APP NOTE

High-Throughput Screening of Gene Edits Using PACE Allele-Specific Genotyping for Agriculture and Life Sciences

Genome editing technologies, particularly CRISPR/Cas systems, have revolutionized genetic research by enabling precise modifications of genes, regulatory sequences, untranslated regions, and intergenic regions. These tools are increasingly being adopted for plant breeding to improve crop varieties and for cell line development in life sciences. Alongside CRISPR, tools like TALENs remain valuable, offering diverse approaches to genome modification.

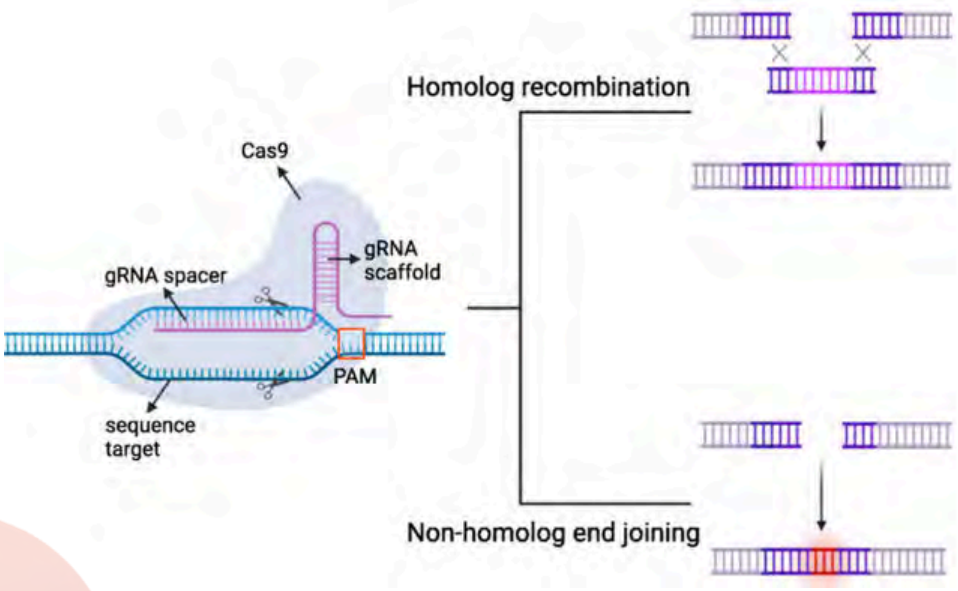
Despite the transformative potential of these technologies, a significant challenge lies in efficiently identifying successful edits efficiently within a population. The current reliance on high-throughput sequencing (HTS) for screening can be time-consuming, expensive, and data-intensive. **PACE® (PCR Allele Competitive Extension) genotyping provides a cost-effective, scalable, and rapid alternative for primary screening of genome edits, applicable to both agricultural and life sciences research.**

CRISPR and the Spectrum of Edits

CRISPR-mediated gene editing can generate a range of modifications, including:

- Single base-pair changes
- Frameshifts
- Gene knockouts
- Small and large insertions or deletions.

These edits occur through either **Non-Homologous End Joining** (NHEJ), which introduces random mutations at the double-strand break site, or **Homology-Directed Repair** (HDR), which uses a template DNA strand for precise changes. Identifying successful edits across populations requires efficient high-throughput screening tools.



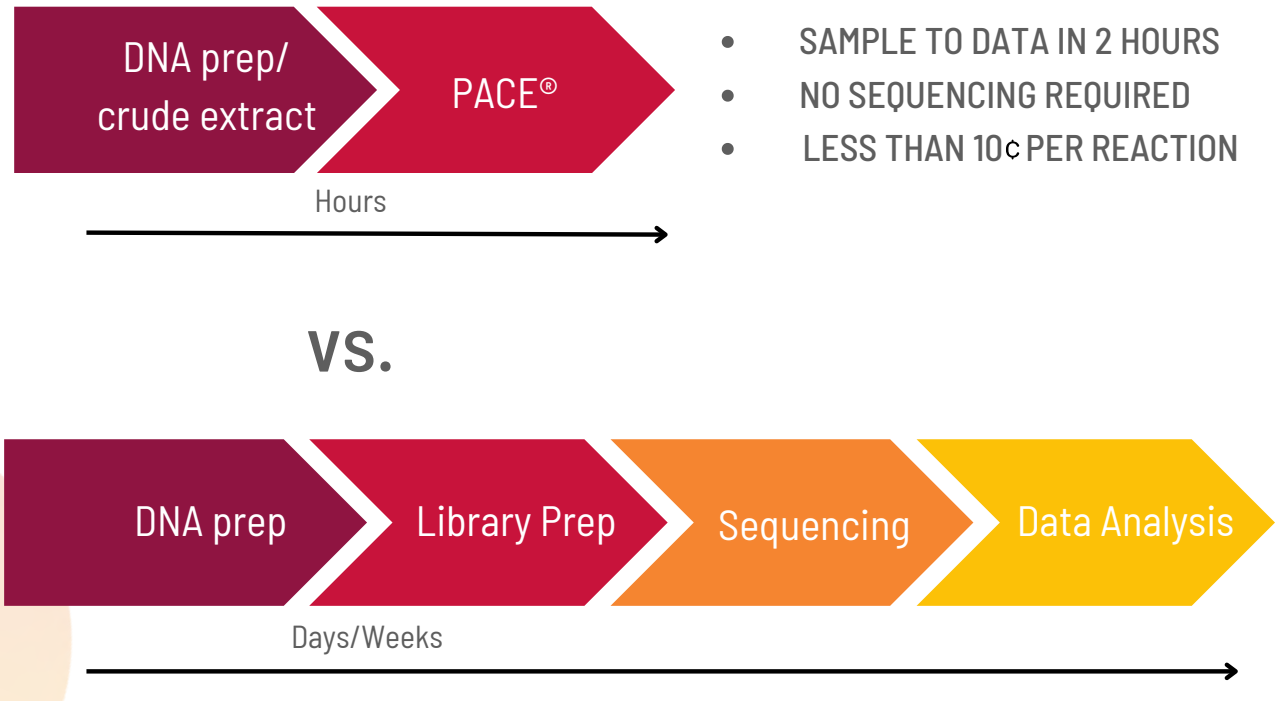
Challenges of Sequencing for Screening

Traditional sequencing methods are often used to screen edited populations, but they present several drawbacks:

- **High Costs:** Sequencing is expensive, especially for large populations.
- **Sample Purity Requirements:** Sequencing demands high-purity DNA extractions, increasing preparation time and costs.
- **Data Overload:** Large datasets must be analyzed to pinpoint successful edits, requiring computational resources and expertise.

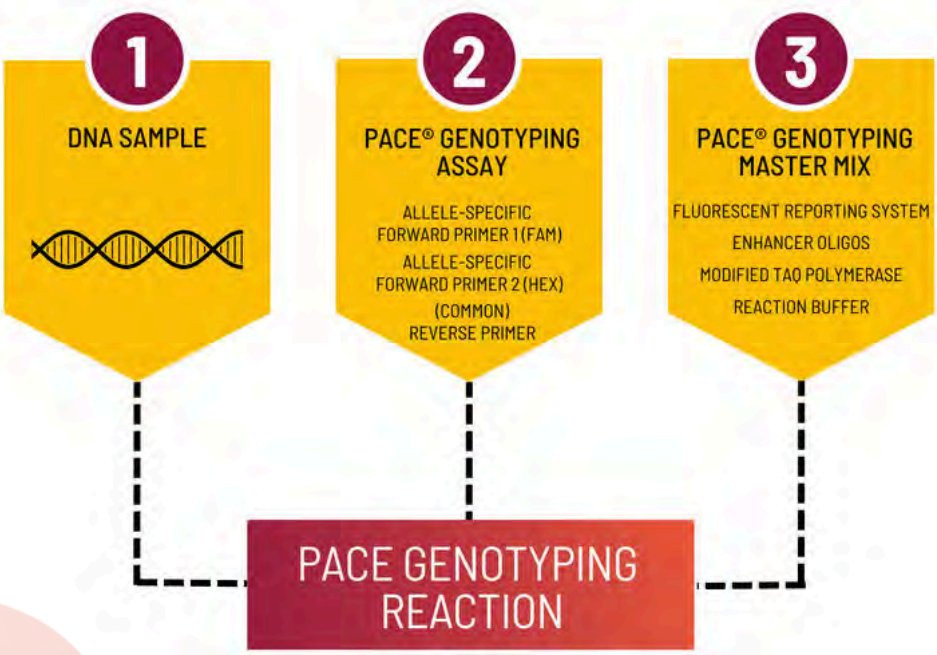
PACE® Genotyping: A Cost-Effective Screening Solution

PACE allele-specific genotyping addresses these limitations by enabling rapid, precise, and scalable detection of genome edits.



Key Benefits of PACE Genotyping

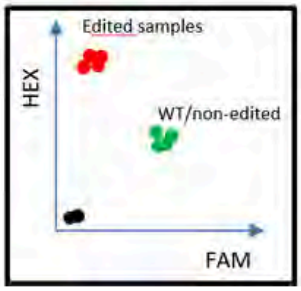
1. **Broad Applicability:**
   - Compatible with plants, animals, tissue cultures, protoplasts, seeds, and more.
2. **Efficiency:**
   - Detects a wide range of edits, including SNPs, knockouts, and both small and large Indels.
   - Uses simple, unlabelled primers customized to specific target sequences. Real-time or endpoint reporting available.
3. **Cost-Effectiveness:**
   - Requires minimal DNA input and works with crude lysates and high-throughput extraction methods.
   - Reduces the need for sequencing by pre-screening populations to identify desired edits.
4. **High Accuracy:**
   - Employs allele-specific primers to distinguish wild-type from edited sequences with precision.
5. **Versatility:**
   - Supports zygosity assays, detection in single cells/plants, and monitoring genetic changes throughout breeding.
   - Enables screening in DNA-mediated and DNA-free editing processes.
6. **Ease of Use:**
   - Combines target amplification and fluorescence detection, eliminating any post-PCR processing.
   - Tailored workflows simplify adoption for new users.



PACE Genotyping: Assays for Every Type of Edit

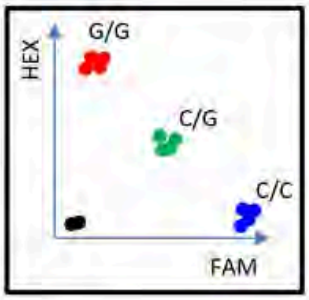
Example 1: Gene Knockouts Using Non-Homologous End Joining

CRISPR induces double-strand breaks, leading to gene inactivation through NHEJ due to random small insertions or deletions.



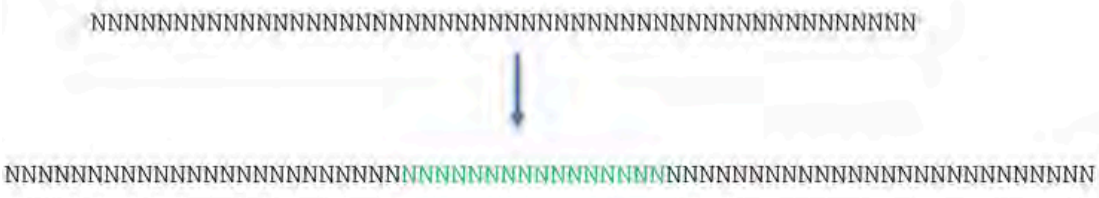
Example 2: Single Base Edits Using Homology-Directed Repair

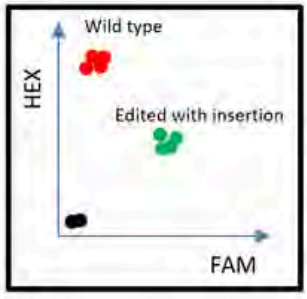
Base editing allows single nucleotide changes. It is used to correct point mutations or introduce single-base changes.



Example 3: Gene Knock-Ins Using Homology-Directed Repair

A precise DNA sequence is inserted at the target site using HDR. Used for correcting mutations or inserting





Spotlight: Applications in Plant Breeding

CRISPR/Cas systems dominate plant breeding, enabling the development of crops with improved traits. PACE genotyping offers critical support at various stages of the breeding pipeline:

- **Optimizing Genome Editing:** Detects edits early to refine transformation protocols.
- **Bulk Screening:** Tests bulk seed or grain populations for purity of edited traits.
- **Regulatory Dossiers:** Provides robust data for regulatory approval.
- **Market Monitoring:** Tracks edits in commercial products.

PACE can detect all classes of edits, from single nucleotide changes (SDN1) to large rearrangements (SDN3), plus monitor potential off-target modifications.

Example Workflow

1. **Assay Design:** Submit the target sequence to 3CR Bioscience, including 100 bases upstream and downstream.
2. **Assay Delivery:** Receive ready-to-use PACE assays, pre-validated using synthetic controls tailored to detect your specific edits or wild-type sequences.
3. **Reaction Setup:**
    - Assemble the reaction using ready-to-use PACE Assays and PACE Genotyping Master Mix.
    - Ensure proper plate layout with non-template controls and wild-type reference samples.
4. **Run and Analyze:** Perform the PCR run, analyze fluorescence data, and identify edited samples.
5. **Follow-Up:** Isolate positive samples for further characterization by sequencing, if required.

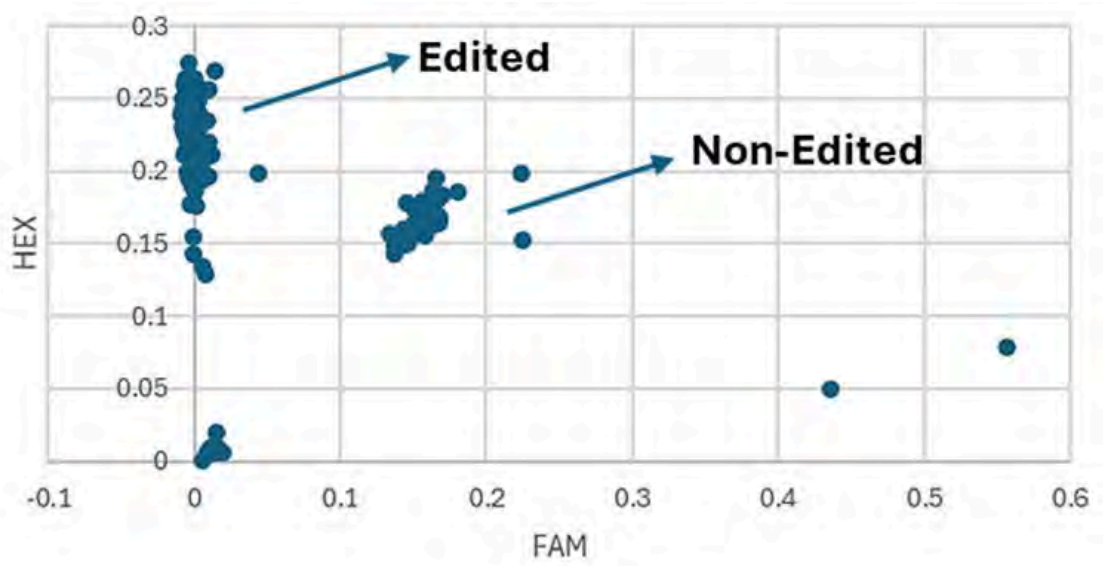
Case Study: CoverCress Inc.

CoverCress Inc. (CCI) is converting field pennycress into a new domesticated variety under the CoverCress® brand as the third crop in standard corn/soybean rotations. This could be used on up to 10 million acres at maturity in the US. To enable the domestication of field pennycress to CoverCress®, the company is using gene editing technology to deploy crucial genetic changes in advanced germplasm identified through breeding programs.

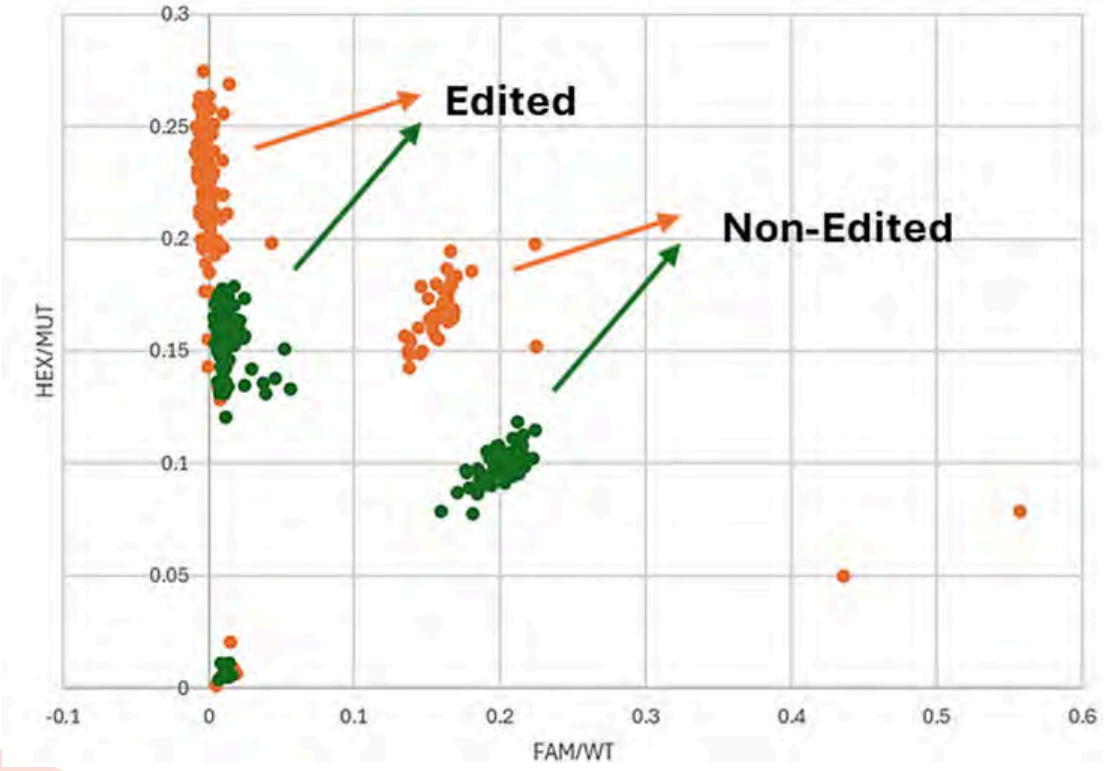
In the CCI product development pipeline, gene editing is used for trait discovery and integration, resulting in the editing of 30-50 unique germplasms with 3-5 gene targets and 2-3 lines with 30-50 unique gene targets every year. Genotyping is one of the major bottlenecks in the edit conversion process. Each DNA repair event can result in a unique DNA modification, limiting the utilization of allele-specific assays in the edit discovery process, and necessitating the use of Sanger sequencing for allele or edit discovery in the lines. While Sanger sequencing is considered the gold standard, its costs can add up significantly when dealing with large volumes of lines, and the turn-around time with Sanger sequencing can delay the decision-making process.

In collaboration with 3CR Bioscience, CCI developed and optimized CRISPR assays that demonstrated remarkable cost efficiencies. Implementing these assays into the pipeline has reduced the total genotyping costs of identifying one edit conversion by 70-80%. These savings highlight the potential for PACE to make gene editing projects not only more cost-effective but also more scalable for a wide range of applications, while reducing delays and limitations associated with Sanger-based genotyping platforms.

Application I: Gene Knockouts using Non-Homologous End Joining for Novel Discovery of Edits



Application II: Selection of Progenies in A Segregating Population with Two Unique Gene Edits using a multiplexed PACE CRISPR assay



Gene editing creates novel alleles in each transformation event, and the edited lines used in breeding programs can pose challenges in marker-assisted selection (MAS) due to the variety of alleles incorporated in the crossing designs. Through continued the collaboration with the 3CR Bioscience, CCI optimized these assays for use in their MAS program, identifying a pathway to multiplex two uniquely edited regions in the genome. In addition to cost savings, these PACE assays play a crucial role in streamlining marker selection with edited germplasm in any crop.

Conclusion

PACE allele-specific genotyping is a valuable tool for high-throughput gene editing workflows. By offering a cost-effective, precise, and scalable alternative to sequencing, it empowers researchers in both agriculture and life sciences to accelerate their genome editing projects. Whether optimizing editing protocols, screening bulk populations, or preparing regulatory data, PACE genotyping is a critical component of the gene editing toolkit.