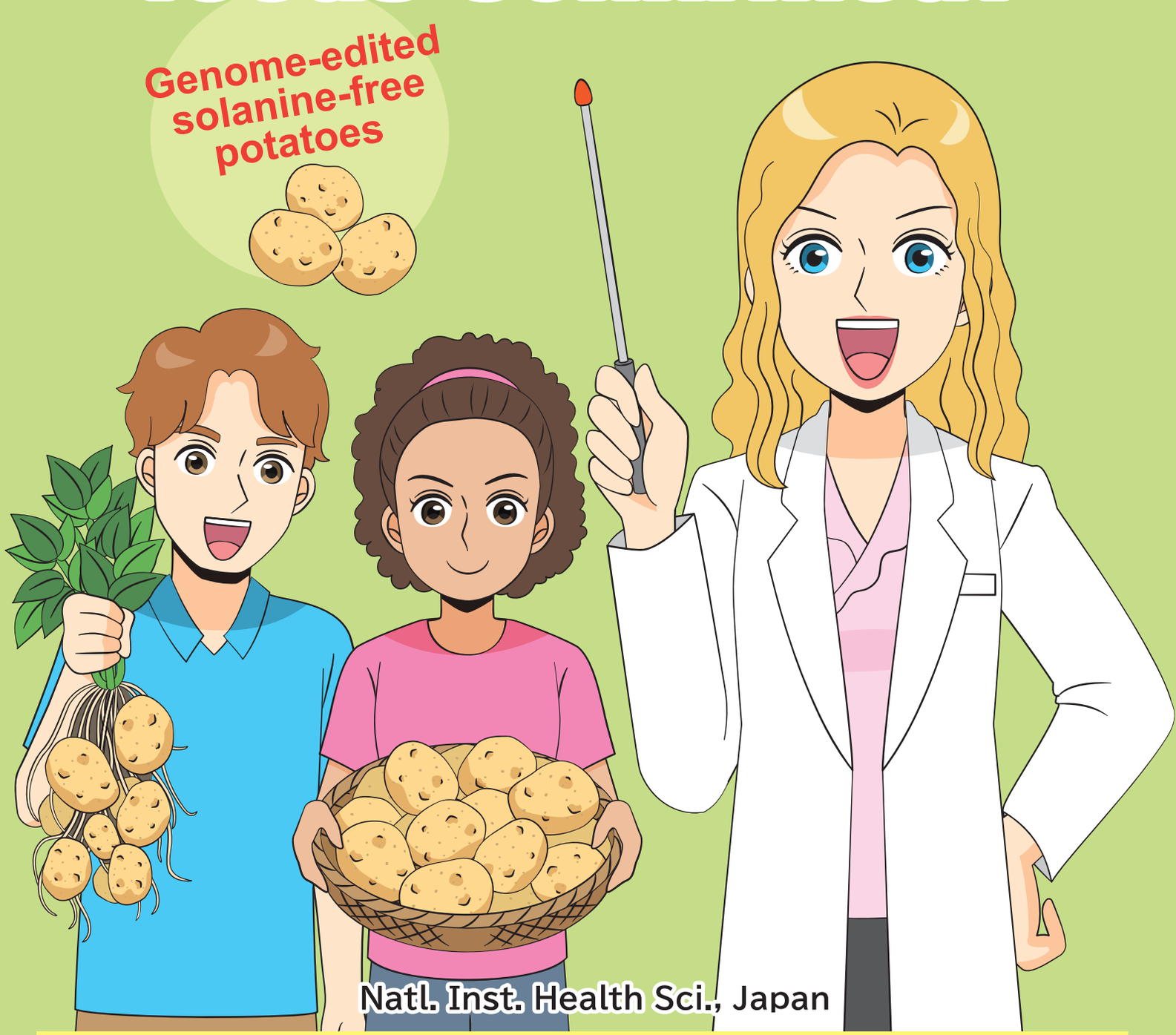
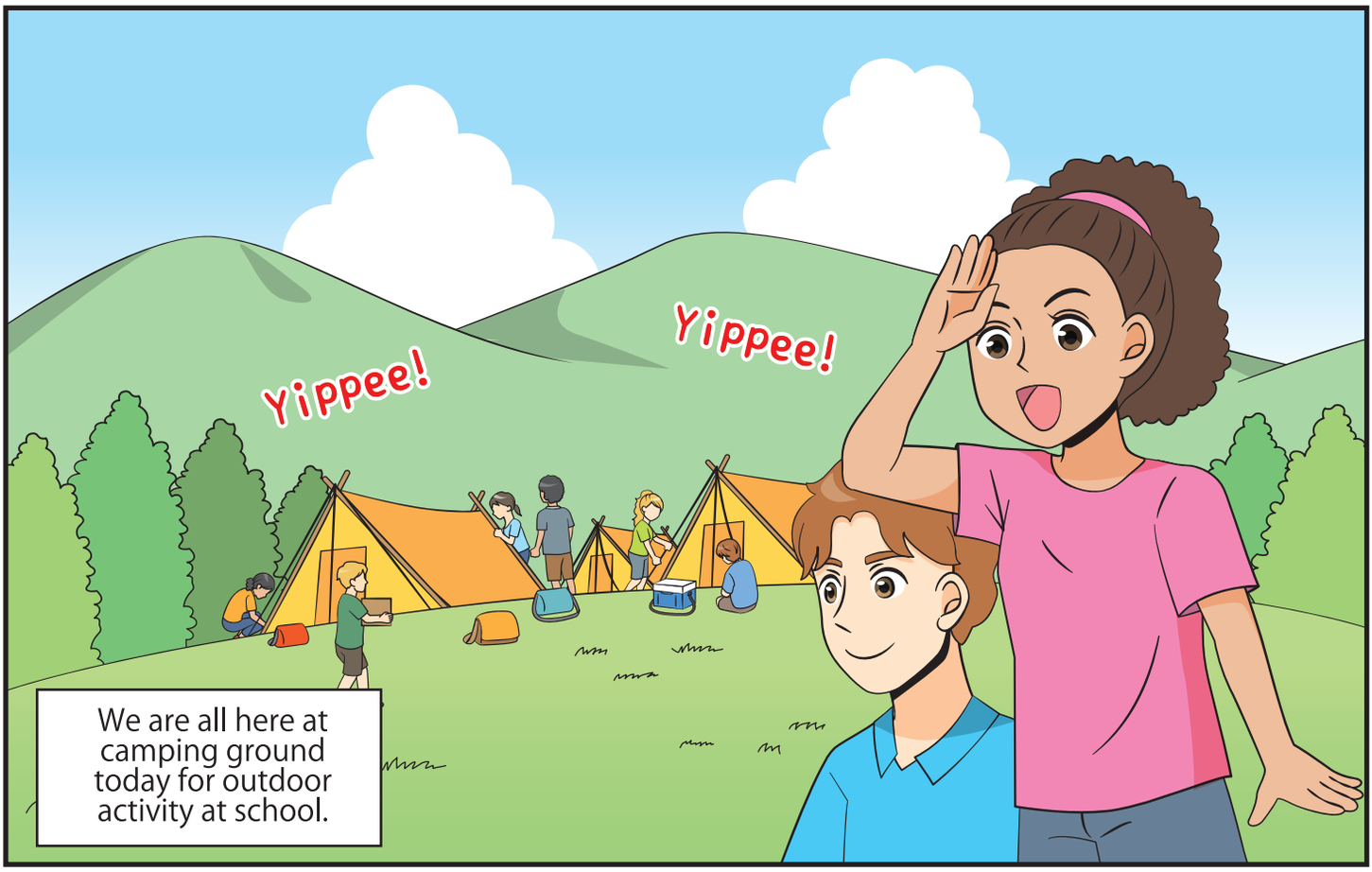
How is the safety of genome-edited foods confirmed?

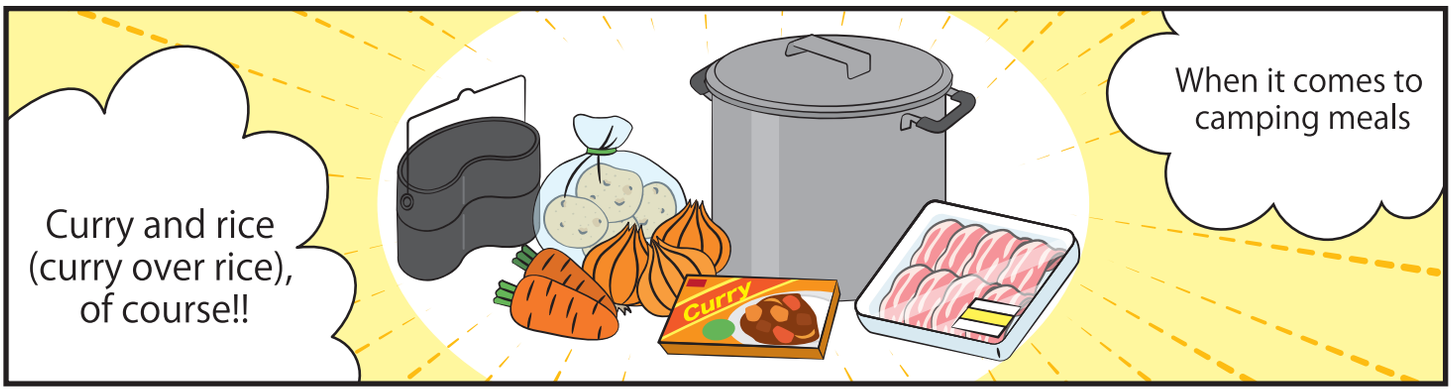


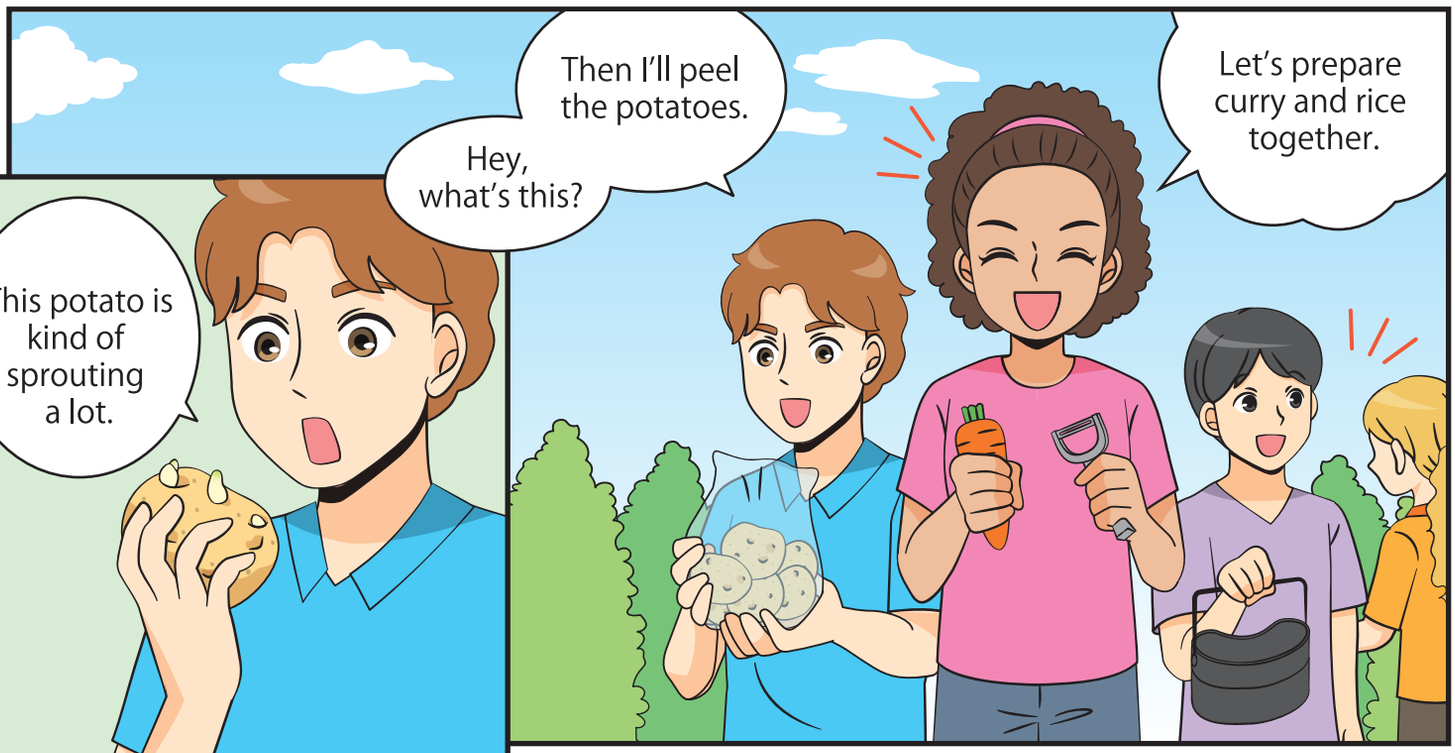
Genome-edited potatoes that do not produce solanine have not been submitted for notification yet. This cartoon was created using the example of the solanine-free potatoes, and assumes how safety is confirmed in the prior consultation phase before notification and distribution.

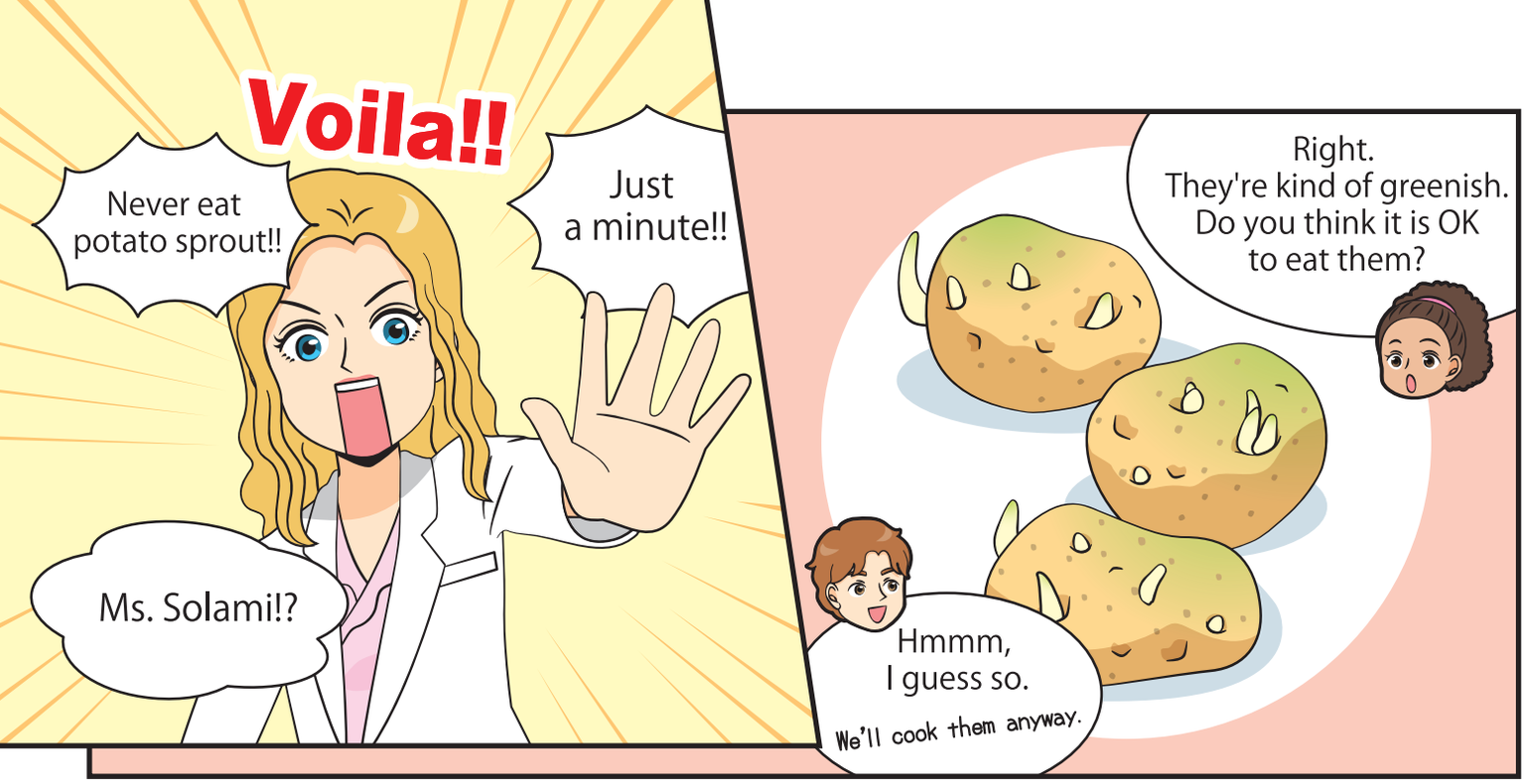
- In Japan, a notification system for genome-edited foods was launched in October 2019. The safety of genome-edited foods is confirmed during the prior consultation phase of the notification system, and a notification will be submitted to and accepted by the Ministry of Health, Labour, and Welfare (MHLW) when it is determined that there are no safety concerns and the requirements for notification are met.
- In this MANGA (cartoon), outline of the prior consultation and notification system for the genome-edited foods is introduced.

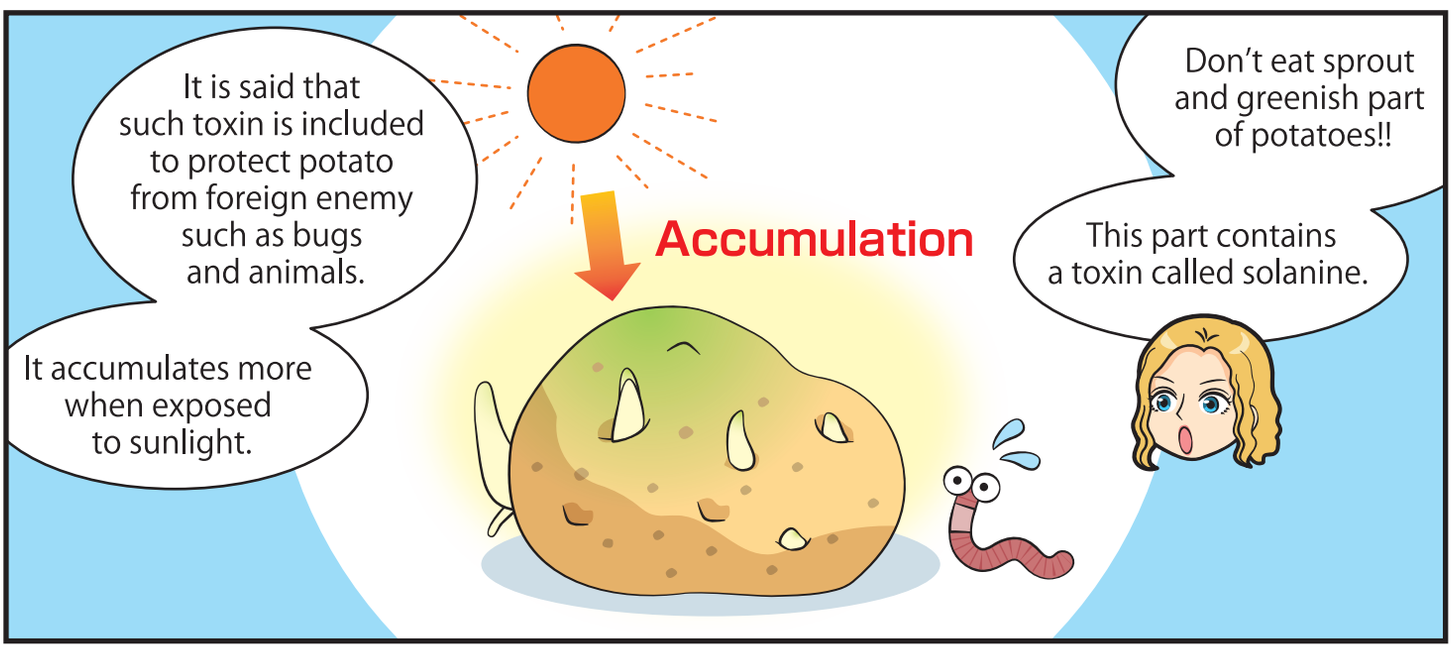
●When reading this MANGA (cartoon), start from the right top corner and move on to the left, then to the boxes below (similarly right to left).

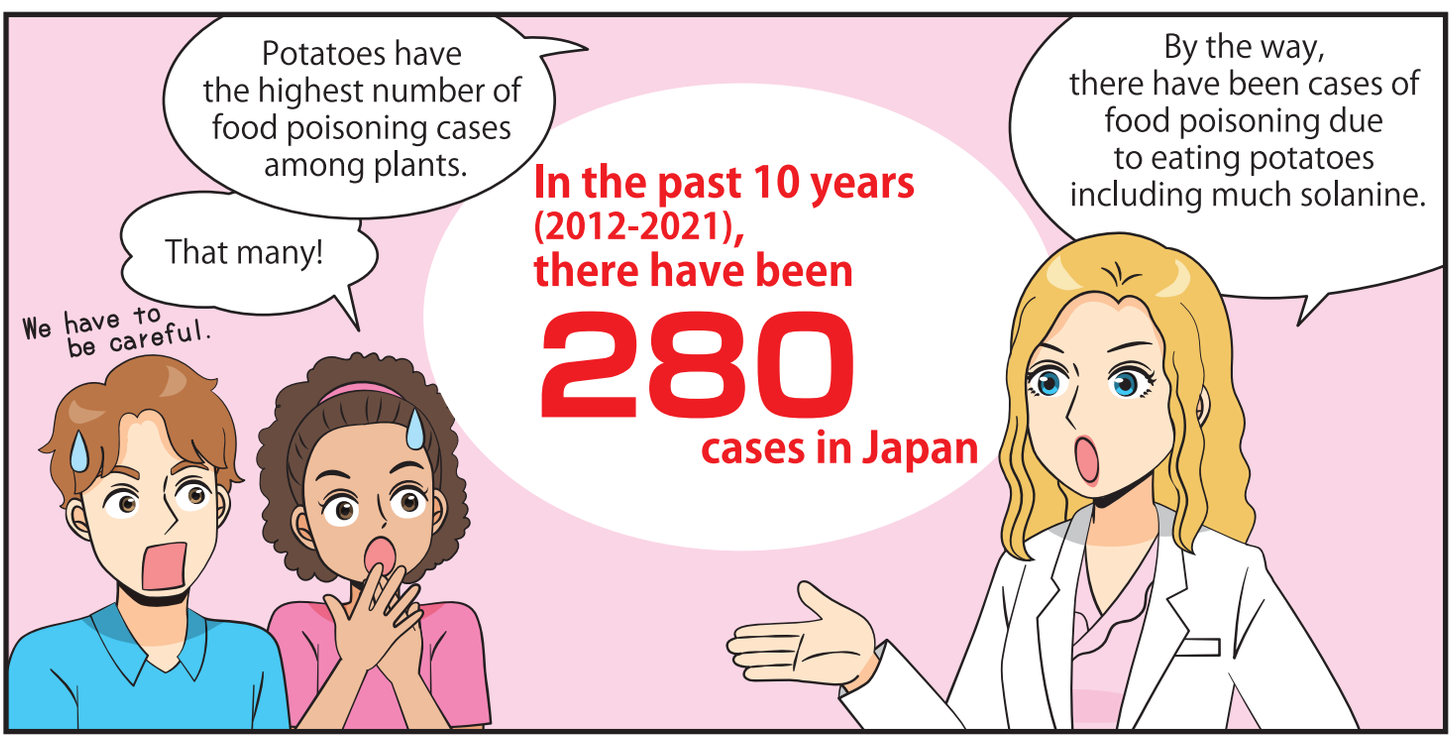







Voila!!
Never eat potato sprout!!
Just a minute!!
Ms. Solami!?
Right. They're kind of greenish. Do you think it is OK to eat them?
Hmmm, I guess so.
We'll cook them anyway.


It is said that such toxin is included to protect potato from foreign enemy such as bugs and animals.
It accumulates more when exposed to sunlight.
Accumulation
Don't eat sprout and greenish part of potatoes!!
This part contains a toxin called solanine.


Potatoes have the highest number of food poisoning cases among plants.
That many!
We have to be careful.
In the past 10 years (2012-2021), there have been
280
cases in Japan
By the way, there have been cases of food poisoning due to eating potatoes including much solanine.

I wish there were potatoes without solanine.

I know, right?

You spend a lot of money managing them, don't you?

As solanine increases by sprouting, people are careful during the distribution process.

We invest in product handling.

Potatoes

Potatoes

Potatoes

SHOP

Radiation

Prevents sprouting

But, in Japan, it is allowed to irradiate potatoes to prevent them from sprouting.

Unfortunately, there is no chance of producing solanine-free potatoes naturally.

Another way is to artificially create potatoes that don't produce solanine!

Can we make such potatoes artificially?

The idea is to induce mutations in the SSR2 gene to stop SSR2 function.

Cholesterol precursor

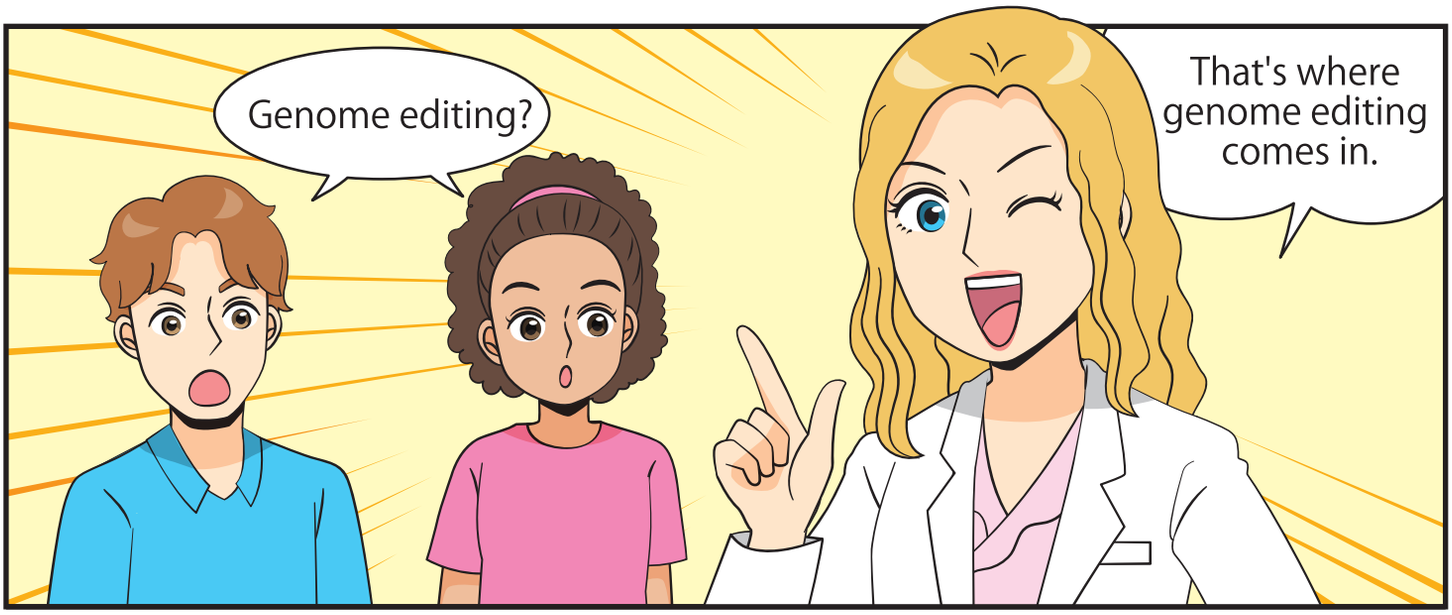
SSR2

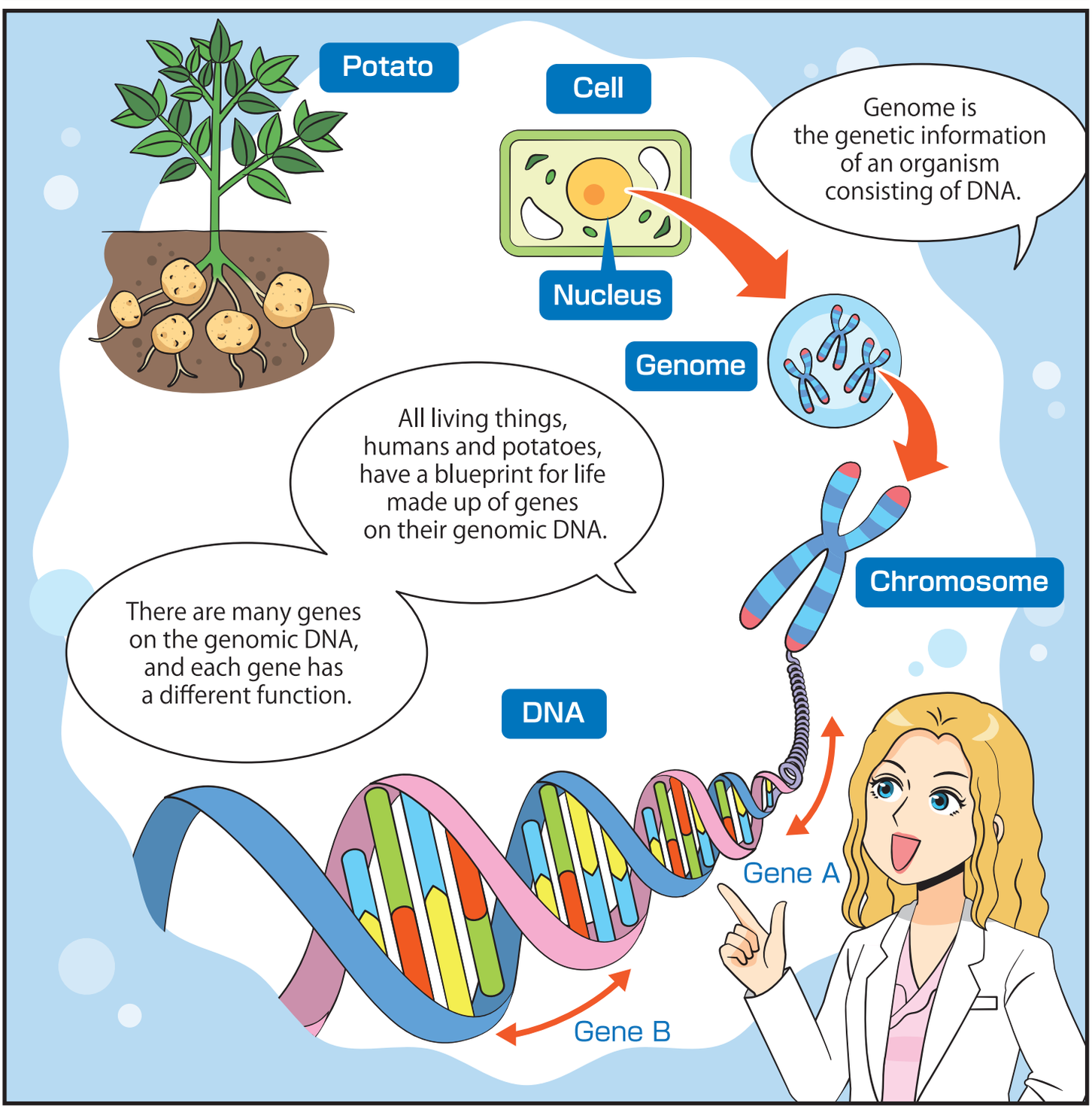
Cholesterol

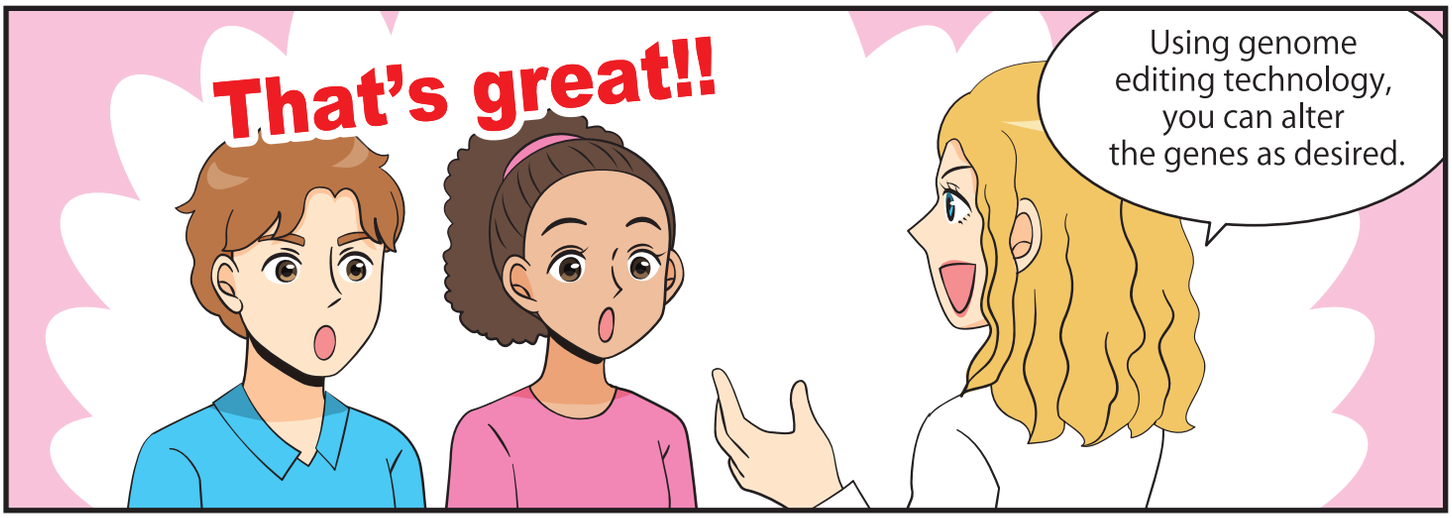
Solanine

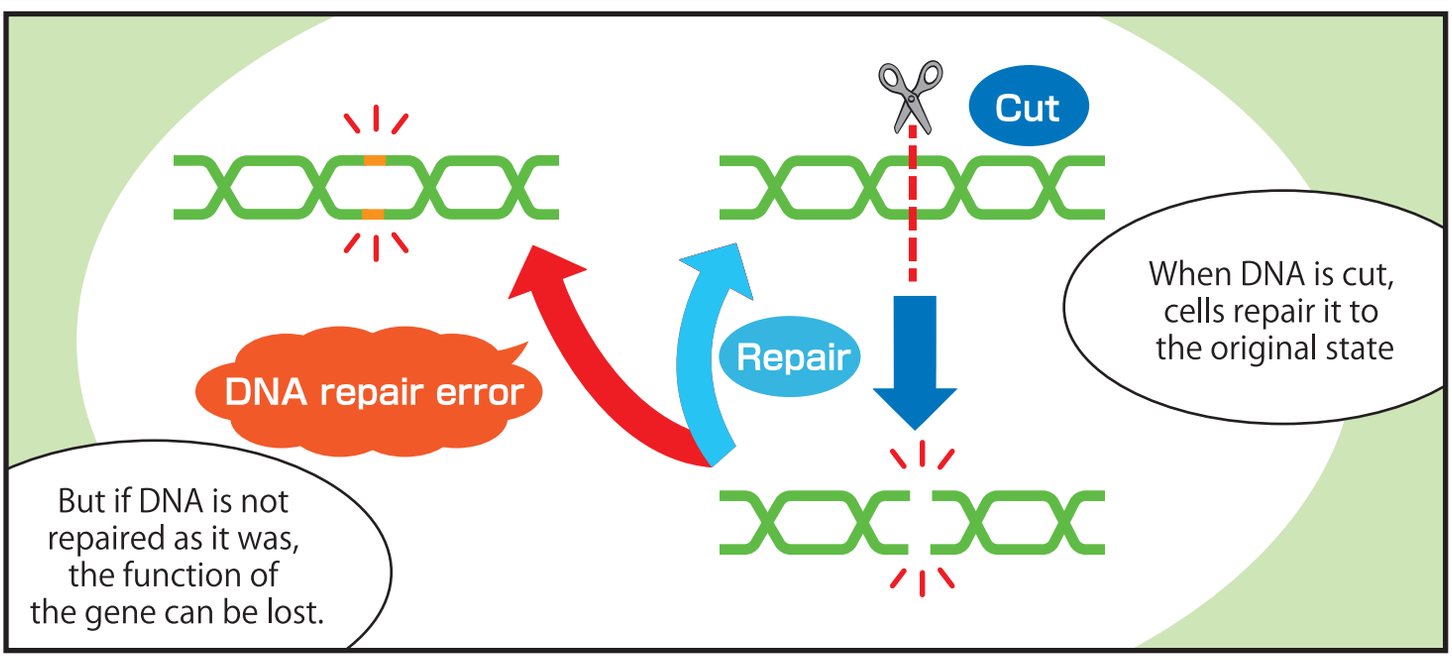
But, how do you induce mutations?

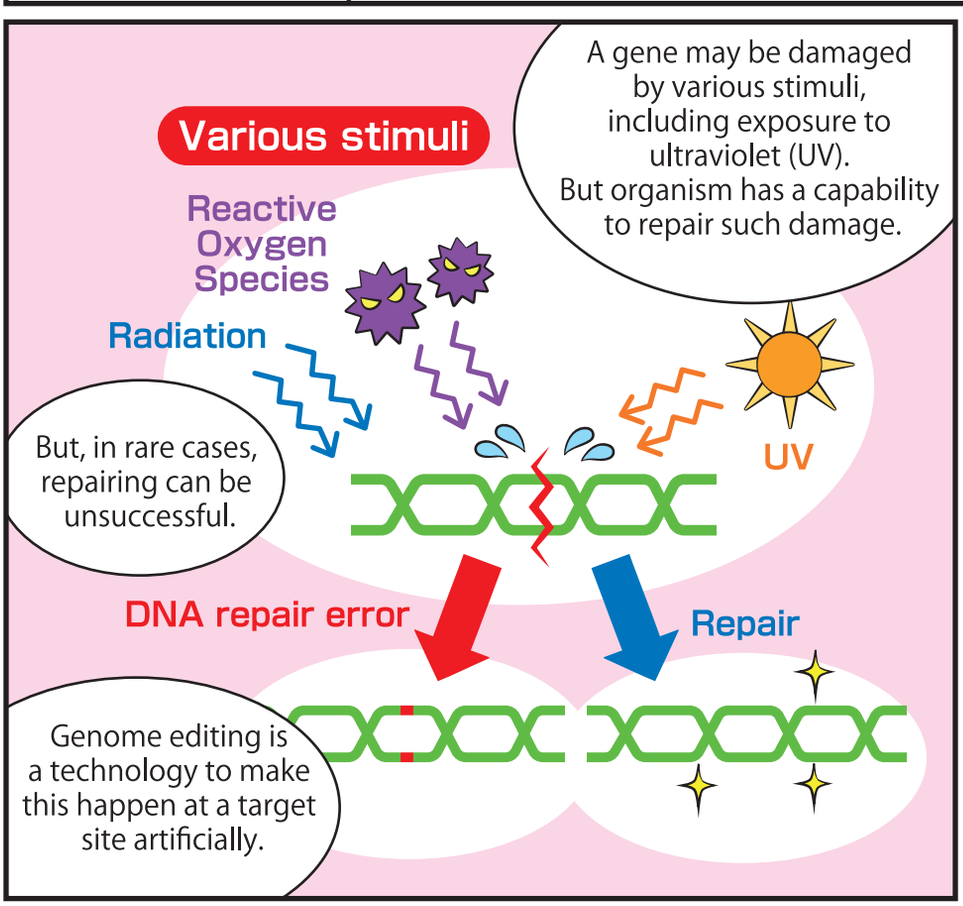
Sounds difficult.

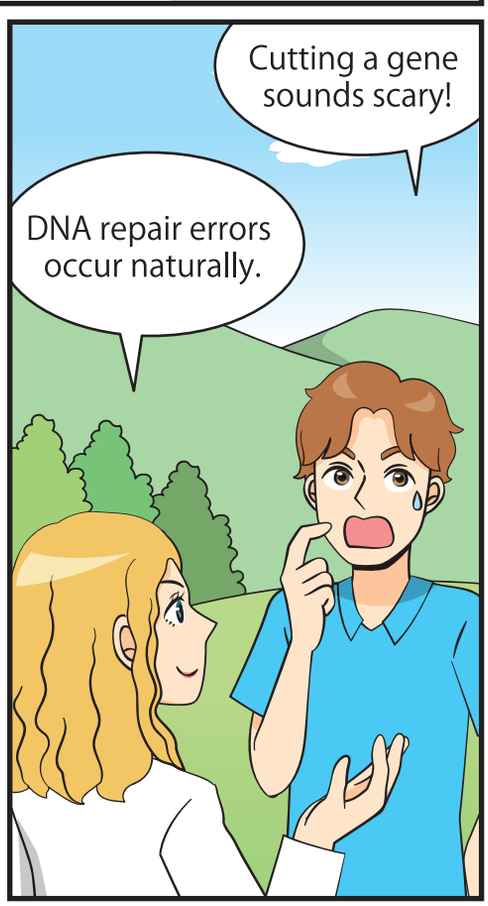

Genome editing?
That's where genome editing comes in.

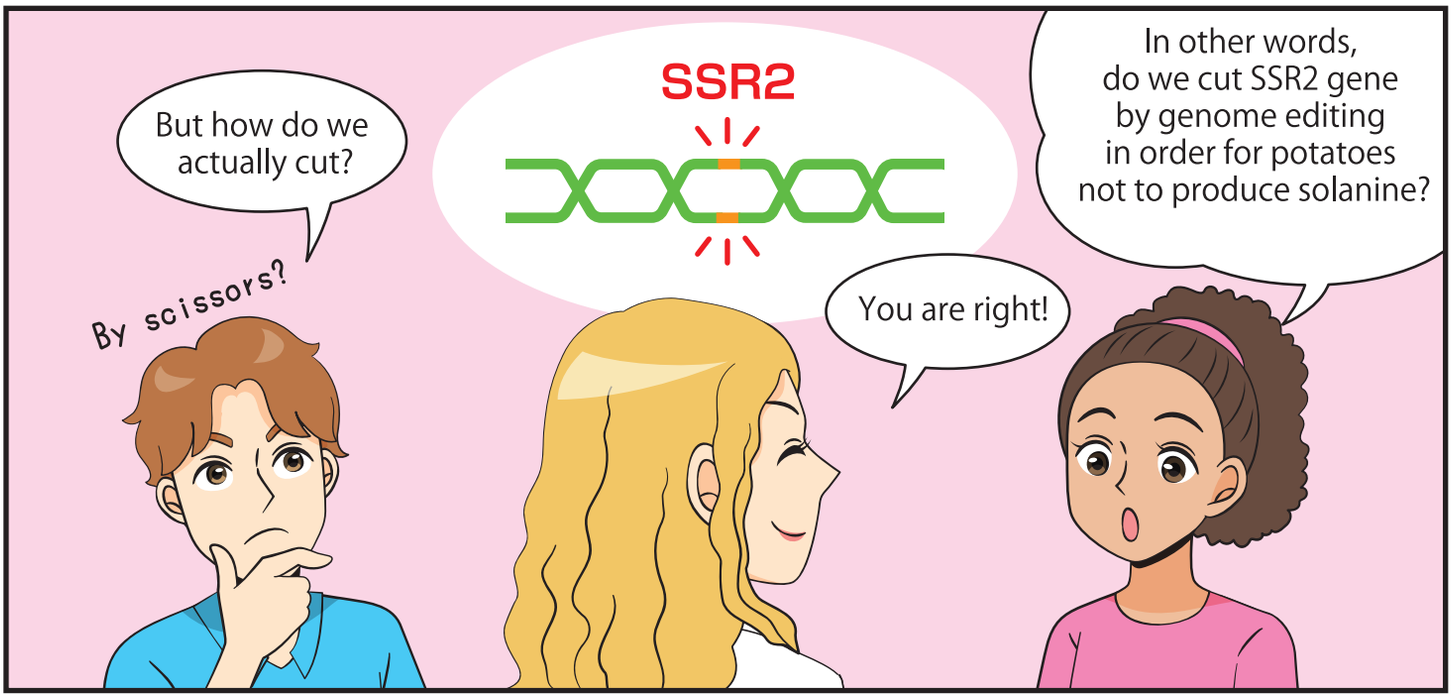

Potato
Cell
Nucleus
Genome
Genome is the genetic information of an organism consisting of DNA.
All living things, humans and potatoes, have a blueprint for life made up of genes on their genomic DNA.
There are many genes on the genomic DNA, and each gene has a different function.
Chromosome
DNA
Gene A
Gene B


That's great!!
Using genome editing technology, you can alter the genes as desired.


Cut
Repair
DNA repair error
When DNA is cut, cells repair it to the original state
But if DNA is not repaired as it was, the function of the gene can be lost.


Various stimuli
A gene may be damaged by various stimuli, including exposure to ultraviolet (UV). But organism has a capability to repair such damage.
Reactive Oxygen Species
Radiation
UV
But, in rare cases, repairing can be unsuccessful.
DNA repair error
Repair
Genome editing is a technology to make this happen at a target site artificially.


Cutting a gene sounds scary!
DNA repair errors occur naturally.

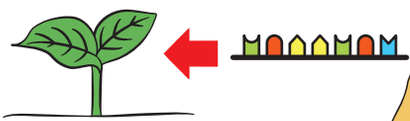


Genome editing procedure

1 Decide a location of SSR2 gene where to cut.

2 Prepare a molecule that binds to the target location.

3 Deliver the molecule to the plant to be genome-edited.



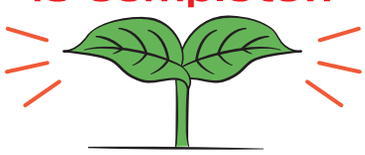
This is the outline of the process.

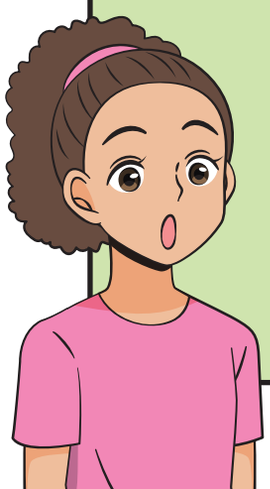
4 That molecule binds to the SSR2 gene.

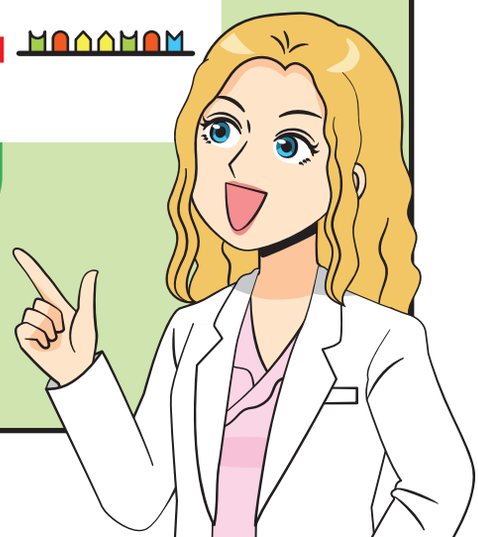
How do you deliver that molecule?

5 SSR2 gene is cut.

Genome editing is complete!!







When this Agrobacterium is delivered to potato nursery plants by infection, the molecule binds to the location to be cut.

Infection

Potato nursery plants

Molecule to bind to the site to be cut

Vector (transporter)

We use a soil bacterium, called Agrobacterium, which can deliver a part of DNA sequence to plants.

Agrobacterium

We connect the molecule, to be combined to the location desired to be cut, to a transporter called a vector, and have the Agrobacterium deliver.

Not yet! Now comes the main part.

Now we can harvest potatoes without solanine!!

Is the genome editing now completed?

SSR2

Then, SSR2 gene will be cut.

I see. It is cut like scissors.

Mutated No change Mutated Mutated

We grow the nursery plants after genome editing, check DNA, and select only the individuals to which mutation has occurred to SSR2 genes.

That means, we select the individuals whose gene was not repaired to its original state. Right?

Deletions

Substitution

Insertion

Repair errors

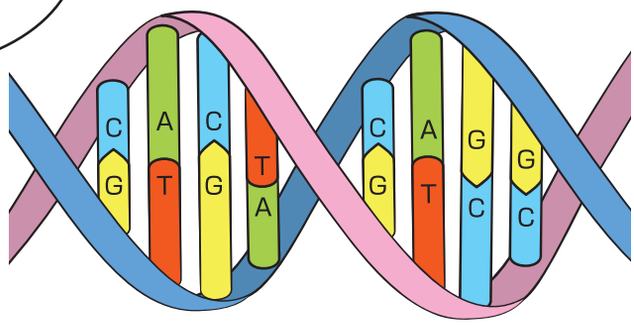
Cut

Repair

We check how the base sequence of SSR2 gene of the selected potatoes has changed.

DNA is composed of four base pairs, A, T, G, and C. The sequence is called a base sequence.

A Adenine
G Guanine
T Thymine
C Cytosine



DNA

What is "base sequence"?

Change in genes due to the change of base sequence.

Threonine	Valine	Tyrosine	Alanine	Arginine	Arginine	
ACT	GTA	TAT	GCC	CGA	CGG	A
TGA	CAT	ATA	CGG	GCT	GCC	T

Cut

Threonine	Valine	Tyrosine	Alanine	Arginine	Arginine	
ACT	GTA	TAT	GCC	CGA	CGG	A
TGA	CAT	ATA	CGG	GCT	GCC	T

The combination of the three base pairs in a sequence determines one amino acids, and the sequence of the amino acids determines the characteristics of the protein.

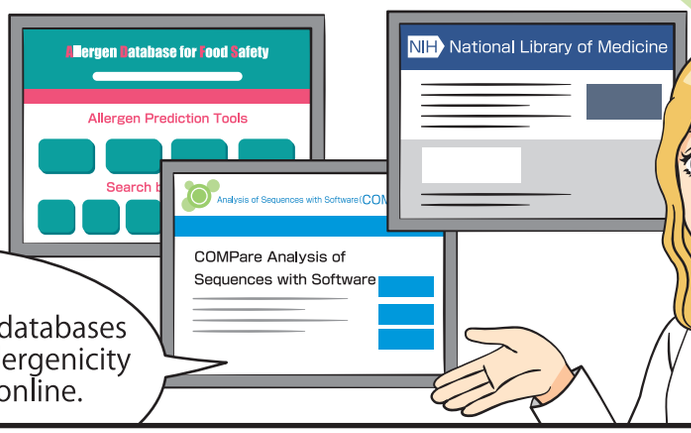
If one base is missing due to a repair error, it will be shifted one by one. As a result, the amino acids will be different from the original.

That results in a loss of function of the original gene. Right?

Unsuccessful repair

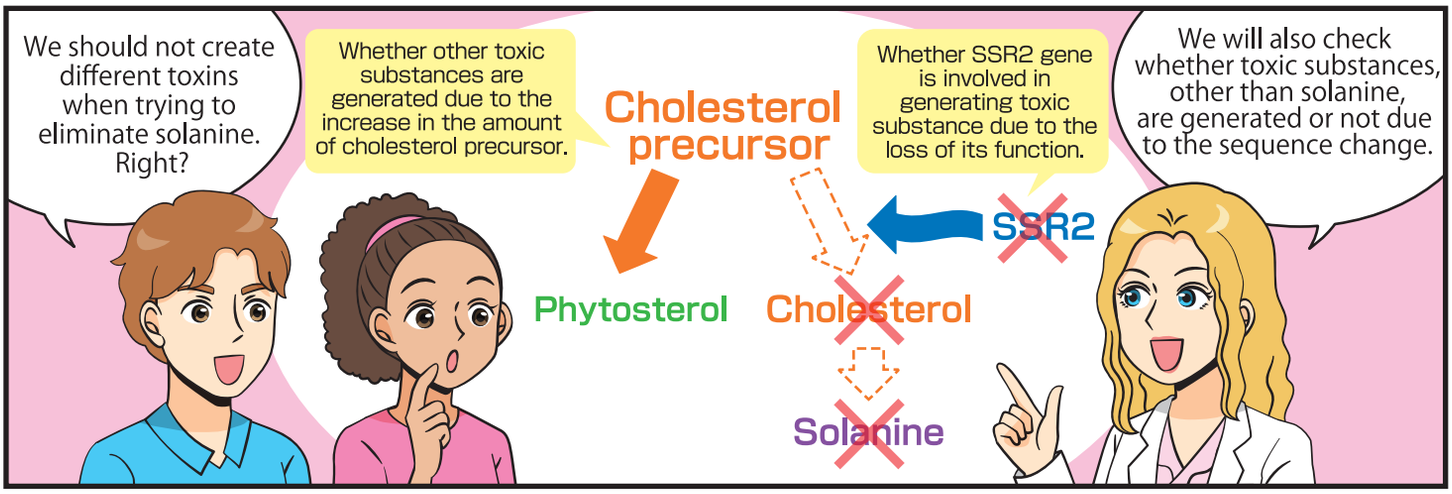
Threonine	Valine	Tyrosine	Proline	Aspartic acid	Glycine	
ACT	GTA	TAT	CCC	GAC	GGA	TAA
TGA	CAT	ATA	GGG	CTG	CCT	

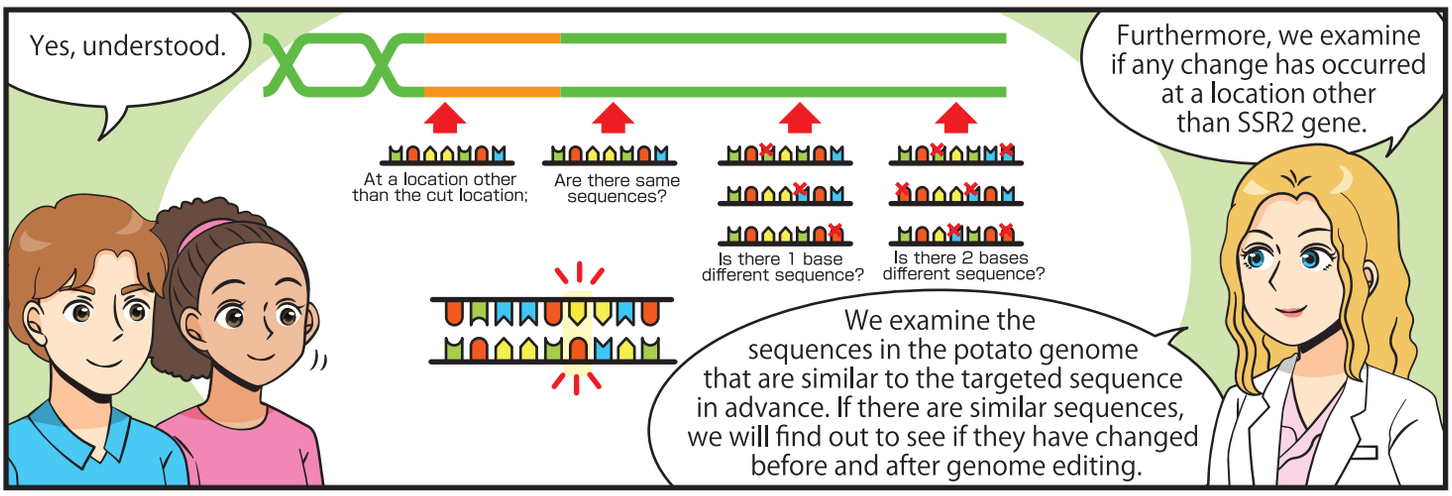
Missing base pair G C The sequence shifts forward one by one.

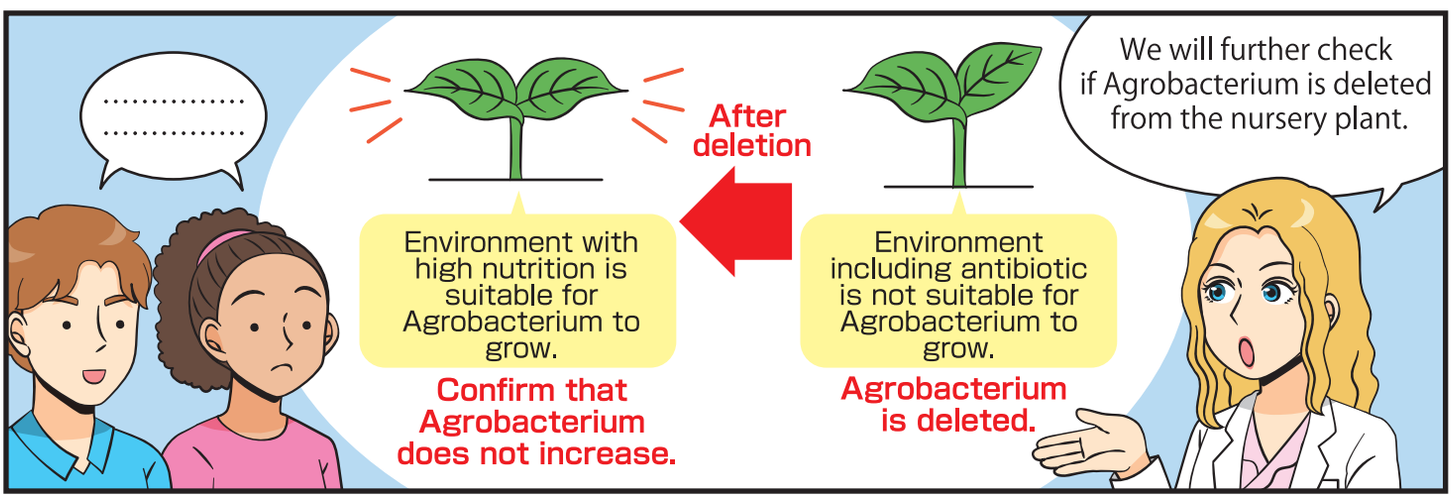


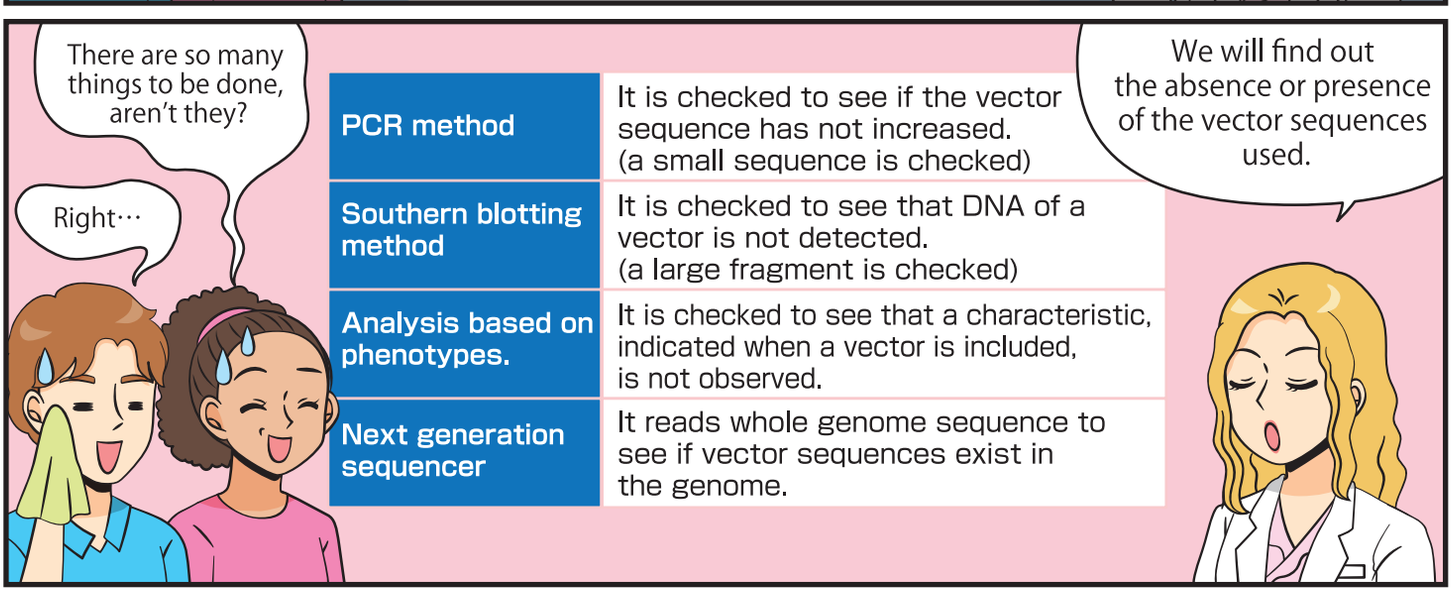
We will also find out whether a substance causing food allergy is generated or not due to the sequence change.

Search tools and databases for toxicity and allergenicity are available online.

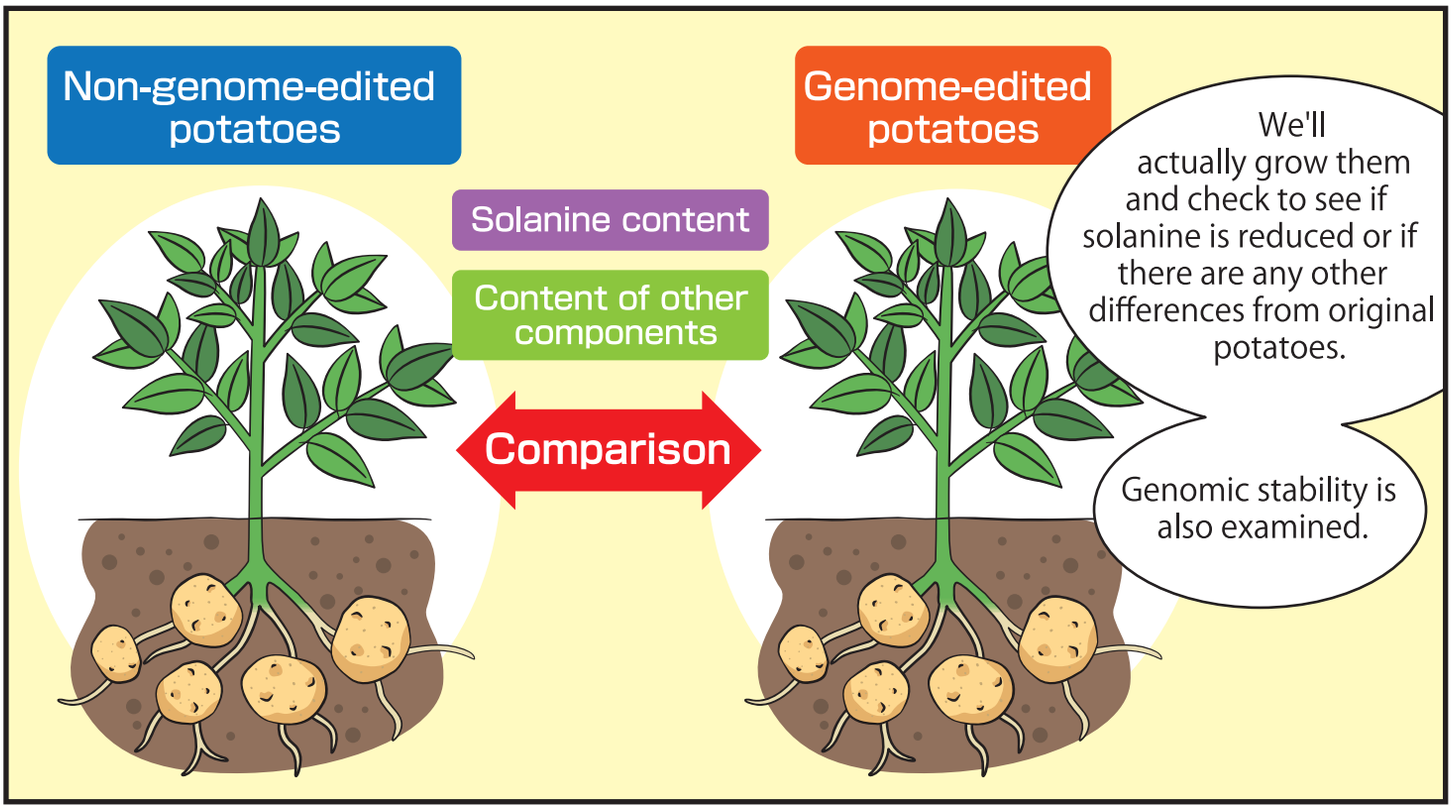


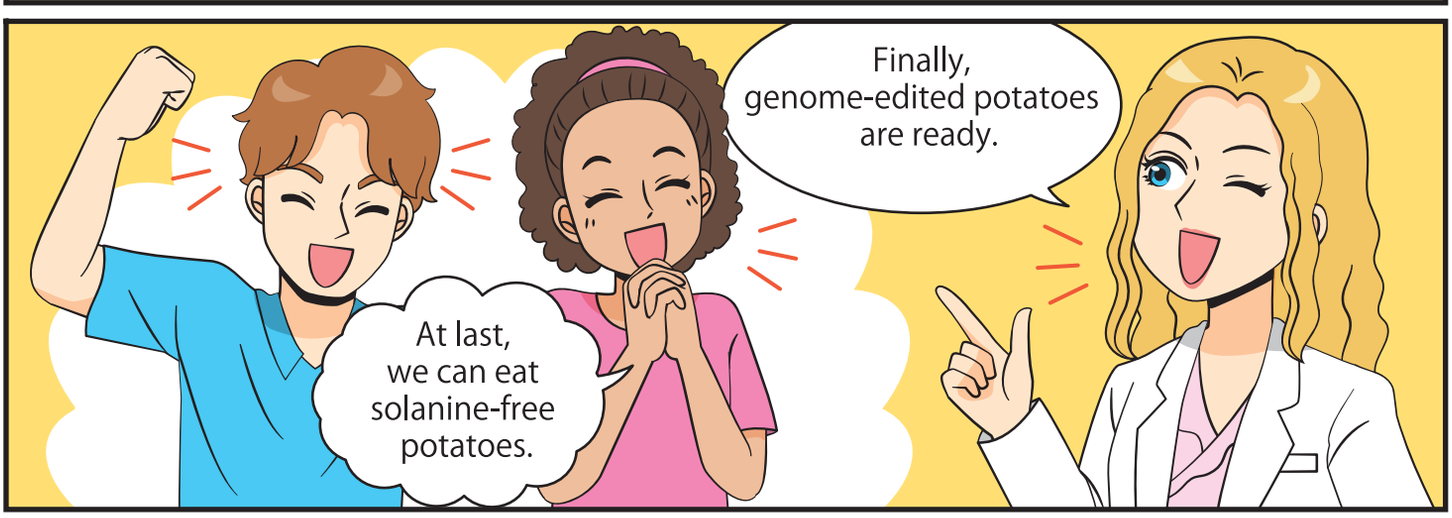


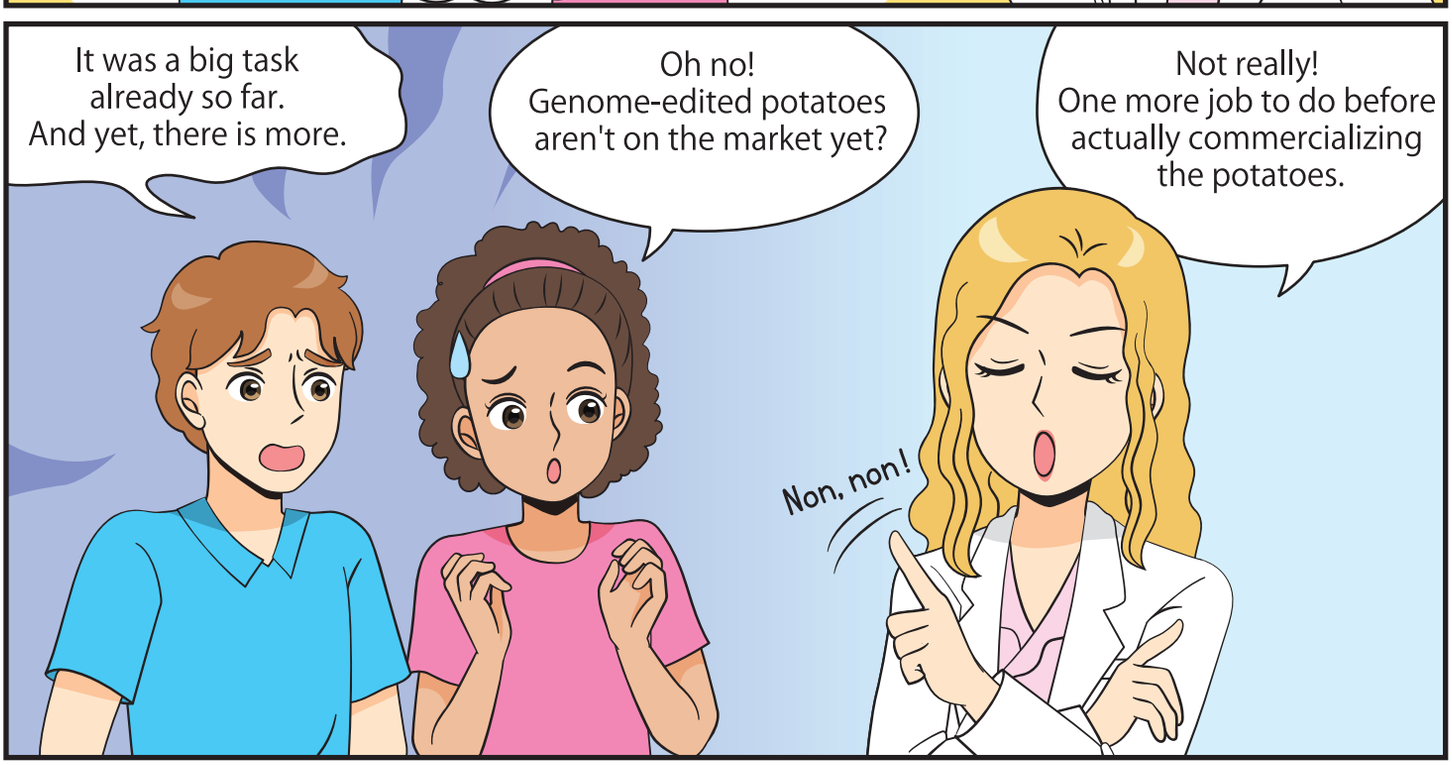




PCR method	It is checked to see if the vector sequence has not increased. (a small sequence is checked)
Southern blotting method	It is checked to see that DNA of a vector is not detected. (a large fragment is checked)
Analysis based on phenotypes.	It is checked to see that a characteristic, indicated when a vector is included, is not observed.
Next generation sequencer	It reads whole genome sequence to see if vector sequences exist in the genome.


Non-genome-edited potatoes
Genome-edited potatoes
Solanine content
Content of other components
Comparison
We'll actually grow them and check to see if solanine is reduced or if there are any other differences from original potatoes.
Genomic stability is also examined.


Finally, genome-edited potatoes are ready.
At last, we can eat solanine-free potatoes.


It was a big task already so far. And yet, there is more.
Oh no! Genome-edited potatoes aren't on the market yet?
Not really! One more job to do before actually commercializing the potatoes.
Non, non!

Content;
(1) Names of item and breed and summary (usage and intended use) of the developed food
(2) Method of genome editing technology used and details of modification
(3) Information on confirmation that there are no remaining foreign genes or their parts
(4) Information on confirmation that confirmed changes in DNA do not cause production of new allergens having adverse effects on human health or increase of known toxic substances contained
(5) For items in which modification affecting the metabolic system was performed in order to increase or decrease specific components, information on changes in major components (nutrient components only) related to the target metabolic system
(6) Year and month of marketing (*Notify the MHLW of it after marketing)

First, developer of the genome-edited potatoes is supposed to prepare pre-submission consultation document, and submit it with the result of analysis to the MHLW.

Developer

Too many!!

Wait a minute!

That's enough!

Examples of the material showing the result of analysis are;
- The result of analysis investigating how SSR2 gene sequence has changed (Example: Electrophoretic image file)
- Result of a search for existence/non-existence of a sequence similar to the one at the designated location.
- Result of a search to see if a new protein is not generated.
- Result of a study to see if allergenicity and toxicity do not exist.
- Result of determination of solanine content etc.
- Details of each measurement and analytical method.

And so on.

Pharmaceutical Affairs ※ and Food Sanitation Council

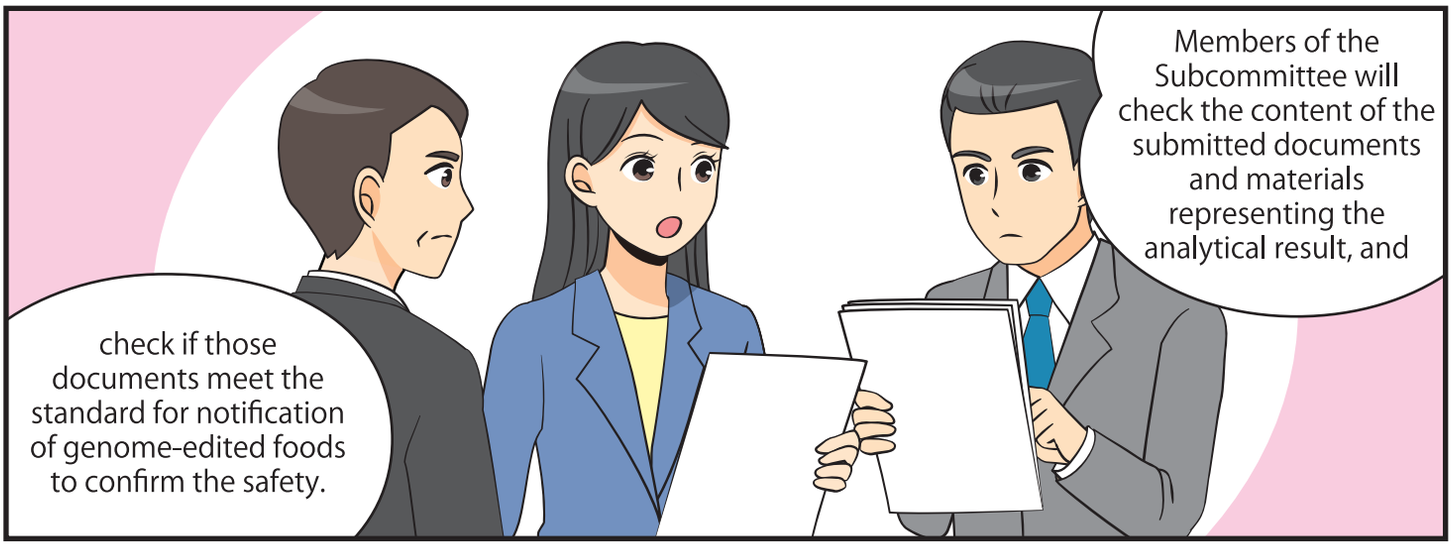
Ministry of Health, Labour and Welfare

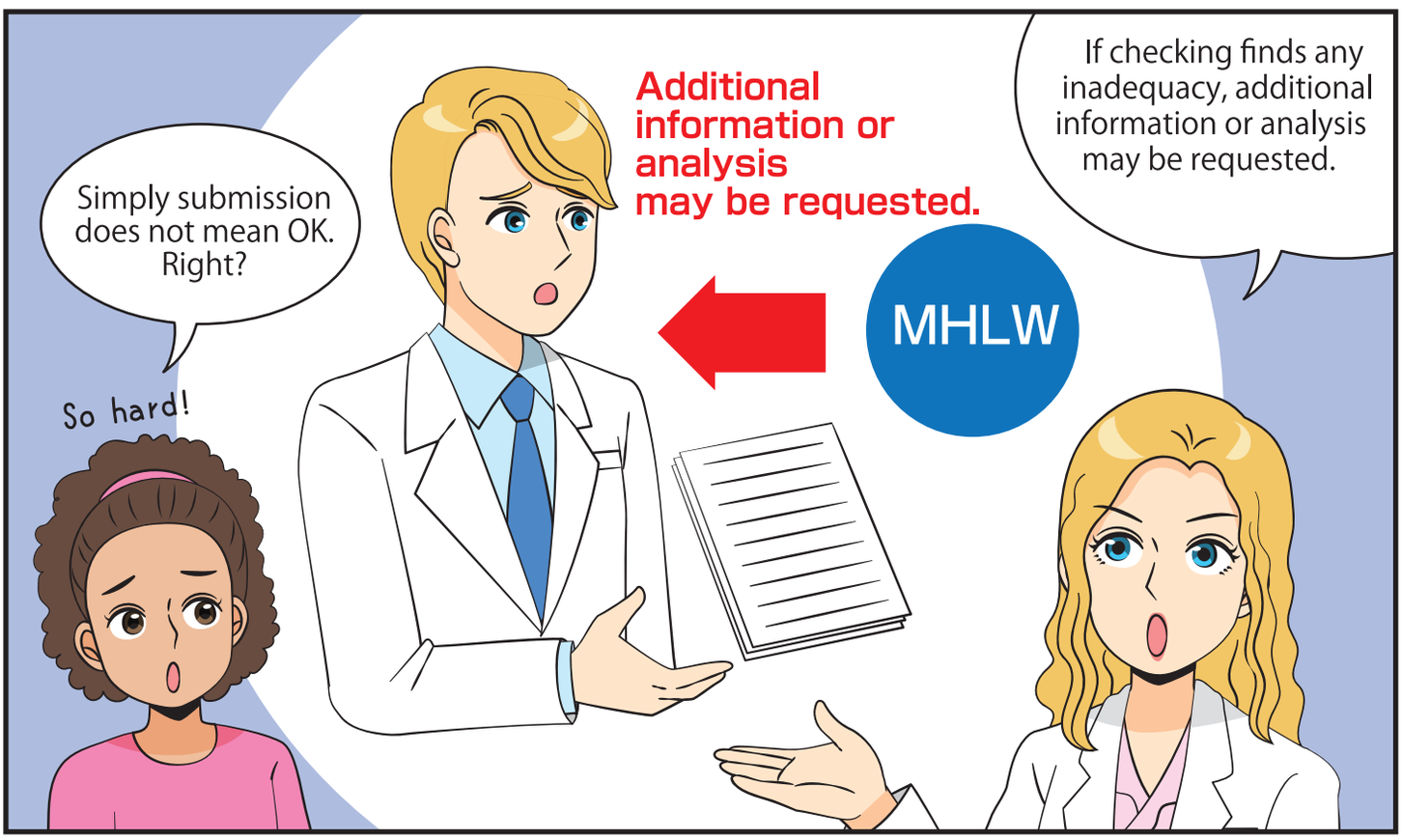
Request for content confirmation

After checking the document,

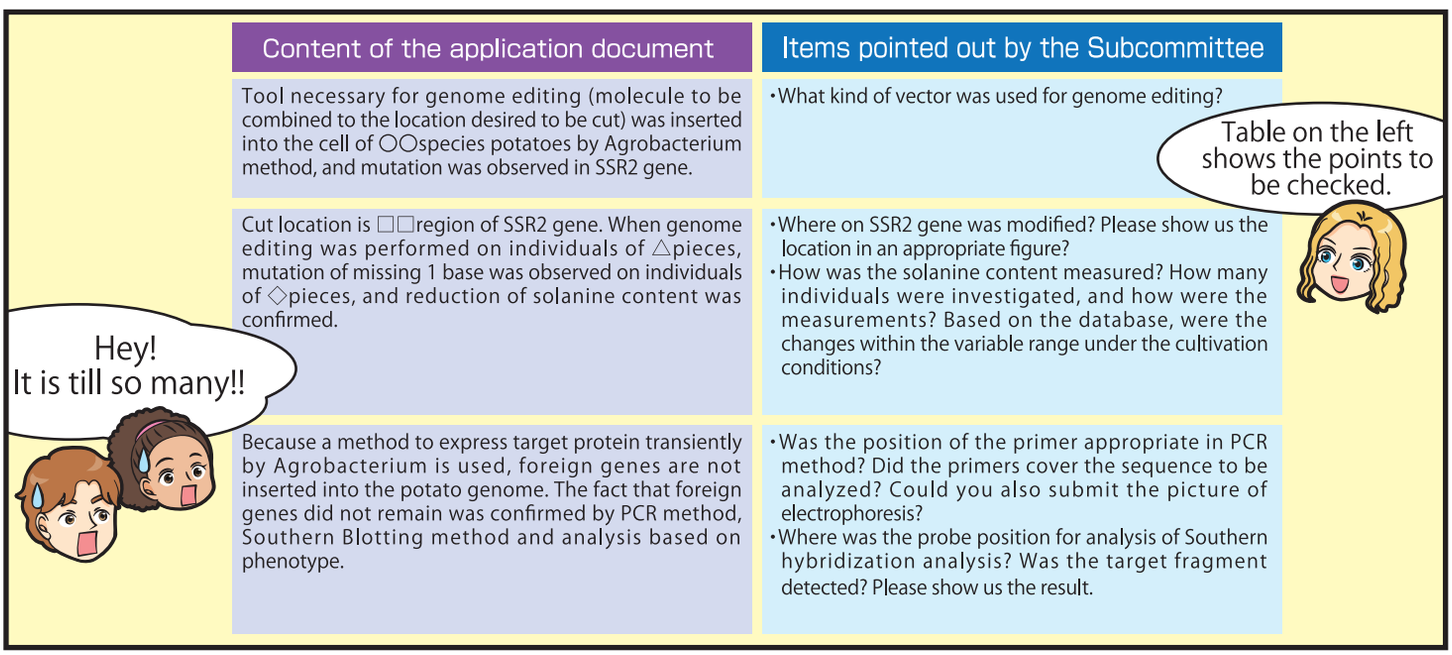
MHLW will request the Pharmaceutical Affairs and Food Sanitation Council (hereinafter referred to as "the Subcommittee"), consisting of experts, to check the content of the document.

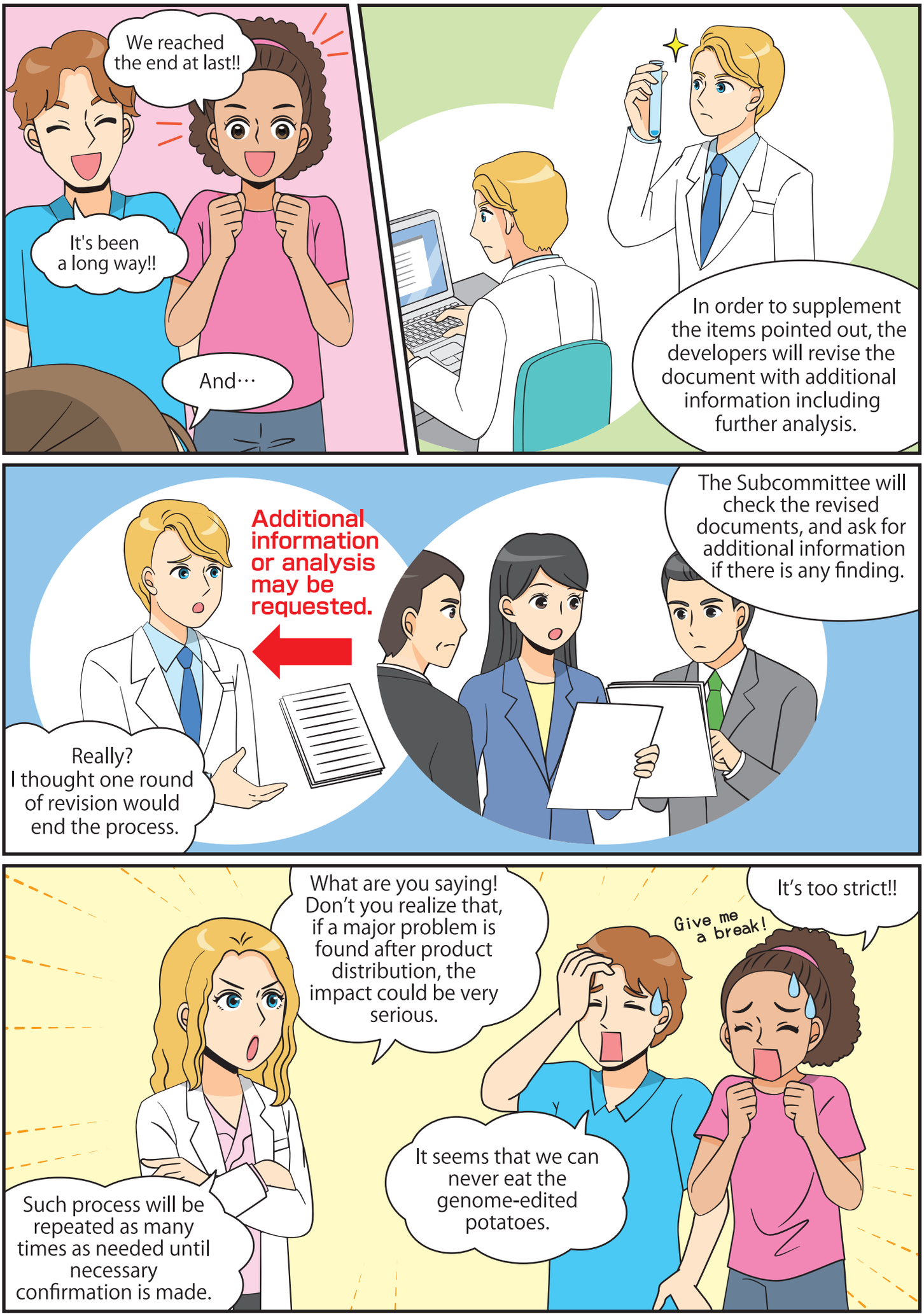
※Subcommittee on Genetically Modified Foods, Newly Developed Food Committee of the Food Sanitation Council



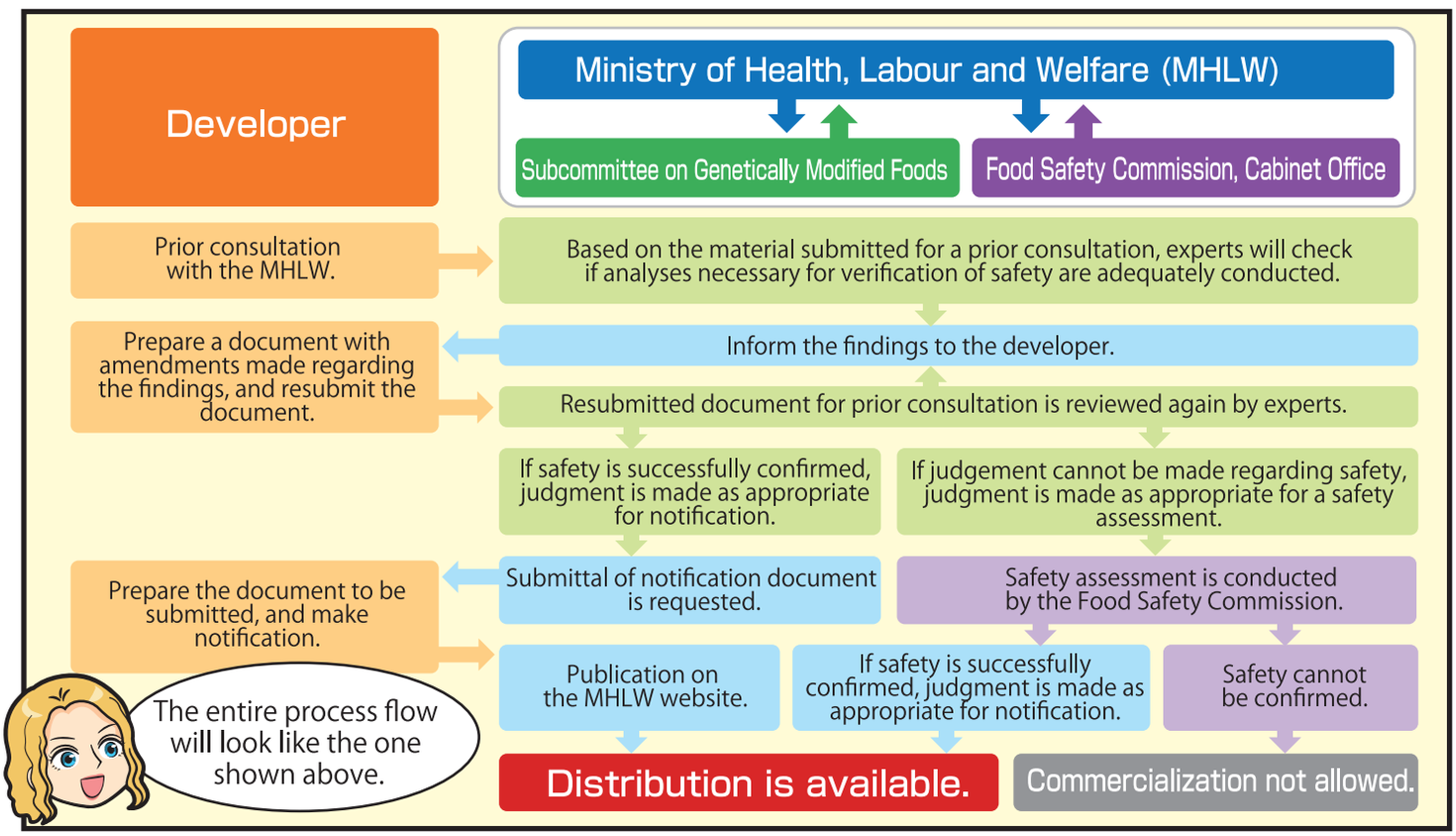


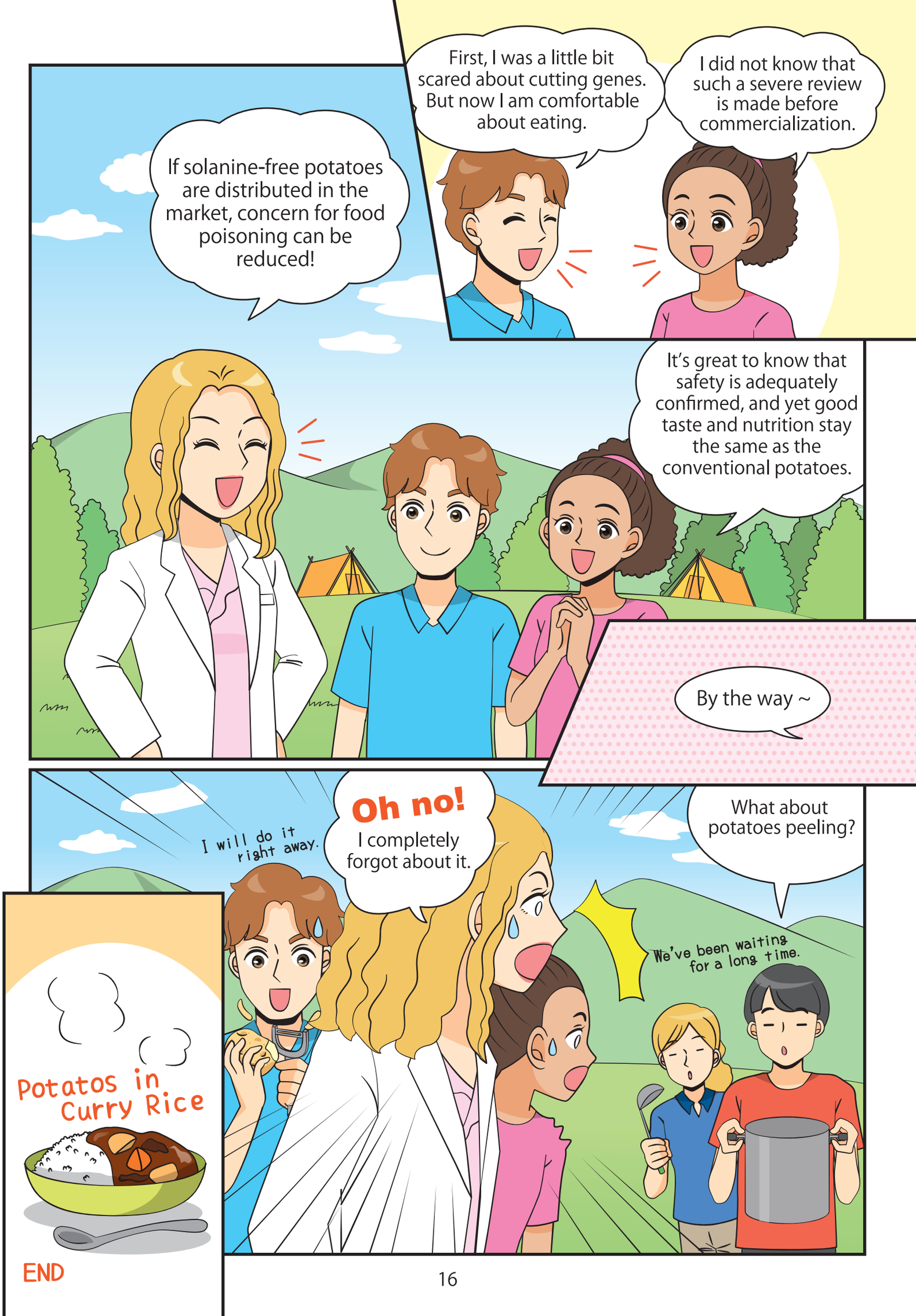
Content of the application document	Items pointed out by the Subcommittee
Tool necessary for genome editing (molecule to be combined to the location desired to be cut) was inserted into the cell of ○○species potatoes by Agrobacterium method, and mutation was observed in SSR2 gene.	・What kind of vector was used for genome editing?
Cut location is □□region of SSR2 gene. When genome editing was performed on individuals of △pieces, mutation of missing 1 base was observed on individuals of ◇pieces, and reduction of solanine content was confirmed.	・Where on SSR2 gene was modified? Please show us the location in an appropriate figure? ・How was the solanine content measured? How many individuals were investigated, and how were the measurements? Based on the database, were the changes within the variable range under the cultivation conditions?
Because a method to express target protein transiently by Agrobacterium is used, foreign genes are not inserted into the potato genome. The fact that foreign genes did not remain was confirmed by PCR method, Southern Blotting method and analysis based on phenotype.	・Was the position of the primer appropriate in PCR method? Did the primers cover the sequence to be analyzed? Could you also submit the picture of electrophoresis? ・Where was the probe position for analysis of Southern hybridization analysis? Was the target fragment detected? Please show us the result.




We reached the end at last!!
It's been a long way!!
And…
In order to supplement the items pointed out, the developers will revise the document with additional information including further analysis.
Additional information or analysis may be requested.
The Subcommittee will check the revised documents, and ask for additional information if there is any finding.
Really? I thought one round of revision would end the process.
What are you saying! Don't you realize that, if a major problem is found after product distribution, the impact could be very serious.
It's too strict!!
Give me a break!
It seems that we can never eat the genome-edited potatoes.
Such process will be repeated as many times as needed until necessary confirmation is made.


When it is judged that the safety is confirmed, notification documents are prepared and submitted to MHLW, and once the documents are accepted,
While research and development are big tasks, document application process is so challenging far more than I thought.
I never imagined that checking for safety is so strict.
Hoo!
We did it at last!!
Finally, the genome-edited potatoes can be introduced to the market!!
We made it!!


Developer
Ministry of Health, Labour and Welfare (MHLW)
Subcommittee on Genetically Modified Foods
Food Safety Commission, Cabinet Office
Prior consultation with the MHLW.
Based on the material submitted for a prior consultation, experts will check if analyses necessary for verification of safety are adequately conducted.
Prepare a document with amendments made regarding the findings, and resubmit the document.
Inform the findings to the developer.
Resubmitted document for prior consultation is reviewed again by experts.
If safety is successfully confirmed, judgment is made as appropriate for notification.
If judgement cannot be made regarding safety, judgment is made as appropriate for a safety assessment.
Prepare the document to be submitted, and make notification.
Submittal of notification document is requested.
Safety assessment is conducted by the Food Safety Commission.
Publication on the MHLW website.
If safety is successfully confirmed, judgment is made as appropriate for notification.
Safety cannot be confirmed.
Distribution is available.
Commercialization not allowed.
The entire process flow will look like the one shown above.


First, I was a little bit scared about cutting genes. But now I am comfortable about eating.
I did not know that such a severe review is made before commercialization.
If solanine-free potatoes are distributed in the market, concern for food poisoning can be reduced!
It's great to know that safety is adequately confirmed, and yet good taste and nutrition stay the same as the conventional potatoes.
By the way ~
What about potatoes peeling?
Oh no!
I completely forgot about it.
I will do it right away.
We've been waiting for a long time.
Potatos in Curry Rice
END