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Solid-phase synthesis of highly repetitive chromatin assembly templates

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ABSTRACT

DNA templates for assembling chromatin model systems typically consist of numerous repeats of nucleosome positioning sequences, making their synthesis challenging. Here we describe a solid-phase strategy for generating such templates using sequential enzymatic ligation of DNA monomers. Using single nucleosome site monomers, we can either generate a twelve-nucleosome site target, or systematically access intermediate-sized templates. Using twelve nucleosome positioning site monomers, longer templates can be generated. Our synthesized templates assemble into well-defined chromatin model systems, demonstrating the utility of our solid-phase approach. Moreover, our strategy should be more widely applicable to generating other DNAs containing highly repetitive DNA sequences.

Introduction

Chromatin is composed of a linear string of nucleosomes [1], DNA wrapped around a spool of histone proteins [2]. Chromatin model systems can be used to better understand the structure of chromatin and its use as a substrate [3,4], and are often assembled from DNA templates that contain head-to-tail repeats of nucleosome positioning sequences [5]. These highly repetitive templates are typically generated by random insertion of individual DNA repeat units into a vector [6,7]. However, with this approach, the size of the resulting template is not readily controlled, nor is it easy to generate templates with sequence differences at specific nucleosome positioning sites. Recently, our group developed a bead-based method for step-wise assembly of trinucleosome model substrates from individual nucleosomes [8]. Here we describe our effort to adapt and extend this approach to make longer DNA templates for chromatin model system assembly.

Materials and methods

Preparation of DNA fragments

Plasmids containing the 601-172-1 and 601-177-12 ligation fragments were made from 601-177-1 [9] and 601-177-12 [7] templates, respectively, using PCR and cloning. Fragments were excised by *Bgl*I digestion (NEB, for this and all restriction enzymes) and gel purified. Primer and vector details are included in the supplementary materials. Biotinylated, double strand DNA adapter and cap fragments with non-palindromic sticky ends were made by annealing synthesized oligonucleotides (IDT) [8]. Oligo details are included in the supplementary materials.

DNA solid-phase ligation

The 601-172-12 ligated DNA template was created as follows: 0.95 pmoles of biotinylated nucleotide adapter, ADT-*Bgl*I′, was immobilized onto either 50 μg or 500 μg of hydrophilic streptavidin magnetic beads (NEB) [8]. Next, a two-hour room temperature ligation on the beads was performed: a 2.25:1 ratio of non-palindromic 601-172-1 fragment to ADT-*Bgl*I′ was mixed to a total volume of 10 μl in 1X Mighty Mix Ligation Kit solution (Takara Bio USA). The beads were then washed with 50 μl of 1X NEB Ligation Buffer to remove any excess, unligated fragment. Ligation and washes were continued, alternately attaching non-palindromic 601-172′-1 and 601-172-1 fragments. After twelve rounds, ligation of CAP-*Bgl*I was performed using the above conditions. The ligated and "capped" fragments were photocleaved from the beads directly into 1 x NEB 3.1 buffer for 10 min with exposure to 312 nm UV light. 20 units each of *Pst*I and *Bam*HI were added and the DNA digested in a total volume of 30 μl for 2 h at 37 °C. The released fragments containing various numbers of 172 bp repeats were gel

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purified and cloned into p601X [10] to create plasmids p601X-172-8, p601X-172-10, and p601X-172-12. DNA templates NPD-172-8, NPD-172-10, and NPD-172-12, respectively, could be excised with *Pst*I/*Bam*HI digestion and gel purified.

601-177-24, 36 and 48 non-palindromic DNA templates NPD-177-24, NPD-177-36 and NPD-177-48 were created as above using non-palindromic 601-177-12 and 601-177′-12 fragments with the following difference: 0.388 pmoles of biotinylated nucleotide adapter was used with 120 μg of beads. Ligations were performed with 1200 U of T4 ligase (NEB) in a final concentration of 1x Ligation Buffer for 4 h with 1.5:1 molar ratio of fragment to ADT-*Bgl*I. For NPD-177-24 and NPD-177-48, ligation with 1CAP-*Bgl*I at a 1.5:1 molar ratio was performed after two and four cycles of ligation, respectively. For NPD-177-36, CAP-*Bgl*I′ was ligated after three cycles. After photocleavage and digestion, fragments were cloned to create plasmids p601X-24, p601X-36 and p601-X48 from which templates NPD-177-24, NPD-177-36 and NPD-177-48 respectively could be excised with *Pst*I/*Bam*HI digestion and gel purification.

Assembly and analysis of nucleosomal arrays

Recombinant *Xenopus laevis* histones were expressed, purified, and then assembled into histone octamers according to standard protocols [11]. Histone octamers were deposited onto various DNA template, via step-wise salt deposition using previously described methods [5,9].

Nucleosomal arrays were digested with restriction enzymes to determine correct assembly, saturation, and stability. The general protocol was as follows: 400 ng of array was digested with 20 U of *Sca*I-HF (or *Bgl*I for 172 array) in a total of 20 μl of digestion buffer (50 mM NaCl, 10 mM Tris pH 7.4, 2.5 mM $MgCl_2$, 0.05% Triton-X) for 2 h at 37 °C. The nucleosomes and free DNA generated by restriction digestion was characterize by 4% native PAGE analysis in 0.5X TBE, according to standard protocols [5]. The absolute molecular weight of nucleosomal arrays was determined by size exclusion chromatography with multi-angle light scattering as previously described [9], with the following changes: In the protein conjugate analysis used to determine array saturation, the array was decomposed into free DNA monomers and mononucleosome components, using the following parameters determined from free DNA monomer and mononucleosome model systems: DNA ε_{260} of 20 ml $mg^{-1}cm^{-1}$ and *dn/dc* of 0.1269 ml g^{-1}. Mononucleosome ε_{260} of 17.74 ml $mg^{-1}cm^{-1}$ and *dn/dc* of 0.1269 ml g^{-1}.

Results and discussion

To generate chromatin assembly templates, we utilized a solid-phase synthesis strategy (Fig. 1A). In this process, a cleavable DNA adapter is attached to a magnetic bead and then undergoes multiple cycles of enzyme-mediated DNA fragment ligation.

After capping and cleaving the full-length DNA, the product is cloned, amplified, and purified from *E. coli*, to give a DNA template suitable for chromatin-model system assembly. In this approach, the DNA ligation fragments contain different non-palindromic overhangs, to enforce head-to-tail ligation, while preventing ligation in the opposite orientation or fragment self-ligation. As few as two different fragment types can be used, limiting the number of different fragments required. However, additional fragments can be used to change the sequence at specific sites in the template. With solid-phase synthesis, an excess of fragment can be used in each ligation step to drive the reaction to completion, and unincorporated reagents can be readily washed away from the beads.

Initial ligations were performed with a 172-base pair DNA template containing a single nucleosome positioning sequence, the

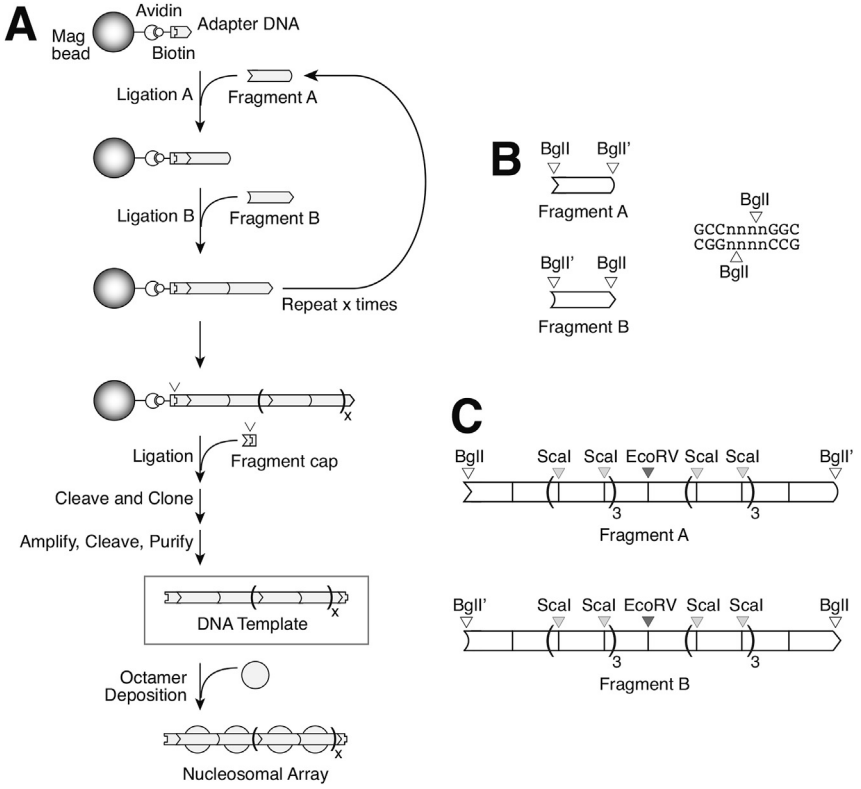

Fig. 1. A) Solid-phase DNA fragment ligation strategy to synthesize DNA templates for chromatin model system assembly. Shown are ligation fragments containing: B) a single nucleosome binding site, or C) twelve binding sites.