



MRT™ for SSRs - Enabling low-cost high-throughput PCR assays

Background

The Molecular Plant Breeding CRC has developed a new technology that permits the low-cost, high throughput analysis of SSRs on an automated DNA fragment analyser. The technology provides a number of advantages over other methods including reduced marker deployment costs, substantially reduced PCR assay costs via multiplexing, and a high amenability to automation. The technology comprises a single-step, closed-tube multiplex PCR assay that combine the principles of the M13-tailed primer method (Oetting et al. 1995) and two-step multiplex PCR amplification (Belgrader et al. 1996).

Multiplex-Ready Marker Technology™ facilitates faster, cheaper and higher-throughput automated multiplex PCR assays . This technology is being used or evaluated by several research groups and cereal breeding programs in Australia.

Technology Value Proposition

The delivery of faster, cheaper and more automated multiplex PCR assays offering potentially significant cost savings through a reduction in labour & consumables.

The flexibility of MRT™ brings advantages to applications where one has a reasonably large set of markers and wants to run different subsets of these for different sets of samples:

- Genetic mapping(because each population will have different polymorphisms)
- Marker-assisted selection
- Any application in a service lab where the client is specifying which marker they want run (or which genomic regions they want to look at)

Disclaimer: The information and claims contained in this document are based on sets of assumptions for particular case scenarios. Individual circumstances may vary between different users of the technology and results may therefore be different.



Relating benefits of MRT™ to applications

Setup Costs

- Lower cost due to fewer dye-labeled primers. Only four dye-labeled primers are required to assay any number of markers in MRT™. In contrast, each primer set is dye-labeled in conventional PCR, i.e. if there are 10 markers then 10 dye-labeling reactions are required.
- Lower setup cost is particularly advantageous for projects in which not all of the primer synthesised is used for PCR. For example, a typical conventional dye-labeled primer costs AUD\$170 (approx. US\$125)¹ from Applied Biosystems and is sufficient for about 10,000 reactions. If only 1000 assays are performed then the cost per reaction is 17¢ (approx US 0.13¢). In contrast, a MRT primer set costs \$AUD20 and typically is sufficient for at least 20,000 reactions. If only 1000 assays are performed then the cost per reaction is 0.1¢ (less in \$US).

PCR Assay

- MRT™ assays (both uniplex and multiplex reactions) are performed under standardized conditions. In contrast, markers amplified in conventional PCR may require different reaction conditions including annealing temperatures and reagent concentrations.
- Markers can be detected in any dye-detection channel during electrophoretic separation as MRT™ products are labeled during PCR amplification. In contrast, reaction products amplified using dye-labeled locus-specific primers in conventional PCR can only be detected in the dye channel corresponding to the fluorescent dye attached to the primer set.
- For all types of projects, the standardised reaction conditions and flexible dye labeling options of MRT™ can improve throughput, reduce consumable and labour costs, and facilitate the development of multiplex assays.
- Multiplex PCR assay development is simplified because the only variable limiting the combination of markers is PCR fragment size. In contrast, the development of conventional multiplex assays is limited by several factors including similarity of reaction conditions, dye-label attached to the locus-specific primers, and PCR fragment size.
- MRT™ can improve throughput and reduce labour and consumable costs because standardised reaction conditions means that PCR setup is more generic, and the entire reaction plate can be used because there is no requirement to partition markers into groups according to their optimal reaction conditions.

Relative Uniformity of PCR Yield

- MRT™ produces a relatively uniform yield of amplification product for each marker within a multiplex PCR assay and between independent reactions. This facilitates automated marker scoring, improves scoring accuracy and call rates, and can increase the number of markers separated per capillary. The latter is achieved because of the potential to pack markers more tightly both within the same, and different, dye detection channels.
- For all types of projects, a relatively uniform PCR yield can reduce separation and labor costs. It is an especially useful property as it enables the reliable separation of multiple DNA samples in the same capillary; i.e. four DNA samples amplified with the same marker panel can be separated in a single ABI3730 capillary by labeling the MRT™ products for each DNA sample with a different fluorescent dye.

¹ Assumes an exchange rate of AUD \$1.00 = US \$0.75



Practical examples

(1) Genetic Diversity Studies

Scenario

- Typically involve a small number of markers and DNA samples per experiment, however many different experiments may be performed
- Typically have a limited potential for multiplex PCR
- Allele size ranges (the size range of PCR fragments amplified for each marker) are often unknown, or “ball-parked”

MRT™ Benefits

- Reduced primer synthesis costs, especially if not all of the synthesised primer is used for PCR.
- Increased throughput and reduced PCR consumable costs because all assays are performed under standardised conditions.
- Flexible dye labeling means that marker panels can be reconfigured if allele size ranges are outside of expected ranges. Additionally, the ability to reconfigure marker panels once exact allele sizes are known provides an opportunity to further reduce separation costs because markers can be optimally packed for each experiment.
- Flexible dye labeling can reduce separation costs by minimizing the number of capillaries required for an experiment. For example, a multiplex marker panel assayed on 96 samples can be separated using 24 capillaries if four sets of 24 individuals are each assayed using a different fluorescent dye. To achieve the same outcome in conventional PCR, each locus-specific primer set in the marker panel would need to be dye-labeled with each of the four fluorescent dyes.
- Data analysis benefits related to relatively uniform PCR yield.



(2) Genetic Mapping Projects

Scenario

- Typically involve large numbers of markers and DNA samples per experiment
- High potential for multiplex PCR
- Typically the markers used in each experiment are different due to the requirement for markers to be polymorphic
- Even if the same set of markers is used in different experiments, a multiplex marker panel may not be transferable due to variation in allele size ranges found between DNA samples.

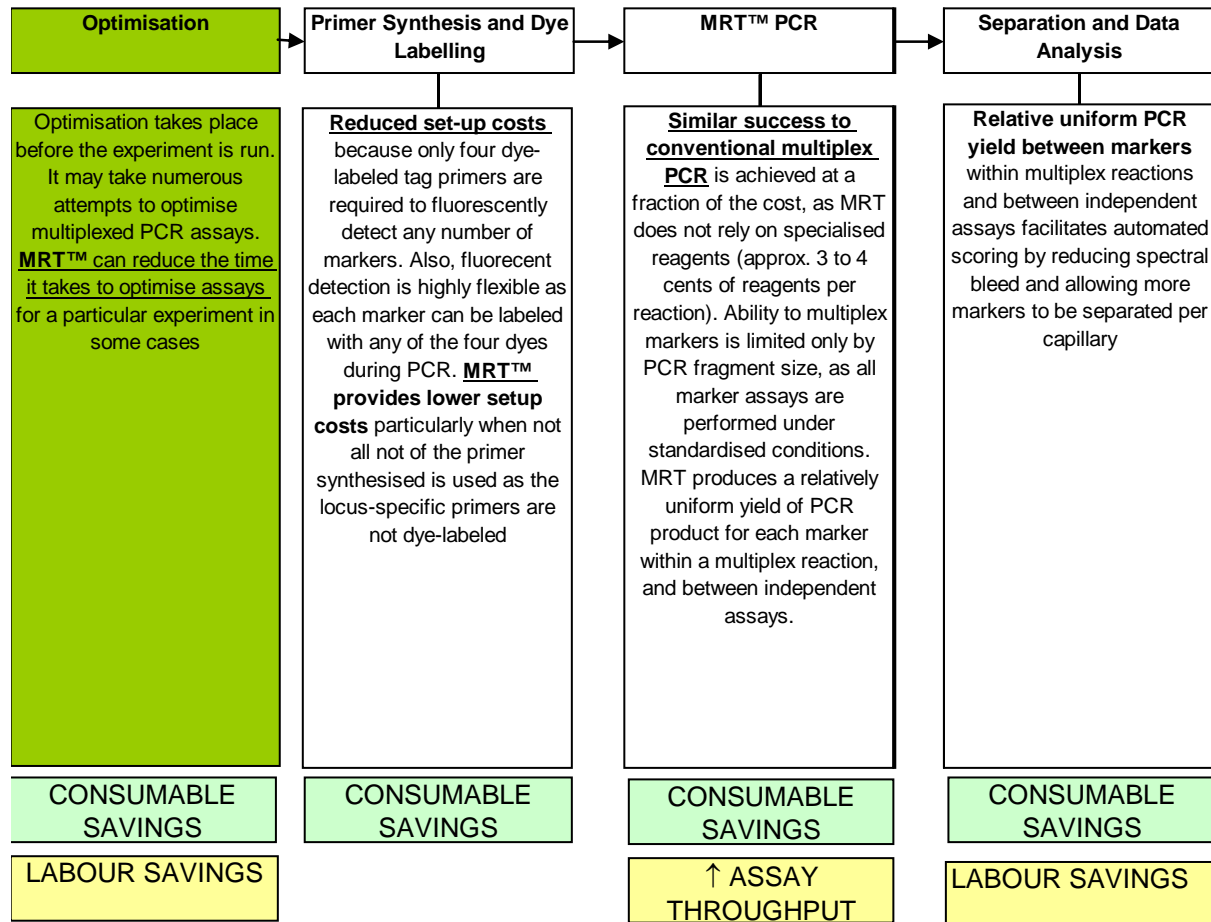
MRT™ Benefits

- Reduced primer synthesis costs may be a consideration, especially if primer sets are synthesised with more than one fluorescent dye (i.e. the synthesis of two dye-labeled locus-specific primers is required for the detection of a marker in two detection channels during electrophoretic separation in conventional PCR)
- Simplified and flexible development of multiplex PCR assays because the combination of markers is restricted only by PCR fragment size.
- Improved assay throughput and reduced PCR consumable costs because all assays are performed under standardised conditions.
- Potential for significant reduction in PCR consumable costs when performing multiplex PCR, as MRT™ doesn't not rely on specialized reagents.
- Potential increase in throughput and reduced separation costs due to uniformity of PCR yield allowing more markers to be separated per capillary.
- Data analysis benefits related to relatively uniform PCR yield.



MRT™ versus conventional approach comparison diagrams

Using MRT™





Conventional Multiplex PCR comparison

