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Direct oligonucleotide synthesis onto super-paramagnetic beads

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ABSTRACT

Super-paramagnetic beads (SPMB)s used for a variety of molecular diagnostic assays are prepared by attaching pre-synthesized oligonucleotides to the surface via a cumbersome and low efficient method of carbodiimide-mediated amide bond formation. To mainstream the process, we describe a novel procedure of direct oligonucleotide synthesis onto the surface of SPMBs (e.g. MyOne Dynabeads). With the many challenges surrounding containment of paramagnetic beads (≤1 μm) during automated oligonucleotide synthesis, we show that by applying a magnetic force directly to the SPMBs we prevent their loss caused by high-pressure drain steps during synthesis. To date we have synthesized 40mers using a Spacer 9 phosphoramidite (triethylene glycol) coupled to the surface of hydroxylated SPMBs. HPLC analysis shows successful product generation with an average yield of 200 pmol per sample. Furthermore, because of the versatility of this powerful research tool, we envision its use in any laboratory working with conventional synthesis automation, as employed for single columns and for multi-well titer plates. In addition to direct synthesis of oligodeoxynucleotides (DNA) onto SPMBs, this platform also has the potential for RNA and peptide nucleic acid synthesis.

1. Introduction

Micro- and nanometer-sized beads with surface-modifications to bind particular ligands (chemical group or compound), have been used extensively for a wide variety of molecular diagnostic assays (Haukanes and Kvam, 1993; Holmerg et al., 2005; Gijs, 2004; Marszałł, 2010). The beads can either be applied to non-target-specific (e.g. carboxyl groups – nucleic acids and streptavidin–biotin) or target-specific purposes via surface coupling of biomarker molecules (e.g. nucleic acids and antibody/antigens) (Nakajima and Ikada, 1995; Gilles et al., 1990; Sehgal and Vijay, 1994; Szajáni et al., 1991). For nucleic acid ligands, the oligonucleotide is pre-synthesized with end modifications to fit the bead-attachment chemistry used; the biomarker ligand will then bind a corresponding anti-target site to be selected for in downstream processing (Nolan and Sklar, 2001; Dunbar, 2006).

Super-paramagnetic beads (SPMB)s compared to other bead-types (e.g. acrylamide, sepharose, silica and polystyrene) have a major advantage in the sample preparation methodology due to the inherent ease of separating particles in solution with an external magnet (Holmerg et al., 2005). Examples where SPMBs have been used for various types of sample preparation and detection platforms include but are not limited to (a) pyrosequencing (Holmerg et al., 2005; Ronaghi, 2001), (b) Luminex (Dunbar, 2006; Ocheretina et al., 2013), (c) Applied Biocode, (d) SOLID (Shendure, 2005; Farias-Hesson et al., 2010), and (e) MagArray (Xu et al., 2008, 2013; Mak et al., 2010).

An alternative to full strand attachment to the SPMB is direct oligonucleotide chemical synthesis onto the bead surface. The advantage of this method lies in streamlining the process, where the middle-step of bead-surface modification is rendered unnecessary. Other benefits include (a) the possibility to scale-down the size of the actual SPMBs allowing for a higher surface display of the synthesized oligonucleotides, (b) higher DNA copy number per bead, (c) lower production costs, (d) consistent quality assessments, (e) increased production speed, and (f) massive parallelization.

Applications using SPMB nucleic acid capture and isolation are becoming more sophisticated often requiring nano-sized particles (Xu et al., 2008, 2013; Mak et al., 2010). Since traditional solid-phase chemical synthesis is a filter-based platform (Pon, 2001), retaining micro- and nanometer-sized beads is not possible. Commercial solid supports for oligonucleotide synthesis (e.g. controlled-pore glass (CPG) particles and polystyrene (PS) beads) have a median diameter of 100 μm, and where filters used to retain these supports have a median porosity of 40 μm allow only passage of reagents during wash and drain steps of the synthesis cycle; however, this is too porous for MyOne beads, for example, which are only 1 μm in diameter. As such, filters with <1 μm porosity greatly restrict

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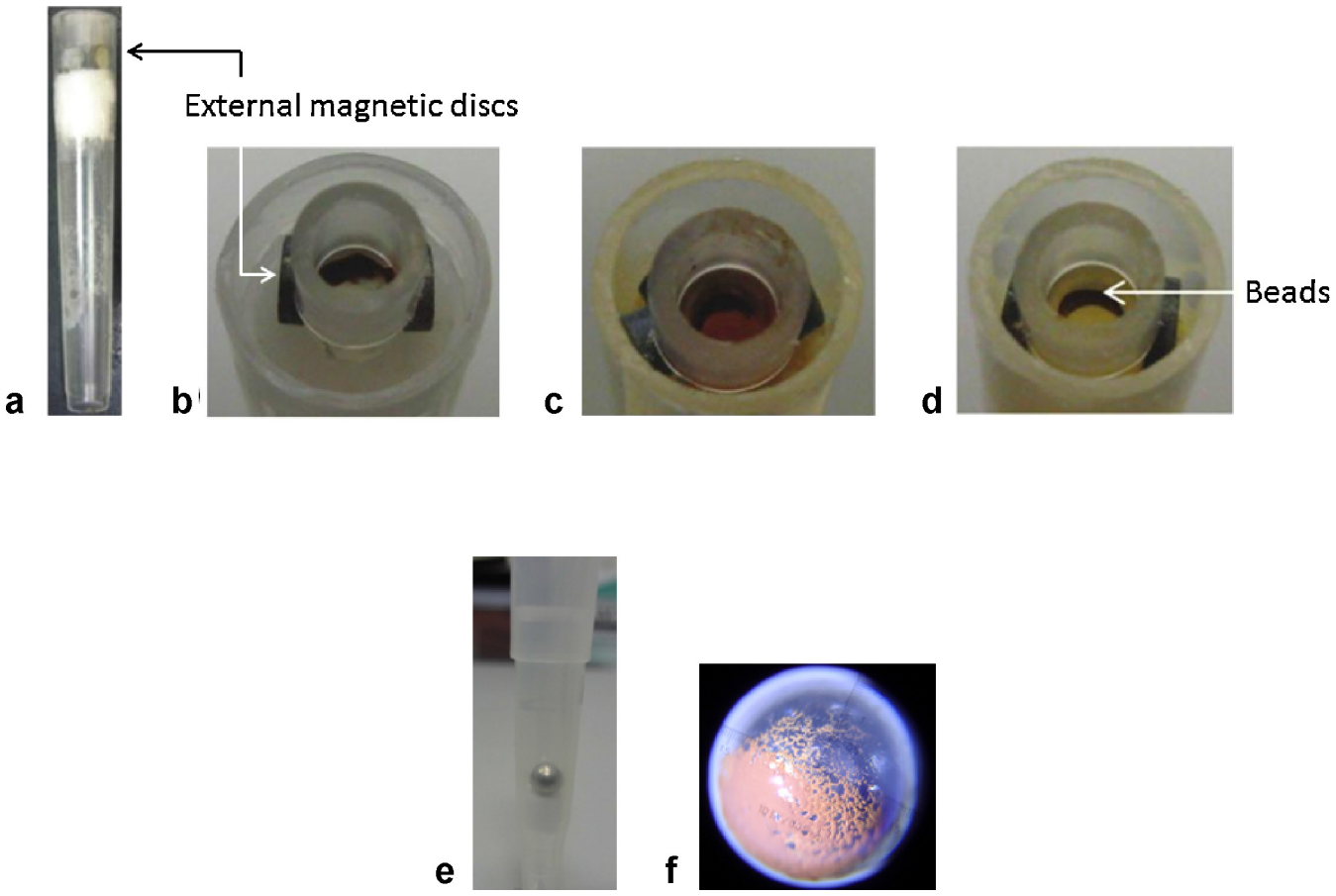

Fig. 1. Shows options for magnetic binding of super-paramagnetic beads (SPMB)s during automated oligonucleotide synthesis. (a) Side view of a modified synthesis column (AB3900-instrument style) with external magnets around an inner column where SPMBs are bound to the walls. (b) Top view of column showing SPMBs bound to the wall before oligonucleotide synthesis. (c) Oligonucleotide synthesis (oxidation step); SPMBs are immersed in oxidizing reagent. (d) Post-synthesis showing SPMBs are still bound to column wall. (e) AB3900-style column with magnetic sphere inside. (f) Sphere coated with SPMBs before synthesis.

reagent flow-through, and therefore cannot be used. Due to these reasons past investigations of chemical synthesis on SPMBs have had only limited, small-scale success proving a major challenge in adapting to higher throughput synthesis automation (Albretsen et al., 1990).

Instead, by applying a magnetic force directly to the SPMBs, we prevent bead loss caused by high-pressure drainage during reagent purge-to-waste steps in the synthesis cycle. This is accomplished by using a permanent magnet (e.g. sphere or disk) to which the beads are attracted and remain bound (Fig. 1).

With readily available on-the-market supplies (e.g. MyOne SPMBs and magnetic spheres/discs) we envision this method for use in any laboratory working with conventional synthesis automation such as those for single columns (Applied Biosystems AB394 and AB3900 instruments) and those for multi-well titer plates (96, 384, and 1536). The option to synthesize directly onto SPMBs will vastly improve workflow in the lab from synthesizer to sequencer; therefore, time-consuming and expensive oligonucleotide pre-functionalization, bead attachment and laborious washing procedures are avoided.

2. Materials and methods

2.1. Direct oligonucleotide synthesis onto super-paramagnetic beads (SPMB)s

Hydroxyl coated MyOne Dynabeads were kindly provided by Life Technologies. The beads were washed three times with 1× wash/storage buffer (PBS, supplemented with 0.2% Tween-20 (pH 7.7)) using a standard DYNAL Invitrogen bead separator magnetic rack (Life Technologies, Carlsbad, CA) to remove any excess bovine serum albumin (BSA); BSA is an additive to prevent clumping during storage. Single magnetic spheres (~3 mm diameter, Neodymium Iron Boron (NdFeB), K&J Magnetics) were then placed inside blank synthesis columns (Biosearch Technologies, Novato, CA); a bottom filter is used only to retain the sphere. Thirty microliters of the original MyOne stock solution (~10^7 beads/μl) were used to coat the surface of the magnetic sphere. Prior to synthesis, magnetic sphere-bound MyOne beads were then washed repeatedly with acetonitrile to remove excess water and residual BSA. Using the manufacturer's recommended synthesis protocol (for Applied Biosystems AB3900 DNA synthesizer) all samples were synthesized onto the hydroxylated surface of the MyOne beads with a non-cleavable spacer (9-O-dimethoxytrityl (DMT)-triethylene glycol,1-[(2-cyanoethyl)-(N,N-diisopropyl)]-spacer 9 phosphoramidite (S9)) (10-1909-xx, Glen Research, Sterling, VA) (Supplementary Fig. S1).

For control samples, 5′-DMT thymidine succinyl hexamide phosphoramidite (CLP-2244, ChemGenes, Wilmington, MA) was added before the first base of each sequence (Supplementary Fig. S2); the succinyl linkage allows for cleavage of the target strand during ammonolysis. This was done for strand characterization using reverse-phase high performance liquid chromatography (HPLC), sequencing analysis (Pyro- and Sanger), and for measuring optical density (OD) to determine final product yield. All sequences were synthesized at the Stanford Genome Technology