kilobaser

QUALITY REPORT of oligonucleotides synthesized by Kilobaser ONE

Samples

#	Name	Oligo type	Modifications	Sequence
1	XNA_B_22mer	5 letter oligonucleotides (XNA)	5 B bases are introduced in the first 10 bases.	5'- CTATCCAGCTAC**B**G**B**T**B**T**BB**AC -3'
2	XNA_S_22mer	5 letter oligonucleotides (XNA)	5 S bases are introduced in the first 10 bases.	5'- ATTCAGTCGAAGT**SS**A**S**A**S**C**S**G -3'

Measurements

Device	Method	Outcome
Advion AVANT™ UHPLC System and Advion expression® Compact Mass Spectrometer (CMS)	Reverse HPLC to separate oligonucleotides from each other by their length and modifications; Separated oligonucleotides are detected via absorption at 260 nm and analyzed in the mass spectrometer.	• Chemical composition of sample • Differentiation of oligonucleotides in length and number of modifications • Identification of modifications • Yield in % of full-length product
Qubit 3.0 Fluorometer	Fluorometric quantification of ssDNA	• Yield of synthesis in pmol To of note: The yield obtained with Qubit Fluorometer might be not exact as fluorescence intensity is not optimized for S ans B bases.
Thermocycler - Open qPCR by Chai	Melting curve analysis and Polymerase Chain Reaction	• Hybridization efficiency • Enzyme interaction

Application

The XNA oligonucleotides are designed to overlap on the 3'-end to form a short double strand with overhangs on both sides. This double strand initiates strand elongation by a polymerase resulting in the formation of a 33mer double strand. The double strand parts can be detected through SYBR green. The size increase through the strand elongation can be detected through determination of the melting point of the double strands.

Strand elongation ← 3'- CABBTBTBGBCATCGACCTATC -5'

5'- ATTCAGTCGAAGT**SS**A**S**A**S**C**S**G -3' → *Strand elongation*

SAMPLE 1 XNA_B_22mer

Sample type 5 letter oligonucleotides (XNA)
Sequence 5'- CTATCCAGCTACBGBTBTBBAC -3'
Length 22mer

Chemical composition, mass and purity

The chromatogram (figure 1) of this sample shows following peaks from left to right:

- Peaks arriving during the first 5 minutes of the HPLC are caused by synthesis reagents.
- **Full-length product** arriving at 15 min is the main product of the synthesis.
- Peak arriving after 18 minutes comes from the change of the mobile phase.

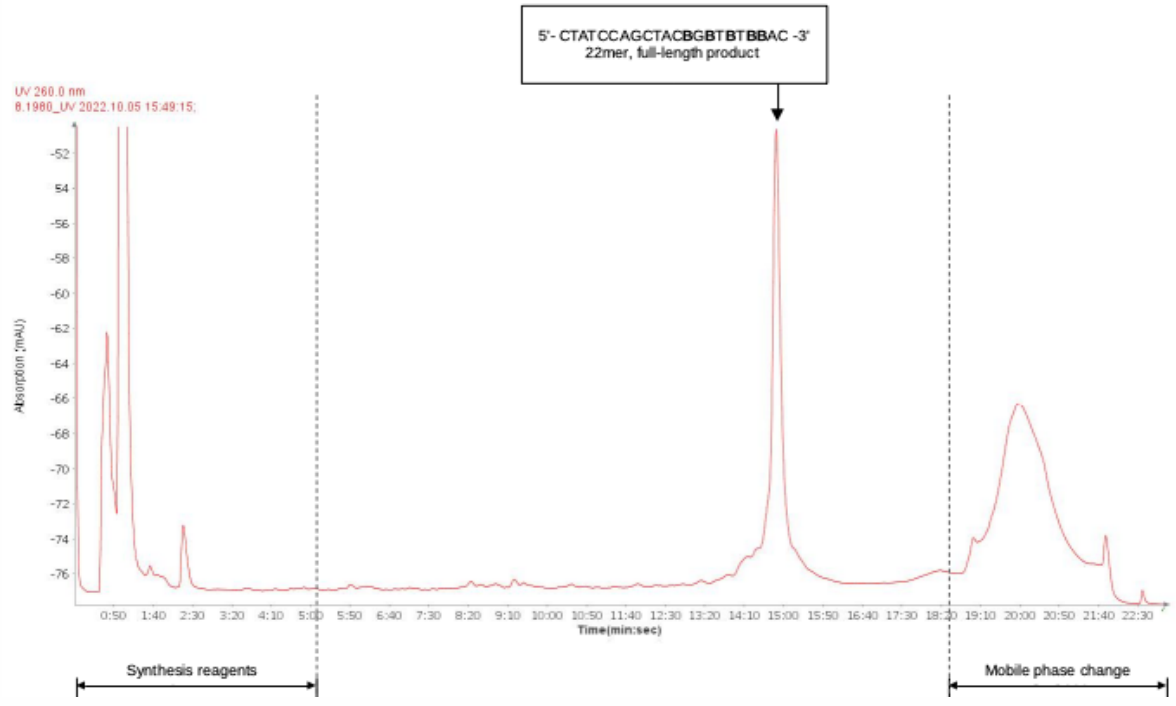

Figure 1: Chromatogram of reverse HPLC collecting absorption at 260nm overtime

The chemical composition and the mass of the full-length strand was further confirmed by the mass spectrometer. The mass proves that no additional protection groups are present.

Measured Molecular Weight: 6749 g/mol
Calculated Molecular Weight: 6751 g/mol

Outcome of synthesis

The synthesis and purification worked well resulting in a final yield of 263 pmol full-length product with hardly any truncated strands visible in the HPLC. Based on this results it can be concluded that the XNA bases were coupled and deprotected efficiently at the applied synthesis conditions.

SAMPLE 2 XNA_S_22mer

Sample type 5 letter oligonucleotides (XNA)
Sequence 5'- ATTCAGTCGAAGT**SS**A**S**A**S**C**S**G -3'
Length 22mer

Chemical composition, mass and purity

The chromatogram (figure 2) of this sample shows following peaks from left to right:

- Peaks arriving during the first 5 minutes of the HPLC are caused by synthesis reagents.
- **Full-length product** arriving at 17 min is the main product of the synthesis.
- Peak arriving after 18 minutes comes from the change of the mobile phase.

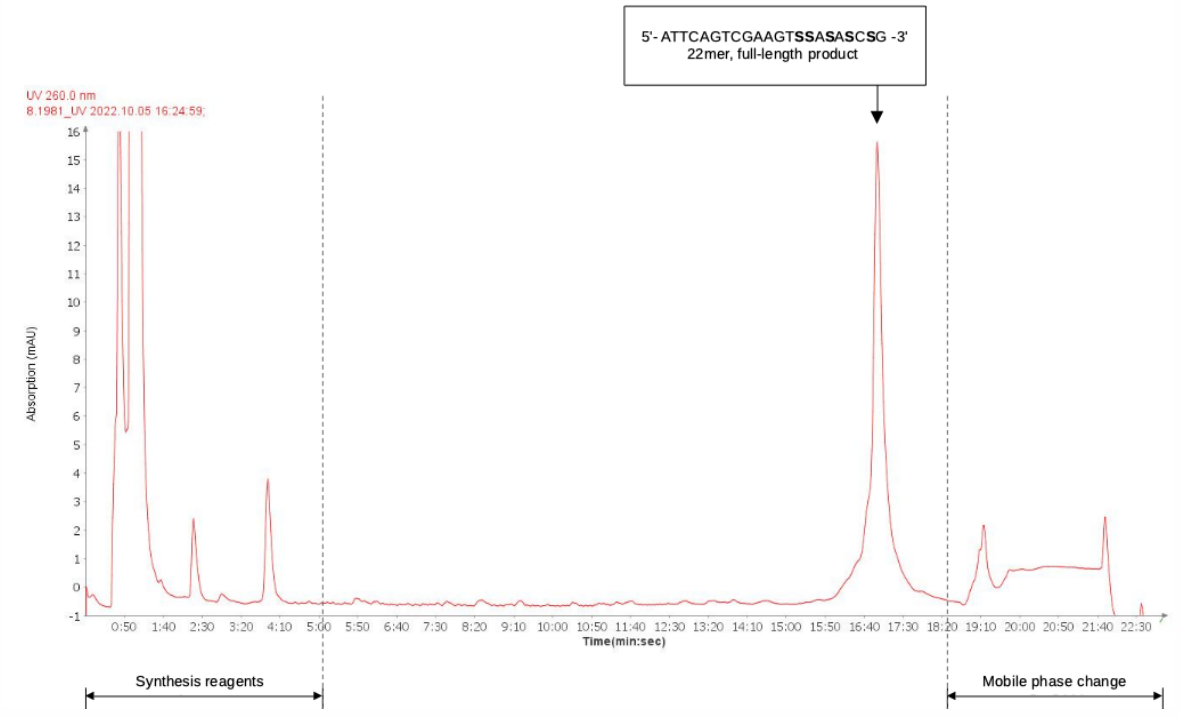

Figure 2: Chromatogram of reverse HPLC collecting absorption at 260nm overtime

The chemical composition and the mass of the full-length strand was further confirmed by the mass spectrometer. The mass proves that no additional protection groups are present.

Measured Molecular Weight:	6733.7 g/mol
Calculated Molecular Weight:	6734 g/mol

Outcome of synthesis

The synthesis and purification worked well resulting in a final yield of 200 pmol full-length product with hardly any truncated strands visible in the HPLC. Based on this results it can be concluded that the XNA bases were coupled and deprotected efficiently at the applied synthesis conditions.

Result of application test

The melting curve analysis allows the detection of double strand formation and its stability against heat represented by the melting point. The melting points of the synthesized DNA fragments – sample 1 and sample 2 - are visible as peaks in the following figure 3, in which dF/dT is plotted against the temperature.

It can be seen that:

- Each fragment alone (F1 in orange, F2 in blue) forms at low temperature unspecific double strands that disappear quickly with the increase of temperature.
- Mixing the fragments together (F1+F2 in green) a significant more stable 11nt long double strand is formed that melts at 60°C and thus similar to primer binding.
- This double strand initiates strand elongation by a polymerase resulting in the formation of a 33nt long double strand (F1+F2 in violet) that melts at 78°C.

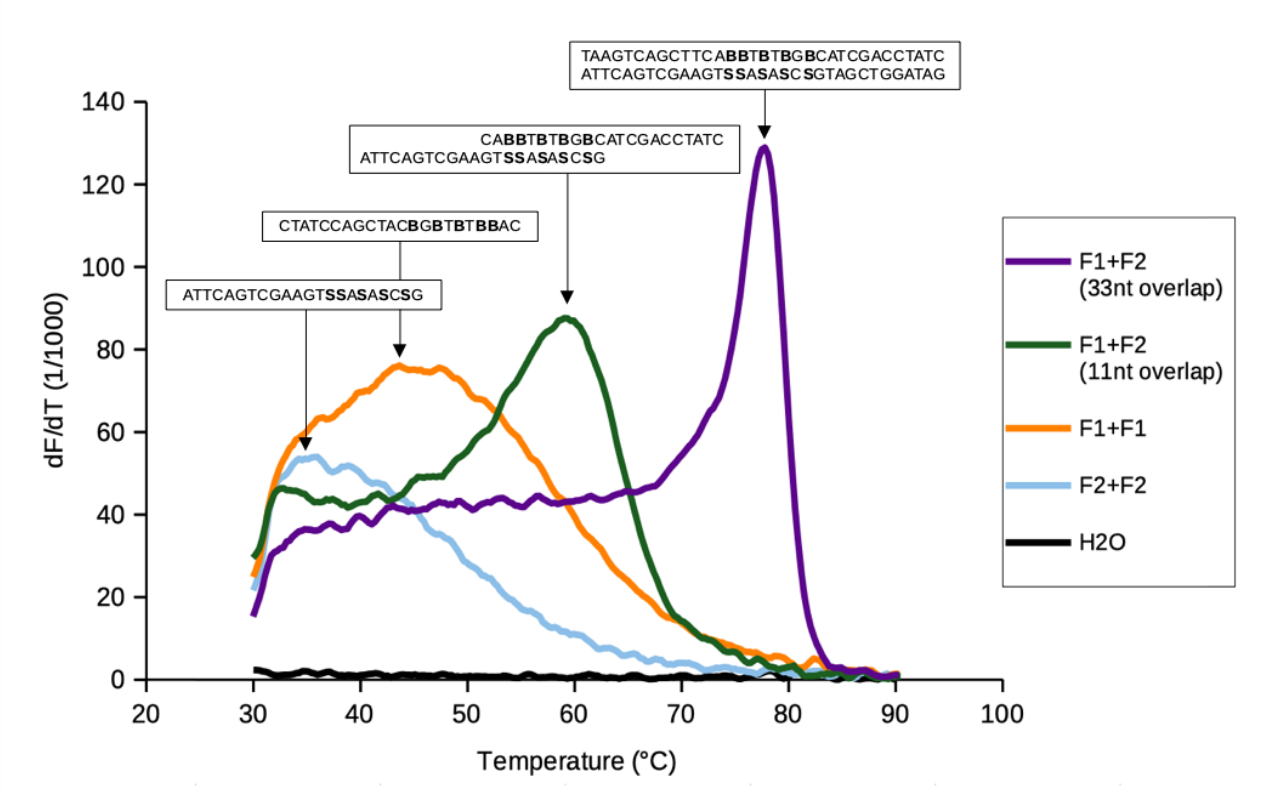

Figure 3: Melting curve analysis of sample 1 (= fragment 1, F1) and sample 2 (= fragment 2, F2) alone, mixed and after PCR

Outcome of application test

The hybridization was even recognized by an polymerase initiating the length growth. Therefore these XNA bases can be used also as primer in PCR assay.