Off-the-shelf designer DNA: sequence-to-order and length-to-fit DNA

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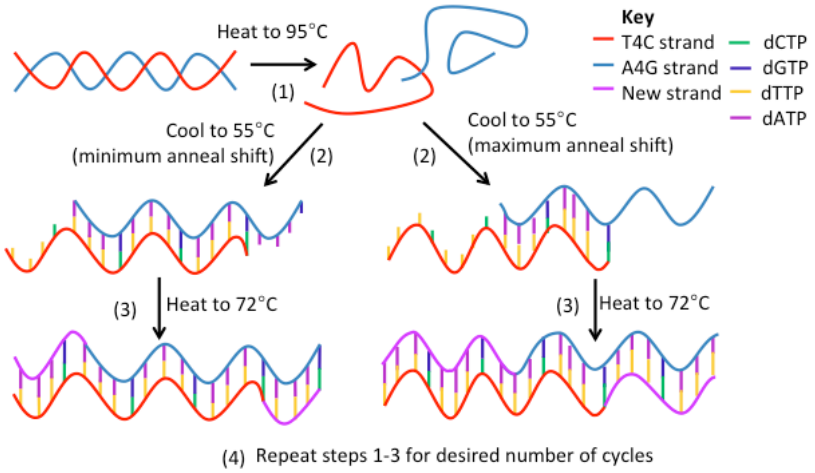
DNA of user-defined sequences, and of lengths over 100 base pairs (bps) has not been easily accessible. Automated DNA synthesis affords great control over the DNA sequence, but longer strands (>100 bps) result in diminishing yields, and of only a single strand. Typically, polymerase based routes for DNA synthesis are not limited to short DNA (<100 bps) but rely on primer-templates being available to give the sequence control. Additionally, non-standard modifications to DNA can lead to reduced yields and truncated sequences. This talk will present the recent developments from the Chemical Nanoscience Laboratories in Newcastle that address these shortcomings in an effort to produce off-the-shelf designer DNA.



We recently reported a protocol for the heat-cool cycle extension of oligo "seeds" that produces long DNA of controlled base pair composition, see figure above.[1] The oligo seeds are typically complementary repeat DNA sequences of the form $[W_aX_bY_cZ_d]_n$ where W, X, Y and Z represent any of the four standard nucleobases, and $n(a+b+c+d) \approx 20$. Upon heating to the $[W_aX_bY_cZ_d]_n$ duplex denatures fully, which on cooling can re-assemble into displaced duplexes providing overhangs as templates for extension by polymerase enzymes. Multiple heat-cool cycles yield longer strands of the repeat sequence where n > 1,000 by using a *Thermococcus gorgonarius* Family B polymerase exonuclease minus variant, Z3.[4]

It is also possible to incorporate modified nucleotides to produce a wide range of functional-DNA, DNA that can be further synthetically modified or used to coordinate metals for nanomaterial fabrication.

Alkyne-modified DNA was produced to demonstrate facile modification via "click chemistry" with an azido ($N=N^+=N^-$)-fluorescein.

The synthesis of thiolated-DNA allowed for metal addition by the titration of Au^+, Cd^{2+} and Au^{3+} with full characterization of the products by UV-Vis and IR spectroscopy, atomic force microscopy and fluorescence microscopy.

The synthesis of DNA with modifications situated at user-defined positions suggests that this method for the synthesis of long designer DNA may be a useful for the controlled fabrication of unique 1-D nanomaterials were composition control is required in sequences over 100 bps.

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*[1] C. J. Whitfield et al, Angew. Chem.. Int. Ed. 2015, **54**. 8971.*
*[2] S. K. Jozwiakowski and B. A. Connolly, ChemBioChem, 2011, **12**, 35-37*