Technical Note

Locked Nucleic Acid

LNA24/05.2005

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LNA Oligonucleotide Synthesis

Contents

Introduction

LNA oligonucleotides are synthesized by the phosphoramidite approach and can thus be synthesized with standard oligonucleotides synthesizers. However, it is recommended to use slightly altered coupling protocols in order to get the best coupling efficiency with the LNA phorphoramidites. In general, a longer coupling time and oxidation time compared to the coupling and oxidation times needed for standard DNA phosphoramidites is recommended.

Exiqon has developed some very efficient protocols for using LNA phosphoramidites on ABI DNA synthesizers – the Expedite™ and the ABI3900 synthesizers that are described in this flyer along with guidelines for synthesizing 3'-end modified LNA-oligonucleotides.

Protocols for Expedite™ synthesizer

The LNA-A, G and T amidites are used as 0.07M solutions in anhydrous acetonitrile.

For LNA-mC the amidite is first dissolved in anhydrous tetrahydrofurane. When a clear solution has been obtained anhydrous acetonitrile is added to a final THF/ACN[1] ratio of 25:75 and an amidite concentration of 0.07M. It is highly recommended to check the THF for peroxides as these oxidize the phosphoramidite and hence irreversibly inactivate it.

It is recommended to increase the coupling time to 250 seconds for all LNA phosphoramidites.

It is recommended to use 0.5M dicyanoimidazole (DCI) as activator[2].

LNA requires a longer oxidation time compared to DNA. 45 Seconds has been found to be optimal when synthesizing standard oligonucleotides.

Synthesis protocols are depicted on the following pages using an Expedite™ DNA synthesizer equipped with and without a MOSS unit. The protocol is given for a 0.2µmol synthesis scale with the LNA-A amidite either at position 5 or at position A.

The user should be aware that these protocols have been developed on one instrument using one type of support and holder. Changing instrument, support or holder might cause a change in the length of the reagents flow and therefore the addition or deletion of one or more pulses might be necessary in order to get the reagents to the support at the right time e.g. the step "slow pulse to couple" should be used when the amidite is at the column and neither when the amidite is before or after the support.

	Molecular weight g/mole	Product Nr.	CAS. Nr.	Dissolve in	To obtain a 0.07M solution	
					100 mg	250 mg
LNA-A^{Bz}	885.9	A-0063-	[206055-79-0]	Anhydrous Acetonitrile	1.6 mL	4.1 mL
LNA-mC^{Bz}	875.9	mC-0066-	[206055-82-5]	THF [1]/Acetonitrile 25/75 (v/v)	1.6 mL	4.1 mL
LNA-G^{DMF}	852.9	G-0082-	[709641-79-2]	Anhydrous Acetonitrile	1.7 mL	4.2 mL
LNA-T	772.8	T-0064-	[206055-75-6]	Anhydrous Acetonitrile	1.8 mL	4.6 mL

Table 1. Dissolution of LNA amidites. The 0.07M concentration of LNA amidites is recommended for the use on Expedite™ DNA synthesizers.

[1] We recommend the use of anhydrous tetrahydrofurane (Aldrich 401757), as an alternative anhydrous dichloromethane (Aldrich 270997) can be used as a substitute for THF.

[2] A 0.5M DCI solution is not commercial available. A 0.5M DCI solution can be made by dissolving 5.9g of DCI (Aldrich 67864) in 100mL of anhydrous acetonitrile.

Expedite™ with MOSS unit

Synthesizing LNA oligonucleotides on an Expedite™ DNA synthesizer with a MOSS unit requires altered protocols compared to the ones used when used without a MOSS unit. Given below are examples of synthesis of LNA oligonucleotides on a 0.2µmol scale with LNA either on position 5 or position A.

Synthesis Cycle for LNA on an Expedite™ Multiple Oligo Synthesis System (MOSS)

The synthesis cycle has been written for position 5 on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

MOSS LNA-cycle (0.2µmole scale)

```
/* ------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)         Description       */
/*                                  /Arg1   /Arg2                              */
/* -------------------------------------------------------------------------*/
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre cycle wash"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
  16 /*Dblk                */ PULSE     20     0  "Dblk to column"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     20    20  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  22 /*5 + Act             */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                 */ PULSE      5     0  "Chase with Act"
   1 /*Wsh                 */ PULSE     12     0  "Chase with Wsh"
   1 /*Wsh                 */ PULSE     10   250  "Slow pulse to couple"
   1 /*Wsh                 */ PULSE      4     0  "Flush with Wsh"
$Capping
  13 /*Caps                */ PULSE     12     0  "Caps to column"
  12 /*Wsh A               */ PULSE     10     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    45  "Slow pulse to cap"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE     12     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    45  "Slow pulse to Ox"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE     10     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    10  "Slow pulse to Cap"
  12 /*Wsh A               */ PULSE     20     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

Synthesis Cycle for DNA on an Expedite™ Multiple Oligo Synthesis System (MOSS)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

MOSS DNA-cycle (0.2µmole scale)

```
/* ------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)         Description       */
/*                                  /Arg1   /Arg2                             */
/* ------------------------------------------------------------------------- */
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre cycle wash"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
  16 /*Dblk                */ PULSE     20     0  "Dblk to column"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      5    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     20    20  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act             */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                 */ PULSE      5     0  "Chase with Act"
   1 /*Wsh                 */ PULSE     12     0  "Chase with Wsh"
   1 /*Wsh                 */ PULSE     10   120  "Slow pulse to couple"
   1 /*Wsh                 */ PULSE      4     0  "Flush with Wsh"
$Capping
  13 /*Caps                */ PULSE     12     0  "Caps to column"
  12 /*Wsh A               */ PULSE     10     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    45  "Slow pulse to cap"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE     12     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    30  "Slow pulse to Ox"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE     10     0  "Chase with Wsh A"
  12 /*Wsh A               */ PULSE     10    10  "Slow pulse to Cap"
  12 /*Wsh A               */ PULSE     20     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

Expedite™ without MOSS unit

Synthesizing LNA oligonucleotides on an Expedite™ DNA synthesizer without using a MOSS unit requires altered protocols compared to the ones given above, as there is a shorter distance from the reagent bottle to the support. Given below are examples of synthesis of LNA oligonucleotides on a 0.2µmol scale with LNA either on position 5 or position A.

Synthesis Cycle for LNA on an Expedite™ Nucleic Acid Synthesis System without MOSS

The synthesis cycle has been written for position 5 on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

LNA-cycle (0.2µmole scale)

```
/* ------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)        Description       */
/*                                  /Arg1   /Arg2                             */
/* ------------------------------------------------------------------------- */
$Deblocking
  12 /*Wsh A               */ PULSE     10     0  "Pre cycle wash"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default            */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk               */ PULSE     10     0  "Dblk to column"
  16 /*Dblk               */ PULSE     50    49  "Deblock"
  38 /*Diverted Wsh A     */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                */ PULSE     10     0  "Flush system with Wsh"
   2 /*Act                */ PULSE      5     0  "Flush system with Act"
  22 /*5 + Act            */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                */ PULSE      1     0  "Couple monomer"
   2 /*Act                */ PULSE      3    75  "Couple monomer"
   1 /*Wsh                */ PULSE      7   175  "Couple monomer"
   1 /*Wsh                */ PULSE      8     0  "Flush system with Wsh"
$Capping
  12 /*Wsh A               */ PULSE     20     0  "Flush system with Wsh A"
  13 /*Caps                */ PULSE      8     0  "Caps to column"
  12 /*Wsh A               */ PULSE      6    45  "Cap"
  12 /*Wsh A               */ PULSE     14     0  "Flush system with Wsh A"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE     10    45  "Slow pulse to Ox"
  12 /*Wsh A               */ PULSE      4     0  "Flush system with Wsh A"
$Capping
  13 /*Caps                */ PULSE      7     0  "Caps to column"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

DNA-cycle (0.2µmole scale)

```
/* ------------------------------------------------------------------------- */
/*       Function              Mode  Amount Time(sec)         Description      */
/*                                   /Arg1   /Arg2                           */
/* ------------------------------------------------------------------------- */
$Deblocking
  12 /*Wsh A               */ PULSE     10     0  "Pre cycle wash"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     10     0  "Dblk to column"
  16 /*Dblk                */ PULSE     50    49  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act             */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                 */ PULSE      1     0  "Couple monomer"
   2 /*Act                 */ PULSE      3    36  "Couple monomer"
   1 /*Wsh                 */ PULSE      7    84  "Couple monomer"
   1 /*Wsh                 */ PULSE      8     0  "Flush system with Wsh"
$Capping
  12 /*Wsh A               */ PULSE     20     0  "Flush system with Wsh A"
  13 /*Caps                */ PULSE      8     0  "Caps to column"
  12 /*Wsh A               */ PULSE      6    45  "Cap"
  12 /*Wsh A               */ PULSE     14     0  "Flush system with Wsh A"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE     15     0  “Flush system with Wsh A"
$Capping
  13 /*Caps                */ PULSE      7     0  "Caps to column"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

Synthesis Cycle for LNA on an Expedite™ Nucleic Acid Synthesis System without MOSS

The synthesis cycle has been written for position 5 on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

LNA-cycle (1µmole scale)

```
/* ------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)         Description       */
/*                                  /Arg1   /Arg2                              */
/* ------------------------------------------------------------------------- */
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                */ PULSE      2    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     15     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  22 /*5 + Act             */ PULSE      5     0  "LNA-A + Act to column"
  22 /*5 + Act             */ PULSE      2    75  "LNA-A + Act to column"
   2 /*Act                 */ PULSE      3    75  "Couple monomer"
   1 /*Wsh                 */ PULSE      7   100  "Couple monomer"
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE      6    45  "Cap"
  12 /*Wsh A               */ PULSE      4     0  "Flush system with Wsh A"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE      8    45  "Slow Ox Wsh A"
  12 /*Wsh A               */ PULSE     10     0  "Flush system with Wsh A"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE      8    10  "Wsh A"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed.

DNA-cycle (1µmole scale)

```
/* ------------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)         Description           */
/*                                  /Arg1   /Arg2                                  */
/* ------------------------------------------------------------------------------- */
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                */ PULSE      2    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     40     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE      5     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act             */ PULSE      5     0  "DNA-a + Act to column"
  23 /*6 + Act             */ PULSE      2    35  "DNA-a + Act to column"
   2 /*Act                 */ PULSE      3    35  "Couple monomer"
   1 /*Wsh                 */ PULSE      7    50  "Couple monomer"
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE      6    45  "Cap"
  12 /*Wsh A               */ PULSE      4     0  "Flush system with Wsh A"
$Oxidizing
  15 /*Ox                  */ PULSE     15     0  "Ox to column"
  12 /*Wsh A               */ PULSE      8    15  "Slow Pulse to Ox"
  12 /*Wsh A               */ PULSE     10     0  "Flush system with Wsh A"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  12 /*Wsh A               */ PULSE      8    10  "Flush system with Wsh A"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act             */
  19 /*C + Act             */
  20 /*G + Act             */
  21 /*T + Act             */
  22 /*5 + Act             */
  23 /*6 + Act             */
  24 /*7 + Act             */
  25 /*8 + Act             */
  26 /*9 + Act             */
```

LNA Phosphorthioates

As LNA amidites can be used as parent DNA amidites it is likewise possible to make LNA phosphorothioates in which one of the non-bridging oxygen atoms has been replaced with a sulphur. The amidites should be used as described above – only change is that the oxidizer should be replaced with Beaucage's reagent on the synthesizer.

We recommend the use of Beaucage's reagent as a 0.2 M (4%) solution:
Dissolve 4g in 100 mL of anhydrous acetonitrile.*
* Do not add sieves as this might promote decomposition of Beaucage's reagent. Use silanized bottles as recommended by the manufacturer.

Synthesis Cycle for LNA phosphorothioates on an Expedite™ (0.2 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```
/*--------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)        Description         */
/*                                  /Arg1   /Arg2                               */
/*----------------------------------------------------------------------------*/
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                */ PULSE      2    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     50     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     15     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act             */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                 */ PULSE      3    75  "Couple monomer"
   1 /*Wsh                 */ PULSE      7   175  "Couple monomer"
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
$Oxidizing
  17 /*Aux                 */ PULSE     15     0  "SOx to column"
  17 /*Aux                 */ PULSE     15   120  "SOx to column"
  12 /*Wsh A               */ PULSE     10    60  "Slow pulse to thioate"
  12 /*Wsh A               */ PULSE     20     0  "Wsh A"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  13 /*Caps                */ PULSE     10    30  "Caps to column"
  12 /*Wsh A               */ PULSE      8    15  "Wsh A"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ (1.0 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third and fourth line in the coupling step has to be changed, see below.

```
/*---------------------------------------------------------------------------- */
/*       Function             Mode  Amount Time(sec)         Description         */
/*                                  /Arg1   /Arg2                                */
/*-----------------------------------------------------------------------------*/
$Deblocking
  12 /*Wsh A               */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.  */ NA         1     0  "Event out ON"
   0 /*Default             */ WAIT       0   1.5  "Wait"
 141 /*Trityl Mon. On/Off  */ NA         1     1  "START data collection"
  16 /*Dblk                */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                */ PULSE      2    10  "Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  16 /*Dblk                */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A      */ PULSE     50     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off  */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.  */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                 */ PULSE     15     0  "Flush system with Wsh"
   2 /*Act                 */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act             */ PULSE      5     0  "LNA-A + Act to column"
  18 /*A + Act             */ PULSE      2    75  "LNA-A + Act to column"
   2 /*Act                 */ PULSE      3   150  "Couple monomer"
   1 /*Wsh                 */ PULSE      7   200  "Couple monomer"
   1 /*Wsh                 */ PULSE     10     0  "Flush system with Wsh"
$Oxidizing
  17 /*Aux                 */ PULSE     15     0  "SOx to column"
  17 /*Aux                 */ PULSE     15   120  "SOx to column"
  12 /*Wsh A               */ PULSE     10    60  "Slow pulse to thioate"
  12 /*Wsh A               */ PULSE     20     0  "Wsh A"
$Capping
  13 /*Caps                */ PULSE     10     0  "Caps to column"
  13 /*Caps                */ PULSE     10    30  "Caps to column"
  12 /*Wsh A               */ PULSE      8    15  "Wsh A"
  12 /*Wsh A               */ PULSE     30     0  "End of cycle wash"
```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ with MOSS (0.2 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```
/*------------------------------------------------------------------------------ */
/*       Function              Mode  Amount Time(sec)         Description          */
/*                                   /Arg1   /Arg2                                */
/*------------------------------------------------------------------------------*/
$Deblocking
  12 /*Wsh A                */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.    */ NA         1     0  "Event out ON"
   0 /*Default              */ WAIT       0   1.5  "Wait"
  16 /*Dblk                 */ PULSE     20     0  "Deblock to column"
 141 /*Trityl Mon. On/Off    */ NA         1     1  "START data collection"
  16 /*Dblk                 */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                 */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                 */ PULSE     10     0  "Deblock"
  16 /*Dblk                 */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                 */ PULSE     10     0  "Deblock"
  16 /*Dblk                 */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                 */ PULSE     10     0  "Deblock"
  16 /*Dblk                 */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                 */ PULSE     10     0  "Deblock"
  16 /*Dblk                 */ PULSE      2    10  "Slow Deblock"
  38 /*Diverted Wsh A       */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A       */ PULSE      2    10  "Slow Deblock"
  38 /*Diverted Wsh A       */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A       */ PULSE     50     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off    */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.    */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                  */ PULSE     10     0  "Flush system with Wsh"
   2 /*Act                  */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act              */ PULSE      5     0  "Monomer + Act to column"
   2 /*Act                  */ PULSE      5     0  "Chase with Act"
   1 /*Wsh                  */ PULSE     12     0  "Chase with Wsh"
   1 /*Wsh                  */ PULSE     10   250  "Slow pulse to couple"
   1 /*Wsh                  */ PULSE      4     0  "Flush with Wsh"
$Oxidizing
  17 /*Aux                  */ PULSE     30     0  "SOx to column"
  12 /*Wsh A                */ PULSE     20   180  "Slow pulse to thioate"
  12 /*Wsh A                */ PULSE     20     0  "Wsh A"
$Capping
  13 /*Caps                 */ PULSE     20     0  "Caps to column"
  12 /*Wsh A                */ PULSE     20    45  "Wsh A"
  12 /*Wsh A                */ PULSE     60     0  "End of cycle wash"
```

Synthesis Cycle for LNA phosphorothioates on an Expedite™ with MOSS (1.0 µmol scale)

The synthesis cycle has been written for position A on the Expedite™. If any other positions are used, third line in the coupling step has to be changed, see below.

```
/*------------------------------------------------------------------------------ */
/*       Function              Mode  Amount Time(sec)         Description          */
/*                                   /Arg1   /Arg2                                */
/*------------------------------------------------------------------------------*/
$Deblocking
  12 /*Wsh A                */ PULSE     15     0  "Pre Wsh A"
 144 /*Index Fract. Coll.    */ NA         1     0  "Event out ON"
   0 /*Default               */ WAIT       0   1.5  "Wait"
  16 /*Dblk                  */ PULSE     20     0  "Deblock to column"
 141 /*Trityl Mon. On/Off    */ NA         1     1  "START data collection"
  16 /*Dblk                  */ PULSE     20     0  "Deblock to column"
  16 /*Dblk                  */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                  */ PULSE     10     0  "Deblock"
  16 /*Dblk                  */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                  */ PULSE     10     0  "Deblock"
  16 /*Dblk                  */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                  */ PULSE     10     0  "Deblock"
  16 /*Dblk                  */ PULSE      2    10  "Slow Deblock"
  16 /*Dblk                  */ PULSE     10     0  "Deblock"
  16 /*Dblk                  */ PULSE      2    10  "Slow Deblock"
  38 /*Diverted Wsh A        */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A        */ PULSE      2    10  "Slow Deblock"
  38 /*Diverted Wsh A        */ PULSE     10     0  "Deblock"
  38 /*Diverted Wsh A        */ PULSE     50     0  "Flush system with Wsh A"
 141 /*Trityl Mon. On/Off    */ NA         0     1  "STOP data collection"
 144 /*Index Fract. Coll.    */ NA         2     0  "Event out OFF"
$Coupling
   1 /*Wsh                   */ PULSE     15     0  "Flush system with Wsh"
   2 /*Act                   */ PULSE      5     0  "Flush system with Act"
  18 /*A + Act               */ PULSE      7     0  "LNA-A + Act to column"
   2 /*Act                   */ PULSE      5     0  "Couple monomer"
   1 /*Wsh                   */ PULSE     10     0  "Couple monomer"
   1 /*Wsh                   */ PULSE     15   425  "Couple monomer"
   1 /*Wsh                   */ PULSE     20     0  "Flush system with Wsh"
$Oxidizing
  17 /*Aux                   */ PULSE     30     0  "SOx to column"
  12 /*Wsh A                 */ PULSE     20   180  "Slow pulse to thioate"
  12 /*Wsh A                 */ PULSE     20     0  "Wsh A"
$Capping
  13 /*Caps                  */ PULSE     20     0  "Caps to column"
  12 /*Wsh A                 */ PULSE     20    45  "Wsh A"
  12 /*Wsh A                 */ PULSE     60     0  "End of cycle wash"
```

Bottle/position codes:

```
  18 /*A + Act               */
  19 /*C + Act               */
  20 /*G + Act               */
  21 /*T + Act               */
  22 /*5 + Act               */
  23 /*6 + Act               */
  24 /*7 + Act               */
  25 /*8 + Act               */
  26 /*9 + Act               */
```

LNA Oligonucleotide Synthesis on an ABI3900 Instrument

The use of LNA phosphoramidites on an ABI3900 instrument follows standard procedures for use of phosphoramidites with a slight modification of coupling protocols and concentration of the amidite.

The LNA-A, G and T amidites are used as 0.05M solutions in anhydrous acetonitrile.

The LNA-mC amidite is first dissolved in anhydrous tetrahydrofurane and when a clear solution has been obtained anhydrous acetonitrile is added to give a final THF/ACN ratio of 25:75 and an amidite concentration of 0.05M, Table 2. It is highly recommended to check the THF for peroxides as these oxidize the phosphoramidite hence irreversibly inactivate it.
To get the optimum coupling efficiency, double couplings are used for LNA, a 2 x 75 seconds coupling time is found to be sufficient for all LNA phosphoramidites. In this manner, the consumption of LNA amidites per coupling can be minimized and only 33% more LNA amidite is used per coupling compared to DNA amidite consumption.

LNA is oxidized slower as DNA and therefore requires a longer oxidation time compared to DNA. Triple oxidation is found to be optimal when synthesizing standard LNA oligonucleotides on the ABI 3900.

	Molecular weight g/mole	Product Nr.	CAS. Nr.	Dissolve in	To obtain a 0.05M solution	
					100 mg	250 mg
LNA-A^{Bz}	885.9	A-0063-	[206055-79-0]	Anhydrous Acetonitrile	2.3 mL	5.7 mL
LNA-mC^{Bz}	875.9	mC-0066-	[206055-82-5]	THF[1]/Acetonitrile 25/75 (v/v)	2.3 mL	5.7 mL
LNA-G^{DMF}	852.9	G-0082-	[709641-79-2]	Anhydrous Acetonitrile	2.3 mL	5.8 mL
LNA-T	772.8	T-0064-	[206055-75-6]	Anhydrous Acetonitrile	2.6 mL	6.5 mL

Table 2. Dissolution of LNA amidites. The 0.05M concentration of LNA amidites is recommended for the use on ABI 3900 DNA synthesizers. [1] We recommend the use of anhydrous tetrahydrofurane (Aldrich 401757). As an alternative to THF, anhydrous dichloromethane (Aldrich 270997) can be used as a substitute for THF.

Synthesis Cycle for LNA/DNA on an ABI3900 Instrument (0.2µmole)

Explanation to Synthesizer Cycle File:
When LNA amidites are used they are placed on the modifier positions 5, 6, 7, 8, 9 and 0.
In the sequence the 5 to 0 positions are written in numbers, but to make modification in the synthesis cycle for these positions, except for the amidites, the modifications has to be made in the "Upper Case" cycle. Therefore it is advisable to use "Lower Case" for denoting DNA monomers in the sequence.

Exiqon A/S

Applied Biosystems Oligonucleotide Synthesizer Cycle File

Program_Name: **200nm, 0.05M, LNA-DNA**

Scale: **200nMole**

		Case UPPER						Case LOWER					
PREPROCESSING	Purges	# Iterations	Wait sec.	Command Code	Param1 Chemical	Param2 Volume	START	# Iterations	Wait sec.	Command Code	Param1 Chemical	Param2 Volume
PREWASH		1	0	DISP	ACN	280	PREWASH1	1	0	DISP	ACN	280
	LONG_PURGE											
START_LOOP												
DETRITYLATION		3	0	DISP	DEBLOCK	140	DETRITYLATION	3	0	DISP	DEBLOCK	140
	LONG_PURGE											
TCA_WASH		1	0	DISP	ACN	280	TCA_WASH	1	0	DISP	ACN	280
	LONG_PURGE											
COUPLING		1	0	DISP	ACTIVATOR	45	COUPLING	1	0	DISP	ACTIVATOR	45
				DISP	AMIDITE	30				DISP	AMIDITE	30
	REACT											
CAPPING		2	0	DISP	CAPB	30	CAPPING	1	0	DISP	CAPB	30
				DISP	CAPA	30				DISP	CAPA	30
	SHORT_PURGE											
OXIDATION		3	0	DISP	OXIDIZER	60	OXIDATION	1	0	DISP	OXIDIZER	60
	SHORT_PURGE											
OX_WASH		1	0	DISP	ACN	280	OX_WASH	1	0	DISP	ACN	280
	LONG_PURGE											
END_LOOP												
TRITYLOFF		2	0	DISP	DEBLOCK	140	TRITYLOFF	2	0	DISP	DEBLOCK	140
	LONG_PURGE											
FINAL_FLUSH		4	0	DISP	ACN	280	LAST_WASH	4	0	DISP	ACN	280
	LONG_PURGE											
DRY_SUPPORT		1	0	DISP	ACN	280	FINAL_FLUSH	1	0	DISP	ACN	280
	DRY_BEADS											
END_PROGRAM												

Coupling Wait Time
40

Coupling of Modified Amidites

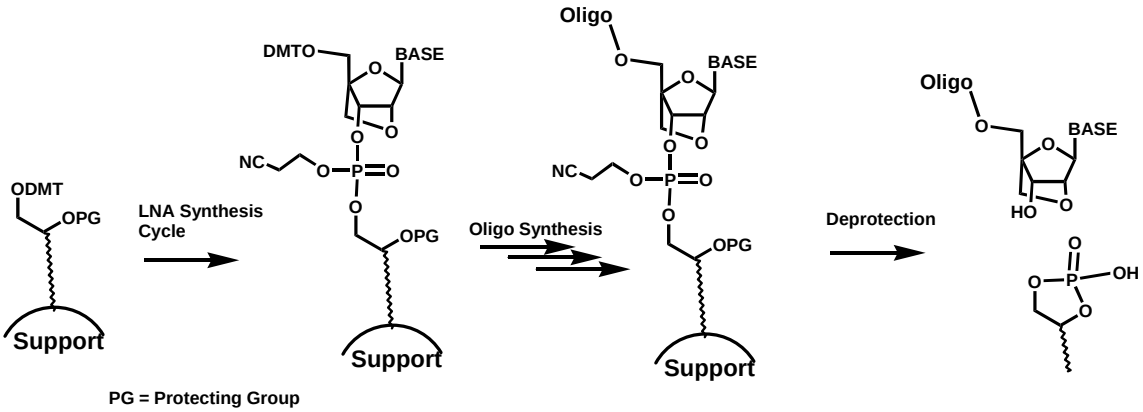
Amidite	Volume	Reps	Wait	Cpl.Wait
5	20	2	0	75
6	20	2	0	75
7	20	2	0	75
8	20	2	0	75
9	20	2	0	75
0	20	2	0	75

Purge Settings

Name	Reps	Seconds	Interim
REACT	1	1	0
LONG_PURGE	1	6	0
SHORT_PURGE	1	3	0
DRY_BEADS	4	10	1

Synthesis of 3'-LNA modified oligonucleotides.

Standard oligonucleotide synthesis use solid support functionalised with the 3'-nucleotide. For synthesis of oligonucleotides containing LNA in the 3'-end a so-called Universal Support must be used. Different types of Universal Supports are available. The general structure of the Universal supports is a 1,2-diol unit, which can be connected to the support in different ways. In the post synthesis deprotection step the 1,2-diol unit is eliminated as a cyclic phosphate.



Several types of Universal Supports have been tried for synthesis of 3'-LNA modified oligonucleotides. All types have resulted in oligonucleotides of similar quality and quantity as compared to oligonucleotide synthesized on LNA modified CPG support.

Different suppliers of universal supports:

Biosearch Technology (www.biosearchtech.com)
- # Universal Support – CPG (Ribose type)
- # "Rapid Cleave" Universal Support – CPG (Ribose type)
- # Universal Support – polystyrene (Ribose type)

Glen Research (www.glenres.com)
- # Universal Support (Ribose type)
- # Universal Support II (3-amino-1,2-propanediol type)
- # Universal Q-Support (Ribose type)

Please follow manufactures protocol for deprotection and note that the use of methylamine for deprotection of oligonucleotides containing LNA-mC should be avoided.

Latest revision May 30, 2005