Making a scanning array: *in situ* synthesis of oligonucleotides on a solid substrate

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Equipment and reagents

- DNA synthesizer (ABI)
- Reaction mask and assembly frame
- Solid support (derivatized glass or polypropylene)
- Standard dA, dG, dC, and T CE phosphoramidites[a]
- Oxidizing agent[b]
- Acetonitrile
- Activator solution
- Deblock solution

Method

1. Program the DNA synthesizer with an appropriate synthesis cycle.
2. Enter the sequence in 5' to 3' direction.[c]
3. Mark the first footprint of the mask on the polypropylene or derivatized glass surface by placing it in the desired starting position (see Mounting polypropylene on a glass plate to provide support during array fabrication).
4. Tighten the plate against the mask with the pressure clamp to produce a seal (see Figure 1).
5. Start the DNA synthesizer to go through the pre- programmed cycle to couple the appropriate nucleotide.[d]
6. After completion of the step during the interrupt, slacken the pressure clamp and move the plate one increment.
7. Tighten the pressure clamp and start the synthesizer for the next nucleotide in the sequence. Continue the process until the full sequence length is synthesized.

Notes

a With the use of standard phosphoramidites in synthesis, the oligonucleotides are attached to the solid support at their 3' ends. Reverse phosphoramidites (Glen Research) can be used to make oligonucleotides that are attached to the solid support at their 5' ends.

b Iodine is used as an oxidizing agent to produce phosphodiester bonds between nucleotides. This can be replaced with a sulfurizing agent (Beaucage reagent, Cruachem) to make arrays of phosphorothioate oligonucleotides.

c The first condensation on the substrate is of the base at the 3' end of the sequence.

d At the start of each synthesis cycle, an ‘interrupt step’ can be introduced to halt the process at the first step for the next nucleotide condensation cycle to allow the operator to move the plate and restart the program.

Figures

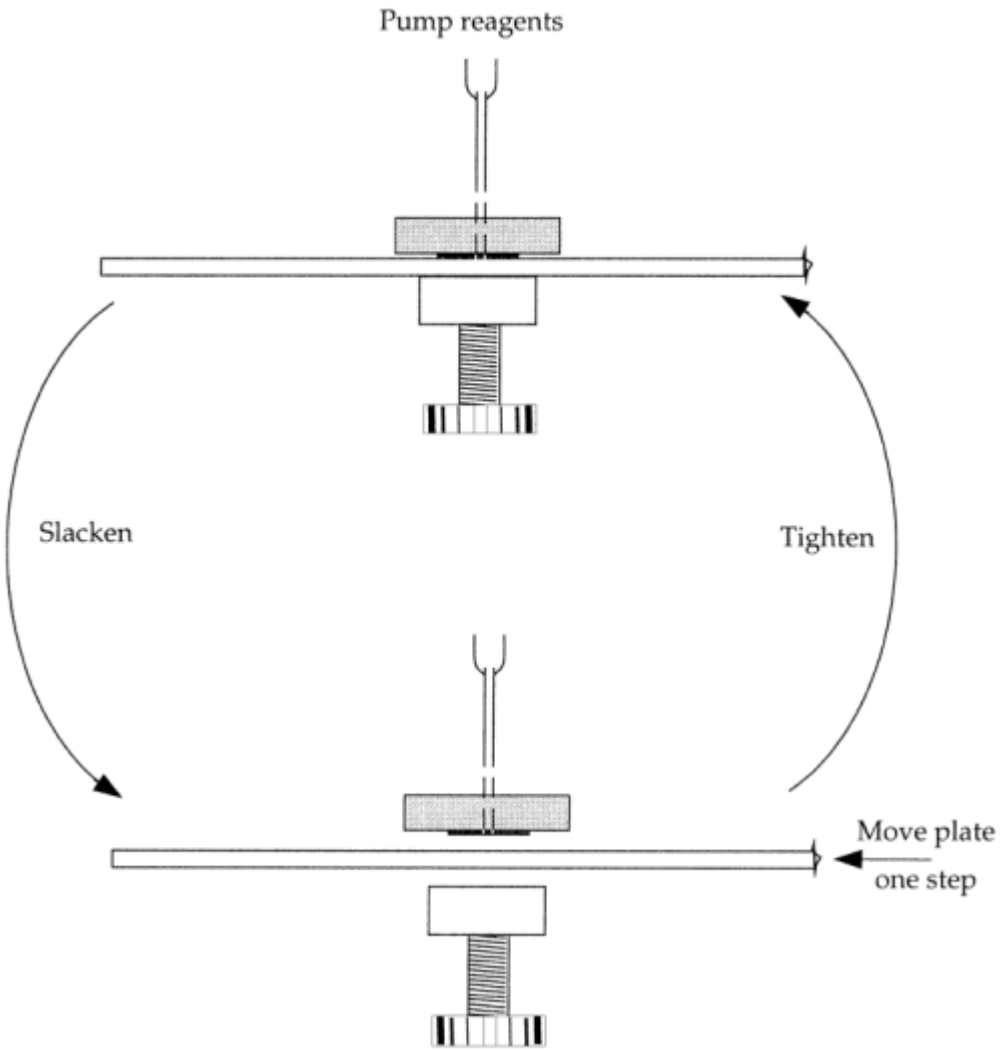

Figure 1

One coupling cycle comprises clamping the plate up to the mask, starting the DNA synthesizer to go through the pre- programmed cycle to couple the appropriate nucleotide, slackening the clamp, and moving the plate one increment by driving the lead screw the required number of whole or half- turns.