

Analytical methods for the detection of NGT products

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- Served on CLI Detection Methods Expert Committee
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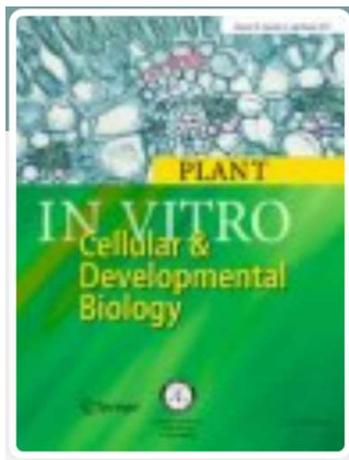
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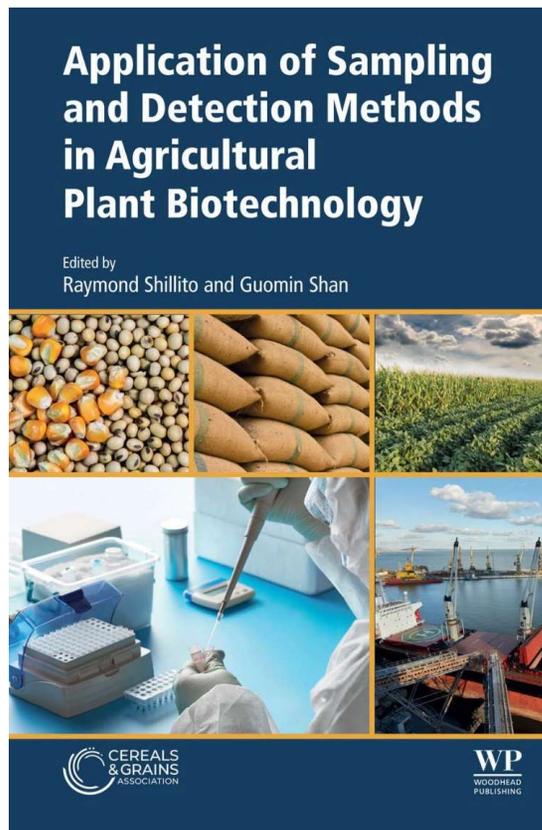
Analytical methods for the detection of NGT products

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Detection of genome edits in plants—from editing to seed



<https://www.elsevier.com/books/application-of-sampling-and-detection-methods-in-agricultural-plant-biotechnology/shillito/978-0-323-99293-0>



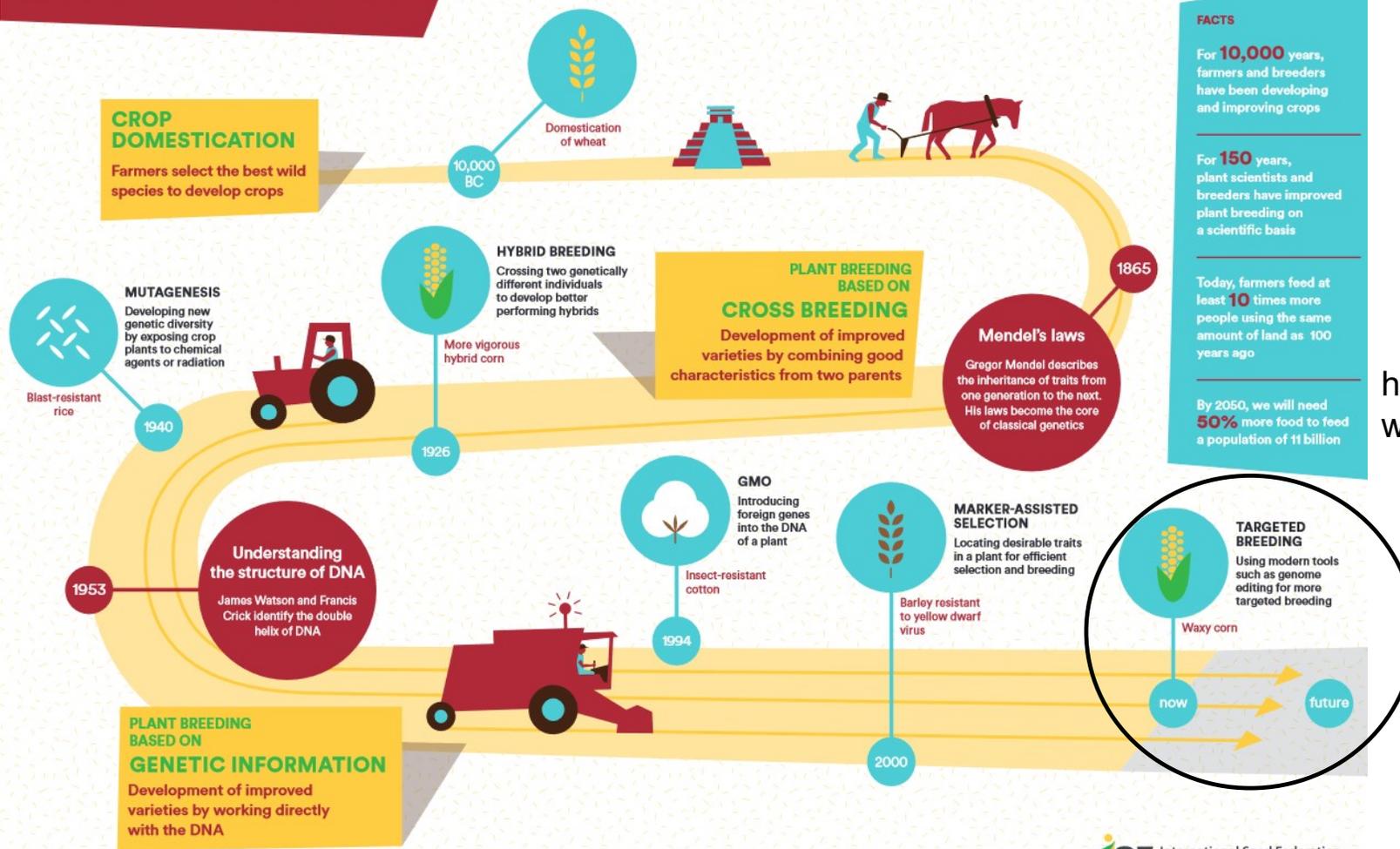
QR code for book

Published July 29, 2022

Includes a chapter on detection of genome edits.

- Impact of genome editing
- Proposed detection methods
- Common questions

MILESTONES IN PLANT BREEDING



FACTS

For **10,000** years, farmers and breeders have been developing and improving crops

For **150** years, plant scientists and breeders have improved plant breeding on a scientific basis

Today, farmers feed at least **10** times more people using the same amount of land as 100 years ago

By 2050, we will need **50%** more food to feed a population of 11 billion

<https://worldseed.org/our-work/plant-breeding/>

I asked ChatGPT: Can you provide me with a poem on New genomic techniques in crops?

In fields of green, a quiet revolution grows
New genomic techniques in crops, nature's foes
Fighting pests and drought, they strive
To feed a world where hunger's alive

CRISPR snips and edits with ease
Traits selected with genetic expertise
Crops engineered to yield more
And thrive where they hadn't before

Disease-resistant, climate-smart
Genomic techniques do their part
To help farmers feed the world with pride
And put food on tables worldwide

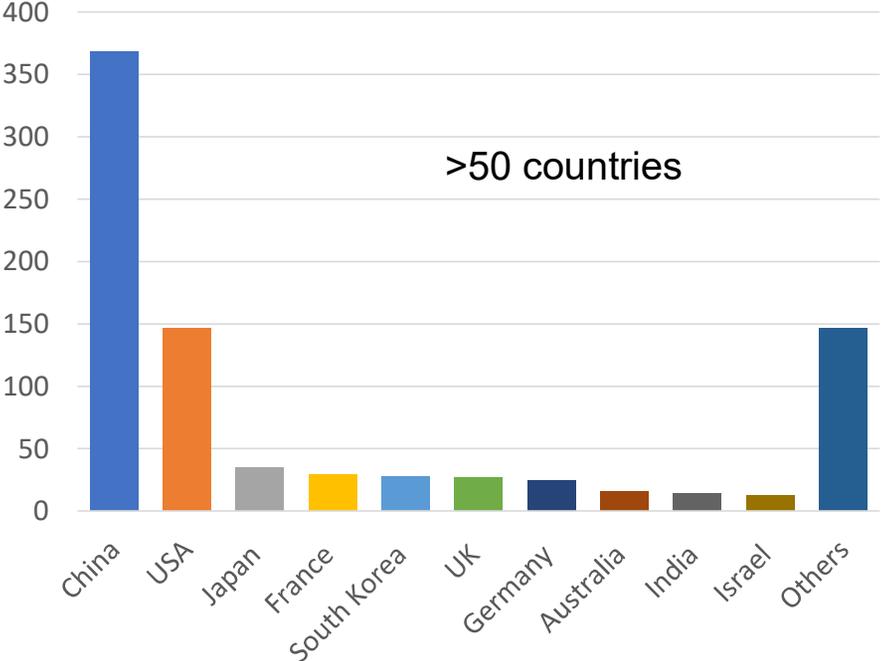
A new era of farming has begun
Thanks to genomics, we're getting things done
In fields of green, a brighter future lies
With new genomic techniques, the world thrives.

CHAT-GPT

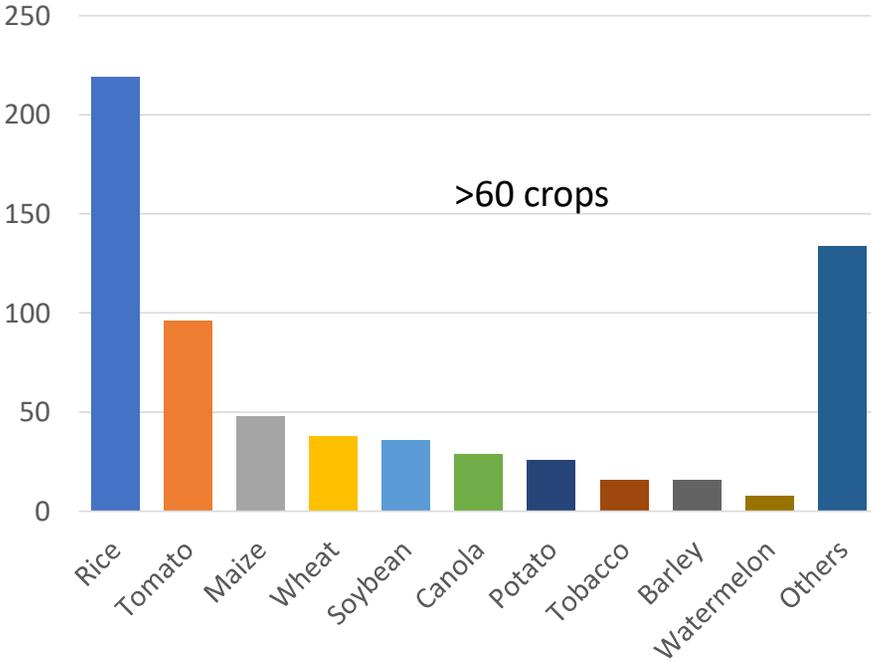
https://www.linkedin.com/posts/petra-jorasch-57120a56_i-asked-chatgpt-can-you-provide-me-with-activity-7031945507958595584-Vl0k

There are hundreds of applications of genome editing to plants

Applications by Country (top 10)



Applications by Crop (top 10)



Data from www.eu-sage.eu/genome-search

Table 1. Summary of potential genome-edited crop products. An “x” indicates relevance to a United Nations Sustainable Development Goal (2 Zero Hunger, 3 Good Health and Well-Being, 13 Climate Action, 15 Life on Land) for those products

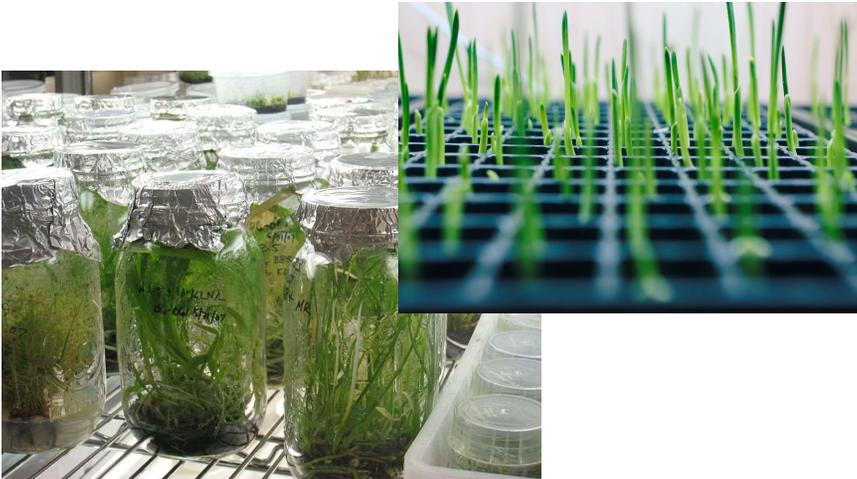
Species	 2 ZERO HUNGER	 3 GOOD HEALTH AND WELL-BEING	 13 CLIMATE ACTION	 15 LIFE ON LAND
Alfalfa				X
Apples	X	X	X	X
Avocado	X	X		
Bananas	X	X		
Berries	X	X		
Canola	X		X	X
Cassava	X	X		
Citrus	X	X		
Cotton	X		X	X
Dandelion			X	
Grapes	X	X		
Hemp		X	X	X
Leafy Greens	X	X		
Maize	X		X	X
Peanuts	X	X		
Peas	X	X	X	
Pennycress			X	X
Poplar			X	X
Potato	X	X	X	X
Rice	X	X	X	X
Soybean	X	X	X	X
Tomato	X	X	X	X
Wheat	X	X		

Genome editing has the potential to contribute to several UN Sustainable Development Goals

Jenkins et al., *In Vitro Cellular & Developmental Biology - Plant* (2021) 57:609–626
<https://doi.org/10.1007/s11627-021-10201-4>
 (with permission)

Detection methods are needed at many stages

Research & Development



Daderot, CC BY-SA 4.0 via Wikimedia Commons



International Trade



The approach to detection depends on the edit

“Larger” insertions (eg. Whole genes)
(SDN3)



Same methods as “classical GMOs”

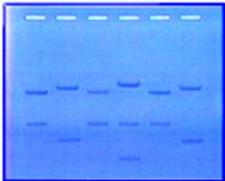
Single nucleotide variants/
polymorphisms, insertions, deletions, or
change of one bp (SDN1)



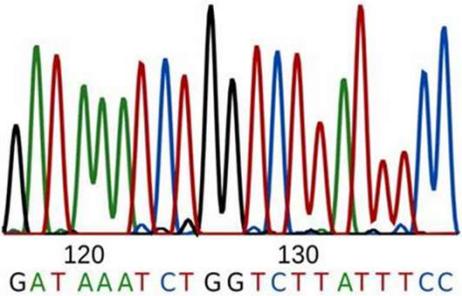
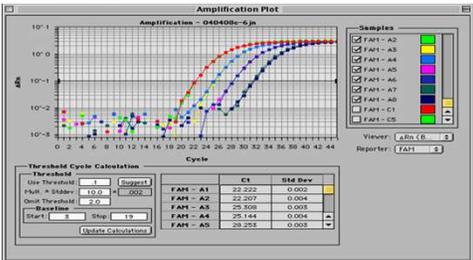
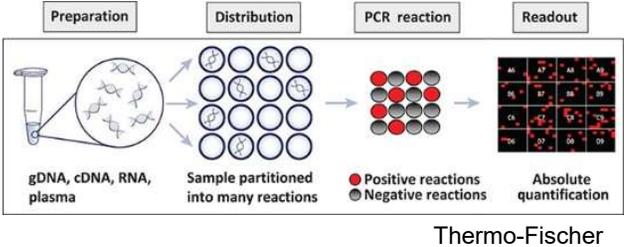
Not as technically straightforward

Insertions or deletions (indels) of a few
bp (SDN2)

Multiple methods are available to detect DNA sequence changes

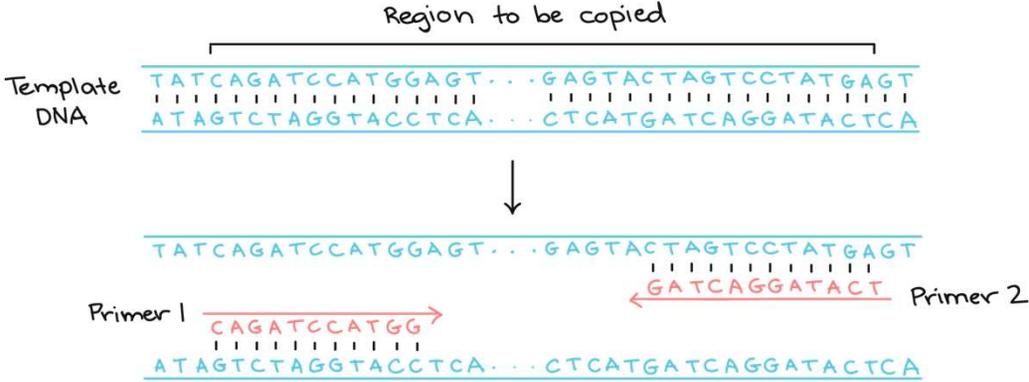
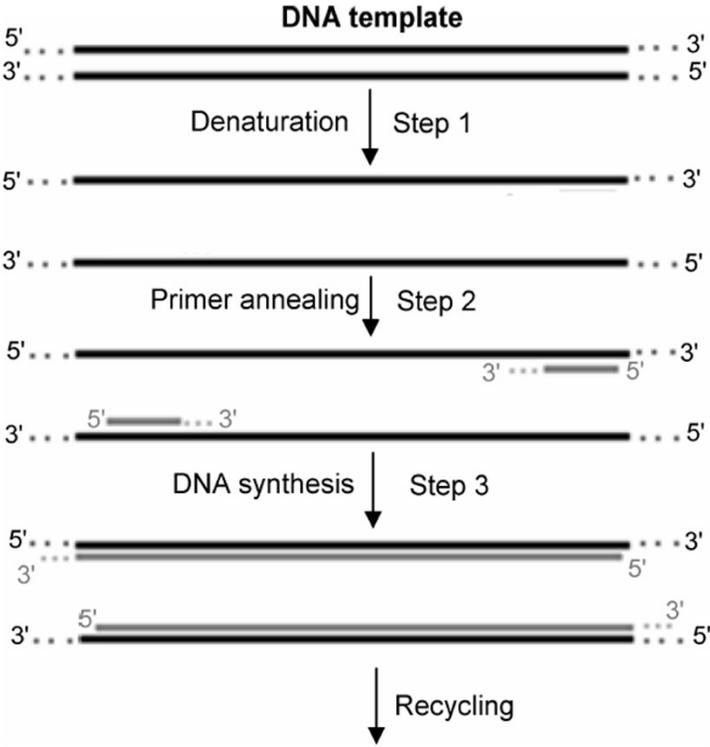


- Selectable marker
- Detection of the Protein (Immunoassay)
- PCR Detection
- Sequencing



- For bulk samples and food, methods will be pushed to the limits, which can lead to ambiguous results.

PCR depends on DNA recognition

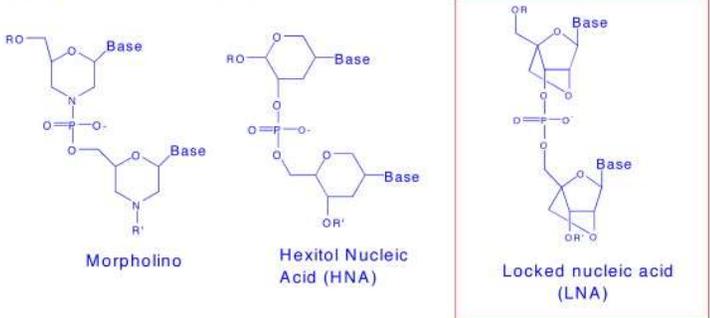
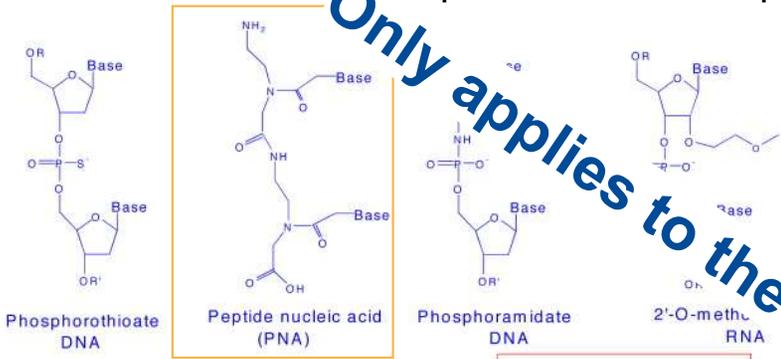


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PCR-based DNA detection and quantification methods rely on primers and probes to recognize the sequence.

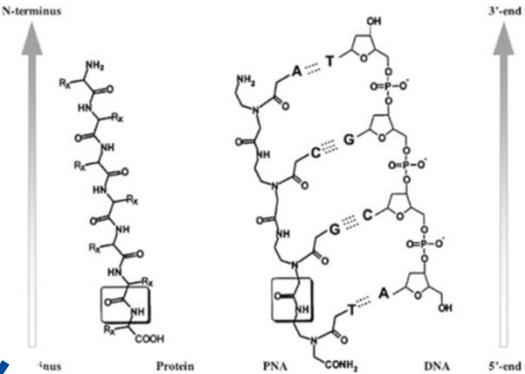
Modified nucleic acids can be used to increase base differentiation in PCR assays.

PNA's bind specific sequences and prevent access to polymerase.



Braasch and Corey (2001)

Only applies to the initial amplification cycle



Pellestor and Paulasova 2004

LNA[®] bases integrated into probes change the conformation of the helix and increase the stability of the duplex.

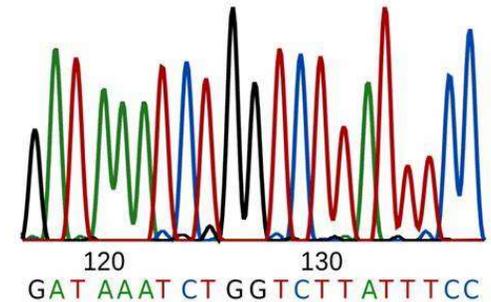
LNA is a registered trademark of Qiagen, Inc.

PCR summary

- PCR is low cost and easy to use
 - ▶ End point or real-Time (qPCR) is widely available
 - ▶ Digital PCR reduces signal to noise ratio and its use is increasing
- Detecting large edits/inserts is similar to GM inserts
- PCR methods might be used to detect small edits
 - ▶ Routinely used in analysis of SNPs in single plants
 - ▶ Small/single base changes can be difficult to detect in bulk samples.
 - ▶ Modified probes like PNA and LNA can increase differentiation
 - ▶ Each case is different due to local sequence

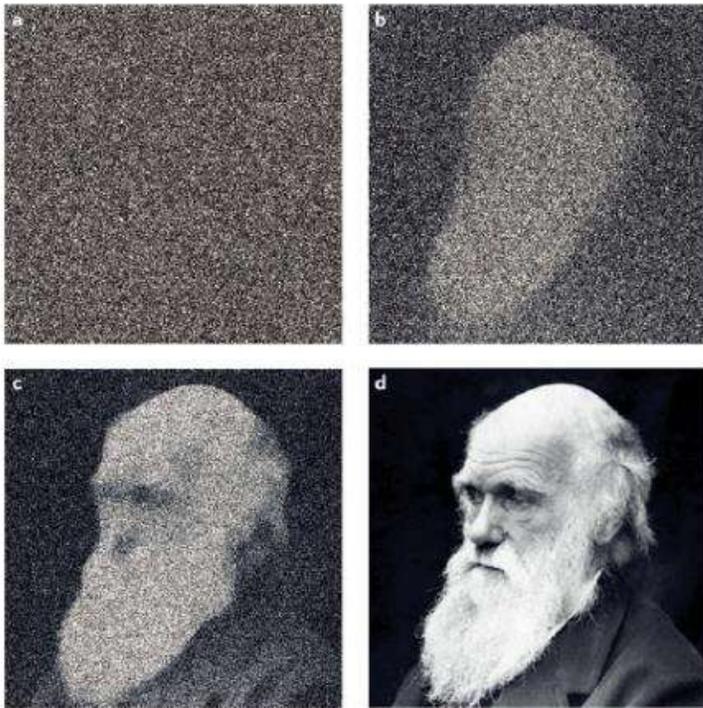
Sequencing for genome edit detection

- Targeted sequencing is useful to characterize tissue cultures or single plants
- Targeted sequencing - amplicon-based can be used when sequence is known
- Targeted deep sequencing may detect low frequency variants at high cost
- Whole genome sequencing is not useful for searching for 'unknown' edits due to plant genome variability
- Sequencing alone is not useful for testing bulk samples



Thermo-Fischer

Signal to noise ratio is a critical issue in sequencing



Salk et al, 2018 (Nature Reviews Genetics)

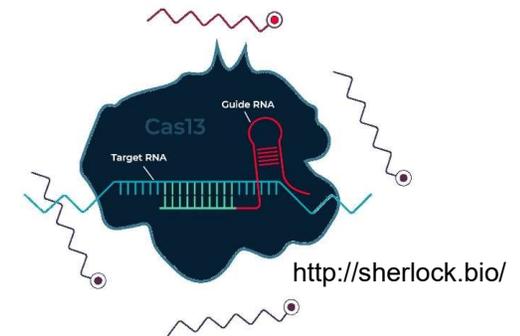
- Technical artifacts can be an issue for sequencing when close to the Limit Of Detection (LOD<1%)
- Recent developments are improving the LOD, but limited by errors inherent in the process (including the PCR amplification of the targeted area)
- Although Nanopore sequencing is increasing in accuracy, it requires very high purity DNA.

Developing technologies

- Isothermal amplification and nanopore methods will compete with PCR
- CRISPR to detect CRISPR? – unLOCKing detection method named SHERLOCK
- Targeting by several different Cas enzymes can be used to drive different isothermal systems
- May have low sensitivity (target concentration dependent) so may need pre-amplification of the target sequences



Oxford Nanopore



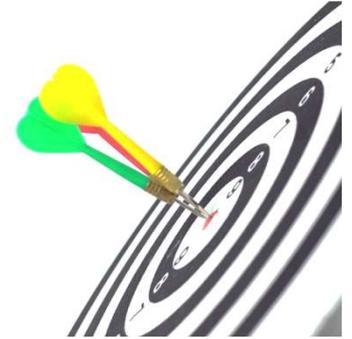
Common Questions



- What about off-target mutations?
- Can I distinguish an edit from 'natural' mutations?
- Can I detect a gene edited grain in a shipment, or food?
- How feasible is it to deploy detection methods?
- Are screening approaches feasible

Off-target editing is of low concern in plants

- Plant reference genomes allow precise design
- Few off-target mutations are observed in plants
- Plants are very resilient to somatic cell mutations
- Plant genomes are very plastic
- Potential off-target mutations are removed through backcrossing
- Commercial varieties are backcrossed, and off-types removed



DNA sequence changes occur often in nature

- Single base and other small (and large) deletions/rearrangements and additions are constantly occurring in plant populations
- The rate of natural mutation from one generation of a plant to the next, ranges from 10^{-5} – 10^{-6} in corn to 10^{-9} in Arabidopsis.
- Transposons (DNA segments that jump around) are extremely active in some species like maize
- DNA sequence will be the same whether the changes are directed or undirected, so the safety profile of such products will be the same.



Detection methods cannot discriminate intentional from background mutations

- A DNA sequence may not uniquely identify a specific technology, product, or developer.
- Differentiation of spontaneous and directed mutations is not possible, and thus may cause regulatory / legal challenges
- “Although it is technically possible to detect specific DNA alterations, without prior knowledge, none of the techniques described are able to distinguish whether the Single Nucleotide Variation or Insertion/Deletion is caused by genome editing, by classical breeding technologies or by natural mutation” *

*Detection of food and feed plant products obtained by new mutagenesis techniques European Network of GMO Laboratories (ENGL) Report endorsed by the ENGL Steering Committee, 26 March 2019

Detection in grain in a shipment is a challenge, and in food even more so.

■ Technical limitations

- ▶ Limited Sensitivity (LOD), specificity and accuracy
- ▶ Methods may not meet the performance criteria of current GMO methods (Wiedner et al: 2022)
- ▶ No common DNA elements like 35S – each edit is unique

■ Detection ≠ identification

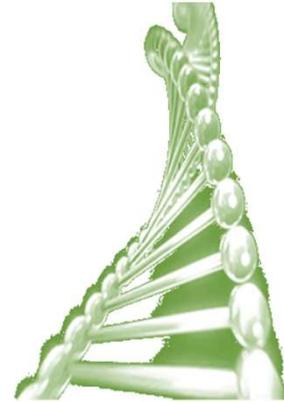
- ▶ Not possible to determine how the sequence change arose
- ▶ The same edit could be made by multiple developers, or due to ‘natural’ or induced mutations
- ▶ Enforcement issue for regulators

Challenges exist in implementation:

- Capacity and infrastructure limitations already impacting detection laboratories for “classic” GMO testing, especially where targets contain multiple events
- Costs are increasing for the laboratories due to complexity
- No Screening methods – case by case testing
- Source of information for methods?
- Expertise may be in short supply

Conclusions

- Many different types of detection tools
- Most applications employ PCR steps
- Cannot differentiate from mutations
- Unknown ability to detect in bulk seed, grain or food products
- Capacity challenges to implementation, especially in less wealthy countries
- Detection tools will continue to evolve



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