

Opinion

Anticipating and Identifying Collateral Damage in Genome Editing

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Genome editing has powerful applications in research, healthcare, and agriculture. However, the range of possible molecular events resulting from genome editing has been underestimated and the technology remains unpredictable on, and away from, the target locus. This has considerable impact in providing a safe approach for therapeutic genome editing, agriculture, and other applications. This opinion article discusses how to anticipate and detect those editing events by a combination of assays to capture all possible genomic changes. It also discusses strategies for preventing unwanted effects, critical to appraise the benefit or risk associated with the use of the technology. Anticipating and verifying the result of genome editing are essential for the success for all applications.

Genome Editing: A Transformative Technology

The application of genome editing is transforming agriculture, biomedical research, and healthcare. The many proposed purposes include the generation of more productive or robust crops and farm animals, animal hosts for the production of tissues for graft purposes and therapies that use *ex vivo* or somatic tissue engineering [1–3]. The promise of applicability is turning into reality, as illustrated by the first nonrandomized Phase I clinical trial[†] in which the use of clustered regularly interspaced short palindromic repeats (CRISPR)-engineered T cells was recently found to be safe [4]. To date, >20 Phase I/II human clinical trials are underway for a broad range of diseases, including cancers, β -thalassemia, sickle cell disease, and Duchenne muscular dystrophy (summarized and discussed in [1,5]).

Genome editing is generally based on either zinc finger nucleases [6], transcription activator-like effector nucleases [7], or the **CRISPR/CRISPR-associated (Cas) system** (see [Glossary](#)) [8]. These molecules act by inducing a double-stranded cut in a specific DNA sequence, which results in a genetic alteration as the gap is being repaired. In the clinic, the initial applications aim for deletions of genomic DNA intervals and do not yet involve precision at the nucleotide level; thus, these can be executed through the sole delivery of a genome-editing nuclease. However, for more precise editing, such as the generation of point mutations or more intricate changes, or even accurate deletion of a genomic segment, single- or double-stranded DNA templates are also delivered, together with the nucleases, to direct the repair to result in a given sequence by homology directed repair (HDR) [9–11] or nonhomologous end-joining [12]. Base editors [13] and prime editors [14] are alternative strategies for more precise editing. Overall, the range of genome editing tools is ever increasing and their transformative potential across a range of fields of application is immense.

Genome Editing: A Disruptive but Still Erratic Technology

The safety of genome-editing technologies is just as critical as their efficiency for their successful application in health or agriculture. Common to all fields of application are the risks associated with undesired genetic changes that can be triggered by genome editing.

Highlights

Genome editing has a transformative potential in healthcare or to improve crops or livestock. However, the use of Cas9 or other nucleases can yield unpredictable events at the target site or off target.

To overcome these challenge, it is critical to understand and accurately predict the whole range of possible editing outcomes.

The key to success is to combine molecular assays to evaluate the sequence changes at the target site and to quantify the number of copies of segments deleted/inserted across the genome.

For all applications, thorough evaluation of these outcomes is essential to identify all collateral damage from nuclease activity and for a real appraisal of the benefits and risks associated with applying this technology.

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The potential for unwanted off-target nuclease activity was recognized early in the application of the CRISPR/Cas9 system as a genome-editing tool [15]. The frequency of such events and the attached risks were the subjects of much debate [16–18]. The general consensus is that, with careful molecular design, off-target events are rare and generally can be segregated away from the allele of interest in genome edited animals, unless the off-target region is in linkage disequilibrium with the target site; however, they are potentially more pernicious in cultured cells or in a somatic delivery system [19–23]. In those cases, it is particularly essential that off-target events are captured [24]. However, on-target effects and ectopic insertions of donor template are less predictable and have often been underestimated.

Nevertheless, on-target effects of genome-editing enzymes are also now better documented. These can take many forms: single nucleotide variations, indels, large and/or complex genomic rearrangements, segmental duplications, chromosomal translocation, terminal chromosomal truncation up to several megabases, or loss of one or both arms of a chromosome [25–31] (Figure 1), and some mutagenic events are not compatible with efficiently populating a cell lineage *in vivo* [30]. These effects are intimately linked to the kinetics of the enzyme's interaction with the DNA, and with the DNA repair pathways [32,33]. This results in multiple cutting at the target site and can lead to the alteration of a larger than expected segment by ~1–2 kb up to 50 kb at a frequency of ~15–20% of the target DNA in somatic cells [25,26,29]. In early embryos, this additionally translates to mosaicism of the mutated alleles [34–36].

Similarly, repair with a template can result in a variety of sequence changes even when the insertion of the repair template is on target (Figure 1). It can yield unpredictable and sometimes complex events at the target site, such as partial insertion of the template, sequence duplications, inversions or rearrangements of the template in combination with endogenous sequences [35,37–39], as well as ectopic insertions of the repair template. The delivery of a template for repair or vectors for nuclease expression can also yield ectopic insertions that could affect the

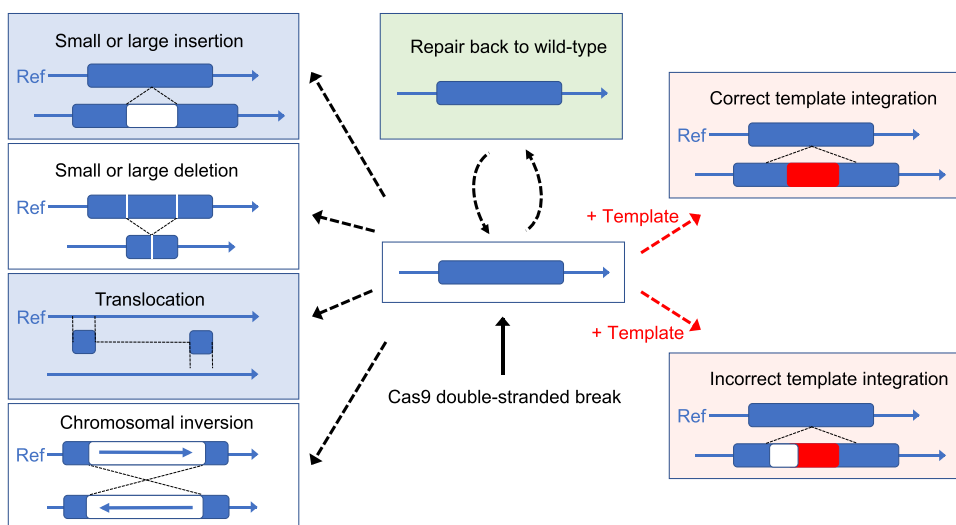


Figure 1. Possible outcomes of Cas9 double-stranded breaks (DSBs) of DNA. Cas9-induced DSBs lead to a series of expected and unexpected outcomes. Final editing outcome can be small insertions, deletions, chromosomal translocations, or incomplete template integration.

Glossary

Biotin-labeled probe: enzymatic incorporation of biotin at the 5' end of a DNA oligonucleotide to label a PCR product in conjunction with fluorophores and enzymes.

Cas9 variants: Cas9 nickases cut a single strand of the DNA. Dead Cas9 (dCas9) is a catalytically inactive Cas9. HFCas9 and eSpCas9 are highly specific Cas9 variants. Base editing is the fusion of a cytidine or adenosine deaminase with dCas9 or a Cas9 nickase. Prime editing is the fusion between a reverse transcriptase enzyme and dCas9.

Cas9-aided capture: use of Cas9 enzyme to enrich a genomic target for high-throughput sequencing without utilizing PCR amplification methods.

CIRCLE-seq and Digenome-seq: unbiased high-throughput genome-wide profiling techniques to detect randomly sheared genomic DNA and DNA cleaved by Cas9 ribonucleoprotein that are circularized (CIRCLE-seq) or not (Digenome-seq).

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems: defense systems in bacteria against phage infection comprising an acquisition system to memorize phage attack and an effector complex that recognizes a protospacer adjacent motif (PAM) of the intruder genome, resulting in a single- or double-stranded break of the DNA or single-stranded break of the RNA.

Type II, V, or VI CRISPR systems with Cas9, Cas12, and Cas13 as effector protein signatures are harnessed as gene-editing tools.

Comparative genomic hybridization (CGH) array: competitive *in situ* hybridization between two different DNA genomic samples in conjunction with a DNA microarray is used to evaluate copy number variation.

Cytogenetic methods: fluorescence *in situ* hybridization (FISH) and fiber fluorescence *in situ* hybridization (fiber-FISH) are fluorescent probe-based methods to visualize a specific region or an entire chromosome within a particular genome. Fiber-FISH (or DNA Combing) uses FISH on mechanically stretched DNA to increase its resolution.

DISCOVER-seq: unbiased high-throughput genome-wide profiling techniques to detect DSB repair protein MRE11-binding sites.

safety of the approach [40,41]. The recent example of genome editing of the *POLLED* allele in cattle [42], in which the full outcome had not been identified from the first analysis of the edited cows [43], illustrates the difficulties involved in thoroughly identifying ectopic insertions of the repair template. Additional studies show that pervasive insertion of the donor template across the genome can remain undetected with conventional methods, such as PCR and Sanger sequencing [39]. These examples underline questions relating to the prevalence and type of on-target modifications and ectopic insertions of the donor DNA following codelivery with nucleases. They also articulate the importance of using the appropriate assays to evaluate the correctness of the resulting genome-editing event(s). (See [Box 1](#)).

Unknown Unintended Consequences of Genome Editing

As genome editing is increasingly used, further unexpected and potentially negative outcomes of its application are still being uncovered. For example, the perdurance and transmission of **DNA double-stranded breaks** (DSBs) is a phenomenon so far overlooked and forms a molecular basis for several mixed alleles arising from a single cutting event [31,44].

Also, the occurrence of potentially extensive gene conversion [45] of edited alleles went unnoticed until recently. This involves the transfer of DNA from one genomic location to another by homologous recombination. Examples of gene conversion are the transfer of the DNA from delta-hemoglobin to beta-hemoglobin in the case of dividing or nondividing cells, or transfer to the use of the paternal allele as a repair template in the case of embryonic cells [46–48]. Gene conversion could result in a partial or full repair of the allele; it was hypothesized that such a mechanism could be utilized as an internal template repair for precision editing, but this is still disputed [47]. In particular, because conversion tracks may expand well beyond the targeted region, the resulting loss of heterozygosity represents an additional risk for clinical application [31,49].

Finally, and importantly, it is now becoming increasingly apparent that genomic segments can be inadvertently altered at comparatively large distances from the cutting site [29,50]. The frequency of such outcomes and the genetic range susceptible to alteration following genome-editing intervention remain to be fully appraised.

All of these poorly understood consequences pertain to changes to the DNA sequence. Other potential unknown consequences of on- and off-target effects could have an entirely different molecular basis, such as deregulation of the chromatin environment or the 3D organization of the nucleus, which could change the genome stability or gene expression. The incidence of such potential consequences is as yet largely unexplored,

The Context of the Genome Editing Application Changes the Question

In all instances, the challenge is to fully apprehend the editing outcomes that may have adverse consequences. This is likely to require the application of a suite of molecular techniques to interrogate the different artefactual features that can be encountered in genome editing. These features can be diverse in scale (single base to megabase) and may involve additional template insertions. All of these outcomes can occur at the targeted site or ectopically. Second, to add more complexity, the extent and nature of lesions vary considerably depending on whether the editing occurs in nondividing somatic, dividing somatic, or germinal (early embryonic) cells. In the case of euploid clonal cell populations or the progeny of founder animals, there are only two alleles for each autosomal locus and, therefore, a maximum of two variants may need to be identified. By contrast, animals born from genome editing of early embryos are generally mosaic [34,35,37]. Modification of pools of cultured cells yield heterogenous cell populations

DNA double-stranded break (DSB): results in cellular machinery repairing DNA breakage via the nonhomologous end-joining pathway, the microhomology end-joining repair pathway based on 3–5-bp microhomology sequences as a repair template, or the homology directed repair (HDR) system, which uses a longer template for repair, such as a single-stranded DNA.

GUIDE-seq: unbiased high-throughput genome-wide profiling technique to detect integration of a double-stranded oligonucleotide cassette in a DSB.

LAM-HTGS: unbiased high-throughput genome-wide profiling technique to detect chromosomal translocations using a 'bait' and 'prey' DSB.

Targeted locus amplification (TLA): amplification of a targeted gene of interest using the physical proximity of the nucleotides within this locus of interest by DNA crosslinking, fragmentation, ligation, and high-throughput sequencing.

UDiTaS™ and CHANGE-seq: harnessing of Tn5 transposon tagmentation followed by a series of PCR amplifications (UDiTaS™) or genomic DNA circularization (CHANGE-seq).

[18], and tissues modified by somatic modification [51] represent yet a larger degree of genetic complexity. In all these examples, multiple and potentially diverse allelic variants are represented at different frequencies. It is critical to elaborate a clear strategy that takes into account these different degrees of genetic complexity to uncover, fully characterize, or ideally prevent, these unwanted events.

The stakes are high because the impact of incomplete characterization of editing effects is potentially important in all areas of application: in the laboratory, the risk is of irreproducible or artefactual research. Therefore, information obtained with genetically edited founder animals (likely to be mosaic) must be interpreted on the basis of the intrinsic genetic complexity of these animals and the genetic content of progeny must be extensively revalidated. Equally, interpretation of data obtained with edited culture cell pools (where repair may result in many different alleles with various rates) requires an understanding of the genetic composition of these complex cell populations, such as mosaicism [25]. When editing is used for the production of agricultural products (plants or animals), it remains unclear whether uncontrolled outcomes may pose a risk to the users. Such variability may prevent licensing for commercialization by regulators or negatively affect the confidence of consumers in the safety of those products [43,52]. For use in the clinic, in tissue engineering or by somatic delivery, the degree of variability of genomic outcome that may be acceptable in terms of safety remains to be appreciated and may not represent an insurmountable obstacle [4]. However, the range of edited sequences that can result from a given therapeutic intervention must still be thoroughly understood to evaluate the associated benefit–risk balance [24]. Finally, an inability to fully validate the consequences of CRISPR/Cas activity throughout the embryo represents a practical barrier to germline editing in the clinic [53].

In all applications of genome editing (whether in biomedical research, agricultural production, or the clinic) a thorough evaluation of these outcomes is necessary for a realistic appraisal of the benefit–risk ratio associated with the use of the technology. The required level of investigation depends on the specific application, but all demand the ability to anticipate the whole range of potential (wanted and unwanted) consequences of each genome-editing intervention.

Strategy for Validation

Capturing the variability of genome-editing outcomes requires increasing investment in resources as the attention extends away from the target site: (i) as a minimum, amplification and sequencing of the target site and of chromosomally linked potential off-target sites should be achieved; (ii) quantification of the number of copies of deleted segments or donor template to capture on target duplications and ectopic integrations should be included. (This is also essential for all applications.); (iii) the use of more elaborate assays to inform on potential larger-scale chromosomal rearrangements is desirable, because an increasing number of examples have been identified in which additional sequence changes away from the cutting site have been found; and (v) where required, analysis should be extended to the whole genome to predict or capture potential off-target sites.

Equally, a pragmatic approach to the interrogation of genome editing takes into account the likely genetic complexity of the edited material (whether all cells have identical genomes or constitute a genetically diverse population) and the context of application (see [Box 1](#)). For example, mosaic founder small laboratory animals will be bred, thus allowing for the segregation of most unwanted edits at the next generation. Therefore, it is only essential to search for the presence of an allele of interest and linked off-target effects. Definitive characterization of the model can await transmission of the allele of interest to the subsequent generation. By contrast, the whole gamut of mutations arising from CRISPR/Cas activity is to be considered when this technique is used in large

Box 1. Factors that Should Be Assessed when Validating Genome-Editing Outcomes

1. What is/are the new genetic modification(s) on target? What is the length of the interval potentially altered by the intervention?
2. Are there sequence changes in potential off-target sites? Are these sites physically linked to the target locus?
3. What is the number of copies of the mobilized segments (deleted or introduced as template)?
4. Can the purpose of the application cope with potential unwanted changes in the genome?

livestock (because associated financial and welfare costs are high and timelines extended by long gestations) or for somatic treatment in the clinic [24,43]. Therefore, depending on the genome-editing application, the strategy for validation will either aim to identify the presence of a specific variant, seek to capture complexity, or definitively ascertain an entire genetic make-up. In summary, the genotyping strategy will take into account the ability of each molecular assay to cope with the genetic complexity of the material and will customize effort for the context of utilization.

Capturing the Variability of Genome Editing Outcome on Target

Appraising the on-target outcome of CRISPR/Cas activity was initially perceived as a straightforward exercise and, therefore, was performed by a simple set of standard molecular biology protocols: surveyor assays or PCR amplification and Sanger sequencing, with, in some instances, prior cloning of the PCR product [9] (see Table 1). Although such approaches are generally sufficient to detect the presence of the desired mutation [36], they do not support capturing the entire range of sequences that arises from the CRISPR/Cas mutagenesis effect on target sites in materials of complex genetic make-up. For example, larger deletions that include the sequences annealed by at least one of the PCR primers used for genotyping are not detected [26]. Equally, low frequency events may be overlooked, or relevant cell lineages may be inaccessible for sampling or under-represented in samples; for example, a variant may not be detected within the somatic cells of a founder animal (ear biopsy) but may be identified in their progeny [37].

Southern blot analysis appraises a wider genetic interval and can identify genomic changes away from the immediate vicinity of the targeted sequence [41,54]. **Cytogenetic methods** and fiber fluorescence *in situ* hybridization (fiber-FISH) [28,55] support the survey of an even broader region and can identify unwanted insertions or deletions of genetic material as well as large-scale

Table 1. Methods for Analyzing Loci Targeted by Genome Editing

Technique	Advantages	Disadvantages	Refs
PCR and Sanger sequencing	Easy to implement	Can be difficult to interpret and may overlook some alleles	[9]
PCR, subcloning, and sequencing	Easy to implement, appropriate to disentangle mosaic cell population	Work intensive and may overlook some alleles	[9]
Surveyor assay (i.e., T7 nuclease assay)	Easy to implement	Does not provide sequence granularity	[9]
Southern blot	Interrogates a large genomic segment	Does not provide sequence granularity	[41,54]
FISH	Interrogates a large genomic segment	Does not provide sequence granularity	[55]
Fiber-FISH	Interrogates a large genomic segment	Does not provide sequence granularity	[28]
dPCR or qPCR	Detects duplications	Does not provide sequence granularity	[27,39,62]
TLA	Interrogates a large genomic segment	Expensive to implement	[56]
PCR and short read based-NGS	Appropriate to analyze many genome-editing samples	Expensive to implement unless large numbers of samples analyzed	[57]
PCR and long read based-NGS	Appropriate to disentangle mosaic cell population and interrogates a large genomic segment	Expensive to implement unless large numbers of samples analyzed	[26,58]

sequence rearrangements. Given the variability of the outcome and the length of the modified segments, a fuller examination requires more elaborate and expensive assays, such as **targeted locus amplification** (TLA) [56] or, in the case of material with a complex genetic make-up, high-throughput short-read [57] or long-read [26,58] sequencing. For the latter two, targeted sequencing can rely on the isolation of the loci of interest by simple PCR [26,57,58], but this limits the size of the interval that can be interrogated. Other approaches for larger template isolation are emerging to lift this constraint (e.g., biotin-labeled probes [59] and Cas9-aided capture [60,61]) (see Table 1).

Scanning the Genome for Wider Consequences of CRISPR/Cas Activity

Genome-editing nucleases are powerful tools to introduce sequence changes at a target locus, but they can also lead to changes in other similar sequences genome wide. Copy counting of a deleted segment will inform on the possibility that an unexpected rearrangement has occurred instead of the simple removal of an interval of interest [27,36]. Equally, copy counting of the DNA template (single- or double-stranded) will identify additional integrations. Digital PCR (dPCR) generally is a straightforward assay for this, but standard quantitative PCR (qPCR) can also be used [62]. On a larger genomic scale, **comparative genomic hybridization** (CGH) arrays and FISH enable a whole genome to be surveyed and can identify large sequence alterations away from the nuclease cutting site [29].

Whole-genome sequencing allows for a broad and unbiased capture of the genome-editing outcome [63] but such an approach is expensive and inadequate for complex genetic materials, such as heterogeneous cultured cell populations, or when genome editing nucleases are used with somatic delivery. The complexity of this question demanded the development of bespoke analysis approaches for more effective identification of off-target effects. Many solutions have evolved, based on sequencing of captured susceptible sites (e.g., **GUIDE-seq** [64], **CIRCLE-seq** [65], **LAM-HTGTS** [66], **UDiTaS™** [67], **Digenome-seq** [68] and **CHANGE-seq** [69]). An alternative method captures off-target CRISPR/Cas activity 'red-handed' by detecting the DSB repair complex MRN, binding to genomic DNA using ChIP-seq, a method called **DISCOVER-seq** [70]. Methods are continuously evolving in particular to address the remaining challenges of capturing the rarer events in samples of high genetic complexity and of eliminating bias towards particular types of sequence modifications.

No Single Assay Captures All the Potential Outcomes of Genome Editing

Crucially, no single technology is able to capture all of the unexpected sequence changes that can result from genome editing. Targeted sequencing using Sanger, or next-generation methods [26,58,71], affords validation of the targeted locus to the single-base level, but only reports on sequence variation at loci that are chosen as relevant and on the integrity of an interval of a limited size; neither do these techniques identify additional sequence changes elsewhere. Droplet digital PCR [27,39] or even Southern blot analysis [41] help to identify unexpected copy numbers of given sequences, but do not report on the exact sequences. Neither of these techniques unravels the complexity of nonclonal materials that contain many genetic identities. Technologies based on the visualization of chromosome segments with fluorescent probes permit the survey of large regions but generate data of low resolution.

All strategies to identify distal or off-target activity also have sensitivity limitations and biases. Sanger sequencing can be applied to many off-target sites, but the loci for analysis must be predicted. Protocols based on Sanger or short-read sequencing do not readily identify structural variations [72]. By contrast, FISH cytogenetic analysis and the elegant variation of DNA combing allow for the documentation of large structural variation at the expense of the granularity of

sequencing information. Methods for capturing potential off-target sites [64–69] may not reveal all events, whereas DISCOVER-seq technology [70] captures events contemporary to the assay, but not those that occurred earlier in the time course of the intervention. Finally, even whole-genome sequencing technologies, when they can be afforded, will have their own biases that leave remaining ambiguities in terms of nuclease activity consequences: short read-based sequencing is likely to miss structural rearrangements, whereas long-read sequencing can lack accuracy if ample coverage is not obtained. With either modality, achieving close to a full genome sequence requires heavy investment, even with genetically homogeneous material.

Nevertheless, characterization of potentially complex genome editing events is essential to establish the reliability of genome edited materials and their suitability for their intended use. Defining the appropriate validation strategy will determine the best possible combination of assays in terms of their scope and available resources, and requires the anticipation of potential outcome, genetic complexity of the edited material, and essential quality criteria for a given application. Understanding the frequency of the different variants that result from each genome-editing application will also underpin the development of refined strategies for a more exact outcome in future attempts [58].

Preventing the Damage

Whilst it is important to identify unwanted events, their prevention seems even more desirable. To alleviate the risk of unwanted outcomes following CRISPR/Cas9 editing, many strategies have been proposed. Early in the development of the technology, predicting the mutagenesis pattern of the guide RNA through its computational design was a major focus for precision editing. Guide efficiency, as well as the prediction of mutagenesis effects and their potential off-target effects, is now better understood. In addition, noncanonical single guide RNA (sgRNA), **Cas9 variants** selected for enhanced specificity [73], and nickases [74] were used in initial strategies to achieve accurate interventions, in many cases to the detriment of efficiency. An alternative approach aims to focus activity on the desired target by increasing protospacer adjacent motif (PAM) selectivity, thereby diminishing activity at some other nontarget sites that harbor alternative PAM variants [75]. In addition, temporally controlling its activity by using ribonucleoprotein or a ubiquitin-proteasome degradation signal could help to restrict extensive DNA cutting [76]. The introduction of spatial control by expressing Cas9 in a specific cell type or targeting its delivery could also reduce the risk of DNA damage [24,77]. Finally, competition with inactive ribonucleoproteins (RNPs) targeting off-target sites has been proposed as a means to focus genome editing onto the target site [78].

Where a DNA template is used, tipping the balance in favor of HDR against nonhomologous end-joining and other repair events is beneficial to ensuring quality. This may be achieved by codelivery of HDR effectors [79], by pharmacological intervention using small-molecule compounds [80] (although this may reduce cell viability), or by directing Cas protein expression to specific cell-cycle phases [81,82]. The choice of the repair template is also of primary importance to reduce or eliminate the prevalence of ectopic insertions. For example, circular DNA repair templates [11] or templates tethered to the CRISPR complex [11,83] could result in a higher proportion of on-target integrations compared with double-stranded and single-stranded linear DNA donors. Equally, delivery of the template at a lower concentration would result in a lower copy number being ectopically inserted across the genome [84], although this may affect overall efficiency of the genome-editing attempt. All of these techniques have been shown to enhance the frequency, or proportion, of desired outcomes, but none of them guarantees it. Thorough monitoring of outcomes remains essential in all cases and for all applications. New generations of genome editing tools, such as base editing [13] and

prime editing [14], represent further progress towards controlling genome editing outcomes, but are not yet capable of absolute precision editing.

Concluding Remarks

Although genome editing is being developed for application across many fields (biomedical research, agricultural production, or in the clinic), much work remains to be done towards understanding the frequency, extent, and mechanisms of the sequence changes that the system produces (see [Outstanding Questions](#)). Existing genome-editing systems all rely on the uncontrolled participation of endogenous DNA repair factors and, consequently, are error-prone and not entirely predictable. An error-free system will await more sophisticated approaches that will both carry out the desired DNA repair and shield the modified locus from the intervention of endogenous DNA repair machineries. An additional possible avenue would be to further develop and engineer additional DSB-free and, ideally DNA nick-free, editing systems, such as transcriptome engineering using Class VI CRISPR enzymes or other classes of effectors [85]. Finally, an important area of development in the gene-editing field involves uncovering and harnessing the tremendous diversity and versatility of antiphage defense systems in bacteria discovered through metagenomic sequencing [86,87]. In the near future, this will lead to improvements in the specificity and efficiency of current gene-editing tools, and to alternatives to these tools.

Meanwhile, existing genome-editing tools are still extraordinarily powerful: while the technology cannot yet aim for one single ideal product, a pragmatic version of precision editing would ensure an outcome of acceptable results when a benefit–risk evaluation is performed. However, in all instances of application, the bottom line is that the potential collateral genetic damage must be anticipated and identified.

Acknowledgments

The authors thank Dr Louise Tinsley for expert assistance with the preparation of the manuscript, and three anonymous reviewers and the Editor for their comments and suggestions. L.T. was supported by Medical Research Council in the UK (grant A410) and the National Institute for Health in the USA (Grant U42OD011174). G.B. was supported by the National collaborative Research Infrastructure (NCRIS) via the Australian Phenomics Network, the National Health and Medical Research Council (NHMRC) of Australia (Grant APP1143008), the Australian Research Council (ARC) (Grant DP180101494), and the National Natural Science Foundation of China (NSFC) (Grant 81772214).

Resource

<https://clinicaltrials.gov/ct2/show/NCT03399448?cond=crispr&draw=2&rank=7>

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Outstanding Questions

What would be the acceptable balance of specificity versus efficiency in editing for health or agriculture application?

Will DSB-free gene-editing tools offer higher specificity and efficiency in targeting than classical CRISPR editing tools?

What would be the place of RNA-editing enzymes or other classes of CRISPR effectors in the expansion of the gene-editing toolbox?

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