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Application News

MALDI-TOF Mass Spectrometry Analysis MALDI-8030

# Negative Mode Analysis of Synthetic Oligonucleotides using the MALDI-8030 Dual Polarity Benchtop MALDI-TOF Mass Spectrometer

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## User Benefits

- Simple analysis of oligonucleotides, in negative ion mode to reduce adducts, on an affordable benchtop MALDI-TOF
- Quality spectra with good mass accuracy offers MALDI-TOF as an alternative to gel-Ethidium bromide detection post PCR
- Genotyping workflow useful for introducing mass spectrometry to students in teaching laboratories

## ■ Introduction

Synthetic oligonucleotides are short DNA or RNA sequences which find different applications in molecular biology, such as primers used in DNA sequencing and amplification by the polymerase chain reaction (PCR). Recently, synthetic oligonucleotides have also been explored for therapeutic and diagnostic purposes, and DNA-based diagnostic test kits, in several conditions.

Cystic fibrosis is an example of a condition which develops at the DNA level (Fig 1). It is the commonest autosomal recessive disorder among Caucasians and caused by mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is located on chromosomal region 7q31.2 and contains 27 exons [1]. Mutations in the CFTR gene disrupts the normal maintenance of hydration of secretions within airways and ducts through the transport of chloride and inhibition of sodium uptake. This occurs mainly in epithelia of airways, the sweat glands, the gastrointestinal tract (comprising the pancreas and biliary system), and the genitourinary system - areas where the CFTR protein is largely expressed [1].

Besides its clinical relevance, cystic fibrosis can be useful for demonstrating genotyping workflows in teaching laboratories because of the multiple mutations (>1500) identified [1], which may involve different molecular assays. These are typically PCR related assays, such as, amplification refractory mutation system (ARMS-PCR), which can be used for mutations Phe508del, Gly542X and Asn1303Lys [2]; restriction fragment length polymorphism (PCR-RFLP), which can be used for mutations Arg1303Lys, Arg347Pro [1] and Arg334Try [3]; and heteroduplex analysis (HA), which can be used for mutations Phe508del, Ile507del, and 1677delTA [3]. However, following PCR amplification in these assays, the products are typically detected by gel electrophoresis and read using ethidium bromide staining in a UV box [1, 3], and this is where the workflow becomes labour intensive, slow and costly. An alternative detection using MALDI-TOF would be easier.

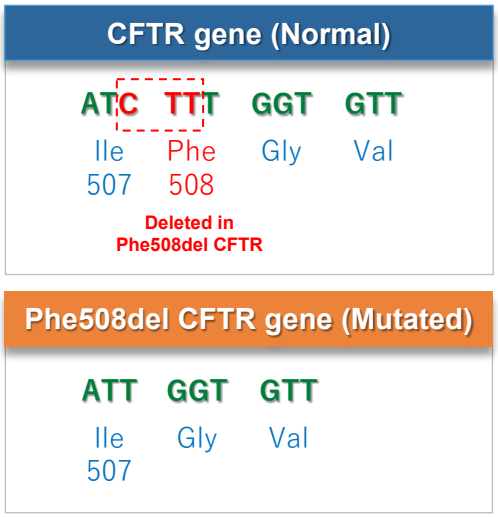


Fig 1 Mechanism of the commonest mutation (Phe508del) of the CFTR gene in cystic fibrosis.

MALDI-TOF mass spectrometry is a well-established and widely used technique for the analysis of oligonucleotides, as it is quick, simple and can provide information on the molecular identity as well as the sequence. One of the challenges in the detection of oligonucleotides by positive ion mode mass spectrometry is the formation of sodium or potassium adducts in solution, which could result in decreased sensitivity and peak resolution if a clean-up step is not performed. However with negative ion mode detection this is circumvented, as the salt adducts would not be detectable.

Here, we present the dual polarity MALDI-8030 benchtop linear mass spectrometer for genotyping, using cystic fibrosis disease (Phe508del mutation) as an example (Fig 2).

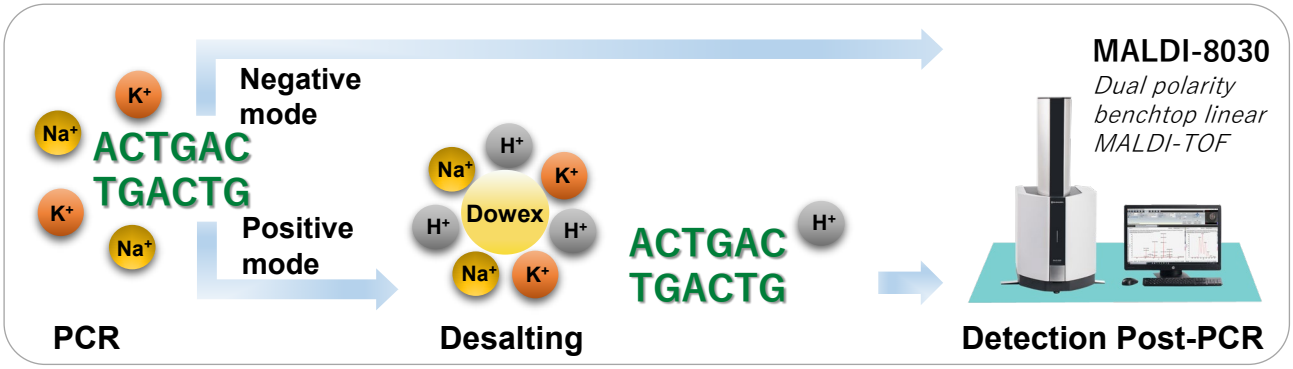


Fig. 2 Sample analysis workflow for synthetic oligonucleotides

We demonstrate the benefits of negative ionisation mode to simplify the sample preparation and interpretation of the mass spectra through elimination of salt adduct interferences.

We also show the separation and detection of oligonucleotides, which could be a useful alternative to gel electrophoresis/ ethidium bromide following PCR in the approaches described below (Fig 3).

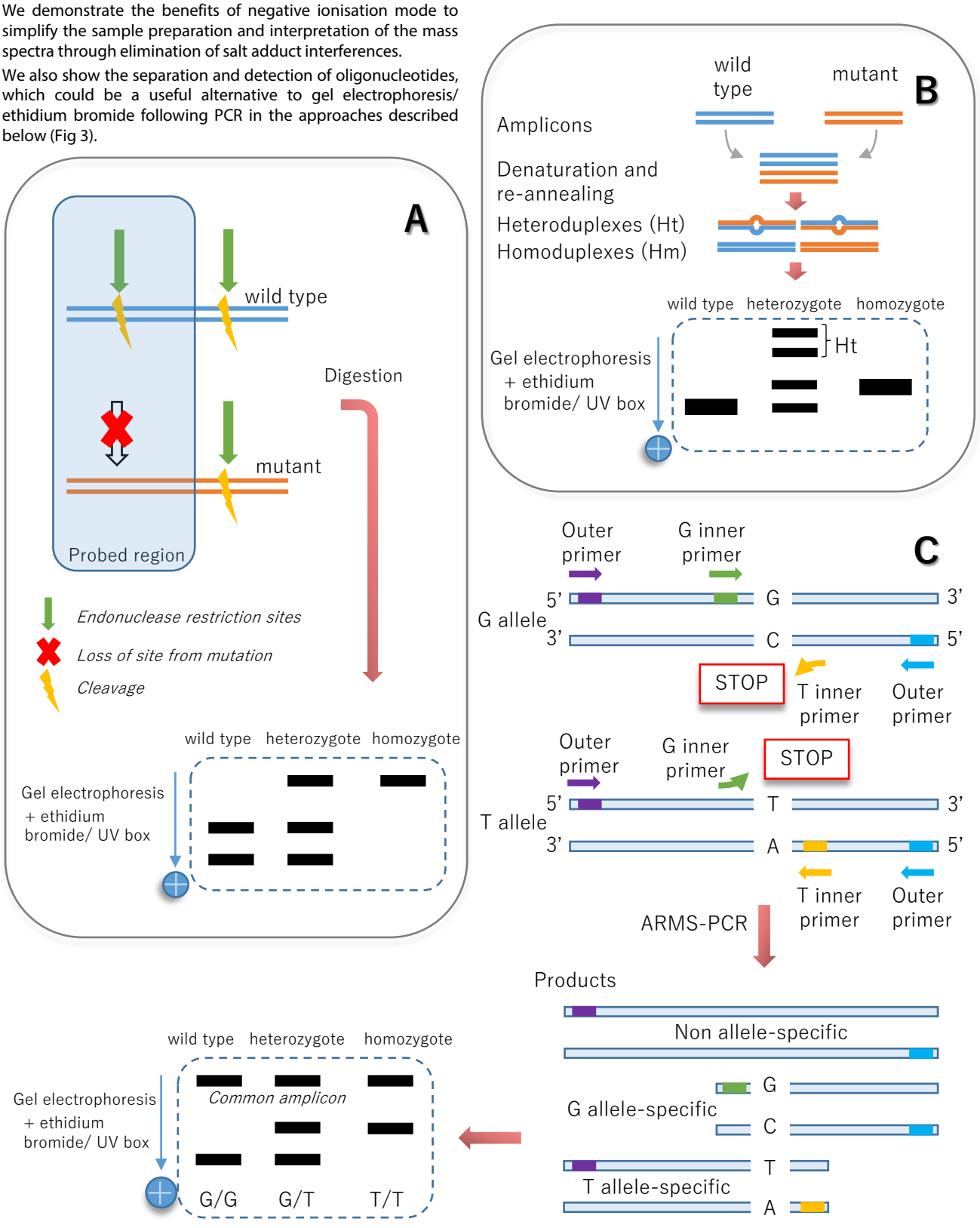


Fig. 3 Some common PCR methods where detection of oligonucleotide products are performed with gel electrophoresis, ethidium bromide/ UV box. A) Restriction fragment length polymorphism (PCR-RFLP); B) heteroduplex PCR analysis; and C) amplification refractory mutation system (ARMS-PCR). Images based on [4], [5], [6].

## ■ Measurement Conditions and Samples

A scenario where oligonucleotides had been generated following PCR was simulated. In this example, it was based on cystic fibrosis alleles. Samples of synthetic oligonucleotides, corresponding to the sequence of the CFTR gene where the mutation causing cystic fibrosis occurs, were purchased from Merck Life Science: ATCTTTGGTGTT (wild type/normal CFTR gene); ATTGGTGTT (Phe508del CFTR mutated gene). Ammonium citrate dibasic, Dowex® ion exchange resin and 3-Hydroxypicolinic acid (3-HPA) MALDI matrix were also purchased from Merck Life Science. Oligonucleotides were prepared at 100 µM in UHQ-water. Ammonium citrate dibasic was prepared at 5 mg/mL in 70:30 acetonitrile/water, which was used to prepare the 3-HPA matrix (45 mg/mL).

Sample desalting was carried out for the analyses in positive ion-mode. Dowex cation exchange resin works through exchange of hydrogens for sodium and other salts. No desalting was performed for the negative mode analyses. Samples were pre-mixed with matrix (1:2) prior to spotting onto the MALDI target.

MALDI analyses were conducted on the MALDI-8030 in positive and negative ion modes using the desalted and non-desalted samples, respectively. It is demonstrated that analysis in negative mode provides an advantage over the positive mode as it simplifies the sample preparation without compromising spectral quality.

## ■ Results of cystic fibrosis genotyping (synthesised oligonucleotides)

Fig 4 shows the MALDI negative mode spectra of the oligonucleotides which are representative of the different genotypes of cystic fibrosis: A) the subject has inherited the normal CFTR gene from both parents (wild type); B) the subject has inherited the mutated Phe508del CFTR gene from both parents (homozygote); C) the subject has inherited one normal CFTR gene and one mutated Phe508del CFTR gene (heterozygote).

The exact m/z values were calculated for the average [M – H]- species: m/z 3656.43 (ATCTTTGGTGTT, normal CFTR gene), and m/z 2758.85 (ATTGGTGTT, Phe508del CFTR mutated gene). All oligonucleotide species were detected with good mass accuracy.

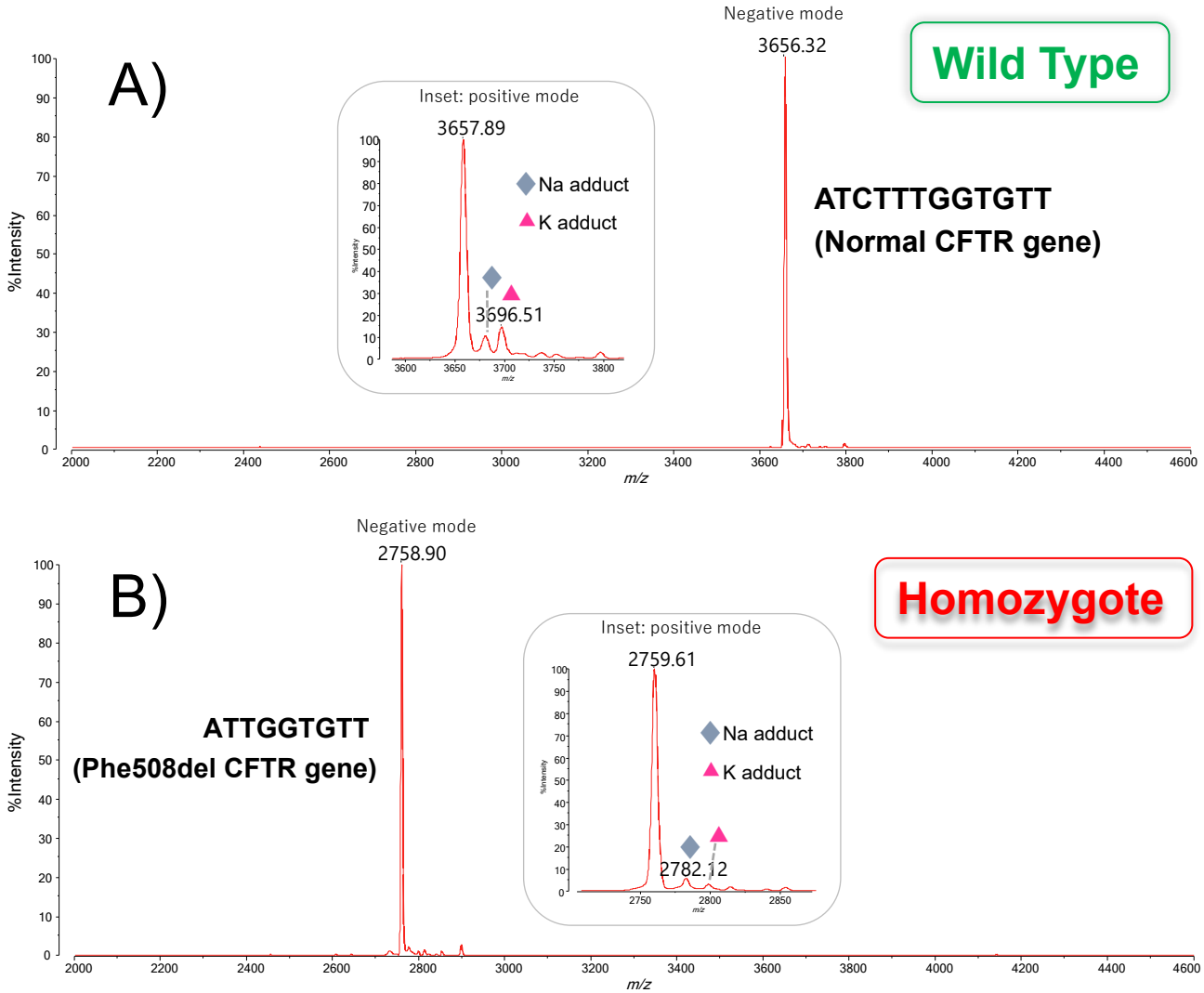


Fig. 4 (continued on next page). MALDI negative mode spectra of the oligonucleotides representative of the three different genotypes of cystic fibrosis: A) normal CFTR gene only (wild type). The inset in A) shows the corresponding oligonucleotide peak obtained with the positive mode analysis. The calculated m/z value for the average $[M + H]^+$ species is m/z 3658.44 (ATCTTTGGTGTT, normal CFTR gene). B) Phe508del CFTR mutated gene only (homozygote), thus expressing the disease. The inset in B) shows the corresponding oligonucleotide peak obtained with the positive mode analysis. The calculated m/z value for the average $[M + H]^+$ species is m/z 2760.86 (ATTGGTGTT, Phe508del CFTR mutated gene).

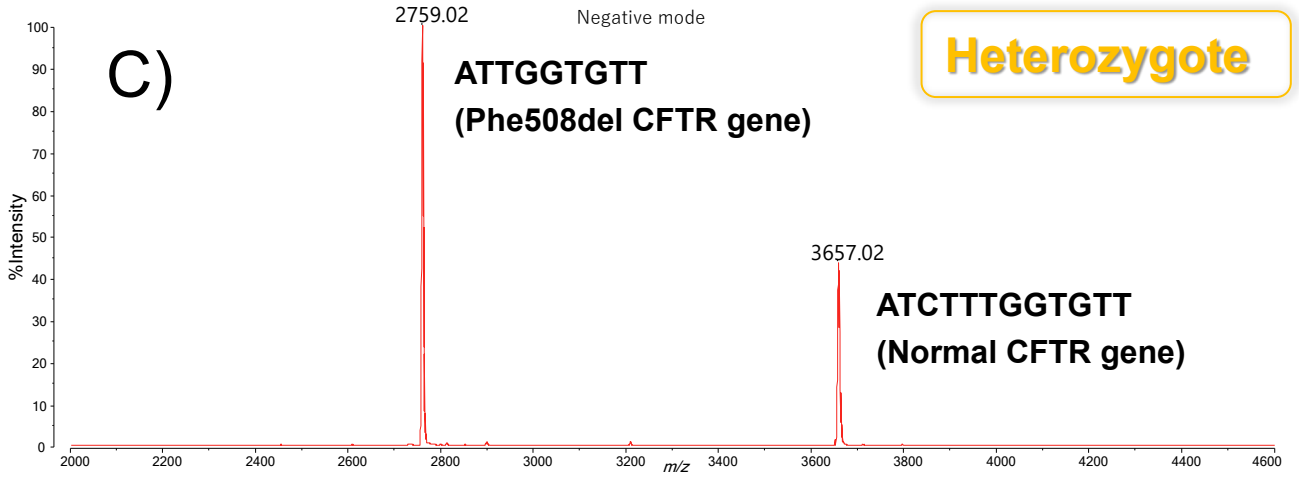


Fig. 4 (continued from previous page). Negative mode MALDI spectra of the oligonucleotides representative of the three different genotypes of cystic fibrosis: C) one normal CFTR gene and one Phe508del CFTR mutated gene inherited (heterozygote), therefore a carrier of the disease.

The insets in Fig 4A and Fig 4B spectra show the corresponding oligonucleotide peaks obtained with the positive mode analyses. As can be observed, even with desalting (cation exchange), some minor sodium and potassium adducts are still detected in positive ion mode. In contrast, the corresponding spectra obtained in negative mode are cleaner and free from salt adducts (no desalting is necessary).

Fig 4 A-C also shows the detection of the oligonucleotides is good using MALDI-TOF, as seen with the strong peak signals. The mass resolution also enables separation of the normal CFTR oligonucleotide and the Phe508del CFTR mutated oligonucleotide in the heterozygote (Fig 4C), therefore genotyping of all three outcomes for wild type, homozygote and heterozygote is easily possible.

## ■ Conclusion

This application demonstrates the capability of the dual polarity MALDI-8030 to easily detect and resolve synthetic oligonucleotides.

We demonstrated the benefits of the negative ion mode detection for eliminating the desalting sample clean up step in the analysis of oligonucleotides, while still producing good signal sensitivity.

The overall analysis workflow is simple and faster than when performed via gel electrophoresis. The technique could therefore be useful in training students in teaching laboratory sessions following PCR amplification, and also in more routine laboratories, for genotyping.

## ■ References

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