Novel DNA Sensor System for Highly Sensitive and Quantitative Retrovirus Detection using Virus Encoded Integrase as a Biomarker.

- Supplementary Materials -

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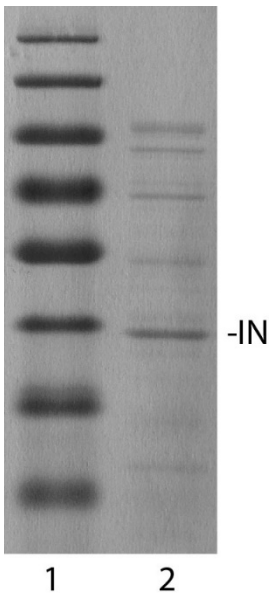
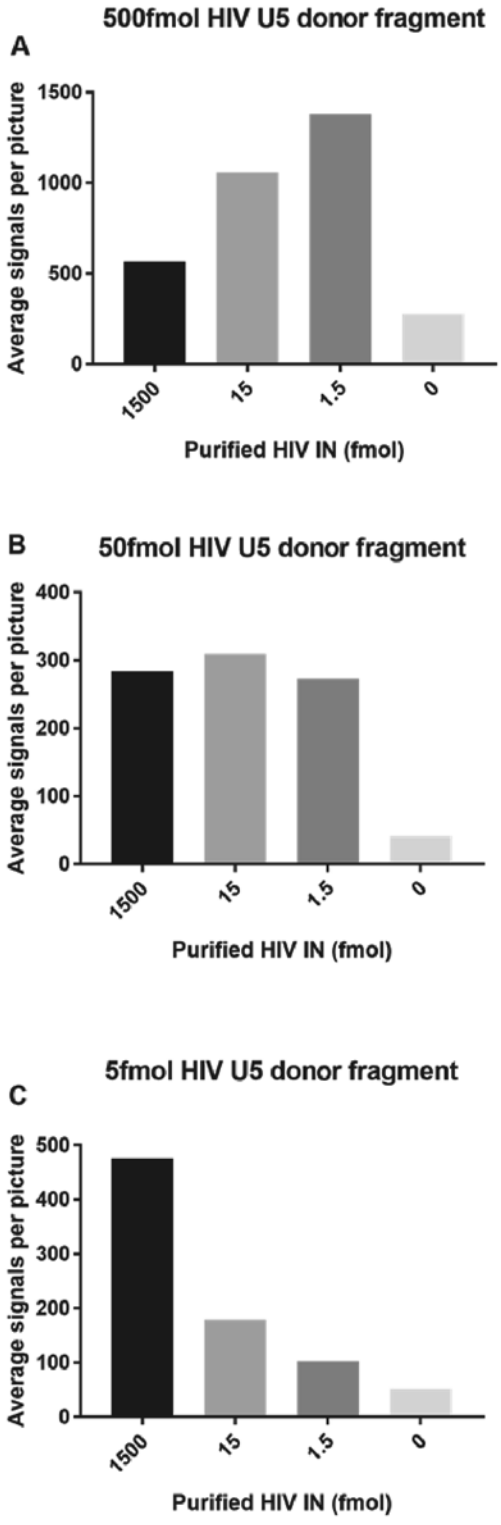


S1: Picture of Coomassie stained SDS polyacrylamide gel. Lane 1: size marker, lane 2: purified recombinant HIV IN.



S2. Optimization of the U5 donor fragment concentration for quantitative detection of HIV IN: As demonstrated in A) and B) printing of high concentrations (500 or 50 fmol total in the printed volume) of the U5 containing donor fragment compromise the expected quantitative relationship between the enzyme

concentration and signal numbers. Since the natural reaction of IN is to act as a tetramer, which integrates both ends of the viral DNA into the host genome, this may best be explained by integration of two or more U5 donor fragments into the acceptor circle at high enzyme concentrations. This would lead to fragmentation of the acceptor circle and result in loss of signals. As expected, considering this mechanism of action of IN, dilution of the U5 donor fragment to 5 fmol per printed volume resulted in the expected quantitative relationship between IN concentration and signal numbers.

To ensure the U5 donor fragment concentration was sufficient to cover the activity level of IN at the utilized concentration, the U5 donor fragment coverage on the printed area was calculated and compared to the number signals observed.

The water droplet is assumed to have a contact angle of 30° with the CodeLink glass slide. Given by the volume used for the printing, that is 2µL, the surface covered was estimated to be around 34 mm^2, where the radius of droplet is approximately 1.65mm. On the other hand, assuming a uniform distribution of U5 donor fragments (5fmol in 2µL) in the water drop, the molecules in contact with the CodeLink slide are estimated to be 17 x 10^8 molecules/mm^2. Taken together, if the coating efficiency is close to 50%, the total amount of U5 donor fragments conjugated on the CodeLink slide is approximately 3 x 10^{10} molecules (17 x 10^8 molecules/mm^2 x 34 mm^2 x 50%).

By the same token, assuming the range of interest (ROI) is approximately 0.1 mm^2, the substrates conjugated within the ROI are approximately 8.5 x 10^7 molecules (17 x 10^8 molecules/mm^2 x 0.1 mm^2 x 50%). The number signals observed are at least 5 order of magnitude lower than the estimated number of U5 donor fragments, which can potentially react with IN.