

Automatic genotyping assay using Scorpion™ primers, MBSR® thermocyclers and Fluoroskan Ascent®

Key Words

- SNP assay
- Scorpions
- Fluorescence
- HTS

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Introduction

Scorpions is a class leading PCR detection technology with significant benefits over comparable approaches. These include stronger signals, lower backgrounds, faster reactions, simplified design and improved Single Nucleotide Polymorphism tests.

Scorpions are bi-functional molecules containing a PCR primer element covalently linked to a probe element. The molecules also contain a fluorophore that can interact with a quencher to reduce fluorescence. When the molecules are used in a PCR reaction the fluorophore and the quencher are separated which leads to an increase in fluorescence from the reaction tube.

The benefits of Scorpions derive from the fact that the probe element is physically coupled to the primer element - this means that the reaction leading to signal generation is a uni-molecular rearrangement. The benefits of a uni-molecular rearrangement are significant - as the reaction is effectively instantaneous it occurs prior to any competing or side reactions such as target amplicon re-annealing or inappropriate target folding. This leads to stronger signals, more reliable probe design, shorter reaction times and better discrimination (Whitcombe, et al. 1999).

Scorpions primers can be used to detect SNPs by making the probe or primer sequences allele specific (Thelwell, et al. 2000). Allelic variants of a SNP can be detected in a single reaction by labeling the different versions of the probe with different fluorophores, typically FAM and ROX, though Scorpions are compatible with any fluorescent dye.

Materials and Method

Automation

Automation of the complete genotyping process is achieved with a Hamilton robotic sample handling system incorporating a Thermo Fluoroskan Ascent and Thermo MBSR thermocyclers.

Polymerase Chain Reaction

PCR is performed in ABgene Thermo-Fast® 384 PCR plates. This has two benefits in that the throughput achievable is increased when compared to 96-well plates and low volume reactions (10µl) can be used.

Plate Reading

Prior to PCR cycling the sealed PCR plates containing the reaction mixes are heated in the MBSR thermocyclers to 95°C, cooled to 22°C and the plate read on the Fluoroskan Ascent. This allows the Scorpions primers and quenchers to melt and reanneal resulting in a lower background fluorescence. The plates are then subjected to optimised cycling conditions.

With 384 well PCR plates, the small (1.5mm) beam facility is set

on the Fluoroskan Ascent. Filter pairs 485nm/538nm and 584nm/612nm are ideal for measuring FAM and ROX in a multiplexed reaction on the Fluoroskan Ascent.

Scoring Genotypes

Genotyping results are scored by examining the change in fluorescence as follows:

$$\frac{(\text{Post-PCR Fluorescence})}{(\text{Pre-PCR fluorescence})} = \Delta F$$

ΔF is normalised on a scale of 0-1 by dividing all ΔF values by the maximum ΔF value for each fluorophore:

$$\frac{(\Delta F)}{(\Delta F_{\text{max}})} = F_n$$

This has the effect of applying equal weight to each fluorophore. Genotyping is scored by plotting the difference in normalised fluorescence divided by the total change in fluorescence. This normalises for between well differences in liquid delivery and fluorescent reading of the plate:

$$\frac{(F_{na} - F_{nb})}{(F_{na} + F_{nb})} \text{ where } a \text{ and } b \text{ are the 2 multiplexed dyes}$$

Reactions that fail to amplify will generally be scored as a heterozygote. It is necessary to be able to distinguish failed reactions from genuine heterozygotes. Failed reactions can be called by total normalised fluorescence:

$$(F_{na} + F_{nb})$$

Results

Multiplexing FAM and ROX labelled allele specific Scorpions primers in a 384-well plate format give very clear and reproducible results

The ability to identify reactions that have failed is essential for genotyping with confidence. Identifying failed reactions is a simple task when using multiplexed Scorpions primers, in a 384-well plate format and measuring fluorescence with the Fluoroskan Ascent.

Conclusions

Stronger signals and lower backgrounds make Scorpions primers ideal for use in assays where the total assay volume is limited i.e. in a 384-well plate format. The ease of multiplexing makes Scorpions the assay of choice for single tube closed format genotyping.

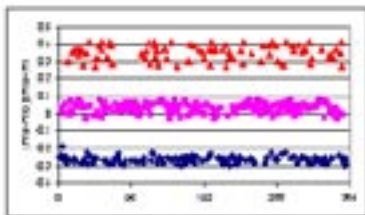


Figure 1. Human DNA mismatch repair protein homologue (hMLH1) genotyping using two labelled Scorpions primers.

References

Thelwell, N., Millington, S., Solinas, A., Booth, J. and Brown, T. (2000) - Mode of Action and Application of Scorpion Primers to Mutation Detection. *Nucleic Acids Research* 28(19), 3752-3761.

Whitcombe, D., Theaker J., Guy, S.P., Brown, T., Little, S. (1999) - Detection of PCR products using self-probing amplicons and fluorescence. *Nature Biotechnology* 17, 804-807.

DxS Ltd is a leading provider of genetic analysis services to the healthcare industry. They offer a full range of pharmacogenomics services using proprietary Scorpions technology. DxS Ltd provides research genotyping services including high throughput genotyping for target identification and validation. Their services are valuable throughout the drug development process and can assist in the identification and selection of new leads, help identify unsuitable targets and candidates early in the process, reduce the cost and increase the effectiveness of clinical trials, give competitive advantage to a therapy.

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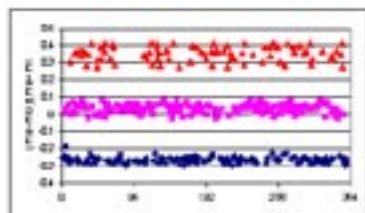


Figure 2. Total normalised fluorescence used to identify wells where no amplification occurred. The first 96 wells of this 384-well plate contained no template the remaining wells contained human DNA at 1 ng/ μ l as a template.