

# Application of the Amplifluor® SNPs Genotyping System using a FLUOstar OPTIMA microplate reader

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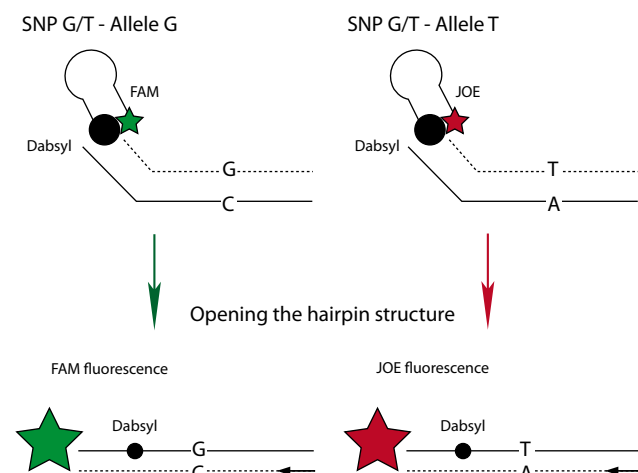
- SNPs were easily detected with the Amplifluor® System from Millipore
- Detailed protocol and instrument settings are shown for this assay
- BMG LABTECH's FLUOstar OPTIMA has proved to be an excellent reader for this assay

## Introduction

SNPs (Single nucleotide polymorphisms) are positions in genomic DNA with a single nucleotide difference between individuals of a species. SNPs are the most frequent mutations found in DNA sequences. These small genetic variations can occur in coding and non-coding regions of the genome and can either have an extreme or no effect on a particular organism. Therefore SNPs and their detection are important for medical research and pharmacology. The detection of SNPs (SNP Genotyping) can be achieved with a number of different methods. In this application note we describe a homogeneous fluorescence assay based on PCR. The Amplifluor® SNPs HT Genotyping System from Millipore was used to screen a number of samples with the help of a FLUOstar OPTIMA.

## Assay Principle

The method utilises two fluorescently labeled oligonucleotides that consist of a specific 3' primer sequence region and a 5' hairpin region that is labeled with either of two fluorophores (FAM or JOE). The presence of the 3' primer sequence enables these fluorescent oligo-nucleotides to work with target specific unlabeled primers designed to the researcher's specific target sequence. Thus, the method can be used for any variation of bases. (Fig.1)



**Fig. 1:** Principle of the Amplifluor® SNPs Genotyping System after addition of FAM and JOE coupled primers. Scheme is based on Millipore's patented method.

The researcher simply designs the allele specific primers containing 5' tails that match the 3' tails on the fluorescently labeled primers. In the example depicted in Fig. 1, the FAM labeled primer is G-specific whereas the JOE labeled primer is T-specific. A quencher dye (Dabsyl) is held within close proximity of each fluorophore by the 5' hairpin region of the oligonucleotide. After successful incorporation of the primers, the hairpin region is opened and the Dabsyl loses its ability to quench the fluorophores, resulting in a large fluorescence signal. The fluorescence produced during each PCR cycle directly correlates to the amount of amplified DNA. A more detailed description of this assay can be found at the Millipore website. (<http://www.millipore.com/techpublications/tech1/74k54f>)

## Materials and Methods

- Amplifluor® SNPs Genotyping System, Millipore
- Platinum® Taq DNA Polymerase, Invitrogen
- ABgene 1100 Thermo-Fast® PCR plate, Thermo Scientific
- FLUOstar OPTIMA microplate reader, BMG LABTECH (Figure 2)



**Fig. 2:** BMG LABTECH's FLUOstar OPTIMA multidetection microplate reader

All reagents needed for the amplification reaction (except polymerase) should be thawed from -20°C storage. DNA (5-150 ng/μL) that has been diluted in distilled water or TRIS-buffer (but not TRIS-EDTA buffer) is placed in a separate rack. All reagents are mixed thoroughly and vortexed briefly before preparing the amplification mixture. The cocktail of reagents is prepared at room temperature in a sterile 1.5 mL microcentrifuge tube. All reagents are added in the order presented in table 1. The reagents should be mixed with a pipette prior to dispensing.

**Table 1:** Amplification Cocktail Preparation: Reaction Volume -10 μL

Reagent	1 Rxn [μL]
dH <sub>2</sub> O	3.15-4.15
dNTPs (2.5 mM each)	0.8
20x amplifluor SNP FAM Primer	0.5
20x amplifluor SNP JOE Primer	0.5
20x SNP specific Primer Mix	1-2
10x Reaction Mix S Plus	1.0
Platinum Taq (5U/μL) <b>add at last minute</b>	0.05
<b>TOTAL</b>	<b>8.0</b>

DNA samples (2  $\mu$ L for 10  $\mu$ L reaction mix) are then transferred into a PCR plate. For mutant amplifluors; mutant, wild type, heterozygote and no DNA controls are used. The polymerase is then added to the amplification cocktail, mixed and spun down. 8  $\mu$ L of the amplification cocktail is added to each DNA sample/control. The plate is sealed with optically clear strips and then placed into a thermocycler and the PCR program is started.

For some assays a total reaction volume of only 5  $\mu$ L will also work. The volumes of individual reagents however, need to be adjusted accordingly.

#### PCR Program

1. 95°C 4 min
2. 95°C 10 sec
3. 60°C 20 sec
4. 72°C 40 sec
5. Go to step 2 for 34 times cycles
6. 72°C 3 min
7. 10°C hold

After completion of the PCR procedure the plate is cooled to room temperature. The plate can either be read stored at 4°C or spun down and placed into the FLUOstar OPTIMA for reading. The instrument is set to read FAM (Excitation 490-10 and Emission 520-10 nm) and JOE (Excitation 530-10 and Emission 560-10 nm) in bottom reading mode.

#### FLUOstar OPTIMA instrument settings

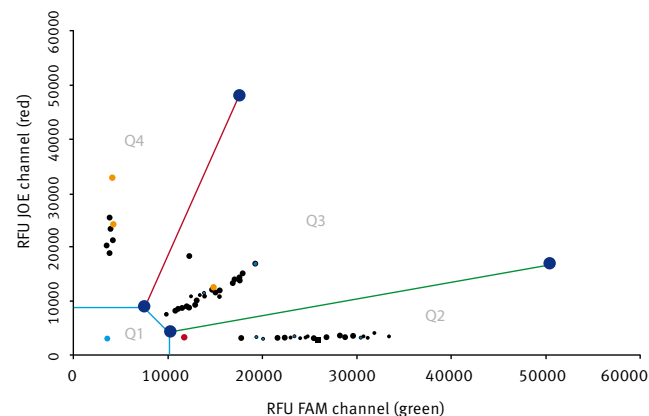
Measurement Mode: Fluorescence intensity  
 Reading Mode: Endpoint / flymode  
 No. of flashes per well: 3  
 Optic Settings: multichromatic; bottom reading

No.	Excitation	Emission
1	490-10	520-10
2	530-10	560-10

NB: You need to use 3 spacers for ABgene 1100 PCR plates.

## Results and Discussion

After measurement the data is transferred into an Excel Macro that can be downloaded at the Millipore homepage. A typical result is shown in figure 3.



**Fig. 3:** A typical pattern of samples. The blue dot in Q1 is buffer. The red dot in Q2 shows the mutant control (green allele), Yellow dots in Q4 are wild type control (red allele) and the orange dot in Q3 represents the heterozygote control (red green allele). Figure 3 clearly shows that all controls that are homozygous for either allele or heterozygous or a mixture only are clearly separated. In addition, samples are precisely located in their clusters.

## Conclusion

High S/B values indicate that the FLUOstar OPTIMA is an excellent instrument for running this assay. It is easily capable of measuring the output from the Amplifluor SNP Genotyping assay kit (Millipore) with high precision and with high throughput.

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