before adoption of the directive include conventional *in vivo* mutagenesis through ionizing radiation and exposure to mutagenic chemicals. Whereas the practice of crop breeding using this form of mutagenesis is indeed considered 'safe'[6], it nonetheless results in many mutations[7], thereby increasing the odds of additional unforeseen effects, as compared with more targeted[8] and effective NPBTs[9].

From a trade perspective, the CJEU's ruling is likely to send ripples through the EU. The major problem in practice is that, unlike transgenic plants, which fall under Directive 2001/18/EC, many mutagenic NPBTs are simply not traceable in the final product and cannot be distinguished from products in which mutations have been introduced in a 'classical' manner considered to be safe according to the law. Although the altered genetic composition can basically be traced, it will not be possible to establish whether the mutated plant product resulted from the application of an NPBT, from the application of an exempted technique of mutagenesis or indeed from natural mutations without well-established identity preservation systems (IPS).

The lack of an IPS, and the difficulty in establishing one, imposes liability risks on the EU agriculture and food sector using imported goods that were potentially created with mutagenic NPBTs. After this judgment, most of these goods will require authorization and labeling and will be subject to strict liability regimes for adventitious presence under the environmental-liability directive and related national coexistence regulations. Some national systems even require a non-GMO declaration for goods before they are admitted to the market. After this judgment, it is almost impossible for businesses to immediately draft such a declaration, because they are unable to preclude the involvement of a mutagenic NPBT in some way at some time in the product's creation.

These complications are not due to the CJEU's interpretation/decision; the CJEU has merely thrown into stark contrast the imperfections and inadequacies of current EU law. If anything, the CJEU's judgment underscores the need for regulatory reform in the EU. Not only member states with a strong crop-breeding sector but also many others will now have an interest in maintaining access for their agriculture and food sector to the international and, even more so, the intra-EU markets.

One wrinkle in these events is that if Brexit takes place, the United Kingdom may well turn out to be a winner of the CJEU judgment. If the United Kingdom decides to exempt NPBT products, Brexit could allow plant breeders and farmers there to invest in and use the technology, thereby increasing their competitive advantage. Taking all the scenarios mentioned above into account, the impetus for regulatory reform in European governments might shift from the present impasse, in which voting behavior at decision-making bodies at the EU level[10] is a major obstacle[1]. It is now time for EU policymakers to pick up the ball and reform the system. Several options are on the table[1].

COMPETING INTERESTS
The authors declare no competing interests

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1. Purnhagen, K.P. *et al. Nat. Biotechnol.* **36**, 573–575 (2018).
2. European Union. Opinion of Advocate General Bobek. *InfoCuria*. http://curia.europa.eu/juris/document/document.jsf?text=&docid=198532&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=240909 (2018).
3. European Union. Judgment of the Court (Grand Chamber). *InfoCuria*. http://curia.europa.eu/juris/document/document.jsf?text=&docid=204387&pageIndex=0&doclang=EN&mode=req&dir=&occ=first&part=1&cid=317008/ (2018).
4. Purnhagen, K. & Rebasti, E. *Eur. J. Legal Stud.* **1**, 13–21 (2007).
5. Purnhagen, K. & Wesseler, J. in *The Coexistence of Genetically Modified, Organic and Conventional Foods* (eds. Kalaitzandonakes, N. *et al.*) 149–165 (Springer, New York, 2016).
6. Ahloowalia, B.S., Maluszynski, M. & Nichterlein, K. *Euphytica* **135**, 187–204 (2004).
7. Shirasawa, K., Hirakawa, H., Nunome, T., Tabata, S. & Isobe, S. *Plant Biotechnol. J.* **14**, 51–60 (2016).
8. van der Wiel *et al. Plant Biotechnol. Rep.* **11**, 1–8 (2017).
9. Fernandez, O. *et al. Metabolomics* **12**, 158 (2016).
10. Smart, R., Blum, M. & Wesseler, J. *Ger. J. Agric. Econ.* **64**, 244–262 (2015).

A call for science-based review of the European court's decision on gene-edited crops

To the Editor: A recent ruling[1] by the Court of Justice of the European Union (CJEU; Luxembourg) classifies genome-edited plants as genetically modified organisms (GMOs) and thus subjects them to prohibitive premarket risk evaluations. This decision not only ignores the science of agricultural improvement but almost certainly will impede developments that would enhance the sustainability of agriculture and world food security. As geneticists who have been involved for many years in genome editing (D.C. and F.D.U.) and crop improvement (P.C.R.), we are gravely concerned by the decision.

The basis for the emergence of new characteristics in living organisms is mutation. Nowhere is this more evident than in agriculture. The crops grown and harvested today—whether in organic or conventional fields—are all products of artificial selection (i.e., the identification of individual plants with desirable traits conferred by variations in genome sequences and further breeding to combine beneficial variants).

For most of human agricultural history, breeding programs have been able to access only naturally occurring variants of crops. Beginning in the first half of the twentieth century, however, the rate of generating new mutations accelerated dramatically. H.J. Muller demonstrated, in Nobel Prize–winning work, that exposing fruit flies to modest doses of ionizing radiation produces high frequencies of new mutations. Many of these mutations had deleterious effects, and some were lethal, but careful breeding-based selection was able to isolate mutations that conferred desirable characteristics.

This approach was adopted enthusiastically by plant breeders. They irradiated seeds, planted them in greenhouses, examined the resulting plants for new traits, and began the laborious process of breeding those traits into existing strains. For example, a common variety of short-grain rice, widely grown in California, was derived through such mutation breeding[2].

DNA-damaging chemicals, which also generate thousands of random mutations, have likewise been used for many years in plant breeding. As a result, some 3,000 varieties of crops produced through chemical and irradiation mutagenesis are widely grown and consumed in the United States, Europe, and Asia (https://mvd.iaea.org/ and ref. 3).

Another approach that breeders have used extensively for the introduction of useful

genetic variation is to combine genes from different plant varieties (or species) and then derive new varieties from those hybrids. For instance, introduction of a submergence-tolerance gene from an ancient rice variety into modern varieties has allowed farmers to harvest grain even in the face of prolonged flooding caused by climate change[4].

From a regulatory perspective, the plants resulting from chemical- or radiation-induced mutagenesis or hybridization are not characterized, or regulated, as 'GMOs' and can be certified 'organic' in both the United States and the European Union.

Although irradiation-based production of new crops continues to this day, the past 20 years has seen the emergence and widespread adoption of new technologies in agriculture that Gregor Mendel, the father of modern genetics, would have found to be in the realm of magic. The complete DNA sequences of all the major food crops—corn, rice, wheat, potato, cassava, and a wealth of others—have been obtained, as well as thousands of diverse varieties for some of these species. This sequencing has allowed scientists to determine the genetic basis and diversity of key traits essential for agronomy and to make directed crosses to combine beneficial characteristics. Incidentally, such efforts indicate that modern crops have been heavily genetically altered by human activity. For example, any given modern rice variety—and there are thousands—differs from its ancestors in hundreds of thousands of small genetic changes (for example, point mutations, or the gain or loss of a few base pairs at specific positions in the genome)[5].

New traits have also been introduced into plants by the process of transgenesis. This approach often involves inserting genes from other species, which some people find objectionable, and which triggers extensive regulatory requirements. Classic examples of transgenic crops are insect-resistant plants created by the introduction of genes from a bacterium called *Bacillus thuringiensis* that encodes an insecticide. Endowing plants with this 'Bt' trait eliminates the need to spray chemical insecticides or a formulation of bacteria producing the toxins onto fields, an approach widely used by organic farmers who are prohibited from planting Bt crops. In fact, the use of Bt crops over the past 20 years has dramatically decreased global spraying of chemical insecticides on corn, soy, eggplant, cotton, and other crops[6,7].

With respect to safety, a rigorous, formal process of review has been in place in the United States long before the first transgenic crop entered the market in 1996. Since that time, not a single documented adverse event to human health or the environment has been traced to planting or consumption of food produced with such transgenic technologies (https://www.aaas.org/sites/default/files/AAAS_GM_statement.pdf).

Today, ~90% of corn, canola, and soybean crops grown in the United States are transgenic; indeed, more than 40% of US agricultural land is used to cultivate transgenic crops. Worldwide, up to 17 million farmers in 24 countries planted 189.8 million hectares (469 million acres) of such crops last year, according to the International Service for the Acquisition of Agri-biotech Applications (ISAAA).

In Europe, however, the picture looks radically different. Currently, only one GMO crop is grown there—Bt corn, which was approved in 1998 before the current European legislation (Directive 2001/18/EC) was passed in 2001. Last year, transgenic cultivation in European Union member countries accounted for only 131,535 ha.

If transgenic crops are as safe as other crops, have massively decreased the use of insecticide sprays, and are widely embraced by farmers in many countries around the world, why do so few EU farmers grow them?

In 2001, the EU introduced Directive 2001/18/EC, which separates crops into two regulatory categories: those produced with 'first-generation technologies' (for example, irradiation- and chemical-based mutagenesis) and those produced via transgenesis. We lack the space to fully describe the severe regulatory burden, costs, and delays that European legislators and policymakers have imposed, but the result is stark: EU farmers are largely prohibited from growing transgenic crops, and most EU farmers do not have access to Bt crops, among many others.

New methods of trait modification by genome editing (by using CRISPR–Cas9 or other platforms[8]) differ substantially from random mutagenesis, cross pollination, and transgenesis. First, genome editing produces genomic alterations that are similar to those that occur through spontaneous and induced mutation—typically small insertions and deletions or single base changes. However, compared with chemical- or radiation-induced mutagenesis, the changes that arise through modern genome editing are precise rather than random. Second, genome editing leaves no foreign DNA in the targeted genome (a major difference from transgenesis). Third, the technology has now advanced to the point that the desired modification is almost always the only one that results from the treatment. Both genomic and phenotypic analyses are carried out to confirm the expected outcome. Fourth, the same modification can be easily introduced into multiple genetic backgrounds in crops adapted and/or selected for other characteristics (for example, climate adaptation, flavor, and disease resistance), thus facilitating the planting of genetically diverse varieties.

Since the initial description of gene editing in crops in 2009 (refs 9,10), modern genome editing has been applied to more than 50 plant species (**Supplementary Table 1**), including not only all the major crop species (for example, maize, rice, and wheat) but also crops as varied as oranges, lettuce, and cassava. What explains the great interest in using genome editing for crop improvement?

To give one clear example, genome editing allows for rapid and precise transfer of natural variants between different varieties of the same crop. In contrast, conventional breeding of natural variants is time consuming and labor intensive, and it results in not only the transfer of desirable mutations between plants but also the incorporation of additional genomic mutations—passenger variants—that are not characterized and often have unpredictable effects. Radiation and chemical mutagenesis produce thousands of random mutations throughout the genome that are carried along with the selected trait on subsequent breeding.

To use genome editing productively in crop plants, specific genetic causes for desirable traits must be known. Fortunately, many years of controlled breeding and contemporary methods of genome sequencing have allowed for the identification of many such genes. Genome editing has already produced soy plants with more healthful oil[11], mushrooms that are nonbrowning, and potatoes that produce less acrylamide (a known carcinogen) upon frying[12]. A particularly impressive example is the generation of disease-resistant wheat[13], which required making targeted mutations in the six copies of the responsible gene in this hexaploid plant—an accomplishment inconceivable through other methods.

The progress of genetics toward a next generation of crops that can meet the needs of a rapidly warming planet has resulted from the joint effort of scientists in Europe, the United States, and many other countries. The reception of these advances, however, has not been uniform. The same genome-edited potato will now be treated differently in the United States, Australia and Argentina on the one hand, and in all of Europe on the other, even though the basic tenets of genetics and the principles of genome editing are universal.

The CJEU ruling does not explicitly ban gene-edited crops. Instead, it categorizes them with transgenic plants and subjects them to such extensive risk evaluation that the cost of gaining approval could be borne by only the

largest corporations. From a scientific perspective, this is, in plain terms, nonsensical: as explained above, thousands of crops produced with radiation carry a wealth of small genetic changes and are deemed safe. Why would a crop in which just one such change has been introduced by genome editing be regulated differently?

We note that in the United States and several other countries, the relevant regulatory authorities have consistently ruled that crops gene-edited to carry small mutations (for example, maize, mushroom, potato, wheat, soybean, and rice) are not regulated as transgenic plants. Because no tenable scientific basis can be provided to justify the CJEU decision, we are left with the sole alternative explanation, namely, that the decision was made without reference to scientific evidence. For example, the court's statement that the "risks linked to the use of these new mutagenesis techniques might prove to be similar to those that result from the production and release of a GMO through transgenesis" has no scientific basis. As was well documented more than 14 years ago, the risks of unintended consequences of transgenic approaches are no greater than the risks of conventional mutagenesis approaches (https://www.nap.edu/read/10977/chapter/1/). In addition, as described here, the precision with which changes can be made with genome editing decreases these risks even further. This process of decision-making by the European court is grounds for grave concern.

Equally concerning is that the decision, although supposedly made in the name of protecting small farmers, would limit the access of those very farmers to a technology that sharply decreases their costs and the environmental impacts of their farms. The ruling will also make it more difficult for small companies, governmental entities, and charitable organizations to develop new varieties.

We support the call (https://www.change.org/p/ipmb2018-immediate-review-of-the-ecj-ruling-on-plant-genome-editing-9ff3df10-9f7d-44de-b379-8a01a1d71ba2/) made by plant scientists at the 2018 International Plant Molecular Biology meeting for an immediate review of the CJEU ruling on plant genome editing. We believe that the benefits of crop improvement, whether through genome editing or other means, should be available to farmers who want them. Furthermore, each new variety should be evaluated on the basis of its specific characteristics—such as decreasing the environmental impacts of farming and enhancing food security—and not on the basis of the method through which it was generated.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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1. Callaway, E. *Nature* **560**, 16 (2018).
2. Vijayagopal, P.D. & Nair, V.G. Genic relationship of induced semi-dwarf rice mutants (*Mutation Breeding Newsl.* no. 34, issue 34) (Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, 1989).
3. Oladosu, Y. *et al. Biotechnol. Biotechnol. Equip.* **30**, 1–16 (2016).
4. Xu, K. *et al. Nature* **442**, 705–708 (2006).
5. Stein, J.C. *et al. Nat. Genet.* **50**, 285–296 (2018).
6. Perry, E.D., Ciliberto, F., Hennessy, D.A. & Moschini, G. *Sci. Adv.* **2**, e1600850 (2016).
7. Klümper, W. & Qaim, M. *PLoS One* **9**, e111629 (2014).
8. Carroll, D. *Annu. Rev. Biochem.* **83**, 409–439 (2014).
9. Shukla, V.K. *et al. Nature* **459**, 437–441 (2009).
10. Townsend, J.A. *et al. Nature* **459**, 442--445 (2009).
11. Chilcoat, D., Liu, Z.-B. & Sander, J. *Prog. Mol. Biol. Transl. Sci.* **149**, 27–46 (2017).
12. Clasen, B.M. *et al. Plant Biotechnol. J.* **14**, 169–176 (2016).
13. Zhang, Y. *et al. Plant J.* **91**, 714–724 (2017).

A case of mistaken identity

To the Editor: Pooled screening assays have enabled the rapid, inexpensive and quantitative assessment of diverse cellular phenotypes. In many implementations, the sequences comprising a library are not directly detected; rather, a short barcode sequence uniquely coupled to each element is sequenced instead, allowing a shorter sequencing read. Likewise, during the sequencing step, different samples are assigned an index, allowing many samples to be multiplexed into a single sequencing run. Several reports, both old and new, have described a potential flaw in these strategies. The phenomenon goes by many names—switching, swapping, shuffling or uncoupling—but the underlying problem is the same: sequence elements are no longer associated with their identifying barcode. Irrespective of the specific content of the library—single guide RNAs (sgRNAs), open reading frames (ORFs), promoters, or other DNA elements—diligence is thus required during the processes of lentiviral production, PCR retrieval and next-generation sequencing to avoid cases of mistaken identity.

In most pooled screening assays, the first potential source of uncoupling is lentivirus production: each lentiviral particle is pseudodiploid, and reverse transcriptase is known to switch between the two co-packaged templates in a distance- and homology-dependent manner, at a rate of approximately once per kilobase[1]. A chimeric product can form during pooled library production when template switching occurs in the region separating two linked sequences (**Fig. 1a**)[2,3]. This phenomenon has the potential to affect a number of experimental techniques that use lentivirus to deliver a vector with two linked elements, including massively parallel reporter assays (MPRA)[4], barcoded ORF screens[3], pooled CRISPR screens assayed by single-cell RNA sequencing (scRNA-seq)[5–8], combinatorial CRISPR screens[9–14], and screens that incorporate unique molecular identifiers (UMIs) to improve quantification[15].

Several strategies to mitigate lentivirus-based shuffling have been described (**Table 1**). First, vector designs should, whenever possible, minimize both the length and homology of intervening sequence between paired sequence elements. Alternatively, vectors may be redesigned to allow the direct detection of the sequence of interest; in the case of pooled CRISPR screens read out by scRNA-seq, for example, one implementation avoids barcodes by placing the sgRNA in the 3′ LTR (long terminal repeat), allowing it to be directly detected during RNA sequencing[2,6]. The addition of nonhomologous carrier DNA during lentiviral production has also been proposed as a potential means of reducing shuffling during this step; however, this method also reduces viral titer by 100-fold, a substantial downside for large-scale pooled screening applications[16]. Finally, although limited in scale, arrayed lentiviral production can be used to avoid the issue of template switching[5,8].

Shuffling can also occur when PCR is used to retrieve two paired sequence elements from genomic DNA before sequencing, such as in combinatorial[9–14] or UMI-based[15] CRISPR screens (**Fig. 1b**). This phenomenon has also been observed in efforts to amplify sequences with substantial homology, such as the