***Supplementary Materials for*:**

# Enzyme Modulated Cleavage of dsDNA for Supramolecular Design of Biosensors

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***DNA Electrophoresis***. Electrophoresis was performed in 1% Agarose (SIGMA), using 1X TAE (0.04 M Tris-Acetate, 0.002 M EDTA pH8.0) supplemented with 0.5 μg/ml ethidium bromide at 5 V/cm for 4 hours [Davis, L.G., Dibner, M.D., and Battey, J.F. Eds. *Basic Methods in Molecular Biology*. Elsevier, New York, **1986**.]. Uncut phagemid dsDNA and linearized phagemid dsDNA after R.E. digestion demonstrate similar transport profile under high-voltage driving force (Figure S1). Only one band could be obtained since there was no fragmentation. Furthermore, biotinylating and non-biotinylating plasmids after enzyme cleavage did not show much difference in eluting speed.

***HPLC Identification*** of uncut and linearized pBluescript II SK (-). HPLC analysis was carried out at room temperature using DIONEX DX 500 chromatography system together with an AD20 absorbance detector and AI 450 Chromatographic software. Peaks

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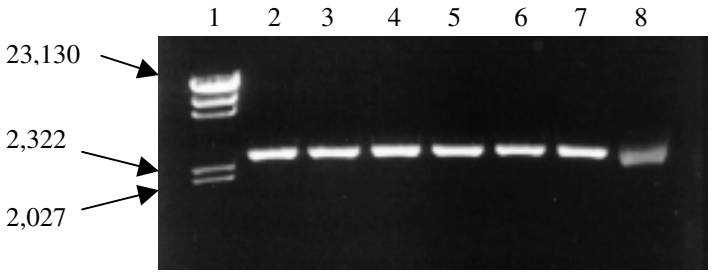
were obtained at 260nm using the absorbance detector. Column, IonPac® AS11 (4 × 250 mm); elution linear gradient from 0.8 to 1.2 M NaCl in 0.03 M sodium phosphate, pH 6.0, 4.2 M urea in 60 min; flow rate, 2 ml/min. [ Pingoud, A.; Fliess, A.; Pingoud, V. at *HPLC of Macromolecules: a Practical Approach* (Oliver, R.W.A. ed.), Oxford University Press: Oxford, 1989. Chapter 7.]

## Supplementary Figure Captions

**Figure S1.** Agarose gel electrophoresis of uncut and linearized pBluescript II SK (-) after enzyme cleavages.

**Figure S2**. Analytical anion-exchange HPLC of a) uncut pBluescript II SK (-) and b) linearized pBluescript II SK (-). A peak with different retention time is shown after overnight enzyme cleavage. Uncut pBluescript II SK (-) is eluted at a time (4.82 min) before linearized pBluescript II SK (-) (at 5.25min).




1. 0.6µg λ/Hind III
2. 0.25 µg plasmid/Kpn I
3. 0.25 µg plasmid/Kpn I
4. 0.25 µg plasmid/BamH I
5. 0.25 µg plasmid/BamH I
6. 0.25 µg plasmid/ BamH I after biotinylation
7. 0.25 µg plasmid/ BamH I after biotinylation
8. 0.125 µg plasmid/uncut

**Figure S1**

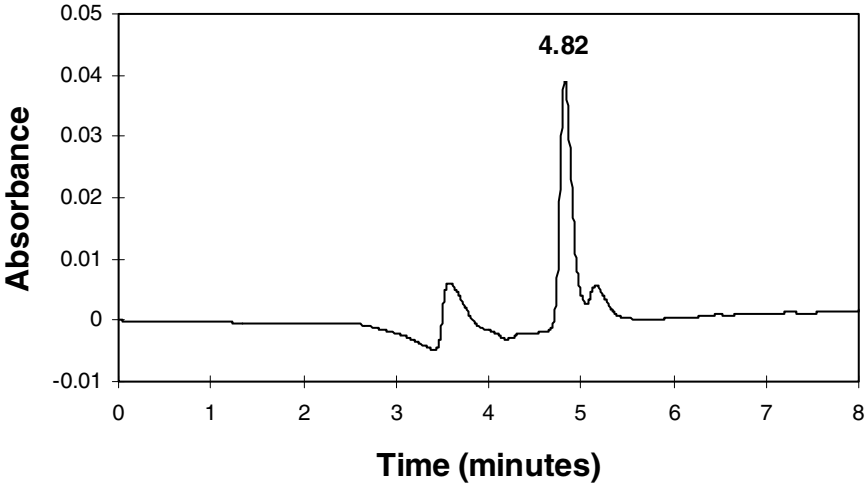



**Figure S2 a)**

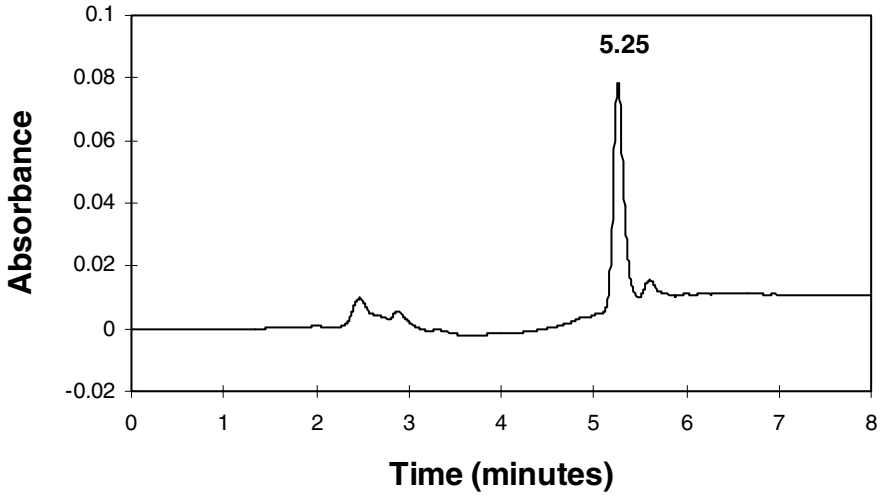



**Figure S2 b)**