Nucleosome positioning sequence

Large scale PCR to obtain the DNA templates

Release of the inserts by specific enzyme combinations

S2 Fig. Illustration of the DNA substrate design used for the nucleosome binding assays. The different DNA substrates were generated by the use of the respective restriction enzyme combinations and the plasmid pPCRSScript_slo1-gla75. A scheme of the DNA templates and restriction enzyme cleavage sites is given. The 601 nucleosome positioning sequence is flanked by structured DNA elements originating from the murine rDNA (80 bp) and the Drosophila Hsp70 (85 bp) genes. By the combination of different restriction endonucleases, symmetrical and asymmetrical DNA overhangs of variable lengths were prepared and analyzed on a 1.3 % agarose gel. The different restriction endonucleases (RI-RV) used here are indicated on top (lanes 2-8). The resulting DNA fragments were amplified by PCR to generate the respective DNA substrates (lanes 9-17) for nucleosome assembly by salt gradient dialysis.