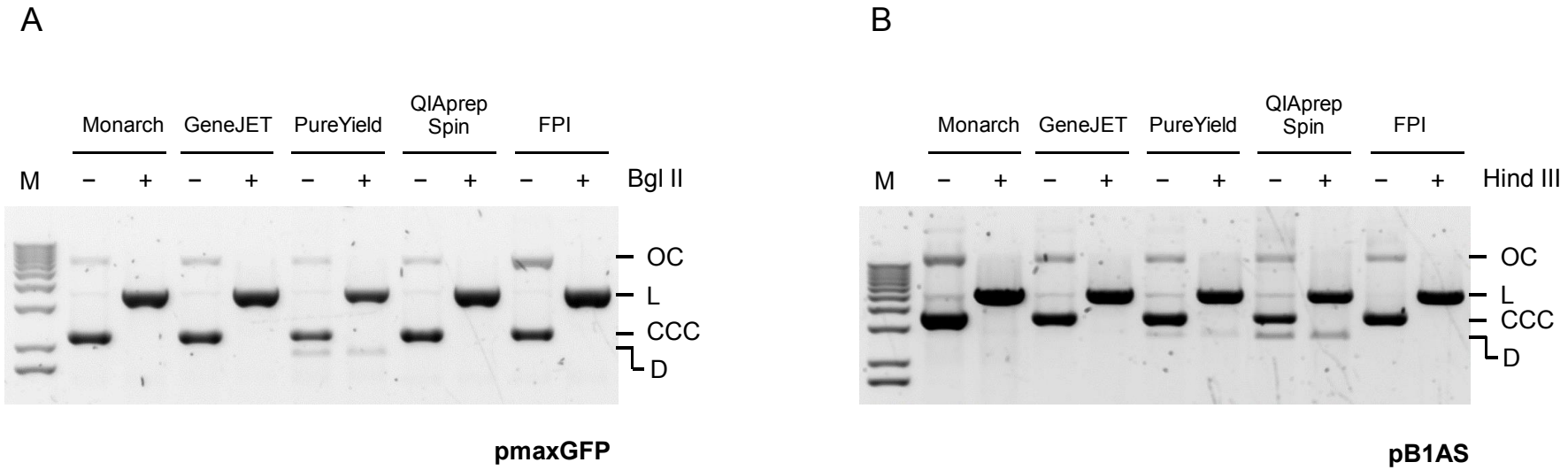
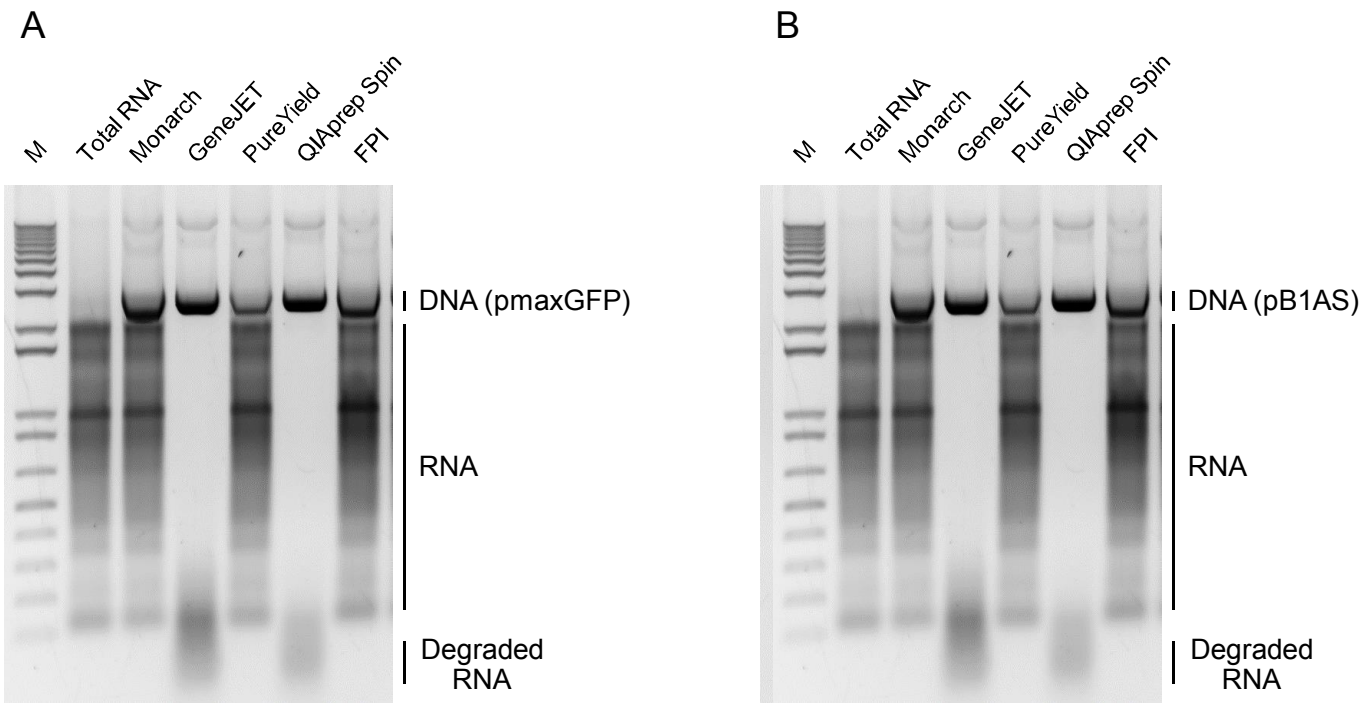
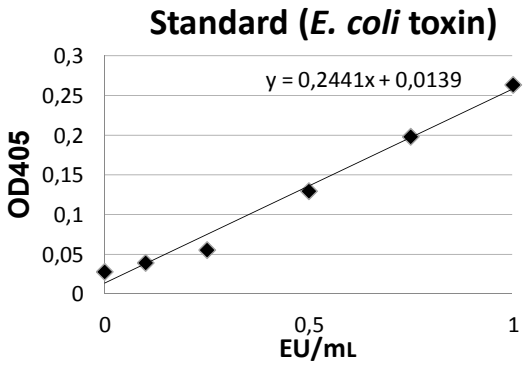


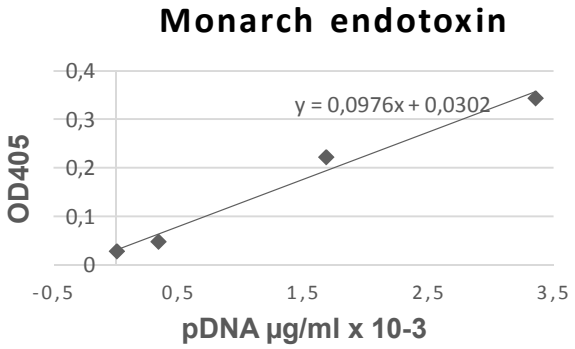
Supplementary Fig. 1 Detection of denatured DNA in commercial pDNA minipreps. (A) Bgl II digestion of pmaxGFP prepared by using four commercial kits (for manufacturers' details see Table 1) and fractional precipitation method (FPI, this study). **(B)** Similar Hind III digestion of pB1AS. All plasmids were incubated with (+) and without (−) the restriction enzyme at 37°C for 1 hr and thereafter analysed by agarose gel electrophoresis. Tris-borate-EDTA (pH 8.3) 0.6% agarose gels, containing 0.1 µg/ml ethidium bromide, were run at low voltage (6 V/cm) for 2 hrs. Electrophoregrams showing different plasmid forms [8, 15]: OC - open circular, L - linear, CCC - covalently closed circular and D - denatured, marked on the right of panels. Note the presence of dpDNA in PureYield miniprep in both panels and QIAprep Spin in panel B. M - 1 kb Plus DNA Ladder (Invitrogen).



Supplementary Fig. 2 Detection of RNase contamination in commercial pDNA minipreps. (A) Three hundred nanograms of pmaxGFP, prepared by using four commercial kits (for manufacturers' details see Table 1) and fractional precipitation method (FPI, this study), were incubated with 1.5 µg of NTera2D1 total RNA in a 5 µl volume at 37°C for 1 hr and thereafter analysed by agarose gel electrophoresis. **(B)** Similar incubation and analysis was performed for pB1AS. Tris-borate-EDTA (pH 8.3) 1.0% agarose gels, containing 0.1 µg/ml ethidium bromide, were run at 5 V/cm for 1 hr. Note the RNA degradation on lanes GeneJET and QIAprep Spin in both panels. M - 1 kb Plus DNA Ladder (Invitrogen).

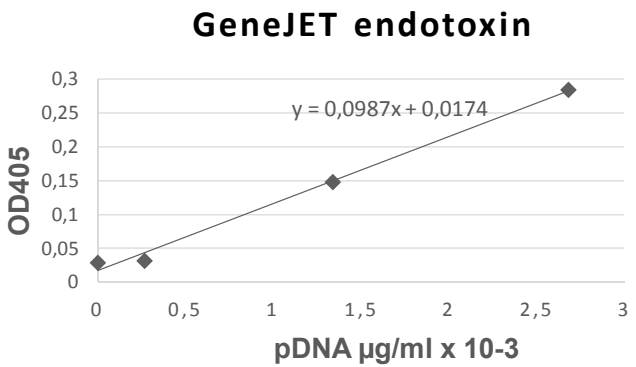


0,1 OD405 = 0,35 EU/ml



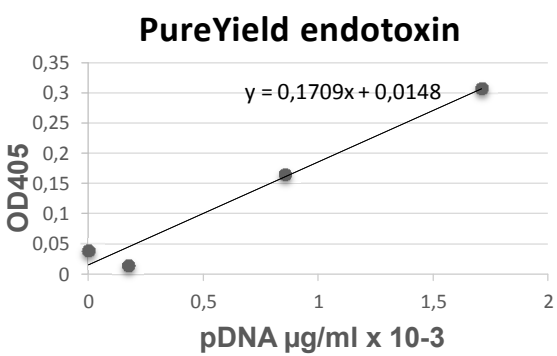
0,1 OD405 = 0,73 x10^{-3}µg

480 EU/µg



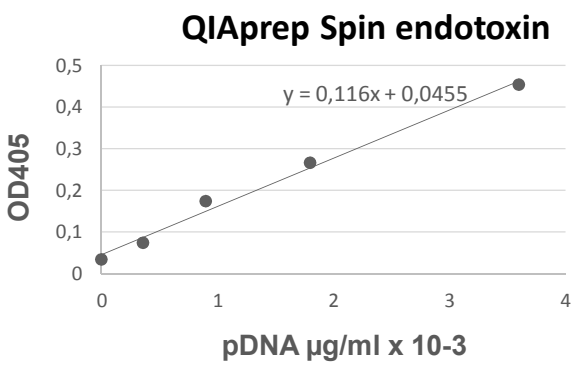
0,1 OD405 = 0,84 x10^{-3}µg

417 EU/µg



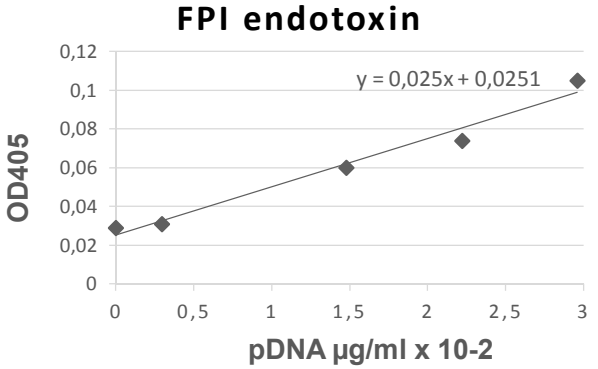
0,1 OD405 = 0,50 x10^{-3}µg

700 EU/µg



0,1 OD405 = 0,47 x10^{-3}µg

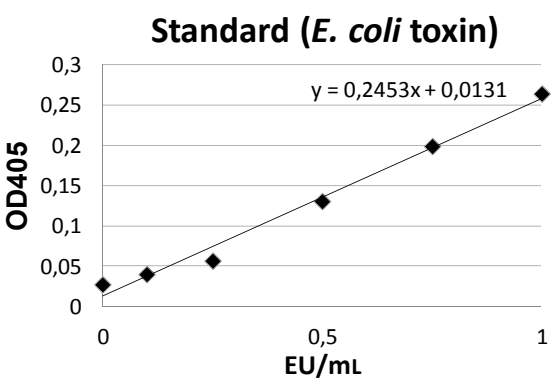
745 EU/µg



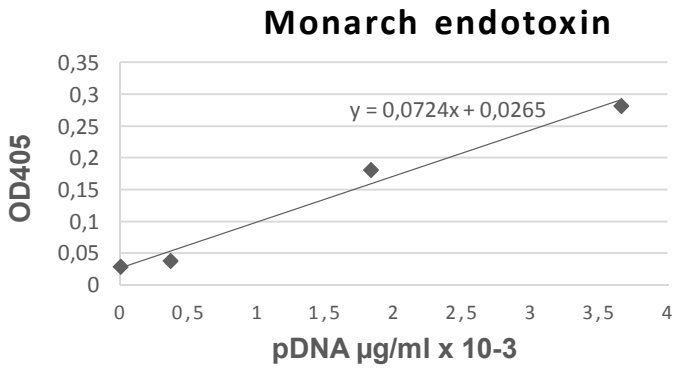
0,1 OD405 = 3,0 x10^{-2}µg

12 EU/µg

Supplementary Fig. 3 Determination of the endotoxin content in pmaxGFP minipreps. The endotoxin content in plasmid minipreps was measured with LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific Pierce). **Two series of measurements of the same plasmid samples were initially carried out independently in parallel by two researchers and only those results were considered matching or reproducible which showed less than 2-fold difference.** Standard curve (first graph) was prepared by using *Escherichia coli* (*E. coli*) Endotoxin Standard (011:B4) according to the manufacturer's protocol. Manufacturers' names of the miniprep kits (on top of each graph) are listed Table 1. For each plasmid, including the one prepared by fractional precipitation method (FPI, this study), endotoxin units (EU) per microgram of pDNA (boxed below the graph), were calculated by using the graph's equation.

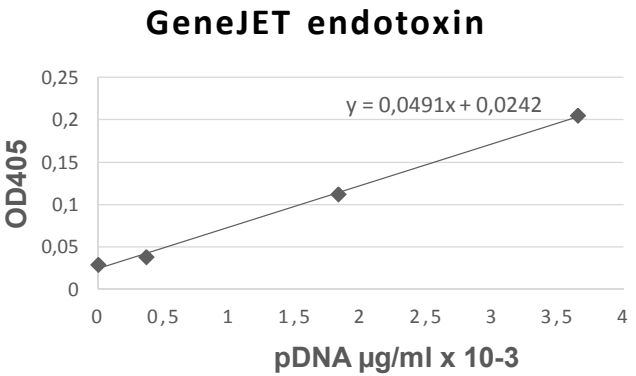


0,1 OD405 = 0,35 EU/ml



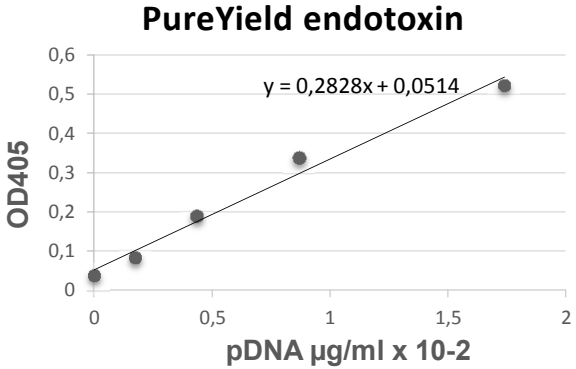
0,1 OD405 = 1,0 x10^{-3}µg

350 EU/µg



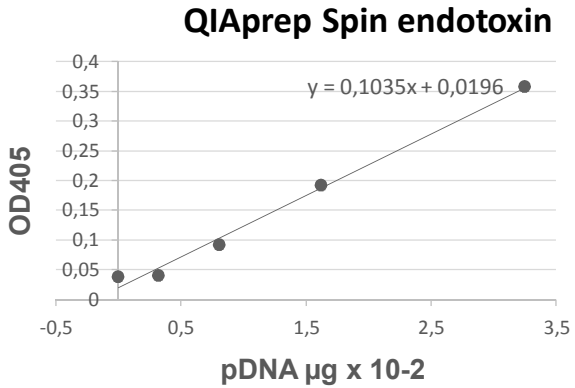
0,1 OD405 = 1,5 x10^{-3}µg

233 EU/µg



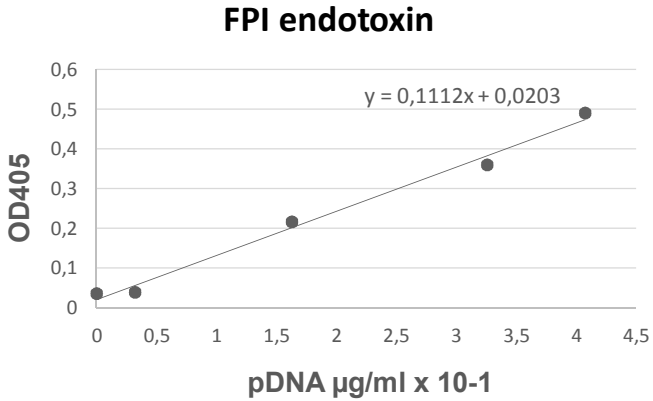
0,1 OD405 = 0,17 x10^{-2}µg

206 EU/µg



0,1 OD405 = 0,78 x10^{-2}µg

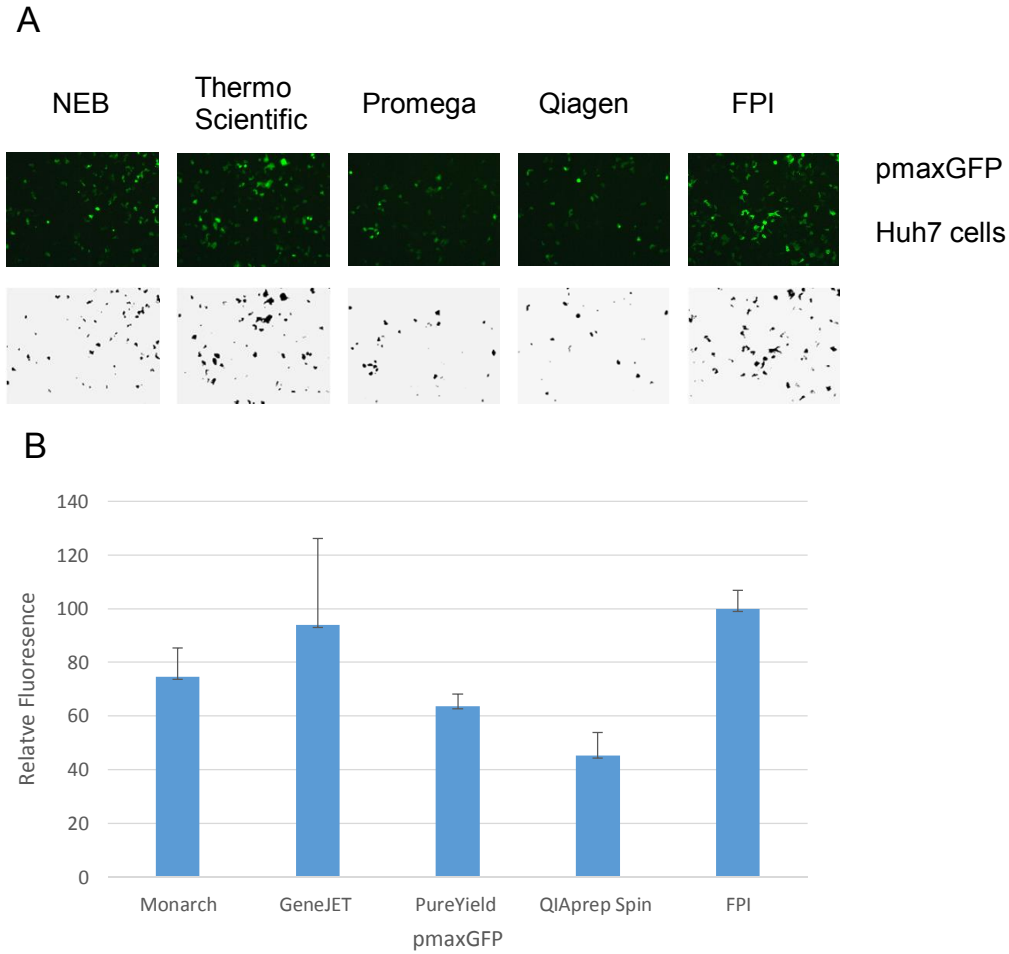
45 EU/µg



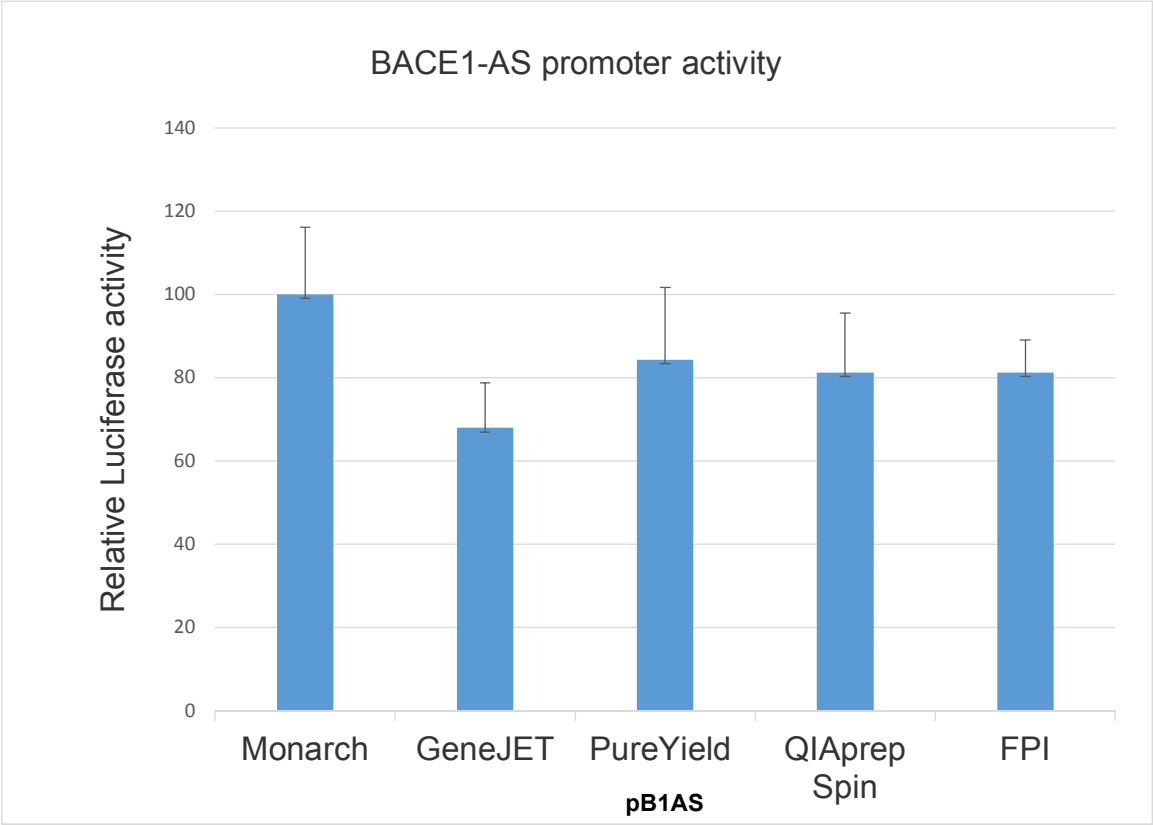
0,1 OD405 = 0,72 x10^{-1}µg

5 EU/µg

Supplementary Fig. 4 Determination of the endotoxin content in pB1AS minipreps. The endotoxin content in plasmid minipreps was measured with LAL Chromogenic Endotoxin Quantitation Kit (Thermo Scientific Pierce). **Two series of measurements of the same plasmid samples were initially carried out independently in parallel by two researchers and only those results were considered matching or reproducible which showed less than 2-fold difference.** Standard curve (first graph) was prepared by using *Escherichia coli* (*E. coli*) Endotoxin Standard (011:B4) according to the manufacturer's protocol. For manufacturers' names of the miniprep kits (listed on top of each graph) see Table 1. For each plasmid, including the one prepared by differential precipitation method (FPI, this study), endotoxin units (EU) per microgram of pDNA (boxed below the graph), were calculated by using the graph's equation.

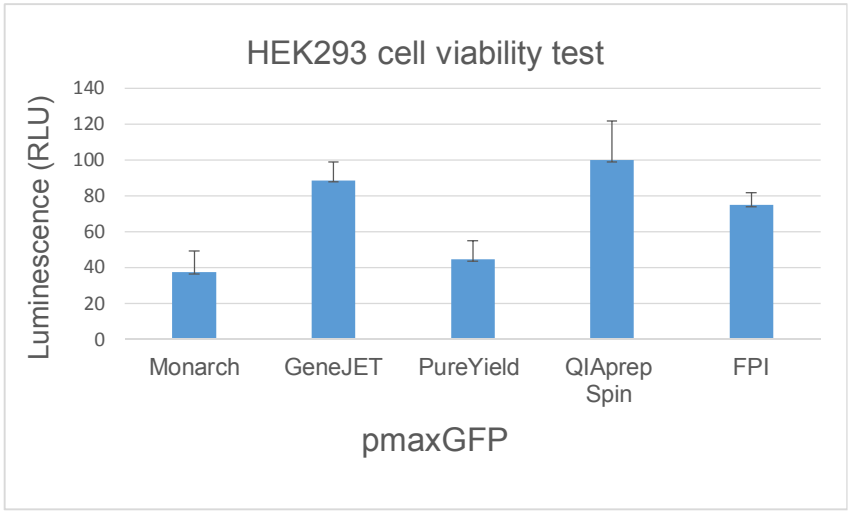


Supplementary Fig. 5 Comparison of the transfection efficiency of different pmaxGFP minipreps. (A) Representative examples of the transfected Huh7 cells recorded by fluorescence microscopy. These micrographs were converted to binary images (shown below) and analysed for particles with ImageJ [16]. Transfection details: plasmids were prepared with commercial kits (manufacturers names shown on top; FPI, this study), One microgram of each plasmid was transfected into Huh7 cells in 12 well plate format using Lipofectamine 3000 Reagent according to manufacturer's protocol (Thermo Fisher Scientific). All transfections were made in triplicate. **(B)** Analysis of transfection efficiency of different plasmids prepared with commercial kits. Corresponding manufacturers' names are listed on panel a. Data shown are the mean ± s.d.

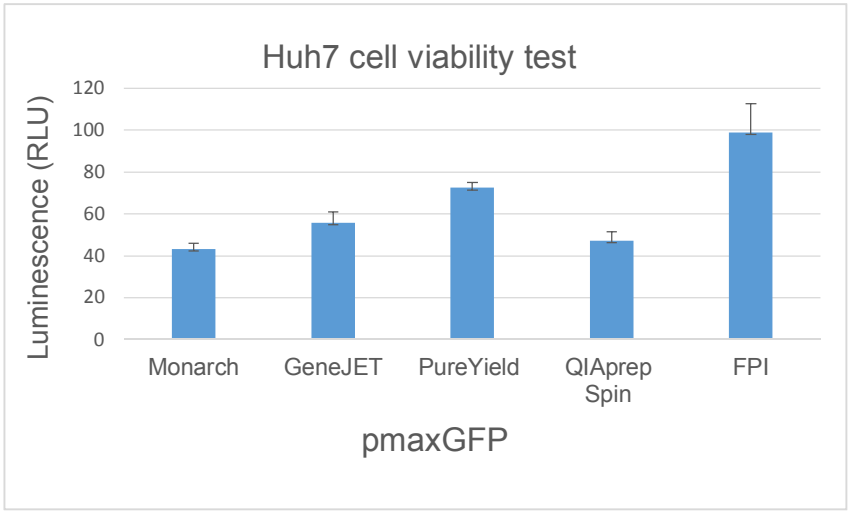


Supplementary Fig. 6 Comparison of the transfection efficiency of different pB1AS minipreps. One microgram of each plasmid, prepared with a commercial kit (for manufacturers' names see Table ; FPI, this study), was cotransfected with 10 ng of pRL into HEK293 cells in 12 well plate format using Lipofectamine 3000 Reagent according to manufacturer's protocol (Thermo Fisher Scientific). All transfections were made in triplicate. Firefly luciferase (pB1AS) and Renilla luciferase (pRL) activities were determined by using Dual-Glo Luciferase Assay System (Promega). Relative firefly luciferase activity normalized to Renilla luciferase is shown. Data shown are the mean ± s.d.

A



B



Supplementary Fig. 7 Characterization of the cell viability determined after transfection of different pmaxGFP minipreps. Five hundred nanograms of pmaxGFP, prepared with commercial kits (manufacturers names are listed in Table 1; FPI, this study), were transfected into HEK293 **(A)** and Huh7 cells **(B)** in three independent experiments. Cell viability was measured 48 hrs after transfection using CellTiter-Glo 2.0 Assay according to the manufacturer's protocol (Promega). Data shown are the mean ± s.d.