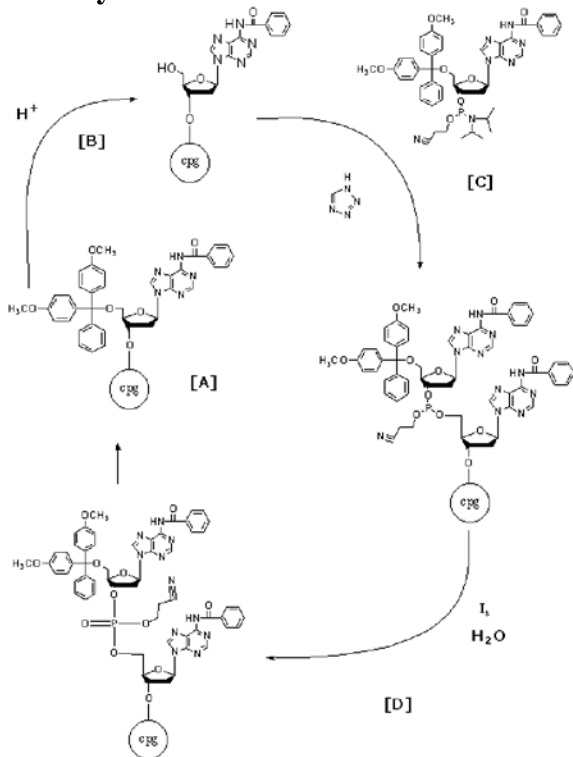


Introduction

Thanks to recent advances in DNA synthesis chemistry, high quality pools of oligonucleotides are available at reasonable concentrations and prices. A quick look around the Internet for companies performing DNA synthesis (companies such as Integrated DNA Technologies or Operon) shows that it's possible to get next day delivery on a 20 or 30 base sequence of your choosing for around 20 dollars. The synthesis chemistry requires only a few things: a solid support on which to build the oligos, nucleotides that can be protected from further nucleotide additions, a way of deprotecting the nucleotides once they've been added to the oligo chain, and finally a way of releasing the finished oligos from the support at the end of the synthesis. An automated synthesizer and a schematic of synthesis chemistry are shown.

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DNA synthesizer



DNA synthesis chemistry

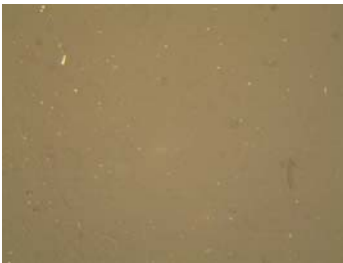
Consider making a 4-mer: GATC. To begin the synthesis, the 3' hydroxyl group of the first nucleotide (guanosine) would be coupled to a 500 angstrom bead ("cpg"). The guanosine would then be treated with a solution (dichloroacetic acid) to remove the cap (a dimethoxytrityl group) protecting its 5' phosphate. The phosphate can then react with the 3' hydroxyl of the incoming nucleotide (adenosine), which would also be capped on its 5' end to prevent the addition of multiple adenines to the growing chain.

Unincorporated adenine would be washed away before the adenine on the bound GA-dinucleotide was deprotected and reacted with a protected thymidine. Next, the GAT-trinucleotide would be washed to remove unincorporated T, deprotected and reacted with cytosine. Ultimately, the final 4-mer would be released from the solid support to be further purified by chromatography or electrophoresis if needed.

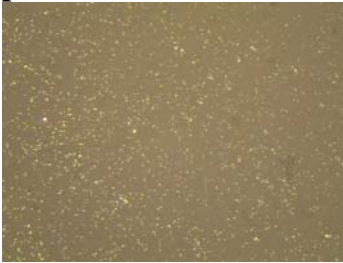
The same chemistry was used to make the library you've been working with but at each nucleotide addition step, an equimolar mixture of all four bases was reacted generating a pool of random DNAs. After uncoupling the oligos from the solid support, the pool was made double stranded with a DNA polymerase and then inserted into the pCT-CON plasmid, with each plasmid getting one version of the oligo. Consequently, the junction of the Aga2 sequence with the 36-base pair oligo is the same in every plasmid but the 12 amino acid sequence fused to each Aga2 protein can be different. Some of these will bind gold and most will not.

You have already screened the library once to isolate some gold-binders from the mix. Your results might resemble the following.

Digital Photographs of Gold Slides after Panning



pCT-CON



pAu1



Library

Yeast Eluted from Slides, growing as colonies on selective media



pCT-CON



pAu1



Library

Sensibly enough, the pAu1 panning gave the most colonies because every yeast from that pool can bind gold. Interestingly, more yeast were isolated from the library pool than from the negative control, a hopeful indication that some novel gold-binding yeast have been identified. Do all these library candidates bind gold equally well? Unlikely. The affinity will depend on the sequence of the Aga2 fusion protein and each yeast colony

from the library could have a different sequence. In fact, some of the candidates may not really bind gold at all. For example cells that were trapped on the glass behind the gold slide would appear to have bound the gold in your initial screen. Today you will choose four library candidates to further evaluate next time, re-examining their gold-binding ability and determining their relative affinity.

Most of your time today will be spent repeating the gold panning from last time, changing one parameter that may improve the protocol. Consider the contents of the solutions you used, the times and temperatures for the binding and washing steps and the mechanics of the yeast-gold incubation. Any one of these can be modified today. Alternatively, you may repeat the panning experiment exactly as before but compare the ability of pAu1 to bind other metals. We have aluminum slides or copper slides for you to try. Based on the results of today's optimization experiment you may choose to rescreen your library candidates differently next time.