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Supporting Information

A novel combined capillary chip for rapid identification of gene mutation

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15 **1. Optimized probe application solution**

To study the effect of dilution buffer on fixation of probes, we diluted Conj.control in PBS or Tris-HCl and at various pH values, or in ddH$_2$O (pH 7.0) at the final probe dilution of 10 μM. The mixture was then added into the glass micro-groove and exposed to UV$_{254nm}$ (0.1 J/cm^2) for 5 min. The developed chromogenic results are demonstrated in Figure 1. The hybridization region showed a regular pattern. An equal positive coloration was achieved with the probe dilution of 0.1 M PBS at pH 7.0~8.0. Thus, PBS with pH 8.0 was
20 selected as the optimal probe dilution buffer.

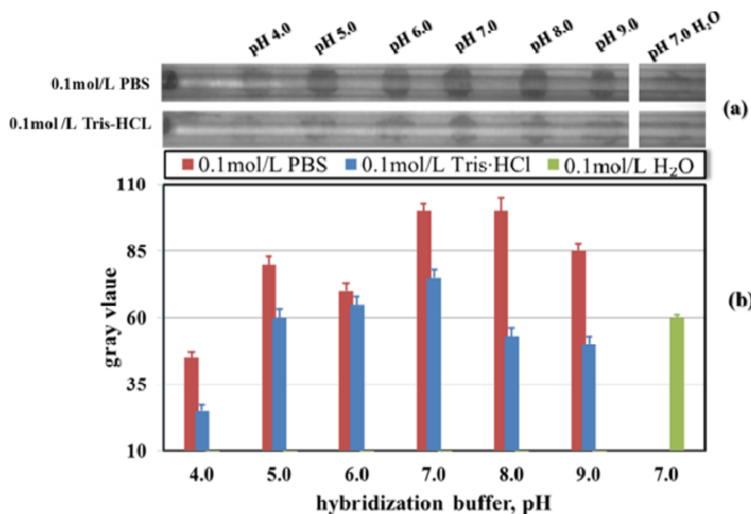

Fig.1 Determination of dilution buffer composition and pH for optimal fixation of probes. (a) Positive chromogenic signals achieved with different probe buffers were analyzed and the 0.1 M concentrations of PBS and Tris-HCl are presented at various pH values. (b) Histogram analysis of gray scale scanning of the positive signals in panel (a) (0.1 M PBS, solid black bars; 0.1 M Tris-HCl, black and white hatched bars). The white bar represents the ddH$_2$O pH 7.0 control. Data shown
25 represents mean (n=3) ± standard error.

2. Optimized probe concentration

To optimize the concentration of the probe, a range of 1~50 μM dilutions of Conj.control, Pro M, and Pro V were applied to the CCC assay. Chromogenic positive signal was developed directly for the Conj.control, and that for the other probes were detected through 1 nM
30 TarM in Figure 2. The signals of Conj.control and Pro M were greatly reduced when the probe concentration was under 10 μM. In contrast, the increase in positive signals plateaued at concentrations equal to or higher than 10 μM. Thus, the 10 μM was selected as the optimal probe dilution.

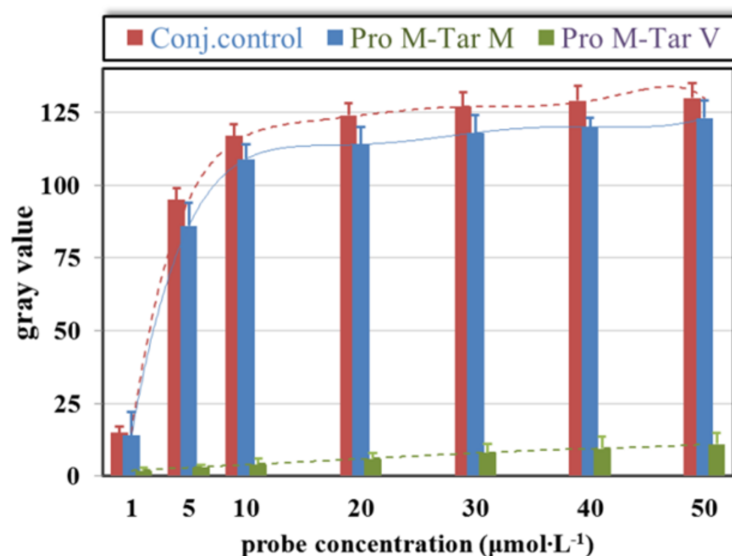

Fig.2 Determination of probe concentration for optimal fixation and hybridization. The reaction between Pro M or Pro V and 1 nM target gene Tar M was significantly enhanced. When the probe concentration was equal to or greater than 10 μM, the hybridization efficiency plateaued and the non-specific reaction between Pro V and Tar M was exponentially enhanced. Data shown represents mean (n=3) ± standard error.

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3. Optimized UV-crosslinking time

To determine the effect of UV-crosslinking time on probe immobilization, a 1~60 min range of UV exposure was applied to the 10 μM Conj.control and Pro M in the capillary micro-groove. The enzyme-linked positive signal was developed directly for Conj.control, and the signal for the other probes was detected through 1 nM Tar M or Tar V in Figure 3. The fixation efficiency of Conj.control increased 10 steadily with UV exposure time up to 10 min, and then decreased. The positive signal of Pro M was highest at 5 min of UV exposure, and decreased thereafter. Furthermore, when the UV exposure time reached 10~20 min, the Pro M probe showed non-specific hybridization.

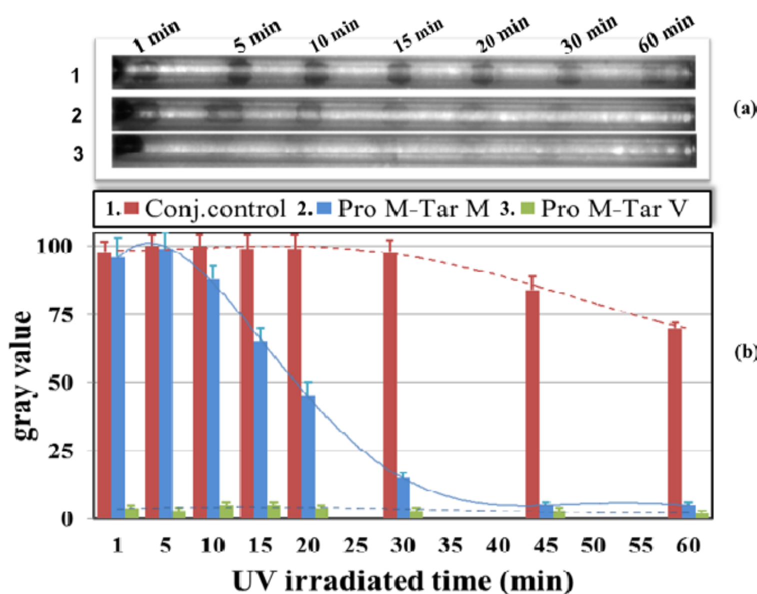

Fig. 3 Determination of UV-crosslinking time for optimal probe fixation. (a) The 10 μM Conj.control diluted in 0.1 μM PBS (pH 7.0) was added into chip No. 1, 15 and 10 μM Pro M was added into chips No. 2 and 3. The chips were exposed to UV from 1 to 60 min, as indicated. The enzyme-linked positive signal was developed directly for the Conj.control on chip No. 1, and that for chips No. 2 and 3 were detected through 1 nM Tar M and Tar V, respectively. (b) Linear graph of gray scale scanning results showing that the positive hybridization signal began to increase at 1 min of UV exposure. The effect of UV-crosslinking time on probe fixation reached 0 at 1 hour of UV exposure. The positive signal of hybridization began to decrease after 10 min of UV exposure. Data shown are mean (n=3) ± standard error.

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