

# Technical Note



## Locked Nucleic Acid

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### LNA Oligonucleotide Synthesis on an ABI3900 Instrument

The use of LNA phosphoramidites on an ABI3900 instrument follows standard procedures for use of phosphoramidites.

The LNA-A, G and T amidites are used as 0.05M solutions in anhydrous acetonitrile, in the case of LNA-mC it is advised to dissolve the amidite in a 25% tetrahydrofuran/acetonitrile<sup>1</sup> solution (0.05M) to avoid precipitation of the amidite.

The best results are obtained when double coupling are used for LNA, a 2 x 75 seconds coupling time is found to be sufficient for all LNA phosphoramidites<sup>2</sup>.

LNA requires more oxidation compared to DNA. Triple oxidation is found to be optimal when synthesising standard LNA oligonucleotides.

	Molecular weight g/mole	Product Nr.	CAS. Nr.	Dissolve in	To obtain a 0.05M solution	
					100 mg	250 mg
LNA-A <sup>Bz</sup>	885.9	A-0063-	[206055-79-0]	Anhydrous Acetonitrile	2.3 mL	5.7 mL
LNA-mC <sup>Bz</sup>	875.9	mC-0066-	[206055-82-5]	THF/Acetonitrile 25/75 (v/v)	2.3 mL	5.7 mL
LNA-G <sup>DMF</sup>	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

<sup>1</sup> We recommend the use of anhydrous tetrahydrofuran (Aldrich 401757), as an alternative anhydrous dichloromethane (Aldrich 270997) can be used as a substitute for THF.

<sup>2</sup> The consumption of LNA amidite is only 33% more compared to DNA since less amidite are used in each "single" coupling step.

## 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	LONG_PURGE	1	0	DISP	ACN	280	PREWASH1	1	0	DISP	ACN	280
<b>START_LOOP</b>												
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											
<b>END_LOOP</b>												
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											
<b>END_PROGRAM</b>												

Coupling Wait Time
40

Coupling of Modified Amidites					Purge Settings			
Amidite	Volume	Reps	Wait	Cpl.Wait	Name	Reps	Seconds	Interim
5	20	2	0	75	REACT	1	1	0
6	20	2	0	75	LONG_PURGE	1	6	0
7	20	2	0	75	SHORT_PURGE	1	3	0
8	20	2	0	75	DRY_BEADS	4	10	1
9	20	2	0	75				
0	20	2	0	75				

### Trademarks and patents

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