

## Supplementary information

### Rapid amplification of *Mycobacterium tuberculosis* DNA on a paper substrate

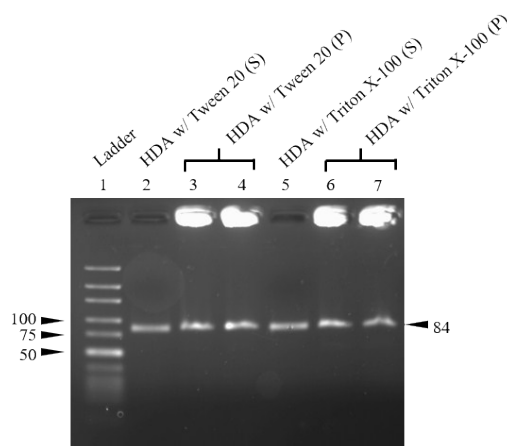
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#### 1. Generating the template DNA for HDA by PCR of MTB genomic DNA

An 84 bp fragment from the insertion sequence 6110 (IS6110) of *M. tuberculosis* (MTB) genomic DNA (H37Rv strain) was first amplified by PCR to generate the template for all HDA reactions. The 84 bp fragment was amplified with the forward primer sequence 5'-CAACAAGAAGGCGTACTCGACCTGA-3' and the reverse primer sequence 5'-CTCGCTGAACCGGATCGATGTGTACT-3'.<sup>24</sup> The main reaction components for PCR were 2X master mix (0.05 U/μl Taq DNA polymerase, reaction buffer, 4 mM MgCl<sub>2</sub> and 0.4 mM of each dNTP), primers (0.2 μM), template DNA (18 nM) and water. The PCR program had an initial denaturation step at 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, 65°C for 30 sec and 72°C for 1 min, and finally an extension step of 72°C for 10 min. 5 μl of the amplified DNA was first run on a 4 % agarose gel stained with ethidium bromide (0.5 μg/ml) to verify successful amplification. The amplified PCR product from the remaining 45 μl amplification mixture was then purified using phenol:chloroform:isoamyl alcohol (25:24:1,v/v) and ethanol precipitation and suspended in 10 mM Tris-Cl. The concentration of the purified DNA was measured by a NanoDrop instrument.

#### Optimization of blocking agents

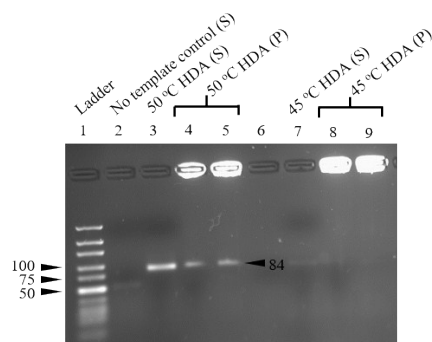
The paper substrate was treated by a blocking agent for reproducible amplification of MTB DNA. Apart from BSA, Tween 20 (0.05%) and Triton X-100 (0.05%) were also explored as blocking agents. After treating the paper with either of these reagents, HDA was performed at 65 °C for 60 minutes. While we could have used any of the three blocking agents, we chose to perform all our experiments with BSA. From fig. S1, it can be seen that Tween 20 and Triton X-100 both act as efficient blocking agents for amplifying MTB DNA on paper.



**Figure S1:** Effect of blocking agents on HDA amplification efficiency. (P) indicates reactions on paper and (S) indicates reactions in solution. The ladder has DNA fragments in 10-300 bp size range (lane 1). HDA in solution were performed as positive controls for 0.05% Tween 20 (lane 2) and 0.05% Triton X-100 (lane 5). Reactions were performed in duplicates on paper treated with 0.05% Tween 20 (lanes 3-4) and 0.05% Triton X-100 (lanes 6-7) at 65°C for 60 minutes with 10<sup>10</sup> copies of template DNA. The bright 84bp bands indicate that treating the paper with these blocking agents had no deleterious effects on the amplification efficiency.

#### Optimization of reaction temperature

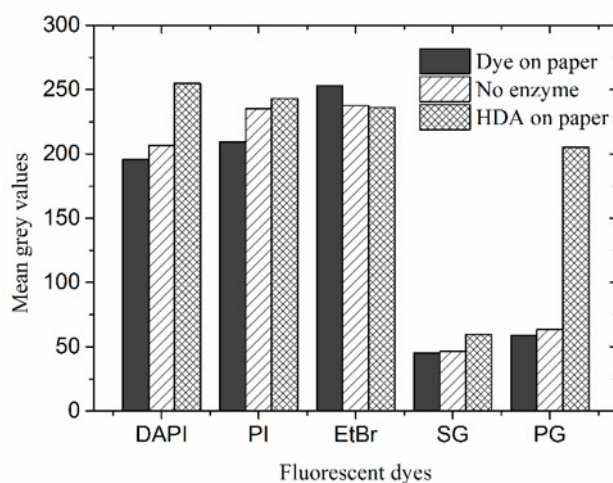
Different incubation temperatures were explored to find the lowest reaction temperature at which successful HDA takes place. Accordingly, incubation temperatures between 65°C and 45°C at intervals of 5°C were explored. Successful amplification occurred until 50 °C. Fig. S2 shows the gel data for HDA at 50 °C and 45°C. Reactions on paper were performed in duplicates



**Figure S2:** Optimization of HDA temperature. (P) indicates reactions on paper and (S) indicates reactions in solution. The ladder is of 10-300 bp range (lane 1). No template control indicates HDA performed without the template DNA (lane 2). This figure shows the results of HDA at 50°C in solution (lane 3) and on paper (lanes 4-5) and at 45°C in solution (lane 7) and on paper (lanes 8-9) with  $10^{10}$  template DNA copies and 60 min incubation time. A faint primer-dimer band can be seen in no template control. Amplification can be seen at 50°C, but no bands are observed at 45°C.

### Screening of fluorescent dyes for nucleic acid detection on paper

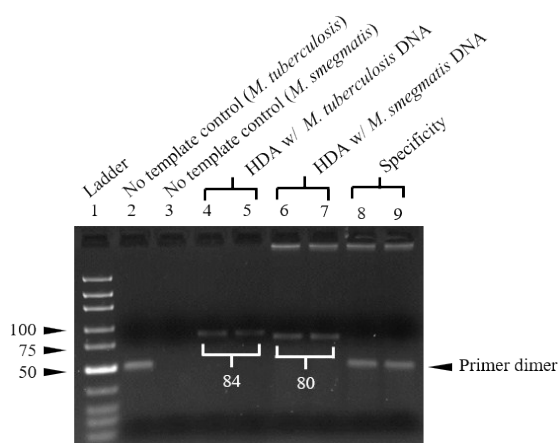
Common fluorescent dyes, such as, DAPI, propidium iodide (PI), ethidium bromide (EtBr), SYBR Green (SG) and PicoGreen (PG) were initially explored to detect the DNA amplified by HDA on paper. Screening of fluorescent dyes was optimized without a wash step with single reactions on paper substrates. The dye concentrations used for optimization are described in the main paper. After amplification on paper at 65°C for 60 min with an initial template of  $10^{10}$  copies, the paper with amplified DNA was dried and then spotted with fluorescent dyes. The incubation period was 5 min for PG and 15 min for the other dyes. Grey scale images of EtBr and SG were taken with a UV gel doc. For, DAPI, PI and PG, an inverted fluorescent microscope was used to capture grey scale images based on their respective ex/em. From fig S3, it can be seen that, there is a significant difference between no enzyme control and positive control when PG is used. For EtBr, the intensity for dye on paper is higher than the positive control. This is because EtBr binds more strongly to cellulose. Although paper has its own auto-fluorescence in the UV-range, the fluorescence of paper is greatly enhanced after binding to EtBr. This can be further confirmed with agarose gel figures (fig. 2-4 and S1-2), where paper can be seen fluorescing inside the well.



**Figure S3:** Screening of fluorescent dyes for HDA on paper. We screened DAPI, propidium iodide (PI), ethidium bromide (EtBr), SYBR Green I (SG) and PicoGreen (PG) for detection of DNA amplified on paper without a wash step. No enzyme control indicates HDA performed without any enzyme mix. Free dye added to the paper substrate was used as another negative control. PG showed a much larger increase in fluorescence compared to the other dyes and was chosen for further experiments.

## Specificity of MTB primers used in HDA

We tested the amplification specificity of the HDA primers to MTB genomic DNA. We performed HDA on *Mycobacterium smegmatis* (mc<sup>2</sup>155 strain) genomic DNA with the MTB primers (specific to IS6110 fragment). *M. smegmatis* was chosen for the specificity test as it belongs to the *Mycobacteria* species and share many sequences with MTB. Since IS6110 sequence is not present in mc<sup>2</sup>155 strain of *M. smegmatis*, this reaction is not expected to give any bands. Two positive control reactions were also performed: (a) amplification of an 80 bp fragment of the *rpoB* gene of *M. smegmatis* genomic DNA with primers specific to *M. smegmatis*, and (b) amplification of an 84 bp fragment of the IS6110 gene of *M. tuberculosis* (H37Rv strain) genomic DNA with MTB specific primers. All reactions were performed at 65°C for 60 min. Figure S4 shows the gel electrophoresis results. While the target sequences from both MTB genomic DNA (84 bp) and *M. smegmatis* genomic DNA (80 bp) could be amplified with their respective primers, amplification of *M. smegmatis* DNA with MTB primers did not give any bands. This shows that the assay we have optimized on paper is indeed specific to MTB.



**Figure S4:** Specificity of primers chosen for HDA of MTB DNA. (P) indicates reactions on paper and (S) indicates reactions in solution. The figure shows the results of HDA performed on *M. smegmatis* genomic DNA using MTB-specific primers. The ladder used is of 10-300 bp range (lane 1). No template control indicates HDA performed without any template DNA, but with specific primers (lanes 2-3). The two positive controls (in duplicates) are HDA performed with specific primers for MTB (lanes 4-5) and *M. smegmatis* (lanes 6-7). MTB genomic DNA gave an 84 bp band and *M. smegmatis* genomic DNA gave an 80 bp band. To check the specificity of the primers used in our assay, HDA was performed on *M. smegmatis* DNA with MTB specific primers (lanes 8-9). However, no bands can be seen in the gel, thus confirming that the assay we used is indeed specific to MTB DNA.