Supplementary Materials for

Using a Multi-junction Microfluidic Device to Inject Substrate into an Array of

Preformed Plugs without Cross Contamination: Comparing Theory and

Experiments

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Figure S-1: (A) Schematic of fluorescence test for cross-contamination. Alternating plugs of fluorescein (green) and buffer (clear) were injected with buffer solution. During injection, if the fluorescein solution contaminated the buffer stream and was injected into the next buffer plug, fluorescence would be detected in the resulting buffer plug. Cross contamination was quantified by determining the ratio of the intensity of the buffer plug to the intensity of the fluorescein plug. Images of a buffer plug and a fluorescent plug, before and after injection, are shown. The average intensity (I) buffer plugs and fluorescein plugs before and after injection are shown below each image, with individual data points presented in (B) and (C), respectively. (B), (C) Graphs of fluorescence intensity of fluorescein plugs (green squares) and buffer plugs (open circles) before injection (B) and after injection (C). Buffer plugs do not show fluorescence, indicating a lack of cross-contamination. Separate arrays of plugs were used to obtain fluorescence

data before and after injection to avoid photobleaching (see Experimental Section in the main text for details).



Figure S-2: A control bioassay performed with the multi-junction device. Thrombin solution was injected into three substrate plugs containing: 1) MCA IIa, 2) MCA Xa, and 3) FDP. Only plug 1 gave rise to fluorescence after injection, indicating successful injection and minimal cross-contamination. Scale bar: 200 µm.



Figure S-3: Comparison of actual injection ratios to theoretical injection ratios predicted by flow rates. Theoretical injection ratio (v_{theo}) is defined as the volume of substrate injected divided by the original volume of the plug. Actual injection ratio (v) was defined as increase of plug length after injection divided by the original length of the plug and was obtained by measuring the actual plug length. Actual injection ratio v was compared

to the theoretical injection ratio v_{theo} controlled by the flow rates of both the red dye and green dye. Error bars for each point indicate an error below 3% for injection consistency.