Technical Note

Locked Nucleic Acid

LNA15/05.2005

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LNA Oligonucleotide Synthesis on an Expedite™ without MOSS

The use of LNA phosphoramidites on an Expedite™ instrument follows standard procedures for use of phosphoramidites.

The LNA-A, G and T amidites are used as 0.07M solutions in anhydrous acetonitrile, in the case of LNA-mC it is advised to dissolve the amidite in a 25% tetrahydrofurane/acetonitrile[1] solution (0.07M) to avoid precipitation of the amidite.

A 250 seconds coupling time is normally found to be sufficient 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 optimal when synthesising standard oligonucleotides.

	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/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

[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.

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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.

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             */
```


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