

---

<b>1. Summary</b>	<b>1</b>
<b>2. Contents</b>	<b>2</b>
<b>3. Abbreviations</b>	<b>5</b>
<b>4. Introduction</b>	<b>7</b>
4.1. <i>In vitro</i> selection	8
4.2. Supercoiling	10
4.3. Z-DNA	12
4.3. ADAR1	17
<b>5. Materials and Methods</b>	<b>20</b>
5.1. DNA constructs	20
5.2. Proteins	21
5.3. Electrophoretic separation of DNA in agarose gels	23
5.4. Electrophoretic separation of DNA in acrylamide gels	23
5.4.1. Gel electrophoresis of DNA minicircles	23
5.4.2. Gel purification of DNA oligonucleotides	24
5.4.3. Detection of DNA in acrylamide-gels by silver staining	25
5.5. Bandshift assays	25
5.6. Circular dichroism spectroscopy	26
5.7. Electroporation	26
5.7.1. Preparation of competent bacteria by CaCl <sub>2</sub> treatment	26
5.7.2. Preparation of competent bacteria for electroporation	27
5.7.3. Electroporation of bacteria	27
5.8. Preparation of DNA	28
5.8.1. Small scale preparation of plasmid-DNA	28
5.8.2. Large scale preparation of plasmid-DNA	29
5.8.3. Precipitation of DNA with ethanol	29
5.8.4. UV-spectrophotometric determination of nucleic acid concentrations	30
5.8.5. Determination of DNA concentration using the fluorescent dye Hoechst 33258	30
5.9. DNA sequencing	31
5.10 DEPC reactions	32
5.11. Enzymatic Reactions	33
5.11.1. Radioactive end labeling of DNA oligonucleotides	33
5.11.2. Restriction of DNA	33
5.11.3. Dephosphorylation of DNA	33
5.11.4. Ligation of DNA	33
5.12. Site directed mutagenesis	34
5.13. Protein methods	35
5.13.1. Gel electrophoresis of proteins	35
5.13.2. Purification of recombinant proteins from <i>E. coli</i>	35
5.13.2.1. Purification of the GST-Z $\alpha$ fusion protein	35
5.13.2.2. Purification of the His-Z $\alpha$ fusion protein	37
5.13.3. Determination of the protein concentration	38
5.14. <i>In vitro</i> selection of plasmids	38
5.14.1. Preparation of the plasmid library	38
5.14.2. <i>In vitro</i> selection of the plasmid library	40

---

5.15. <i>In vitro</i> selection of minicircles	41
5.15.1. Production of radioactively labeled minicircles	41
5.15.2. Preparation of the minicircle library	41
5.15.3. <i>In vitro</i> selection of minicircles	43
5.15.4. Amplification of selected minicircles	44
5.16. Commonly used buffers and solutions	46
5.17. Bacterial strains	48
5.18. Computer methods	49
<b>6. Results</b>	<b>50</b>
6.1. Circular dichroism-Spectroscopy	50
6.2. Bandshift assays	53
6.2.1. Bandshift assays with different oligonucleotides	53
6.2.2. Competition experiments with DNA and RNA	54
6.2.3. Competition with plasmid DNA	55
6.2.4. Competition with RNA	56
6.2.5. Methylated DNA	57
6.2.6. Mutations of the hairpin sequence	58
6.2.6.1. Phased AT mutations	58
6.2.6.2. Phased GC mutations	58
6.3. <i>In vitro</i> selection	60
6.3.1. <i>In vitro</i> selection of plasmids	60
6.3.1.1. Generation of a plasmid library	60
6.3.1.2. <i>In vitro</i> selection of plasmids with GST-Z $\alpha$	63
6.3.2. <i>In vitro</i> selection using DNA minicircles	66
6.3.2.1. Generation of a minicircle library containing a N <sub>16</sub> insert	66
6.3.2.2. Development of the selection assay	72
6.3.2.2.1. Development of the 2 PCR protocol	72
6.4.2.2.2. Primer dimers	72
6.3.2.3. <i>In vitro</i> selection of minicircles with GST-Z $\alpha$	74
6.3.3. Analysis of the selected sequences	80
6.3.3.1. Band shift assays	80
6.3.3.2. DEPC footprinting	81
<b>7. Discussion</b>	<b>83</b>
7.1. Circular dichroism spectroscopy	83
7.2. Bandshift assays	84
7.3. <i>In vitro</i> selection	87
7.3.1. <i>In vitro</i> selection of plasmids	87
7.3.2. <i>In vitro</i> selection of Minicircles	92
7.3.3. Sequences obtained through minicircle selection	94
7.3.4. Analysis of the selected sequences by bandshift assays and chemical modification	99
7.4. Is the binding of the Z $\alpha$ -peptide to DNA conformation- or sequence dependent ?	100

<b>8. Appendix</b>	<b>102</b>
8.1. Lists of sequence words	102
8.1.1. List of sequence words after 5 rounds of minicircle selection	102
8.1.2. List of sequence words after 8 rounds of minicircle selection	109
8.2. Predicting the propensity of a DNA sequence to adopt the Z-DNA conformation	112
8.3. Statistics of randomized libraries	114
8.4. Computer programs	117
8.4.1. The McSelex program	117
8.4.2. The Shuffle program	118
<b>9. References</b>	<b>120</b>
<b>10. Publications</b>	<b>127</b>
<b>11. Zusammenfassung</b>	<b>128</b>
<b>Lebenslauf</b>	<b>129</b>