Supplementary Figure 4

Blockage of restriction endonuclease cleavage at overlapping sites on DNA by enzymatic incorporation of extended groups.

Phage lambda DNA was modified with M.HhaI (GC\textsuperscript{GC}) and cofactors 1 (R = -CH\textsubscript{3}), 4 (R = -CH\textsubscript{2}CH=CH\textsubscript{2}) or 5 (R = -CH\textsubscript{2}C≡CCH\textsubscript{3}) and then analyzed for cleavage with the restriction endonucleases R.Hin6I (GCGC, control), R.BspLI (GGNNCC) and R.BglII (GCCN\textsubscript{5}GGC) followed by agarose gel electrophoresis. Among 82 recognition sites for R.BspLI and 29 sites for R.BglII (GCCN\textsubscript{5}GGC), one and two of these sites, respectively, partially overlap with the GCGC sites modified by M.HhaI. Altered fragmentation patterns (indicated by arrows) resulted from blocking R.BspLI and R.BglII cleavage at the overlapping sites by incorporation of the bulky but-2-ynyl group from cofactor 5.