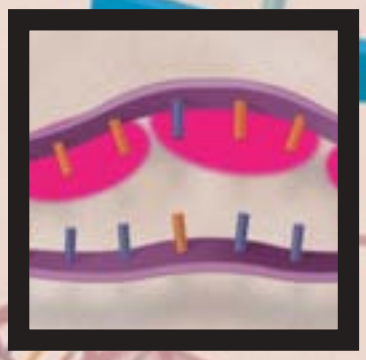
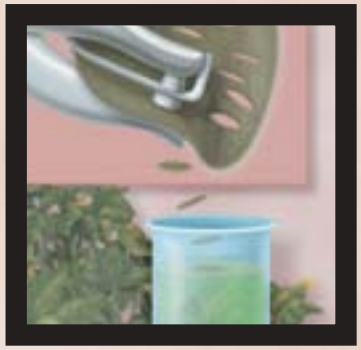
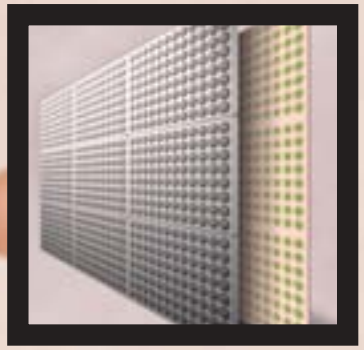
Using Molecular Markers in Plant Genetics Research

Unlocking genetic potential for increased productivity







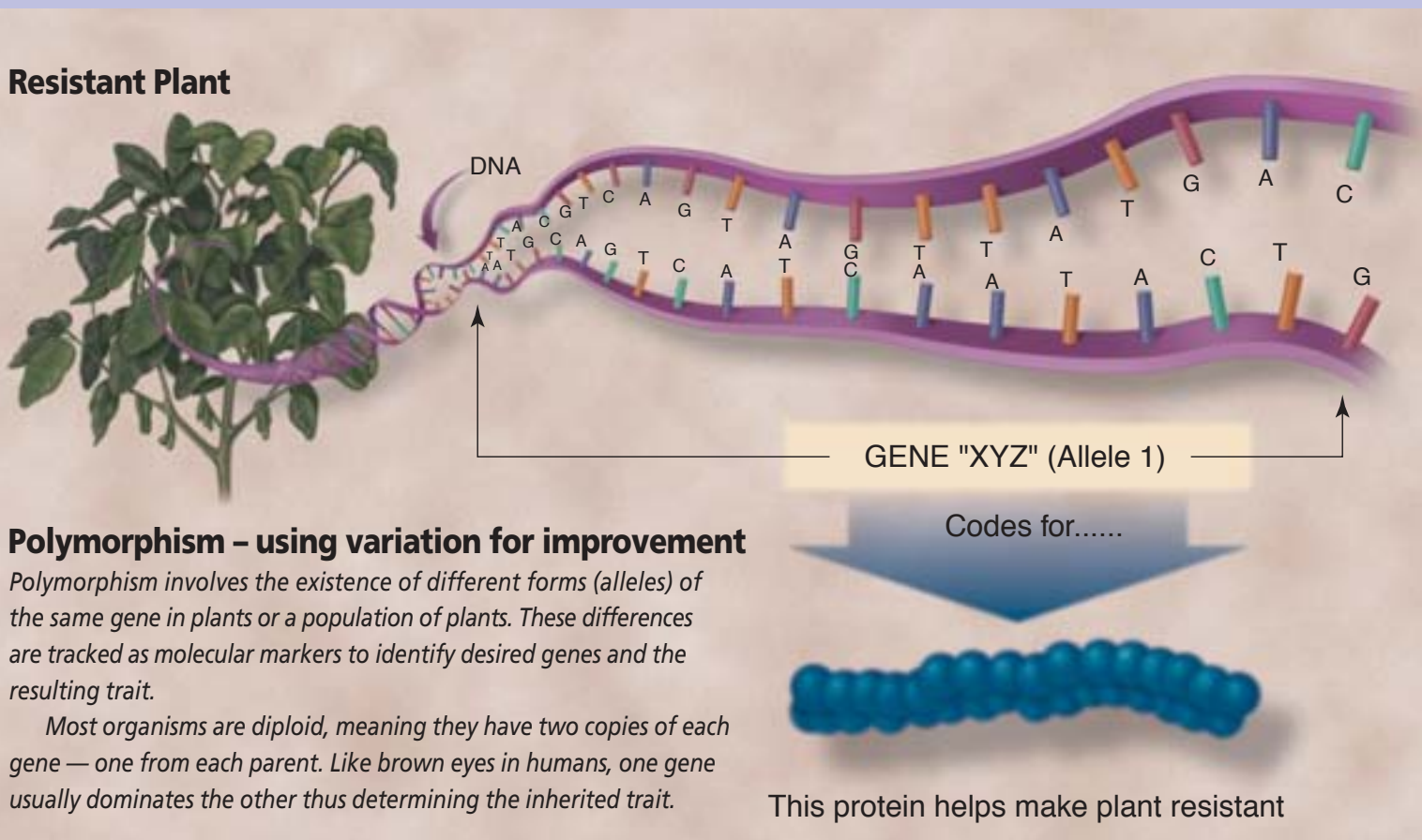
Molecular Markers

Researchers at Pioneer blaze a new genetic trail.

Identifying molecular markers is like blazing a trail through a plant's genetic makeup and putting up road signs along the way.

Molecular markers, sometimes called DNA markers, should be thought of as signs along the DNA trail that pinpoint the location of desirable genetic traits or indicate specific genetic differences. Just as smoke rising into the sky makes it easier to locate a forest fire, a gene of interest is easier to locate when a researcher starts with a nearby marker.

"To successfully use a specific marker to follow a specific trait, the marker must be found close enough to the gene of interest that variations (alleles) of both the marker and the gene can be inherited together," says Dr. Jim Register, research coordinator of analytical nucleic acid technologies at Pioneer. "We study these differences and use them to identify the genes we want. Then, we track those identifiable differences to verify we're making progress in developing a particular trait."



Polymorphism – using variation for improvement

Polymorphism involves the existence of different forms (alleles) of the same gene in plants or a population of plants. These differences are tracked as molecular markers to identify desired genes and the resulting trait.

Most organisms are diploid, meaning they have two copies of each gene — one from each parent. Like brown eyes in humans, one gene usually dominates the other thus determining the inherited trait.

Following the Road Signs to Improve Product Performance

1 Look for genetic clues to find specific genes of interest.

2 Identify markers for specific genes and determine if they can be inherited.

3 Select plants with markers/genes for evaluation.

What's the difference?

Molecular markers are first identified as short fragments or "strings" of DNA located in a specific position on a chromosome. "We are able to use a particular fragment of DNA as a marker when we can detect differences in that fragment's DNA sequence between multiple plants or plant lines," Register says.

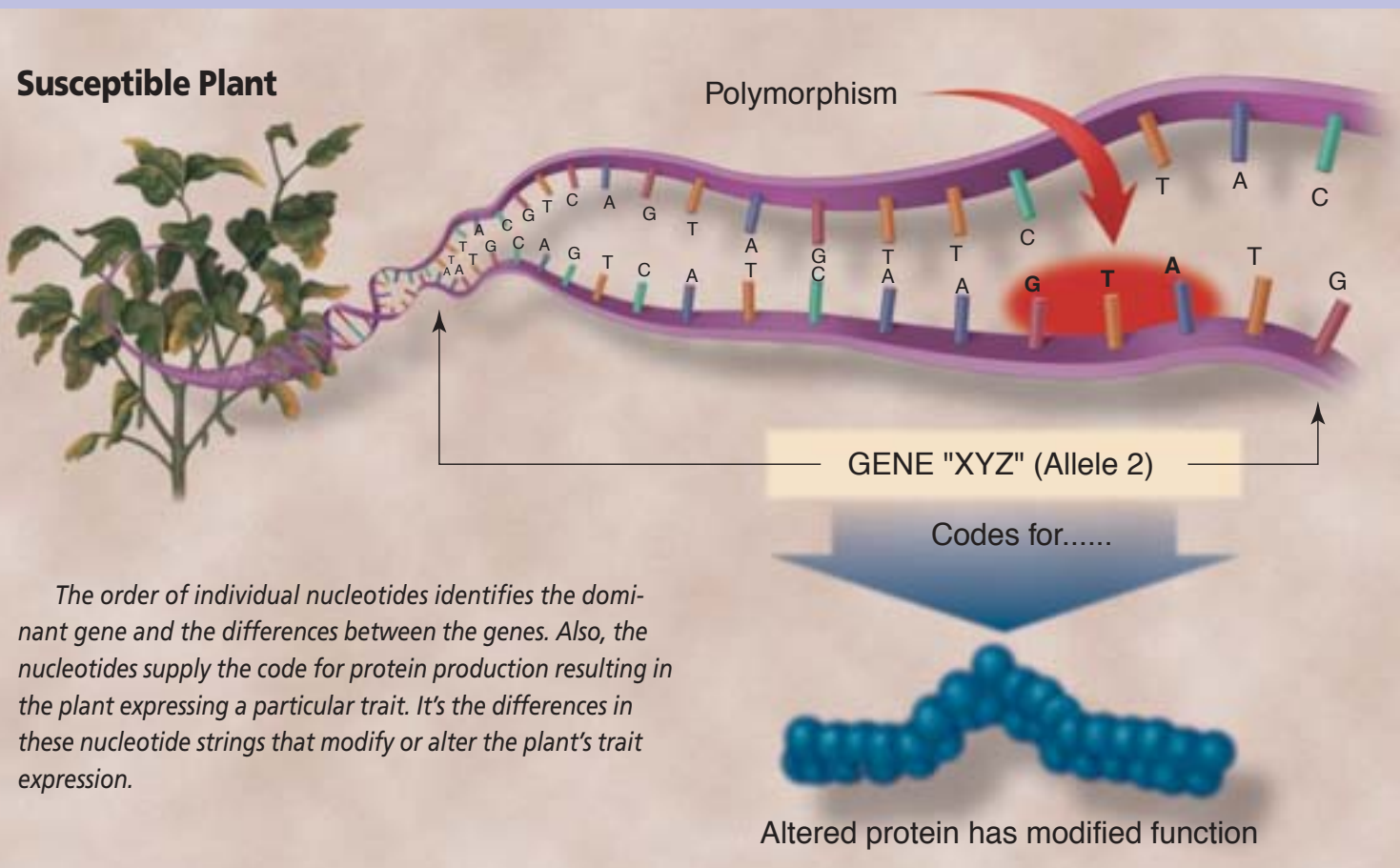
According to Register, these variations in DNA sequence, called polymorphisms, can be associated or linked with different forms (alleles) of nearby genes involved with particular traits. The polymorphism, or difference, is the clue researchers need to find the gene of interest.

For example, markers associated with genes involved in disease resistance have been identified in corn and soybeans. Differences between the DNA sequences of these genes can be responsible for making a plant sensitive or resistant to a particular disease. And differences in DNA sequences near the gene can be used as markers to locate the gene and track the desired results in breeding programs.

Besides disease resistance research, this same technology can be used to localize genes and follow markers associated with other traits such as maturity, plant height, insect resistance, grain oil content, etc.

Getting the desired results

"Using the latest marker technologies, scientists are able to determine right in the lab which plants have economically beneficial traits faster," Register says. "This allows us to select plants based on the traits they possess even before going to field trials. The time saved allows us to move improved products to the market faster."



The order of individual nucleotides identifies the dominant gene and the differences between the genes. Also, the nucleotides supply the code for protein production resulting in the plant expressing a particular trait. It's the differences in these nucleotide strings that modify or alter the plant's trait expression.

4 Develop hybrids and varieties from these parents.

5 Test hybrids and varieties to determine if markers/genes were inherited.

6 Test plant product performance that includes desired gene.

7 Release improved products to market.

Repeat that Again

Repetition in DNA sequences leads researchers to meaningful genes.

Simple Sequence Repeats (SSR) markers, or microsatellite markers, are one of the most advanced marker technologies available in genetic research today and are a key part of research efforts underway at Pioneer. SSR markers are stretches of DNA in which the same short nucleotide sequence is repeated over and over.

Polymorphism, or variation, among SSR markers is determined by the number of times the base sequence repeats (e.g. AGTTAGTT vs. AGTTAGTTAGTTAGTT).

"This variation in DNA sequence can be used just like other types of DNA sequence variation to locate nearby genes and follow specific forms of those genes through product development," says Dr. Jim Register, research coordinator of analytical nucleic acid technologies at Pioneer. "SSR markers are considered highly polymorphic as the number of repeats can vary greatly among plants. This allows us to detect many different alleles for that marker.

"These highly polymorphic SSR markers are also excellent tools for comparing our germplasm with other competitors'," he says. "We use this technology to protect our intellectual property and the investment Pioneer puts into product development research for its customers."

With SSR markers, Pioneer researchers are routinely able to analyze the location of genes on chromosomes (positions of genes on chromosomes) hundreds of plants at one time. The millions of data points generated annually help them develop improved products faster and with added precision.

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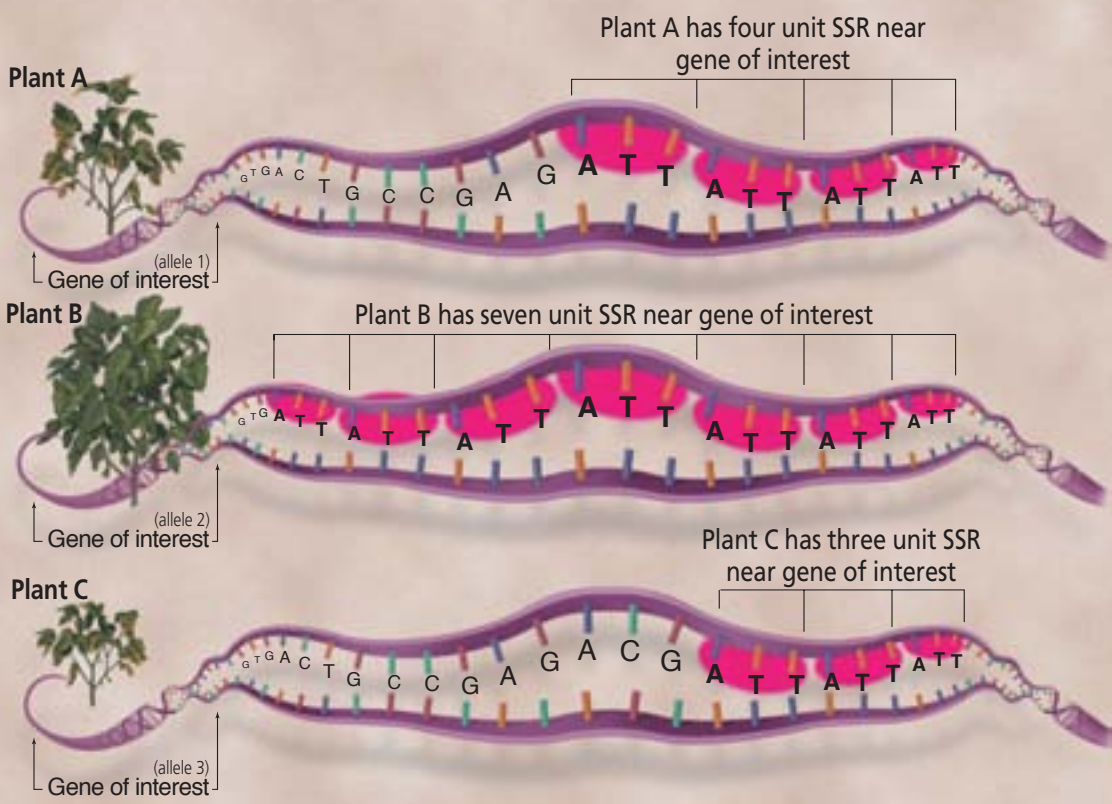
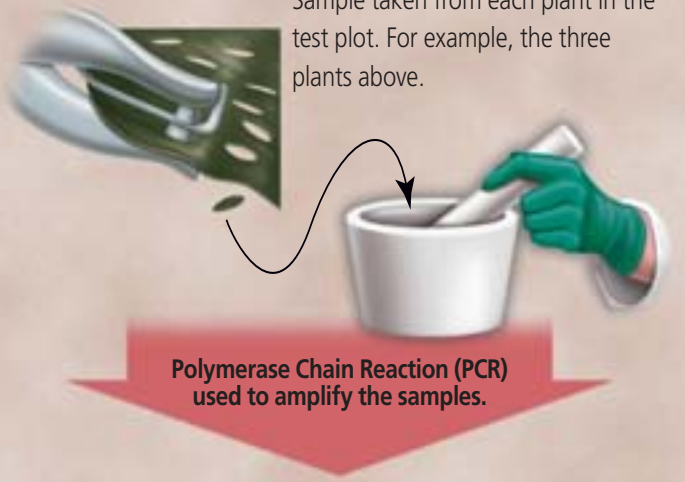
Single Sequence Repeats Markers or Microsatellites.
SSR markers are one to six nucleotide repetitions of the DNA code. These repeats, like an argyle sock pattern, are found most often between genes and don't alter cell function. These are generally found near a gene of interest and serve as a "marker."



Large population of inbred plants, descended from a known male/female cross, is examined for desirable characteristics.

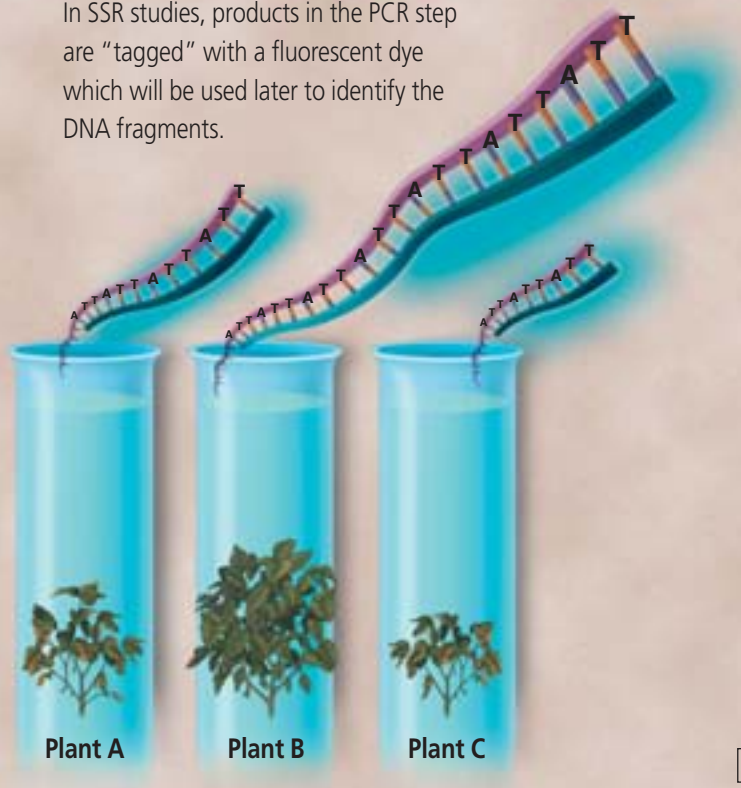
Plant A: Susceptible **Plant B: Resistant** **Plant C: Susceptible**



Sample taken from each plant in the test plot. For example, the three plants above.

Polymerase Chain Reaction (PCR) used to amplify the samples.

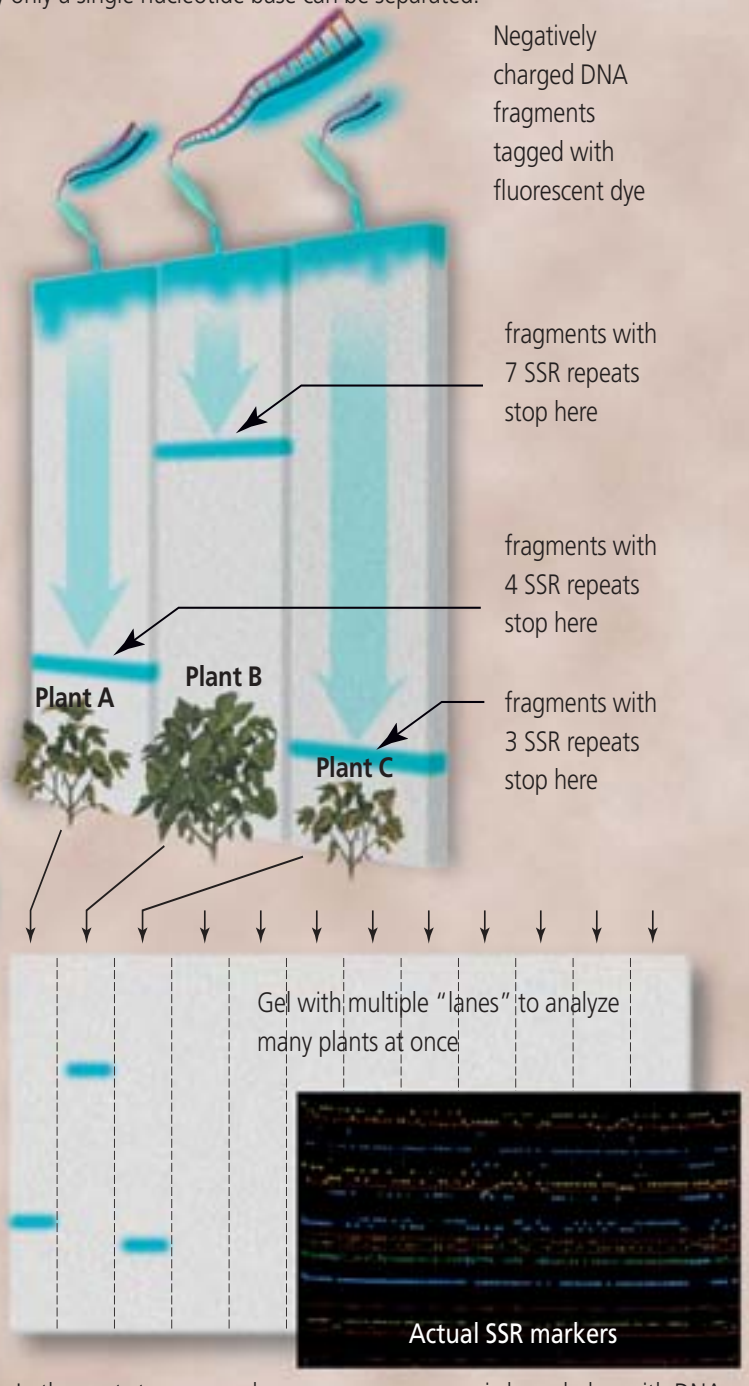
In SSR studies, products in the PCR step are "tagged" with a fluorescent dye which will be used later to identify the DNA fragments.



Because DNA fragments have different lengths due to the number of SSR repeats, Pioneer researchers can separate the fragments through Gel Electrophoresis.

DNA fragments are placed into the top of a gel that acts as a molecular filter. DNA fragments carry a negative charge and the bottom of the gel has a positive charge. Just as positive and negative magnets attract, the DNA moves through the gel matrix towards the positive charge.

As DNA fragments move through the gel, it sorts according to size with the smallest fragments moving farther. This way even fragments that differ by only a single nucleotide base can be separated.



In the next step, researchers compare agronomic knowledge with DNA knowledge via computer analysis. If plant B with an 7-unit SSR repeat has shown resistance to the disease and this same 7-unit SSR repeat has shown up often in other plants demonstrating disease resistance, then this region of DNA can serve as a marker to track and develop the trait.

The One and Only

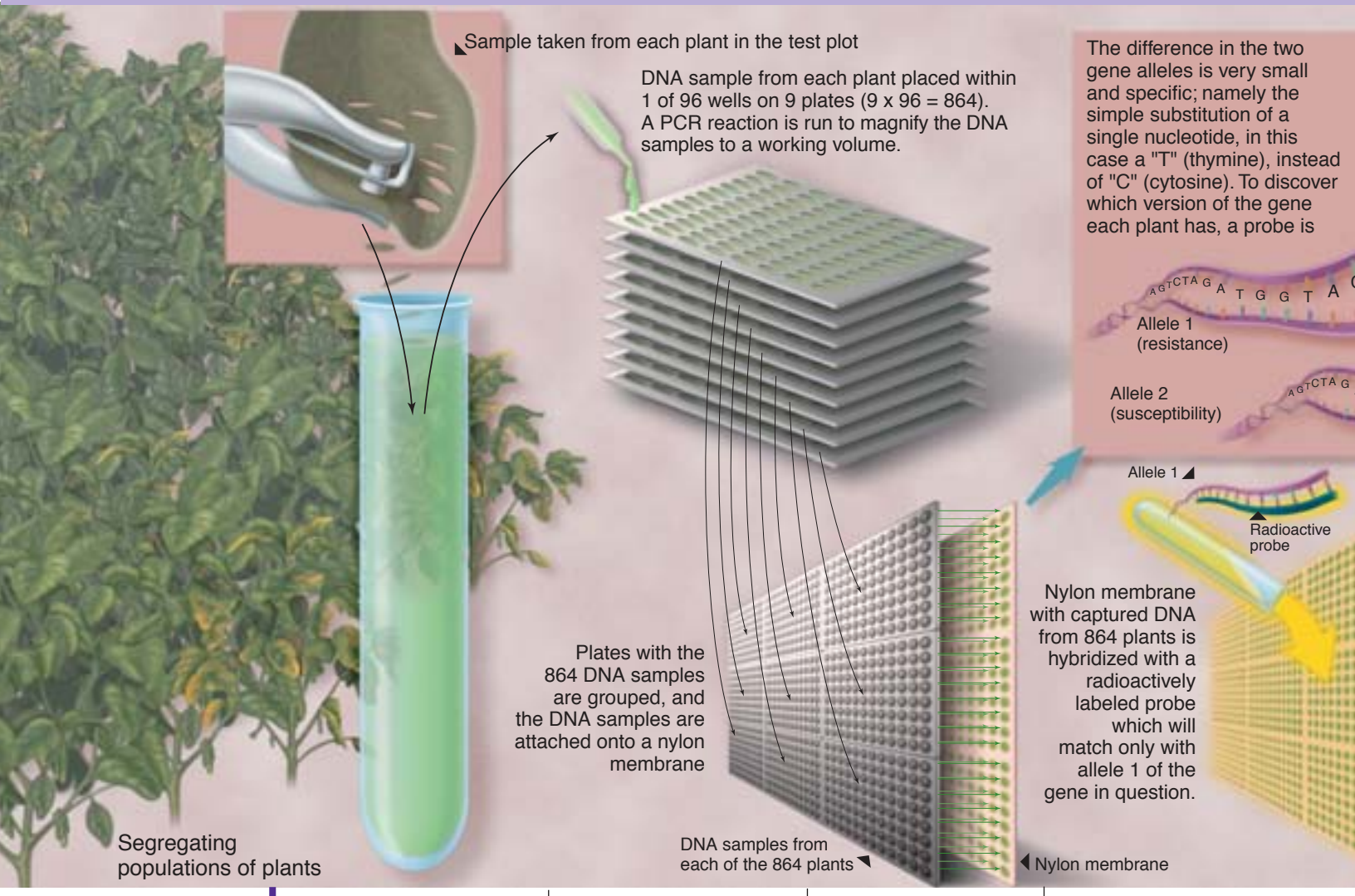
A single base nucleotide substitution can be the key to important crop traits.

Allele Specific Hybridization (ASH) focuses on the most common form of genetic variation in plants: single nucleotide polymorphism (SNP). Because SNPs occur frequently throughout the plant genome, they offer enormous potential in the discovery and detection of important genes in crops.

Just as the name indicates, SNPs are identified by a single nucleotide base change in the genetic code at a specific location on the chromosome. Once discovered, these single-base differences can be used as molecular markers for genes of interest.

Promising possibilities

In recent years, scientists have begun to



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recognize the promise SNPs offer for targeting important genes. Because they represent the most common type of genetic variation in plants, SNPs can have significant effects on resistance to disease, performance under adverse environmental conditions and other economically important traits.

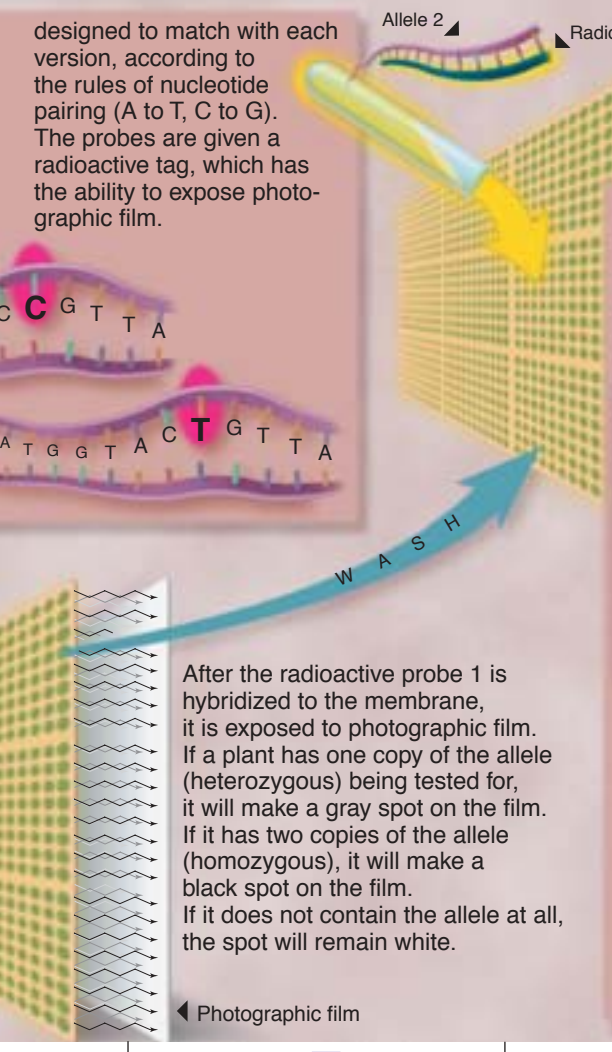
"SNPs are particularly valuable because they offer the potential of ultra-high throughput and highly automated analysis," says Dr. Jim Register, research coordinator of analytical nucleic acid technologies at Pioneer. "SNP analysis is a 'yes' or 'no' proposition (Is the sequence of interest there or not?). SSR markers measure a varying number of sequence repeats."

Resistant or not?

SNP has been a key technology used in Pioneer researchers' work to develop soybean varieties with resistance to soybean cyst nematode (SCN).

"Using ASH analysis of SNPs, we routinely differentiate soybean experimental lines by a difference of one base nucleotide in a region of interest associated with SCN resistance," says Dr. Daria Schmidt, research coordinator in soybean product development at Pioneer. "With it, we're able to identify soybean plants with SCN resistance before we begin field trials. For producers that means getting improved varieties a couple of years faster than field trials alone would allow."

designed to match with each version, according to the rules of nucleotide pairing (A to T, C to G). The probes are given a radioactive tag, which has the ability to expose photographic film.



After the radioactive probe 1 is hybridized to the membrane, it is exposed to photographic film. If a plant has one copy of the allele (heterozygous) being tested for, it will make a gray spot on the film. If it has two copies of the allele (homozygous), it will make a black spot on the film. If it does not contain the allele at all, the spot will remain white.

Photographic film

The membrane is then washed to remove any trace of probe 1. A second probe to match to the second possible allele is then hybridized to the membrane. A final pattern emerges indicating each plant's inheritance status of the 2 alleles in question.

Actual Audorad from ASH study

ASH Analysis: What does it tell us?

Here are examples of results after hybridization with 2 probes.

The photographic films from each hybridization procedure are scanned into a computer, which compares the two films and then tabulates the results. The final product of a series of ASH analyses is a computer spreadsheet of various plant traits, giving breeders a shopping list to choose from.

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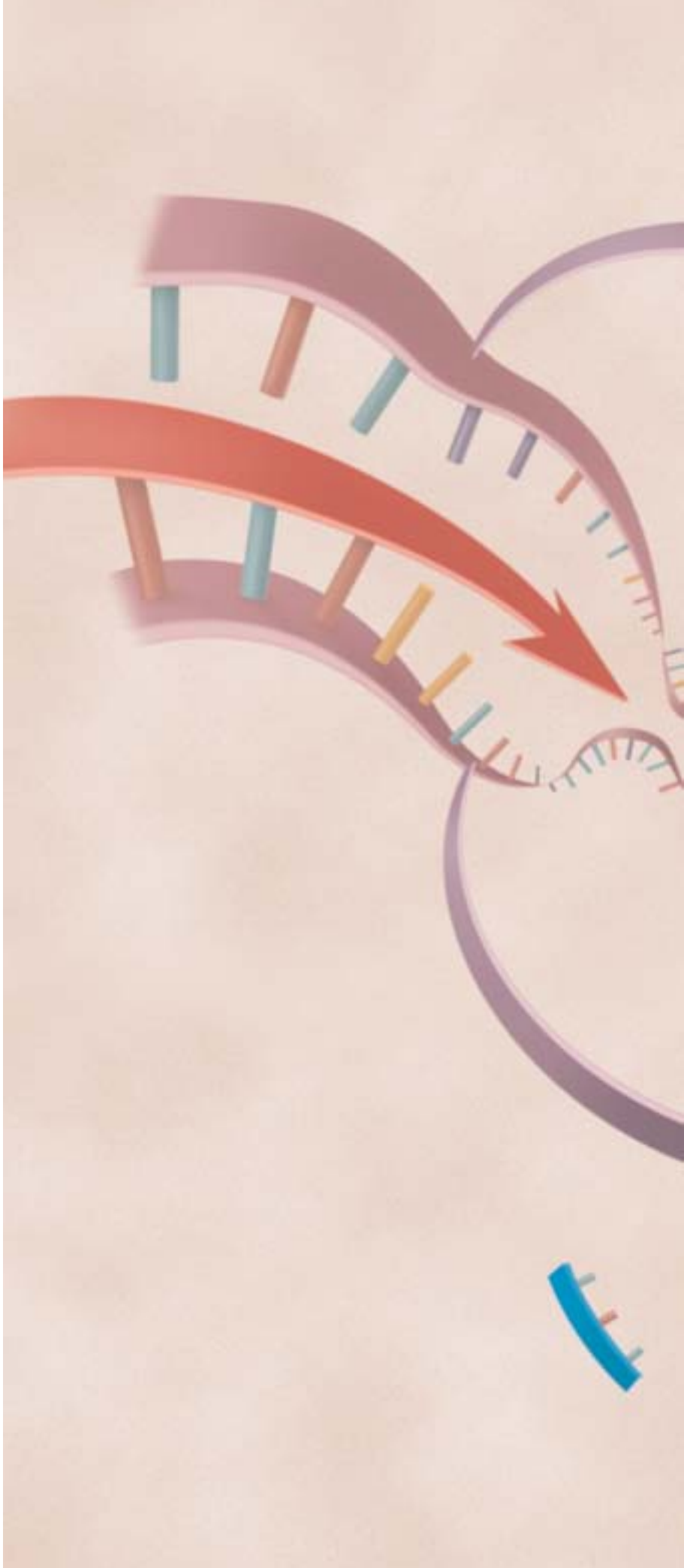

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