::GFP11	0/32	0	1100N 1705	s	glege, place and a confidence of the confidence
Lamin A/C	1629	3xFlag::GFP11	0/32	0	HODN 1706	AS	as sidon 1705 but AS
Lamin A/C	1629	GFP11 at cut and tagRFP 33bp downstream (non-recoded) GFP11 at cut and tagRFP 33bp	23/23	0 and +33 0 and +33	PCR 1948/1949 (on plasmid 1892)	diDNA	geigenasetigengeratgagsentegter –GP11 – engeging-genatergengengggegnaget – bagis P – geigenasengenggeratgagsgennigte –GP11 – engeging-genatere geigenasengenggeratgagsgennigte – GP11 – engeging-genatere
Lamin A/C	1629	downstream (recoded)	13/23	(recoded)	PCR 1948/1949 (on plasmid 1893)	diDNA	agdiceacting tight general parkets
Lamin A/C	1629	estra-sequence::GFP11-Myc::estra- sequence	33/32	0	HOON 1799	s	Reflected are annual reflected than the analytic factor of the alternative for the analytic content of the analytic factor of the analyti
		extra-sequence::GFP11-Myc with 1/6					griprossortgropgrestgysgeroregter gigertgrynstessortgytega" gerest"siggig "ettese" gagtae "gtasse" gripro" g
Lamin A/C	1629	mutations: subra-sequence	33/32	0	HOON 1835	s	ggata "acagga "gg-cgg" gaacag" aaactc "atatct" gaagaa" gatete" etgaagtigta catggaggjeac ca ag-ggog-geaace gea
Lorento A-IC	1629	extra-sequence: GFP11-Myc without mutations: extra-sequence	no HA but homologous sequences to the insert		MODN 1813	5	głącotgoganicana otgęt optąco cacategiosticateja gtatęt anatęc tę otgępatta cagitągo gęcgan cananactejatat cag angag
Lamin A/C	1629				SIDON 1814	,	Entrification of the state of t
Lamin A/C	1629	extra-sequence::GFP11-Myc with 1/3	no HA but 23/24 homologous sequences to the insert on each side, and homeologous sequence (1/2 mutations) to the insert in the middle	non-applicable	HOON 1804	s	głącdącanicanodegłąca "gat" att stęgię" dta "ac "gan" ta "gat" ata "gco" gan" gat" nat "gan" gat" gat" gat "ga "cag" nag" cto" at "tto" gag" gan "gat" oto" diganactigae atgangaga acc
						-	_cod_and_ctc_atc_ttc_fand_fann_fanc_ctc_ctdandctdarcatdfadfficacc
Lamin A/C	1629	extra sequence: GFP11-Myc with 1/6	no HA but 23/24 homologous sequences to the insert on each side, and homeologous sequence (1/6 mutations) to the insert in the middle	non-applicable	1100N 1805	s	gtgcctgcgaatcaaactggtcga*gaccat*atggtg*cticac*gagtac*gtaaac*gctgca*gggata*acagga*ggcggt*gaacag*aaac tc*atatct*gaagaa*gatct*dgaaggatgsacatggaaggagcacc
			(1/6 mutations) to the insert in the middle				
Lamin A/C	1629	extra-sequence: GFP11-Myc with 1/12 mutations: extra-sequence	no HA but 23/26 homologous sequences to the insert on each side, and homeologous sequence (1/12 mutations) to the insert in the middle	non-applicable	HOON 1806	s	$c_{i,} \\ \\ \\ radial description \\ c_{i,} \\ \\ radial description \\ c_{i,} \\ radial description \\ \\ c_{i,} \\ radial descriptio$
Lamin A/C	1629	GFP11	(1/12 musecons) to the insert in the middle	-12 (recoded)	INDON 1678	s	criscocopycopolycoscolycopycolycopolytecocolytecticalpylatylastyletyletylyathropylytelycyn*sca
Lamin A/C	1629	GFP11	38/35	-12 (recoded)	HODN 1679	AS	"cet" ten" engoge gegenecegangegangegange as suDDN 1678 but AS
Lamin A/C	1629	GFP11	13/21	+12 (recoded)	HODN 1793	5	$\\ \textbf{Self-conscribed Secretarian Secretarian}, \textbf{Co.}, \textbf{Cit.}, \textbf{Set.}, \textbf{Cit.}, \textbf$
Lamin A/C	1629	GFP11	23/23	+12 (recoded)	1100N 1794	AS	eggcaccegragegggggggggcaggccacccg as siDDN 1793 but AS
Lamin A/C	1629	GFP11	13/21	+33 (recoded)	HIGON 1795	s	$gistginssig(eggestiggsgscocgliccess^ege^ege^ge^ge^ssgl^eggsgciccsccegestracc$
Lamin A/C	1629	GFP11	23/23	+33 (recoded)	INDON 1796	AS	as sxDDN 1795 but AS
Lamin A/C	1729	GFP11	38/33	-32 (recoded)	HODN 1736	s	ccigcccqcqqqqcqccaaccigccqqcaigcqqacacatqqctctcatqqqttttatqaaatqctqctqqqqttacqqqqqqqcqac
Lamin A/C	1729	GFP11	38/23	-32 (recoded)	HODN 1737	AS	as siGDN 1736 but AS
Lamin A/C	1729	GFP11	23/23	+1	11DDN 1734	s	enforcemproficilitation of the second of the
Lamin A/C	1728	GFP11	38/34	-31 (recoded)	HOON 1736	s	see cr1729-32bp insertion
Lamin A/C	1728	GFP11 GFP11	38/34 32/33	-31 (recoded) +2	MODN 1737 MODN 1734	AS S	see cr1729-12bp insertion see cr1729-1bp insertion
AMELIA	1648	GFP11	22/22	-11 (recoded)	HODN 1778	· ·	gricogcorittigetorioggroupgeautg og innovatigetori og sparintanag gjange acpacypytacypotacyttinang gjange acpacypytacypotacyttinang gjange
RARIIA	1648	GFP11	13/21	-11 (recoded)	HODN 1779	AS	acgacgagtacgactaccictttaaaggtgaggc as sation 1778 but AS
RARIIA	1648	GFP11	22/22	-11 (recoded)	PCR 2008/2009 (on ss00N 1778/1779)	diDNA	as ssiDON 1778 but dsDNA
AARIIA	1648	eGF)	0/0	-11 (recoded)	PCR 1630/832 (on plasmid 1698)	diDNA	see Lorsin A/C with eGFP PCR without HA
RABILA	1648	eCE9	15/15	-11 (recoded)	PCR 1838/1839 (on plasmid 1791)	dyDNA	ctoggcograph—+GSP—ggs*scs*qgt*gsogsogsgtsogscts
AARIIA	1648	eGEP	22/22	-11 (recoded)	PCR 1652/1653 (on plasmid 1698) or PCR 1840/1841 (on plasmid 1791)	diDNA	griccogeocittogriccinggeogogeasig eGFP gg s*ses* egi* gaeg acgagtacgactaccictti saaggigagge
					PCK 1840/1841 (on positind 1791)		
RARIIA	1648						
		eCEP	6)/63	-11 (recoded)	PCR 1842/1843 (on plasmid 1791)	diDNA	$acgeocottggtocoacquatacoactgctgctocogcotttegctcctoggccgcgcastg\\ -eGFP$
		eCEP	G/G	-11 (recoded)	PCR 1842/1843 (on plasmid 1791)	diDNA	ggsacegos egualgigigagidgoting grigistsahotraga gasecoogeocoah casaasoos castranaggeggioogodgytino
		eCFP	ଇ/ଘ	-11 (recoded)	PCR 1842/1843 (on plasmid 1791)	diDNA	ggsacrgosacgsalgitgiagetigothicgitgictautochunga paacoogcococutecacasaccoscocicaceacgetocgcitgginoc agrapagocgsthocggsacggiagetoppa aargagoaagcggocactaagachatgitgstagpagticong actoagthocottigagecicotti
AMELIA	1648	eGEP				dices.	ggsacrgosacgsalgitgiagetigothicgitgictautochunga paacoogcococutecacasaccoscocicaceacgetocgcitgginoc agrapagocgsthocggsacggiagetoppa aargagoaagcggocactaagachatgitgstagpagticong actoagthocottigagecicotti
RABIIA	1648	eCF)	63/63 463/432	-11 (recoded)	PCR 1842/1843 (on plasmid 1791)  PCR 1846/1847 (on plasmid 1791)	diCNA diCNA	Light, and, in the dependence control and
AMBIIA	1648	eGEP eGEP				dsCRA.	Light, and, in the dependence control and
AMBILA	1648	eco				diDNA diDNA	ggsacrgosacgsalgitgiagetigothicgitgictautochunga paacoogcococutecacasaccoscocicaceacgetocgcitgginoc agrapagocgsthocggsacggiagetoppa aargagoaagcggocactaagachatgitgstagpagticong actoagthocottigagecicotti
AADIA	1648	6GP 6GP			PCR 1846/1867 (on plannid 1791) Plannid 1791	diCNA diCNA diCNA	Light, and, in the dependence control and
RABIIA RABIIA	1648 1648 1648	GFP11: heling	464/422 464/422 33/0	-11 (recoded) -11 (recoded) +1	PCR 1846/1847 (on planned 1791)  Planned 1791  siCON 1816	daCNA daCNA circular S	in the control of the
RABIIA RABIIA	1648 1648 1648 1648	GFP11:hding GFP11:hding	464/432 464/432 23/0 33/0	-11 (recoded) -11 (recoded) +1 +1	PCR 1864/1840 (on plasmed 1791)  Pleased 1791  solicit significant	duCNA.  duCNA circular  S  AS	Description (Inc.)  Inc. (Inc.)  Inc. (Inc.)  Inc.)  Inc. (Inc.)  Inc.)
RABIIA RABIIA RABIIA	1648 1648 1648 1648 1648	GF911:hdug GF911:hdug hdug-GF911	461/432 461/432 33/0 4/33	-11 (recoded) -11 (recoded) +1 +1 -2	PCR 1844/2647 (on planned 1792)  Planned 1792  solon 3106  solon 3116  solon 3117	diGNA. diGNAA circular S AS S	PROPERTY OF THE PROPERTY OF TH
AADIIA AADIIA AADIIA AADIIA AADIIA	1648 1648 1648 1648 1648	GF91::Asflag GF91::Asflag Asflag::GF911 Asflag::GF911	463/432 463/433 33/0 233 6733	-11 (recoded) -11 (recoded) +1 +1 -2 -2	PCR 1844/2847 (as plasmed 1792)  Plasmed 1791  scCOM 3816  scCOM 3810  scCOM 3810	diGNA Graber  5  A5  5  A5	Description (Inc.)  Inc. (Inc.)  Inc. (Inc.)  Inc.)  Inc. (Inc.)  Inc.)
RABIIA RABIIA RABIIA	1648 1648 1648 1648 1648	GF911:hdug GF911:hdug hdug-GF911	461/432 461/432 33/0 4/33	-11 (recoded) -11 (recoded) +1 +1 -2	PCR 1844/2647 (on planned 1792)  Planned 1792  solon 3106  solon 3116  solon 3117	diGNA. diGNAA circular S AS S	Proceedings:    Proceedings   Proceedings   Proceedings   Proceedings
AMBITA AMBITA AMBITA AMBITA AMBITA AMBITA AMBITA	1648 1648 1648 1648 1648 1648 1648	GF91::Astag GF91::Astag Astag::GF911 Astag::GF911 GF911	441/432 441/432 33/0 473 4733	-11 (recoded) -11 (recoded) -11 (recoded) +1 +1 -2 -2 -2	PCR 1844/1847 (on placed 1793)  Filled 1791  MCON 1816  MCON 1817  MCON 1810  MCON 1810	dicNA creature  5 A5 5 A5 5	The control of the co
AMBILA AMBILA AMBILA AMBILA AMBILA AMBILA AMBILA AMBILA	1648 1648 1648 1648 1648 1648	GF91:2afug GF91:2afug Nafug-GF911 Nafug-GF911 GF911 GF911	464/432 464/432 33/0 33/0 973 973 3473 3473	-11 (recoded) -11 (recoded) -11 (recoded) +1 +1 -2 -2 -2	PCR 1866/2867 (on prisoned 1790)  Flammed 1791  widow stats	diGNA  diGNA creatur  S  AS  S  AS  AS  AS	Bender standing of standings and december of the control of the co
AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA	1648 1648 1648 1648 1648 1648 1648 1648	CFF11: holing CFF11: holing holing: CFF11  CFF11 CFF11 CFF11 CFF11	46,913 46,913 33,0 33,0 913 673 3,23 3,23 3,23 3,24 3,24	-11 (recoded) -11 (recoded) +1 +1 -2 -2 -2 -2 +19 (recoded) +19 (recoded)	PG3 184C(167 jm planet 1770)  Planet 1771  ROOM 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127	didRA cinular  S S S S S S AS S AS S AS	generation and griphic principles and productions are productions and productions and productions are productions are productions and productions are productions are productions and productions are productions are productions are productions and productions are productions are productions and productions are productions and productions are productions are productions and productions are productions and productions are productions and productions are productions are productions and productions are productions are productions and productions are productions are productions are productions and productions are productions are productions and productions are productions and productions are producti
AABIIA	1648 1648 1648 1648 1648 1648 1648 1648	GP91: Juling GP91: Juling Juling: GP91 Juling: GP91 GP91 GP91 GP91 GP91 HTD SEPECTORY SEPECTORY STORTH SEPTORY STORTH SEPTO	44,9432 44,9432 34,0 34,0 973 34,73 34,73 34,73 34,74 34	-11 (recoded) -11 (recoded) +1 +1 -2 -2 -2 +19 (recoded)	PG 185/160 pm phrend 1710;  Pleand 1731;  #600 HE  #600 H	didNA didNA circular S AS S AS S AS	Proposition Pro-  Transport Contraction Co
AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA AABIIA	1648 1648 1648 1648 1648 1648 1648 1648	GP91: hdfug GP91: hdfug hdfug: GP911  GP911 GP911 GP911 GP911	46,913 46,913 33,0 33,0 913 673 3,23 3,23 3,23 3,24 3,24	-11 (recoded) -11 (recoded) +1 +1 -2 -2 -2 -2 +19 (recoded) +19 (recoded)	PG3 184C(167 jm planet 1770)  Planet 1771  ROOM 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127  4000 8127	didRA cinular  S S S S S S AS S AS S AS	Proposition Pro-  Transport Contraction Co
AABIIA	1648 1648 1648 1648 1648 1648 1648 1648	GFF11.3ding GFF11.3ding Jahling.GFF11 Jahling.GFF11 GFF11 GF	44,9432 44,9432 34,0 34,0 973 34,73 34,73 34,73 34,74 34	-11 (recoded) -11 (recoded) +1 +1 -2 -2 -2 -2 +19 (recoded) +19 (recoded)	PG 185/160 pm phrend 1710;  Pleand 1731;  #600 HE  #600 H	didRA cinular  S S S S S S AS S AS S AS	The control of the co
AGITA SGITA	1648  1648 1648 1648 1648 1648 1648 1648	GFF1: Juding GFF1: Juding GFF1: Juding GFF1: Juding: GFF1: G	44,9432  44,9432  32,0  33,0  973  4973  2473  2473  2473  24743  24744	-11 (recoded) -11 (recoded) -11 (recoded) -11 -1 -2 -2 -2 -19 (recoded) -10 (recoded) -0	PG 188(C107 jos planet 1710)  Planet 1710;  Vector 816:  4000 817:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:	additionaler  1  46  46  5  46  5  46  5  46  5  46  5	weeks (Filters wild (Billicities week) in a design (Billicities week) in the best (Billicities week) in the best (Billicities wild) in the best (Billicitie
AADIIA AADIIA AADIIA AADIIA AADIIA AADIIA AADIIA AADIIA AADIIA	1648 1648 1648 1648 1648 1648 1648 1648	GFF11: Juding GFF11: Juding Juding: GFF11 Juding: GFF11 GFF1	445/432  445/432  340  340  973  473  3473  3473  3474  3474  48 bit but SCSC Whenhington respectors  will but SCSC Whenhington respectors	-11 (recoded) -11 (recoded) +1 +1 -2 -2 -2 -3 -15 (recoded) 0 non-applicable	PGS 14MC/15M2 (ms planed 1770)  Planed 1775  4000 HEL	didRA cinular  S S S S S S AS S AS S AS	The control of the co
AGITA SGITA	1648  1648 1648 1648 1648 1648 1648 1648	GFF1: Juding GFF1: Juding GFF1: Juding GFF1: Juding: GFF1: G	44,9432  44,9432  32,0  33,0  973  4973  2473  2473  2473  24743  24744	-11 (recoded) -11 (recoded) -11 (recoded) -11 -1 -2 -2 -2 -19 (recoded) -10 (recoded) -0	PG 188(C107 jos planet 1710)  Planet 1710;  Vector 816:  4000 817:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:  4000 818:	additionaler  1  46  46  5  46  5  46  5  46  5  46  5	weeks (Filters wild (Billicities week) in a design (Billicities week) in the best (Billicities week) in the best (Billicities wild) in the best (Billicitie
MASILA MA	1648 1648 1648 1648 1648 1648 1648 1648	GP11-bring GP11-bring GP11-bring GP11-bring GP11 GP11 GP11 GP11 GP11 GP11 GP11 GP1	46,0432  46,0432  310  310  673  473  3473  3473  3474  3474  3474  3474  3474  3474	-11 (wcoded) -11 (wcoded) -11 (wcoded) +1 +1 -2 -2 -2 -19 (wcoded) -0 non-applicable -0 -0 -12 (wcoded) -13 (wcoded)	PG 188(C107 jos planet 1710)  Planet 1710  **CON 884  **CON 884  **CON 884  **CON 884  **CON 884  **CON 885  **CON 887  **CON 987	######################################	weeks (Filters wild (Billicities week) in a design (Billicities week) in the best (Billicities week) in the best (Billicities wild) in the best (Billicitie
AGELA	1648  1648  1648  1648  1648  1648  1648  1648  1648  1648  1648  1776and 1777	OFFI John OFFI J	460/433  460/433  300  300  913  913  3273  3274  3274  3274  3274  3274  3274  3274  3274  3274	-11 (recoded) -11 (recoded) -11 -12 -2 -2 -2 -19 (recoded) -10 (recoded) -10 (recoded) -12 (recoded) -13 (recoded) -14 (recoded) -14 (recoded)	PGS 184(CNE) (on planet 1710)  Planet 1711  **CON IRIS  **CON IRIS	additionale  1  AG  1  AG  1  AG  1  AG  1  AG  1  AG  1  1  1  1  1  AG  1  1  1  AG  1  1  AG  1  1  AG  1  1  AG  AG	HOUSE THE WAS AND
MARITA AGRICA MARITA	1648 1648 1648 1648 1648 1648 1648 1648	GF11 July GF11 J	440,432  440,432  300  300  673  673  2023  3024	-11 (weeked) -11 (weeked) -11 (weeked) -11 -12 (weeked) -13 -2 -2 -2 (weeked) -16 (weeked) -17 (weeked) -17 (weeked) -17 (weeked) -17 (weeked) -17 (weeked)	PGS 188C/180 (ms planet 1710)  Planet 173;  width 818	### ##################################	## 1900/1817 PM  *** *** *** *** *** *** *** *** *** *
MARIEA	1448  1448  1448  1448  1448  1448  1448  1448  1448  1778-ad 1777	GFTE Index John GFTE JOHN	#60/133 #60/133 #60/133 #300 #310 #613 #613 #613 #613 #613 #613 #613 #613	-11 presided  -11 presided  -11 presided  -11 -12 -13 -13 -13 presided  -0 -0 -0 -12 presided  -17 presided	PGS 184(CMS ) per planes 1790;  Planes 4 1791  scotto 816  scotto 817  scotto 818  scotto	######################################	**************************************
MASIA	1448  1448  1448  1448  1448  1448  1448  1448  1448  1448  1448  1477  1778-add 1777	GF11 bring GF11 bring GF11 bring GF11  GF11 GF11 GF11 GF11 GF11 GF11 GF1	440,422  440,422  320  320  320  472  472  3272  3272  3272  3274	-11 proded -11 -11 proded -11 -11 -11 -11 -11 -11 -11 -11 -11 -11	PGS 1882/180 jam jalanesi 1710 j  Plesmid 1731 v  1000 1884 v  1000 1885 v  1000 18	6,000 conder  5  65  5  65  5  5  5  5  6  7  6  7  7  7  7  7  7  7  7  7  7	THE OWN TEAM WAS AND ADMINISTRATED TO THE OWN TEAM OF THE OWN
AGETA	1448 1448 1448 1448 1448 1448 1448 1448	GFTE infeg begger GFTE begger GFTE	### ##################################	-11 proofed -11 proofed -11 -11 -11 -1 -1 -1 -1 -1 -1 -1 -1 -1	PGS 184(C167 (on planet 179))  Planet 1791  **CON #16  **CON #17  **CON #16  **CON #17	600A male  3 46 46 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6	particular production of the p
MASIA	1448  1448  1448  1448  1448  1448  1448  1448  1448  1448  1448  1477  1778-add 1777	GF11 bring GF11 bring GF11 bring GF11  GF11 GF11 GF11 GF11 GF11 GF11 GF1	440,422  440,422  320  320  320  472  472  3272  3272  3272  3274	-11 proded -11 -11 proded -11 -11 -11 -11 -11 -11 -11 -11 -11 -11	PGS 1882/180 jam jalanesi 1710 j  Plesmid 1731 v  1000 1884 v  1000 1885 v  1000 18	6,000 conder  5  65  5  65  5  5  5  5  6  7  6  7  7  7  7  7  7  7  7  7  7	THE OWN TEAM WAS AND ADMINISTRATED TO THE OWN TEAM OF THE OWN
MODIFIE MODIFIES AND THE MODIFIES AND TH	1448 1448 1448 1448 1448 1448 1448 1448	GF11 July GF11 J	#60/422  #60/422  200  200  973  973  3073  3073  3073  3073  3073  3073  3073  3073  3073  3073  3073  3073  3074	-11 presided -11 presided -11 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1	PGS 1882/180 join planed 1710 j  Planed 1711 i  1000 HS ii  1000 H	### ##################################	## 1900 HB PM PM  *** PART HB PM  **
MAGINA MA	1448 1448 1448 1448 1448 1448 1448 1448	GFTE infeg begger begger GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	### ##################################	-11 presided -1-11 presided -1-1 -1-1 -1-1 -1-1 -1-1 -1-1 -1-1 -	PGS 845(185) por planes 1793;  Planes 1793;  4000 mills	### ##################################	Handblack planeters  Handblack
AGESTA AG	1446  1446  1446  1446  1446  1446  1446  1446  1446  1446  1446  1446  147778-401777  1778-401777  1778-401777  1778-401777  1778-4017	GFT1 bring GFT1 bring GFT1 bring GFT1 GFT1 GFT1 GFT1 GFT1 GFT1 GFT1 GFT1	46,0432  46,0432  310  320  473  473  3274	-11 prodel) -11 prodel) -11 prodel) -11 prodel) -12 prodel) -13 prodel) -14 prodel) -17 prodel]	PGS 185(7167 jost planes (1710)  Planes (1710)  CODO 8155  CODO 81	### ##################################	THE STATE OF THE PROPERTY OF THE STATE OF TH
MARIE	1448  1448  1448  1448  1448  1448  1448  1448  1448  1778-ad 1777  1778-ad 1777  1778-ad 1777  1779-ad 1779  1779	GPTS afting GPTS afting Manage GPTS APPEN GPTS GPTS GPTS GPTS GPTS GPTS GPTS GPTS	#45/422  #45/422  32/0  32/0  32/0  47/3  47/3  47/3  32/4  32/5	-11 proded) -11 proded) -11 proded) -11 -12 -2 -3 -3 -13 proded) -13 proded) -14 -13 proded) -15 proded) -17 proded) -18 -18 proded) -18 -18 proded) -19 -19 proded)	PGS 188C/180 (ms planed 1710)  Planed 1731  **CON 888  **CON 888  **CON 888  **CON 887	### ##################################	particular programment of the control of the contro
AGESTA AG	1666  1666  1666  1666  1666  1666  1666  1666  1666  1666  1776  1776  1776  1777  1776  1777	GFTE bring GFTE bring GFTE bring GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	#60/132  #60/132  #60/132  300  300  973  473  3074  3075  3075  3075  3075	-11 proded -11 proded -11 proded -11 -12 -2 -3 -3 -4 -17 proded -12 proded -13 proded -14 proded -14 proded -15 proded -1	PG 185(195 ) on planed 1710  Planed 1791  **CON BIE  **	### ##################################	The control of the co
AGESTA MASSIA	1446  1446  1446  1446  1446  1446  1446  1446  1446  1446  1474  1774  1777	GPTS article GPTS article Service GPTS Service GPTS GPTS GPTS GPTS GPTS GPTS GPTS GPTS	#45/422  #45/422  300  300  973  973  3073  3073  3073  3073  3073  3073  3074  3075  3075  3075  3075  3075  3075  3075  3075  3075  3075  3075  3075  3075	-11 pecoled -11 pecoled -1 -12 pecoled -1 -13 -2 -2 -13 pecoled -0 -0 -0 -3 -10 pecoled -0 -0 -3 -10 pecoled -0 -13 pecoled -0 -13 pecoled -17 pecoled -18 pecoled -18 pecoled -19 pecoled -19 pecoled -19 pecoled -19 pecoled	PGS 188C/180 pm planed 1710;  Planed 173;  wichow disk	### ##################################	particular processing strategies and process
AGESTA AG	1446  1446	GFTE infeg GFTE infeg GFTE infeg GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	#40/432  #40/432  300  300  903  403  303  403  303  303	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 proceded	PG 185(150 pm planet 1710)  Planet 1711  **CON 815  **C	### ##################################	**NOTION TO SET OF THE PARTY OF
AGESTA AG	1446 1446 1446 1446 1446 1446 1446 1446	GFT1 bridge GFT1 bridge GFT1 bridge GFT1 GFT1 GFT1 GFT1 GFT1 GFT1 GFT1 GFT1	#40/432  #40/432  33/0  33/0  47/3  47/3  32/3	-11 proceded -12 proceded -13 -14 proceded -14 -14 -14 -14 -14 -14 -14 -14 -14 -14	PGS 1882/180 jam jahaned 1710 jam jahaned 1710 jam jahaned 1710 jam jahaned 1710 jam	### ##################################	The control of the co
AGESTA AG	1446  1446	GFTE infeg GFTE infeg GFTE infeg GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	#40/432  #40/432  300  300  903  403  303  403  303  303	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 proceded	PG 185(150 pm planet 1710)  Planet 1711  **CON 815  **C	### ##################################	**NOTION TO SET OF THE PARTY OF
AGELIA AG	1446 1446 1446 1446 1446 1446 1446 1446	GFTE infeg GFTE infeg GFTE infeg GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	#40/432  #40/432  300  300  903  403  303  403  303  303	-11 proceeds -12 process -2 -2 process -2 proces	PG 185(150 pay planet 1710)  Planet 1711  scott 1815	### ##################################	The control of the co
MASSIA MA	1446 1446 1446 1446 1446 1446 1446 1446	GPTL Safety GPTL Safety Manage GPTL	#40,422  #40,422  300  300  673  473  4723  3073  3073  3073  3073  3073  3073  3073  3073  3073  3074  3074  3073  3073  3073  3073  3073  3073  3073  3073  3073  3074  3074  3074  3074  3074  3074  3074  3074  3074  3074  3074  3074  3074  3075  3076  3076  3077  3077  3078	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 p	POS 1882/180 pm planed 1770;  Planed 1771;  wichow disk wichow dis	### ##################################	THE CONTRACT OF THE CONTRACT O
AGELIA AG	1446 1446 1446 1446 1446 1446 1446 1446	GFT1 bring GFT1 bring GFT1 bring GFT1  GFT	#40/422  #40/422  23/0  23/0  23/0  33/0  47/3  34/23  34/23  34/23  34/23  34/23  34/24  34/25	-11 proceded -12 proceded -13 proceded -14 proceded -2 -2 -2 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3	PGS 1882/180 jam jahrand 1710 j  Planted 1711 i  1000 1815 i  1000 181	### ##################################	The control of the co
MASSIA MA	1448  1448	GFTE bring GFTE bring GFTE bring GFTE  GFTE GFTE GFTE GFTE GFTE GFTE GFTE	46,0432  46,0432  30,0  30,0  47,3  47,3  30,7	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 p	PG 185/167 join planed 1710  Planed 1731  **CON 985  **	### ##################################	THE CONTRACT OF THE CONTRACT O
MARIAN MA	1446 1446 1446 1446 1446 1446 1446 1446	GFTE Infeg Delty GFTE Delty GFTE Delty GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	### #### #############################	41 proded) 41 proded) 41 proded) 42 proded) 5 0 43 proded) 43 proded) 43 proded) 43 proded) 43 proded) 44 proded) 44 proded) 45 proded) 46 proded) 47 proded) 48 proded) 48 proded) 48 proded) 48 proded) 49 proded) 40 proded) 41 proded) 42 proded) 43 proded) 44 proded) 45 proded) 46 proded) 46 proded) 47 proded) 48 proded) 48 proded) 48 proded) 49 proded) 49 proded) 40 proded) 40 proded) 41 proded) 41 proded) 42 proded) 43 proded) 44 proded) 45 proded) 46 proded) 47 proded) 48 proded) 48 proded) 48 proded) 49 proded) 49 proded) 49 proded) 49 proded) 40 proded) 40 proded) 40 proded) 41 proded) 41 proded) 41 proded) 42 proded) 43 proded) 44 proded) 45 proded) 46 proded) 46 proded) 47 proded) 48 proded) 48 proded) 48 proded) 49 proded) 49 proded) 40 proded) 40 proded) 40 proded) 41 proded) 41 proded) 41 proded)	PGS 185(CSG) por planes (TTS)  Planes (173)  400 HS  4	### ##################################	The control of the co
AGELIA AG	1448  1448	GFTE bring GFTE bring GFTE bring GFTE  GFTE GFTE GFTE GFTE GFTE GFTE GFTE	46,0432  46,0432  30,0  30,0  47,3  47,3  30,7	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 p	PG 185/167 join planed 1710  Planed 1731  **CON 985  **	### ##################################	A DESIGNATION OF THE PROPERTY
MASSIA MA	1448  1448	CFTE Infeg  Service CFTE  SERV	### ##################################	41 proceded 41 proceded 41 proceded 42 proceded 43 proceded 44 proceded 45 proceded 46 proceded 47 proceded 48 pro	PAGE 185(CSE) (on planes 179);  Parent 1791;  **CON HIS **  **  **CON HIS **  **CON HIS **  **  **CON HIS **  **  **  **  **  **  **  **  **  **	### ##################################	Description of the control of the co
AGELIA AG	1446  1446	GFTE string GFTE string GFTE John GFTE JOHN GFTE GFTE GFTE GFTE GFTE GFTE GFTE GFTE	#40/432  #40/432  300  300  403  403  403  304  304	-11 perioded -12 perioded -13 perioded -14 perioded -15 perioded -16 perioded -17 perioded	PG 185(150 pa planet 1710)  Planet 1751  **COD III III **COD III III **COD III III **COD III III III III III III III III III I	### ##################################	Physical content of the content of t
AMERIA AM	1448  1448	GFTE bring GFTE bring GFTE bring GFTE  GFTE GFTE  GFTE	46,0433  46,0433  30,0  30,0  47,3  30,0  30,0  47,3  30,0	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 proceded -17 proceded -17 proceded -17 proceded -17 proceded -17 proceded -18 p	PGS 185(185) per juliante (1710)  Placente (173)  **CON 1856  **CON 1856  **CON 1850  **CO	### ##################################	Description of the control of the co
MASSIA MA	1446  1446	GFTL Safety  GFTL Safety  Annual GFTL  GFT	#40/432  #40/432  300  300  403  403  403  304  304	-11 perioded -12 perioded -13 perioded -14 perioded -15 perioded -16 perioded -17 perioded	PG 185(150 pa planet 1710)  Planet 1751  **COD III III **COD III III **COD III III **COD III III III III III III III III III I	### ##################################	Hermonistics in the control of the c
AMERIA AM	1448  1448	GFTE bring GFTE bring GFTE  GFTE GFTE  GFT	46,0433  46,0433  30,0  30,0  47,3  30,0  30,0  47,3  30,0	-11 proceded -12 proceded -13 proceded -14 proceded -15 proceded -16 proceded -17 proceded -17 proceded -17 proceded -17 proceded -17 proceded -17 proceded -18 p	PG 185(150 pa planet 1710)  Planet 1751  **CON BILL  *	### ##################################	Transport care for the control of th
AMERIA AM	1448  1448	GFTL Safety  GFTL Safety  Annual Control of the Con	#60/432  #60/432  32/0  32/0  32/0  47/3  32/3	-11 personal   -12 personal   -13 personal   -14 personal   -15 personal   -16   -17 personal   -18 personal   -19 personal   -10 personal	PGS 185(CHG) per planes (TTI);  Planes 4 1751  scotto 815  scotto	### ##################################	Section of the control of the contro
MARIAN MA	1448  1448	GFTE bring GFTE bring GFTE  GFTE GFTE  GFT	## ## ## ## ## ## ## ## ## ## ## ## ##	41 pecided 41 pecided 41 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	PG 185(150 pa planet 1710)  Planet 1751  **CON BILL  *	### ##################################	Secure deficience de company de la company de company d

			TABLE 53: Plasmids used in this study
Plasmid name	Backbone	Insert	Sequence
1698	pUC19	eGFP	gtgagcaagggrgaggagtigttcaccggggtgigtccatctggtgagctgaaggggargtaaaaggccacaagttcaggtgtcgggaggggag
2050	pUC19	GFP11 with extra-sequence (GFP11 with linker::TEV::3xFlag::Ollas::Myc::V5::HA:: other linker)	cgtgaccacatggtccttcatgagtatgtaaatgctgctgggattacaggtggcgggagaacctttattttcagggagactataaggaccatgacggggattataaagatcacgacattgattacaaggacgacgacgacgataagtccggcttcgctaacgaattggggccaaggctcatgggca aggaacagaaattgatctctgaggaggacctgggtaaacctatcccaaatcccctgttgggtttggactcaacttacccctacgacgttcctgactatgctctggctgg
2042	pUC19	eGFP with extra-sequence (TEV::3xFlag::Ollas::Myc::V5::HA::other linker::eGFP)	gagaactttattttattgaggagactataaggacatgaaggggattataagatcagacatgattacaaggaagg
1894	pUC19	eGFP with extra-sequence and tagRFP (TEV::eGFP::other linker::partial mNeonGreen::3xFlag::tagRFP)	gagaactctacttccaggagtgagcaagggcgaggagtittactggtgtcgttccgsttctcgtgaattgaatggagattaaatggcataaattttctgtttccgggaagggcgaggcgaggcgaggctgaattgaaatttattgcacaactggtaagtccgtgcctgtgcctgtgcctgtgcctgtgcctgtgcctgtgcctgtgcctgtgcctgtgcctgaggtcaattgaaactgaaggatgagatgagatgagatttaaattgaaccgaacaaggaggaggaggaggaggaggaggaggagg
1716	pUC19	eGFP with HAs for <i>Lamin A/C</i>	ctrtcctcttiggtragccraagaaggtragaggcaatgggggcaagtttgagccgacagtgtgagcaggaggccaagaagggggaagtttaggcctacgcagttaggggtggggtggtgggggggg
1791	pUC19	eGFP with HAs for <i>RAB11A</i> (recoded *)	ggaaccgccacgtatgtagctgctttcggctgtttaatctctagagaaccccgcccccatcacaaacccactcacaggcggttccgctggttcaggagccggttccggcaggagaggagcaggcag
1892	pUC19	Insertion of GFP11 at cut tagRFP 33bp downstream in Lamin A/C	Cgtgaccactggtccttcatgagtatgtaaatgctgctgggattacaggtggcggcagcggcgccacccgcagggggctctctaaaggggaagaaccatcaaggggaatatgcactgaaggctctatatggaaggtacagtaaacaatcaccacttcaaatgcacttct gaaggtgaaggaaagccatcgagggtacacagacgtggagaattaaagtgttgagggaagcccgccgccttttgcttttgcaacctcattatgtatg
1893	pUC19	Insertion of GFP11 at cut tagRFP 33bp downstream (recoded *) in Lamin A/C	cgtgaccactggtctttcatgagtatgtaaatgctgctgggattacaggtggcggcagcgcf*cgt*gct*acg*cga*gct*caa*gggg*gct*caa*ggggtcttcaaa*ggggaagaactcatcaaggagaatatgcacatgaagctctatatggaaggtacagtaaacaacacacttcaatgcaagttgcaaggaggaagga
sgPYM1	pX458	sgRNA for PYM1	see Table S5
pBS-AC3CtermGenomic- mCherry	pBlueScript-KS	mCherry (with STOP and extra- sequence) with HAs for mouse <i>Adcy3</i>	tgiggcattaggtogigttggcagigccacagggacaaggacaaggactgaggcctctaaggctttgattcaccaaaacatttaagattaggttgggggaaacggcctcgttcggtagagttaacaactaaactgtttgaacctgtatcagtgccatgaaatggtagttgctgttataagtgcctgtgggttccctttatgtggggggcaggctccctttatgtgggggctgtatctacatactgctactgaggcttcccttttatgtacaggtgtgtgggaaacggcttctgatctttttttgaagggggggg

Primers name	F/R	TABLE S4: Primers used in Description	this study  Sequence (5° to 3")
832	R	amplification of eGFP without HA	cttgtacagctcgtccatg
1630	F	amplification of eGFP without HA	gtgagcaagggcgaggagc
1676	F	genotyping and sequencing of eGFP insert	caagacccgcgcgaggtg
1677	R	genotyping and sequencing of eGFP insert	gtcgcctcgaacttcacc
2014	R	genotyping and sequencing of eGFP insert	tagttgccgtcgtccttg
2044	R	genotyping and sequencing of eGFP insert	ctccttgaagtcgatgccc
2045	F	genotyping and sequencing of eGFP insert	gccctggcccacctcgtg
2047	F	genotyping and sequencing of eGFP insert	gttcatctgcaccaccgg
3001	F	genotyping and sequencing of eGFP insert	accactacetgageacceag
1762 / HK13	F R	genotyping and sequencing of mCherry insert	cacgagttcgagatcgaggg
1763 / HK14	R	genotyping and sequencing of mCherry insert  genotyping and sequencing of mCherry insert	ggtgtagtcctcgttgtggg
1767	F	genotyping and sequencing of mchery insert genotyping and sequencing of mchery insert	caggatgtcccaggcgaagg ggacggcgccactacgacg
1768	R	genotyping and sequencing of mCherry insert	ccttcagcttggcggtctg
1769	F	genotyping and sequencing of mCherry insert	cgtgcagctgcccggcgcc
1773	F	genotyping and sequencing of mCherry insert	tcaagtgggagcgtgatg
1858	F	amplification of Lamin A/C eGFP repair template (cr1629) with~15bp HAs	catggagaccccgtccgtg
1859	R	amplification of Lamin A/C eGFP repair template (cr1629) with~15bp HAs	gggtggcgcgctgcttg
1618	F	amplification of Lamin A/C eGFP repair template (cr1629) with~35bp HAs	getgecaacetgeeggecatggagacecegteggtgagcaagggegaggagc
1619	R	amplification of Lamin A/C eGFP repair template (cr1629) with ~35bp HAs	ggcctgcgcccgctgcgggtggcgcgctgcttgtacagctcgtccatg
1743	F	amplification of Lamin A/C eGFP repair template (cr1629) with ~35bp HAs	gctgccaacctgccggccatg
1744	R	amplification of Lamin A/C eGFP repair template (cr1629) with ~35bp HAs	ggcctgcgcccgctgcgg
2003	F	amplification of Lamin A/C GFP11 repair template (cr1629) with ~35bp HAs	gctgccaacctgccggcc
2004	R	amplification of Lamin A/C GFP11 repair template (cr1629) with ~35bp HAs	gcctgcgcccgctgcgg
2058	F	amplification of Lamin A/C eGFP repair template (cr1629) with~240bp HAs	ggcgttggttgactcagtg
2059	R	amplification of Lamin A/C eGFP repair template (cr1629) with~240bp HAs	ctcggcctcgtaggcggcc
1741	F	amplification of Lamin A/C eGFP repair template (cr1629) with~500bp HAs	ctctcctcttggctctg
1742	R	amplification of Lamin A/C eGFP repair template (cr1629) with $\sim$ 500bp HAs	gacaattccccttgacactg
2051	F	amplification of Lamin A/C GFP11::extra-sequence (336 bp insert) repair template (cr1629) with ~35bp HAs	gctgccaacctgcrggccatggagaccccgtccgtgaccacatggtccttc
2052	R	amplification of Lamin A/C GFP11::extra-sequence (336 bp insert) repair template (cr1629) with ~35bp HAs	gcctgcgccccgctgcgggtggcgcgctgactcgttcttgatctgcgg
2049	F	amplification of Lamin A/C extra-sequence::eGFP (993 bp insert) repair template (cr1629) with ~35bp HAs	gctgccaacctgccggccatggagaccccgtcgagaacctttattttcagg
2015	R	amplification of Lamin A/C TEV::eGFP::extra-sequence (1112 bp insert) repair template (cr1629) with ~35bp HAs	ggcctgcgccccgctgcgggtggcgcgctgctgtgagccttcatacgtgtacc
2005	F	amplification of Lamin A/C TEV::eGFP::extra-sequence::tagRFP (2229 bp insert) (cr1629) with ~35bp HAs	gctgccaacctgccggccatggagaccccgtcgagaacctctacttccaag
2006	R	amplification of Lomin A/C TEV::eGFP::extra-sequence:tagRFP (2229 bp insert) (cr1629) with ~35bp HAs	ggcctgcgcccgctgcgggtggcgcgctgattcagtttgtgccccagc
1948	F	amplification of Lamin A/C GFP11 at cut and tagRFP 33bp downstream (cr1629) with ~35bp HAs	$gctgccaacctgccggccatggagaccccgtc \underline{\alpha} gtgaccacatggtccttc$
1949	R	amplification of Lamin A/C GFP11 at cut and tagRFP 33bp downstream (cr1629) with ~35bp HAs	ggtgatgcgggtgggcgacagcggagtggagctattcagtttgtgccccagc
1965	R	Lamin A/C Illumina sequencing with barcode 5	caagcagaagacggcatacgagatacagcagtgactggagttcagacgtgtgctcttccgatcggcgacagcggagtggagc
1966	R	Lamin A/C Illumina sequencing with barcode 6	caagcagaagacggcatacgagatcaccgigtgactggagttcagacgtgtgctcttccgatcggcgacagcggagtggagc
1967	R	Lamin A/C Illumina sequencing with barcode 7	caag cag aag ac g g catac g ag at g t g at g g t g act g g ag t cag ac g t g t ct t c c g at c g g ca a g c g ag t g ag c g cag a g c g ag t g ag c g cag a g c g ag c g
1968	R	Lamin A/C Illumina sequencing with barcode 8	caag cag aag ac g g catac g ag at t g track g ac g t g ac g t g t c t t c c g a t c g c g a c g c g ag t g a g c g a
1969	R	Lamin A/C Illumina sequencing with barcode 9	caagcagaagacggcatacgagatagatccgtgactggagttcagacgtgtgctcttccgatcggcgacagcggagtggagc
1970 390	R	Lamin A/C Illumina sequencing with barcode 10  pre-amplification of the edit for Illumina sequencing(in insert)	caagcagaagacggcatacgagatcccggagtgactggagttcagacgtgtgctcttccgatcggcgacagcggagtggagc
		pre-amplification of the edit for multima sequencing in fiser()	gtgcctgcgaatcaaactgg
		are amplification of the ordit for the mine commercially (amin 4 fc)	
1849	R	pre-amplification of the edit for Illumina sequencing(in Lamin A/C)	cagccgggtgatgcgggtgg
1849 1928	F	illumina sequencing (in insert)	$a at gatac ggcg accaccg agaic tacacic tttccct accac gacgct cttccg at {\tt cgcctgcg} aat {\tt caactggtcg}$
1849 1928 1712	F	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing	aalgatacggcgaccaccgagatctacacttttccctacacgacgtcttccgatcgctcttccgatcgat
1849 1928 1712 1713	F	illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  Lamin A/C genotyping and sequencing	aalgataegggaccacqagaictacacictitecctacagagcictitecgaictgcqaalcaaactggicg gaaggicraagcaarigggg gaaggicraagcaarigggg gatgtagaccgccaagcgat
1849 1928 1712 1713 2076	F F R	illumina sequencing (in insert)  Lamin A/C genotyping and sequencing	aalgataegggaccaccgagatctaccicticcctacaegacgtettrcgatig-cg-gaalcaac-tggicg gaaggt-cgagca-atggag gatgtagaccgccaagcgat ggccggcgcacccgacc
1849 1928 1712 1713	F R F	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing	aalgataegegaccacgagatciacatciticcctacaegegictitrcgatig:cgcgaatcaactggicg gaaggirtgaggcaatgggg gategagcaccaaggat ggergagactccgactc caagggatcattgagctc
1849 1928 1712 1713 2076 2077	F F R F	illumina sequencing (in insert)  Lamin A/C genotyping and sequencing	aalgataegggaccaccgagatctaccicticcctacaegacgtettrcgatig-cg-gaalcaac-tggicg gaaggt-cgagca-atggag gatgtagaccgccaagcgat ggccggcgcacccgacc
1849 1928 1712 1713 2076 2077	F F R F	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of if-ABIJA ocf/P repair template (x1648) with 155p MAs	aalgataegegaccacqagatctacatctitocctacaqaegegictitocgatiqocqaacaaactggicg gaaggictgaegecatgggg gattapaccqccaagqgt ggrayactopactc caagqagacatocpactc ccagqagacatocgacaagqtg
1849 1928 1712 1713 2076 2077	F F R F	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of if ABILIA GGP repair template (zr1648) with 150p IMAs  amplification of if ABILIA GGP repair template (zr1648) with 150p IMAs	aalgataegegaccacqagatctacatctitocctacaqaegegictitocgatiqocqaacaaactggicg gaaggictgaegecatgggg gattapaccqccaagqgt ggrayactopactc caagqagacatocpactc ccagqagacatocgacaagqtg
1849 1928 1712 1713 2076 2077 1838 1839 1652	F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of frikBLTA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of Fregari template (cr1648) with "150p IMA amplification of RABITA of	anigntanggegancacqagantchacatchitectanacqasquetteteqantqueqqanacqaactgging gnagginqaeganatgggg garginqaeqacaaqqas ggreggaracteqate caaqqantchinqaete ctoggarqaatgganaggig tagtingtactgitgtoo gciccogcoctitogtoctoggarqaaggaqaagga-aaggaraagga-aaggaraagga-aaggaraagga-aaggaraagga-aaggaraggaraag
1849 1928 1712 1713 2076 2077 1838 1839 1652	F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of RAB11A nGFP repair template (cr1648) with "15bp HAs  amplification of RAB11A nGFP repair template (cr1648) with "15bp HAs  amplification of RAB11A nGFP repair template (cr1648) with "15bp HAs  amplification of RAB11A nGFP repair template (cr1648) with "15bp HAs  amplification of RAB11A nGFP repair template (cr1648) with "15bp HAs	asignianggegaccacquaghtiacaticiticctacacquaghtiticquiq-qualicaaciggicg  pasgiriquagecaniggag  gargingacqccaaqqui  ggergapecactequate  caaqquateningatec  cteggecqcaanquiq  talqquateqquict  quicqqcctitiqqtecac  griccqccctitiqctcacqqqqcqcaaqqqqaaqqqaqqqqqqqqqq
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840	F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs  amplification of RABIIA ecif Prepair template (cr1648) with "15bp HAs	asigntanggegaccacquaghtchacdctiticctchacquaghtchtcpatigl.cgg.patcaactgging pasgiriquagecantgggg gargariquagecantgggg gargariquagecantggggt ggorgggactregaete caspegatentgaetec ctsggorggcactregaete ttsggorggcastregaete getccgccttttgetgetae getccgccttttgetgetae getccgccttttgetgetaeteg
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840	F R F R F R	Illumina sequencing (in insert)  Lamin A/C genohyping and sequencing  amplification of RAB11A eGFP repair template (cr1648) with "15bp HAs amplification of RAB11A eGFP repair template (cr1648) with "15bp HAs amplification of RAB11A eGFP repair template (cr1648) with "15bp HAs amplification of RAB11A eGFP repair template (cr1648) with "35bp HAs amplification of RAB11A eGFP repair template (cr1648) with "35bp HAs amplification of RAB11A eGFP repair template (cr1648) with "35bp HAs amplification of RAB11A eGFP repair template (cr1648) with "35bp HAs amplification of RAB11A eGFP repair template (cr1648) with "35bp HAs	asigniangegencaccpagniciacutciticciacacpagniciticpaticpaticpaticpaticpaticpaticpati
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 1841 2008	F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repair template (cr1648) with "15bp IAA amplification of IAB21A coff repai	anigniangegaccaccpagatciacciciticccicacaquagegiciticgatiq-rig-ganicaactigging  pangiriquagecarigage gatignapecqccaapgat ggoragegacatcqaeci cangingatcattqaetic ctogocqqqaanaggaqecatcqaeci tagicqqqqaanaggaqaqaqqqqqqqqqqqqqqqqqqqqqqqq
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 1841 2008	F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair template (cr1648) with "15tp 14As amplification of fix821.A ccfiP11 repair t	anigntanggegaccacegagatciacaciciticcicacacegageteticcgatiquequeaniggeg gnaggirquequeaniggeg gnaggirquequeaniggeg gardingaccqccasegat ggreggagactecqaceaegat ggreggagactecqaceaegat ggreggagacteqacequeaegat teggegagaatgipgactecqueaegagagagagagagagagagagagagagagagagaga
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 1841 2008 2009	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IRABILA eGFP repair template (cr1648) with "15bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "15bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs  amplification of IRABILA eGFP repair template (cr1648) with "35bp IAAs	anigntaegggaccacquagatciacactiticcicacacquagegiciticquiqquanicaactiggicg gnaggiriquagcaniguag garignapccqccaaqqui ggcrggacactcquac ggcrggacactcquac caaqquatrampapctc ctcgacqquaaqqui taqicqiactqicqtac gcicccgccctticqtcctqqqqqaaqqqqqaqqqqqqqqqqqqqqq
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 2008 2009 1846	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of 6AB211A coFP repair template (cr1648) with "15sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "15sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplification of 6AB21A coFP repair template (cr1648) with "35sp HAs  amplifi	asigniangegpaccaccagagatciacaciciticoctacacagagatcitropatique of pasagoriquegocarigaga  pasagoriquegocarigaga  gergangarcagocarigaga  gergangarcagocaria  georgangarcagocaria  cangegatratingarcac  cangegatratingarcac  cangegatratingarcac  cangegatratingarcac  cangegatratingarcac  georgacariagagate  georgacciticagatraca griccogocuticagatracac griccogocuticagatracacacacacacacacacacacacacacacacacaca
1849 1928 1712 1713 2076 2077 1838 1652 1653 1840 1841 2008 2009 1846	F R R F R F R F R F R F R F R F R F R F	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464  amplification of IABILA # Coff Prepair template (cr1648) with "15bp 1464	anigntanggogaccacquaghtchacdciticocdacquaghtchrophicyclgopatchacdgogag gnagorquaghtchacdciticocdacquaghtchacdgogag gnagorquaghtchacdgogagagagagagagagagagagagagagagagagagag
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 1841 2008 2009 1846 1847 2054	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in insert)  Lomin A/C genotyping and sequencing  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs  amplification of A/BJIJA coff Prepair template (cr1648) with "15bp HAs	anigatanggogaccacqagatciacaciciticoctacaqagagocticopaticqaiqquantagacqqqqqqqqqqqqqqqqqqqqqqqqqqqqqqqqqqq
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 2009 1844 1847 2054 2054	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IRABITA GFP repair template (cr1648) with "15bp 14As amplification of IRABITA GFP repair template (cr1648) with "15bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP repair template (cr1648) with "35bp 14As amplification of IRABITA GFP IR (repair template (cr1648) with "35bp 14As amplification of IRABITA GFP IR (cr1777) with "35bp 14	anigntanggegancacqaagatciacaciciitocctanacqacgiciticopatiquiquiquanticaactigatiquiquiquiquiquiquiquiquiquiquiquiquiqui
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 2009 1846 1847 2055 1604 1605 1554	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in invert)  Lamin A/C genotyping and sequencing  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1648) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair template (cr1553) with "15bp HAs  amplification of IAB21A of Prepair	anigntanggegaccacquagniciacaciciticoctacacquagniciticquiquanicaaciggicg  pasgiriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacicitigagica caggaricitigagica caggaricitigagica caggaricitigagica gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicic gericcagoctiticgicic gericcagoctititigic gericcagoctititigic gericcagoctitigic gericcagoctitigic gericcagoctitigic gericcagoctitigic gericagoctitigic gericagocticagocago gericagocaacagotigicagocaacagotigicagocagogoc gericagoccaacagotigicagocaacagotigicagocaagocago gericagoccaacagotigicagocaacagotigicagocaagocago gericagoccaacagotigicagocaacagotigicagocaagocagogocaacagocagagocaagagotigiagocaaagoctigicagocaagocagogocaacagocagagocaagagotigiagocaaagoctigicagocaagoctigicagocaagagocaagagoctigiagocaaagocagagoca
1849 1928 1712 1713 2076 2077 1838 1839 1652 1652 1652 1840 1841 2008 2009 1846 1847 2054 2055 1604 1605		Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IAB21A cell* prepait template (cr1648) with *15bp IAS amplification of IAB21A cell* prepait template (cr1648) with *15bp IAS amplification of IAB21A cell* prepait template (cr1648) with *15bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1648) with *35bp IAS amplification of IAB21A cell* prepait template (cr1633) with *35bp IAS amplification of IAB21A cell* prepait template (cr1633) with *15bp IAS amplification of IAB21A cell* prepait template (cr1633) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of IAB21A cell* prepait template (cr1533) with *15bp IAS amplification of	anigntanggogaccacquagatciacaciciticoctacacquagatcicropaticquagcaniguaga  panagoriquagcaniguaga gariquagatcaniguaga gariquagatcaniguaga gariquagatcaniguaga gariquagatciagatcia canggogatciagatgotac canggogatciagatciac gotcocgoccillicpticac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicptic gotcocgo
1849 1928 1712 1713 2076 2077 1838 1839 1652 1653 1840 1841 2008 2009 1846 2055 1604 1605 1554 1555 1483	F R F R F R F R F R F R F R F R F R F R	Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of RAB11A coFP repair template (cr1648) with "15bp MAs  amplification of RAB11A coFP repair template (cr1648) with "15bp MAs  amplification of RAB11A coFP repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP1 repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP1 repair template (cr1648) with "35bp MAs  amplification of RAB11A coFP1 cepair template (cr1648) with "35bp MAs  amplification of RAB11A coFP1 cepair template (cr1648) with "35bp MAs  amplification of RAB11A coFP1 cepair template (cr1653) with "35bp MAs  amplification of RAB11A coFP1 cepair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs  amplification of SAM1C coFP repair template (cr1553) with "35bp MAs	anigntanggegaccacquagniciacaciciticoctacacquagniciticquiquanicaaciggicg  pasgiriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacanigusg geriquegacicitigagica caggaricitigagica caggaricitigagica caggaricitigagica gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicicac gericcagoctiticgicic gericcagoctiticgicic gericcagoctititigic gericcagoctititigic gericcagoctitigic gericcagoctitigic gericcagoctitigic gericcagoctitigic gericagoctitigic gericagocticagocago gericagocaacagotigicagocaacagotigicagocagogoc gericagoccaacagotigicagocaacagotigicagocaagocago gericagoccaacagotigicagocaacagotigicagocaagocago gericagoccaacagotigicagocaacagotigicagocaagocagogocaacagocagagocaagagotigiagocaaagoctigicagocaagocagogocaacagocagagocaagagotigiagocaaagoctigicagocaagoctigicagocaagagocaagagoctigiagocaaagocagagoca
1849 1928 1712 1713 2076 1838 1839 1652 1653 1840 2009 1846 1847 2054 2055 1604 1605 1555 1483 1485		Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "15tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1648) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGIP repair template (cr1553) with "35tp 14As  amplification of IAB21.A cGI	anigntanggogaccacquagatciacaciciticoctacacquagatcicropaticquagcaniguaga  panagoriquagcaniguaga gariquagatcaniguaga gariquagatcaniguaga gariquagatcaniguaga gariquagatciagatcia canggogatciagatgotac canggogatciagatciac gotcocgoccillicpticac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicpticacigatciac gotcocgoccillicptic gotcocgo
1849 1928 1712 1713 2076 1838 1839 1652 1653 1840 2009 1846 1847 2054 2055 1604 1605 15554 15554 15554 15554 15554		Illumina sequencing (in insert)  Lamin A/C genotyping and sequencing  amplification of IABLIA of Prepair template (cr1648) with "15tp IAS  amplification of IABLIA of Prepair template (cr1648) with "15tp IAS  amplification of IABLIA of Prepair template (cr1648) with "15tp IAS  amplification of IABLIA of Prepair template (cr1648) with "15tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of Prepair template (cr1648) with "35tp IAS  amplification of IABLIA of IABLIA of IABLIA with "35tp IAS  amplification of IABLIA of IABLIA of IABLIA with "35tp IAS  amplification of IABLIA of IABLIA of IABLIA with "35tp IAS  amplification of IABLIA of IABLIA of IABLIA with "35tp IAS  amplification of IABLIA of IABLIA with "35tp IASLIA wit	anigntanggogaccacquagactacactciticctacacquagcetticcpatiquequantesactigateq  pangiriquegocantgugg  patginguequeangque  gorcapequantesacque  gorcapequantesacque  gorcapequantesacque  tagicquantesacque  gorcapequantesacque  gorcapequantesacque
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	TABLE S5: crRNA/sgRNA used in this study						
Guide Name	Target	Туре	Polarity	Sequence			
1629	Lamin A/C	crRNA	S	ccatggagaccccgtcccag			
1648	RAB11A	crRNA	AS	ggtagtcgtactcgtcgtcg			
1728	Lamin A/C	crRNA	S	gcggcgcgccacccgcagcg			
1729	Lamin A/C	crRNA	AS	agctggcctgcgcccgctg			
1776	RAB11A	crRNA	AS	ccatggcctcacctttaaag			
1777	RAB11A	crRNA	S	gagtacgactacctctttaa			
1909	RAB11A	crRNA	S	aaccactgaaaacaagccaa			
1910	RAB11A	crRNA	AS	ttctgacagcactgcacctt			
1553	SMC3	crRNA	AS	attttccaattaaccatgtg			
1747	SMC3	crRNA	S	tgatgtgatcacagcagaga			
1748	SMC3	crRNA	AS	atcatcttctacaaagtctt			
sgPYM1	PYM1	sgRNA	S	gcgtcaacacagcgacctga			
crAdcy3	mouse Adcy3	crRNA	AS	gtggagccagaggtcgctca			

	TABLE S6: Classification of reads from the Illumina sequencing experiment							
Sample	Total sequencing reads	Do not fully map to template	Below quality threshold	Unexpected mutation at diagnostic position	Used in downstream analysis	Reads with switching detected (% of the previous column)		
No mutation	3,369,768	21.60%	42.30%	0.20%	36.00%	0.00%		
PCR control	3,241,689	20.10%	56.40%	0.20%	23.50%	0.02%		
1/3	3,411,796	20.80%	42.30%	0.40%	36.60%	0.02%		
1/6	5,680,820	21.20%	42.40%	0.20%	36.20%	0.50%		
1/12	6,414,459	21.00%	42.40%	0.10%	36.50%	1.40%		