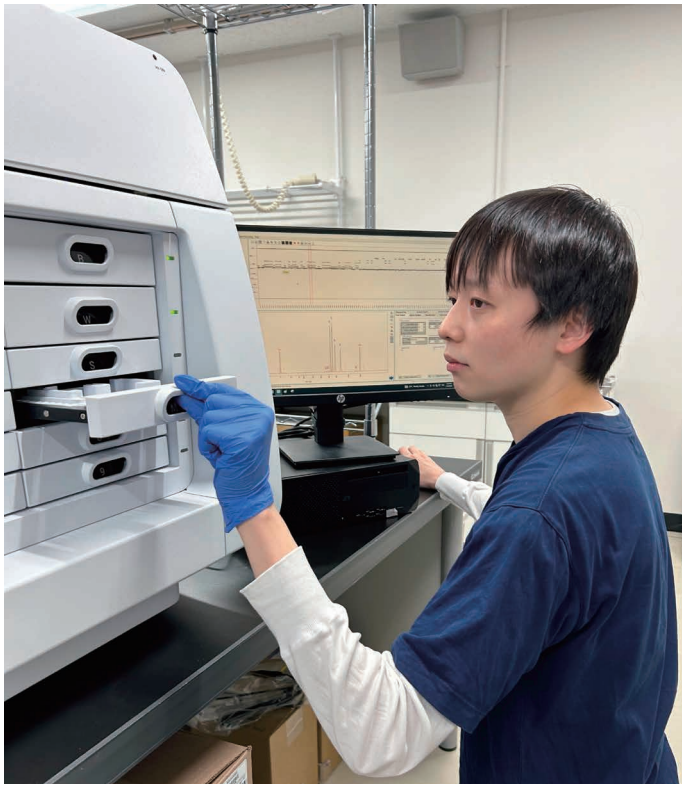
Case study: Agilent Fragment Analyzer system/Agilent ZAG DNA Analyzer system

Screening Genome-Edited Aquaculture Products Using Automated Capillary Electrophoresis Systems

Introduction

Genome editing is a technology for the specific modification of target genes, which, unlike conventional genetic engineering, does not introduce exogenous genes. Such advantages have led to an increase in its application across various fields in recent years. We interviewed Dr. Atsushi Kunii, Senior Researcher at Regional Fish Institute, Ltd. R&D Fish Breeding Group, about the use of the automated capillary electrophoresis systems, the Agilent Fragment Analyzer and Agilent ZAG DNA Analyzer (ZAG) for the screening of aquaculture products produced by high-speed breeding.

Agilent 5300 Fragment Analyzer system



Dr. Atsushi Kunii and the Agilent ZAG DNA Analyzer system

Agilent

Trusted Answers

Q: Please tell us about Regional Fish Institute, Ltd.

Our company aims to contribute to a more efficient, sustainable Japanese aquaculture industry and offers solutions to global challenges of food problems, such as the protein crisis, by combining super-high-speed breeding of aquaculture products. This involves techniques such as genome editing and smart aquaculture where we use IoT and other technologies. In 2021, we launched our “22nd Century Sea Bream”, the first genome-edited animal food product in the world, followed by our “22nd Century Fugu” and our “22nd Century Flounder” in 2024. Our group focuses on the selection of target genes relating to the breeding of aquaculture products, especially fish. We also develop new varieties through genome editing and study genome editing techniques.

Q: Please tell us how you utilize Fragment Analyzer/ ZAG and about the kits you are using.

For high throughput analysis of large sample quantities, we use the 5300 Fragment Analyzer, which is capable of processing 96 samples in parallel, and the ZAG, which can hold up to nine 96-well plates at a time. To check for mutations in the target gene regions after genome editing, we perform a heteroduplex mobility assay (HMA)* which requires PCR and electrophoresis. We utilize the Fragment Analyzer and ZAG for purposes such as observing band patterns (Figure 1). In almost all cases, we use the Agilent dsDNA 905 Reagent kKit (1-500 bp) and Agilent ZAG 105 dsDNA Kit (1-500 bp) to obtain electrophoresis images in a range of up to 500 bp. Depending on the type of mutation (e.g. 3-base deletion and 4-base deletion), the heteroduplex banding pattern differs from that of the wild-type. With long amplified fragments, these may not be clearly distinguishable, or the heteroduplex band may interfere with the upper marker (500 bp). Conversely, short, amplified fragments cannot be used for direct sequencing to examine mutations. Therefore, we aim for an amplification length of 200 bp to fit within the range of the analysis kits. Depending on our objectives, which include confirmation of ordinary PCR products and quality assessment of NGS libraries in addition to HMA, we also use kits with a size range of over 500 bp.

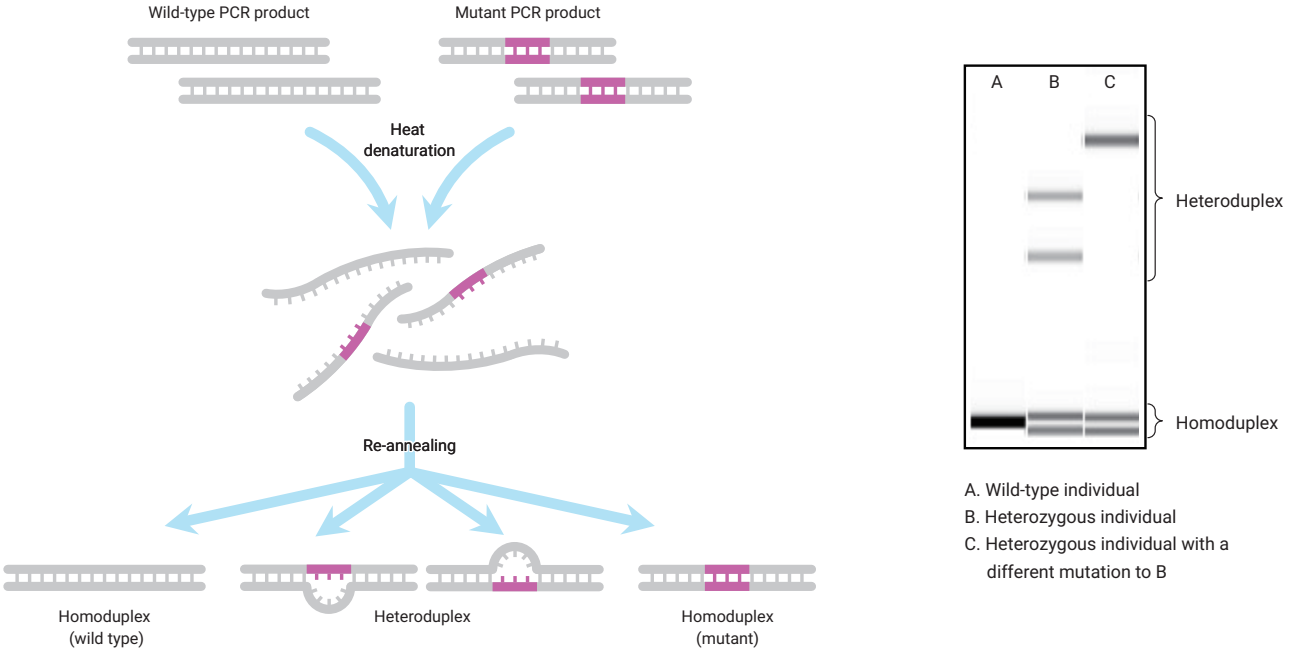


Figure 1. Outline of heteroduplex mobility assay (HMA) and band patterns

* Heteroduplex mobility assay (HMA): After PCR amplification of DNA regions of the genome editing target site, the DNA is denatured by heat and allowed to reanneal, forming a heteroduplex between mutant and wild-type DNA (sometimes mutant and another type of mutant DNA). Heteroduplexes differ in structure to homoduplexes and have markedly slower electrophoretic mobility. A band appears in the high molecular weight area, allowing for visual verification of the presence of a mutation.

Q: How do you differentiate between the Fragment Analyzer and ZAG in terms of usage?

We decide which system to use for analysis based on sample size and running cost. We also make a point of using separate devices for analysis when we are using different kits on the same day. This is to reduce hassle and avoid operator error when switching between gels on the software and when replacing reagent plates (required for 96 capillary array).

Q: How many samples do you process per day?

Because fish have fixed spawning seasons, analysis must be carried out within certain periods. Sometimes we analyze up to five or six 96-well plates per day for days on end. Why such extremely high volumes? This is because fish lay large numbers of eggs, and because there is a need to check for mutations at multiple stages of fish development. The workflow of new variety development starts with the introduction of a genome editing tool by microinjection into the fertilized egg to obtain the F0 generation. After tool injection, mutations begin to occur independently in each cell in the cleavage process, leading to diverse mosaic mutations overall. Therefore, we check for the presence of mutations by comparing band patterns with those of nonedited samples. The sample size is not very large in this generation, as we are only analyzing a maximum of several hundred individuals. In the F1 generation, which is a cross between a successful mutant and a wild-type, a mutation in a germ cell of the F0 organism is inherited and becomes heterozygous. We therefore use band patterns to identify mutations in F1 individuals. As a preliminary step, we take a partial sample of the F1 generation embryo (egg prior to hatching) and consider which F0 organism to use as the parent. We need to examine the embryo after analyzing the fin because mutation manifestations are not exactly the same in the fin and germ cell. We analyze 30 to 40 F1 embryos per F0 individual to estimate the types and frequencies of mutations in the F1 cohort. If an individual is deemed a promising parent, it is crossed with wild-type in an additional step to breed the F1 generation. Next, when the F1 individuals have grown enough to allow for fin harvesting, we check the mutations in each of them. From this generation onward, we are dealing with enormous sample sizes until complete homozygosity for the mutation is achieved. In addition, when we are aiming for mutations in multiple gene regions, we need to examine multiple PCR products for each sample, which calls for a further increase in electrophoresis runs. High-throughput systems are therefore an absolute necessity.

Q: Can you share with us the benefits of using the Fragment Analyzer and ZAG DNA Analyzer as well as any requests or suggestions for improvement?

The high throughput is the biggest benefit because we have to analyze enormous sample quantities at a time, as I mentioned. Another benefit which we had not expected is the high-resolution separation, being able to clearly distinguish between band patterns. Usually, we target deletions of 4 - 5 or as high as 20 bases, but due to the properties of the tool, single-base deletions or insertions sometimes occur. Although not with certainty, we are often even able to tell these single-base mutations apart from wild-type. For a while, we had a problem with the same sample showing a different heteroduplex banding pattern depending on the electrophoresis run. As for suggestions for improvement, we noticed higher accuracy and better reproducibility when we measured the gel volume in a 50 mL tube instead of a 250 mL tube. In terms of usability, we have no particular issues and are satisfied. Any cost reduction would be greatly appreciated.

Agilent Fragment Analyzer System

Using automated parallel capillary electrophoresis, the Agilent Fragment Analyzer system offers nucleic acid quality control for a range of applications, including DNA fragment analysis, NGS libraries, cell-free DNA, and RNA QC.

Simple sample preparation, automated operation, and intuitive analysis software contribute to efficient and accurate measurement.

Key benefits

- High-resolution separation
- Broad range of DNA and RNA kits
- Seamless switching between applications
- Minimal instrument preparation
- Unattended operation

Learn more at www.agilent.com/genomics/fragment-analyzer

Agilent ZAG DNA Analyzer System

The Agilent ZAG DNA Analyzer system relieves bottlenecks in automated high-throughput DNA fragment analysis workflows.

Using parallel capillary electrophoresis, the system can process thousands of DNA fragment samples per day, eliminating barriers associated with agarose gel use.

Key benefits

- High resolution separation
- High sample throughput
- Fast separation time
- Broad sizing range
- Intuitive data analysis

Learn more at www.agilent.com/genomics/zag

Website

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