T7 amplification for generating single stranded target for hybridization to allele-specific DNA microarrays
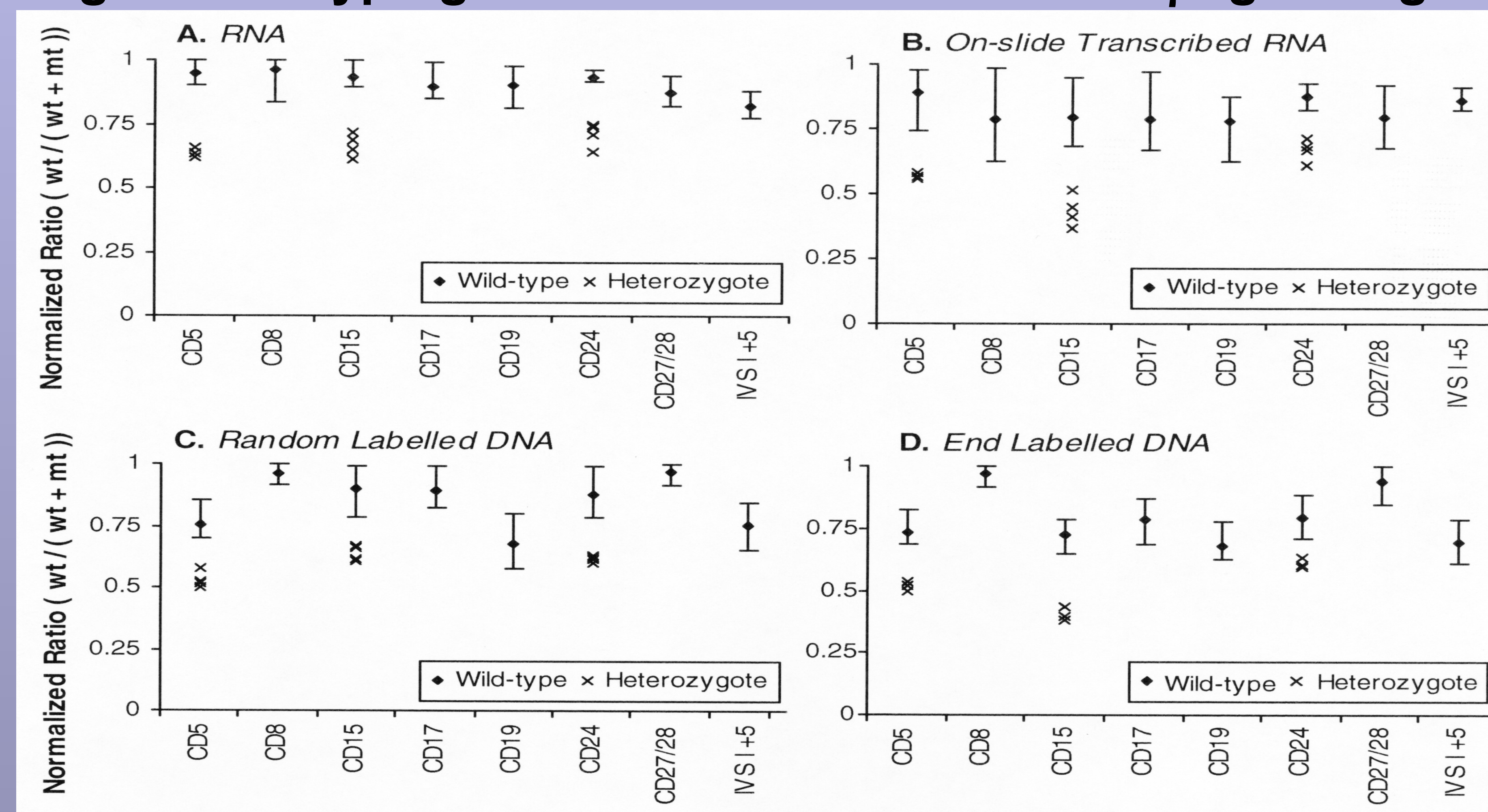
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Introduction

Amplified RNA (aRNA) synthesized by T7 linear amplification is frequently used for gene expression analysis on microarrays. Single stranded DNA (ssDNA) is however mostly used as target in microarray based genotyping assays, although there are several advantages with aRNA. Here, we compared the performance of aRNA and ssDNA as targets in a genotyping assay, comprising a DNA microarray of allele specific oligo probes specific for thalassemic mutations.

Fig 1. Comparison of hybridization signal

The signal intensities from the four differently prepared targets were normalized to an average of four individual observations from the in-tube RNA transcription (RNA). Each bar represents the normalized average of four individually hybridized arrays containing a total of 128 spots. Error bars represent SEM.

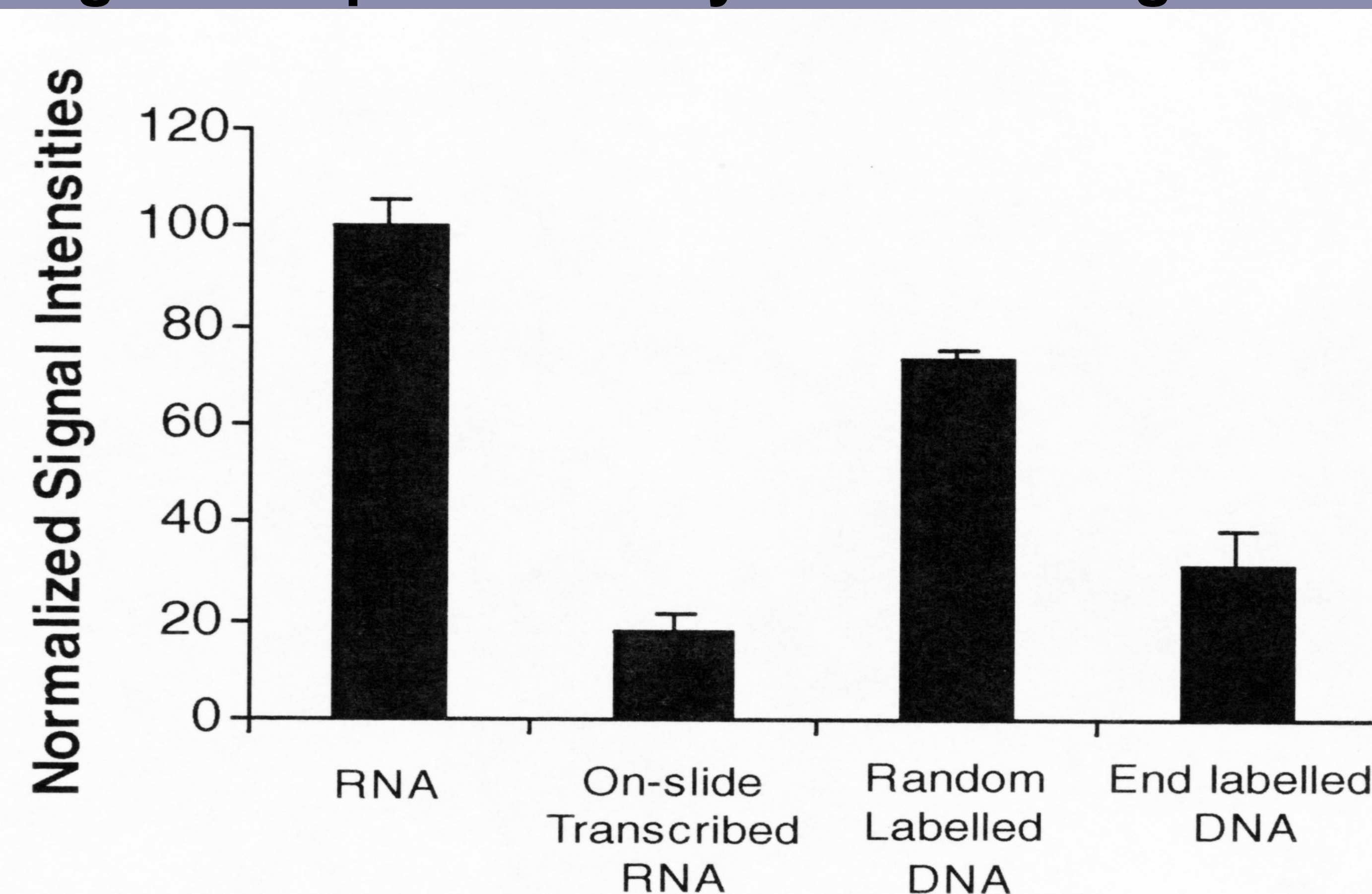## Fig 2. Genotyping carriers of mutations in the β -globin gene

Genotyping of twelve carriers of mutations in the β-globin gene using DNA microarrays hybridized with four differently prepared targets (A-D). Normalized ratios were calculated as the relation between the wild-type signals compared to the sum of the wild-type and mutant-type signals. Error bars represent the interval in which wild-type samples were observed.

Results

A five-fold increase in signal intensities was observed when comparing microarrays hybridized with T7 amplified RNA to end-labelled ssDNA and a 50% increase compared to random-labelled ssDNA (Fig 1). The differently prepared targets were all used to correctly genotype all tested β-thalassemia carriers (Fig 2). Using a t –tests we calculated, that target prepared by T7 in vitro transcription (IVT) in tubes resulted in an error frequency in genotyping in only about one in four million for each mutation investigated. For comparison on-slide target preparation by T7 IVT had an error frequency of one in ten thousands for each mutation investigated, end-labelled ssDNA about one in two thousands and random-labelled DNA one in six thousand.

Methods

Single stranded target for hybridization reactions was produced by four different target preparation methods. T7 IVT yielding aRNA was synthesized in PCR tubes or on-slide under a cover slip. ssDNA was obtained by single primer elongation using random-labelling or end-labelling. No special precautions were taken for working RNase free.

Conclusion

The preformance of genotyping using amplified RNA was 700-fold to 2000-fold better compared to hybridization with ssDNA, indicating that T7 in vitro transcription is the superior method to produce target for hybridization on microarrays. Other advantages using aRNA generated by T7 IVT include
• Easy to multiplex
• Simple chemical fragmention of RNA possible
• Isothermal reaction that can be integrated in lab-on-a-chip devices.