

Supplementary method to make nucleosomal DNA with linkers, gaps and nicks

DNA for nucleosomes with variable linker lengths were prepared by PCR amplification using radiolabeled oligonucleotides, followed by purification in a 2.5% metaphor gel. For the gap/nick experiments, DNA templates were prepared using oligonucleotide hybridization and ligation. Oligonucleotides (100 µg, gel purified by denaturing urea gel) were hybridized by heating at 95°C for 3 minutes, followed by slow cooling in the presence of 50 mM NaCl. They were then ligated at room temperature for 4-6 hr using 10 units of DNA ligase (Invitrogen), followed by gel purification in a 8% native polyacrylamide gel. The double stranded DNA was then extracted using standard crush and soak method, followed by ethanol precipitation. A fraction of the double stranded DNA was then separated in a denaturing urea gel to verify the DNA composition. The double stranded DNA was then labeled with ³²P-γATP using T4 polynucleotide kinase, and assembled into nucleosomes using recombinant yeast histones as described previously (Wittmeyer et al., 2004).

List of Oligonucleotides

Oligonucleotides for making 5S nucleosome substrates with variable linker length

BC1410 (24-mer)
CAACGAATAACTTCCAGGGATTTA

BC 1411 (24-mer)
TGCTGCTTGACTTCGGTGATCGGA

BC 1412 (24-mer)
AGCCCTATGCTGCTTGACTTCGGT

BC 1413 (24-mer)
CAAGTACTAACCGAGCCCTATGCT

Oligonucleotides for making TPT nucleosome substrates with variable linker length

BC 2746 (25-mer)

ACGCGTCGGTGTAGAGCCTGTAAC

BC 2747 (25-mer)

GACAGTGTCCCAGGGAACCTCGAGG

BC 2748 (25-mer)

GTTGTAAAACGACGGCCAGTGAATT

Oligonucleotides for making gapped (or nicked) nucleosome substrates

N5T+7 nucleosomal DNA

BC 2193 (74-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATC
CCTGACCCTTTAAATAGCTTAA

BC 2194 (73-mer)

pCTTTCATCAAGCAAGAGCCTACGACCATACCATGCTGAATATAACCGGTTCTC
GTCCGATCACCGAAGTCAAGC

BC 2324 (27-mer)

AGCATAGGGCTCGGTTAGTATGCCAGG

BC 2196 (33-mer)

pCATCGGCTTATAAATCCCTGGAAGTTATTCGTT

BC 2197 (70-mer)

pGCATGGTATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGGG
TCAGGGATGTTATGACGT

BC 2198 (65-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCA

N5B+7 nucleosomal DNA

BC 2199 (33-mer)

AACGAATAACTTCCAGGGATTTATAAGCCGATG

BC 2200 (70-mer)

ACGTCATAACATCCCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAGC
CTACGACCATAACCATGC

BC 2201 (65-mer)

TGAATATAACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGGGCTCGG
TTAGTATGCCAGG

BC 2202 (74-mer)

TTAAGCTATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATCCC
TGGAAGTTATTCGTTGGAATT

BC 2203 (73-mer)

GCTTGACTTCGGTGATCGGACGAGAACCGGTATATTCAGCATGGTATGGTTCG
TAGGCTCTTGCTTGATGAAAG

BC 2325 (27-mer)

CCTGGCATACTAACCGAGCCCTATGCT

G5B+7 nucleosomal DNA

BC 2199 (33-mer)

AACGAATAACTTCCAGGGATTTATAAGCCGATG

BC 2200 (70-mer)

pACGTCATAACATCCCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAG
CCTACGACCATAACCATGC

BC 2201 (65-mer)

pTGAATATAACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGGGCTCG
GTTAGTATGCCAGG

BC 2202 (74-mer)

pTTAAGCTATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATCC
CTGGAAGTTATTCGTTGGAATT

BC 2203 (73-mer)

GCTTGACTTCGGTGATCGGACGAGAACCGGTATATTCAGCATGGTATGGTTCG
TAGGCTCTTGCTTGATGAAAG

BC2317 (22-mer)

CCTGGCATACTAACCGAGCCCT

G5T+7 nucleosomal DNA

BC 2193 (74-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATC
CCTGACCCTTTAAATAGCTTAA

BC 2194 (73-mer)

pCTTTCATCAAGCAAGAGCCTACGACCATACCATGCTGAATATAACCGGTTCTC
GTCCGATCACCGAAGTCAAGC

BC 2316 (22-mer)

AGGGCTCGGTTAGTATGCCAGG

BC 2196 (33-mer)

pCATCGGCTTATAAATCCCTGGAAGTTATTCGTT

BC 2197 (70-mer)

pGCATGGTATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGGG
TCAGGGATGTTATGACGT

BC 2198 (65-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCA

G5T+6 nucleosomal DNA

BC 2193 (74-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATC
CCTGACCCTTTAAATAGCTTAA

BC 2511 (58-mer)

pCTTTCATCAAGCAAGAGCCTACGACCATACCATGCTGAATATAACCGGTTCTC
GTCCGA

BC 2512 (37-mer)

GAAGTCAAGCAGCATAGGGCTCGGTTAGTATGCCAGG

BC 2196 (33-mer)

pCATCGGCTTATAAATCCCTGGAAGTTATTCGTT

BC 2197 (70-mer)

pGCATGGTATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGGG
TCAGGGATGTTATGACGT

BC 2198 (65-mer)
CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCA

G5T+5 nucleosomal DNA

BC 2193 (74-mer)
AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATC
CCTGACCCTTTAAATAGCTTAA

BC 2509 (47-mer)
pCTTTCATCAAGCAAGAGCCTACGACCATAACCATGCTGAATATACCGG

BC 2510 (48-mer)
GTCCGATCACCGAAGTCAAGCAGCATAGGGCTCGGTTAGTATGCCAGG

BC 2196 (33-mer)
pCATCGGCTTATAAATCCCTGGAAGTTATTCGTT

BC 2197 (70-mer)
pGCATGGTATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGGG
TCAGGGATGTTATGACGT

BC 2198 (65-mer)
CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCA

G5T-5 nucleosomal DNA

BC 2621 (27-mer)
AATTCCAACGAATAACTTCCAGGGATT

BC 2622 (83-mer)
GCCGATGACGTCATAACATCCCTGACCCTTTAAATAGCTTAACTTTCATCAAG
CAAGAGCCTACGACCATAACCATGCTGAATA

BC 2625 (57-mer)
pTACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGGGCTCGGTTAGTA
TGCCA

BC 2626 (67-mer)

pATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATCCCTGGAAG
TTATTCGTTGGAATT

BC 2627 (29-mer)

pGTAGGCTCTTGCTTGATGAAAGTTAAGCT

BC 2628 (78-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCAGCATGGTATGGTC

G5T-4 nucleosomal DNA

BC 2623 (38-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGAT

BC 2624 (72-mer)

CATAACATCCCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAGCCTAC
GACCATAACCATGCTGAATA

BC 2625 (57-mer)

pTACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGGGCTCGGTTAGTA
TGCCA

BC 2626 (67-mer)

pATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATCCCTGGAAG
TTATTCGTTGGAATT

BC 2627 (29-mer)

pGTAGGCTCTTGCTTGATGAAAGTTAAGCT

BC 2628 (78-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCAGCATGGTATGGTC

G5T-2 nucleosomal DNA

BC 2394 (47-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATA

BC 2395 (52-mer)

CCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAGCCTACGACCATAC

BC 2397 (70-mer)

pCATGCTGAATATAACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGG
GCTCGGTTAGTATGCCAGG

BC 2398 (75-mer)

pGTAAAGCTATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATC
CCTGGAAGTTATTCGTTGGAATT

BC 2399 (56-mer)

pCGGACGAGAACCGGTATATTCAGCATGGTATGGTCGTAGGCTCTTGCTTGAT
GAAA

BC2400 (43-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGAT

G5T+2 nucleosomal DNA

BC 2394 (47-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATA

BC 2396 (52-mer)

pACATCCCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAGCCTACGAC

BC 2397 (70-mer)

pCATGCTGAATATAACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGG
GCTCGGTTAGTATGCCAGG

BC 2398 (75-mer)

pGTAAAGCTATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATC
CCTGGAAGTTATTCGTTGGAATT

BC 2399 (56-mer)

pCGGACGAGAACCGGTATATTCAGCATGGTATGGTCGTAGGCTCTTGCTTGAT
GAAA

BC2400 (43-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGAT

G5T-2/+2 nucleosomal DNA

BC 2394 (47-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATA

BC 2744 (47-mer)

CCTGACCCTTTAAATAGCTTAACTTTCATCAAGCAAGAGCCTACGAC

BC 2397 (70-mer)

pCATGCTGAATATACCGGTTCTCGTCCGATCACCGAAGTCAAGCAGCATAGG
GCTCGGTTAGTATGCCAGG

BC 2398 (75-mer)

pGTTAAGCTATTTAAAGGGTCAGGGATGTTATGACGTCATCGGCTTATAAATC
CCTGGAAGTTATTCGTTGGAATT

BC 2399 (56-mer)

pCGGACGAGAACCGGTATATTCAGCATGGTATGGTCGTAGGCTCTTGCTTGAT
GAAA

BC2400 (43-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGAT

G5T 0 nucleosomal DNA

BC 2973 (74-mer)

AATTCCAACGAATAACTTCCAGGGATTTATAAGCCGATGACGTCATAACATC
CCTGACCCTTTAAATAGCTTAA

BC 2974 (45-mer)

ATCAAGCAAGAGCCTACGACCATAACCATGCTGAATATACCGGTTC

BC 2975 (50-mer)

pTCGTCCGATCACCGAAGTCAAGCAGCATAGGGCTCGGTTAGTATGCCAGG

BC 2976 (50-mer)

pTGTTATGACGTCATCGGCTTATAAATCCCTGGAAGTTATTCGTTGGAATT

BC 2977 (52-mer)

pATGGTCGTAGGCTCTTGCTTGATGAAAGTTAAGCTATTTAAAGGGTCAGGGA

BC 2978 (72-mer)

CCTGGCATACTAACCGAGCCCTATGCTGCTTGACTTCGGTGATCGGACGAGA
ACCGGTATATTCAGCATGGT