

Large-Scale Syntheses of Long RNA Oligonucleotides

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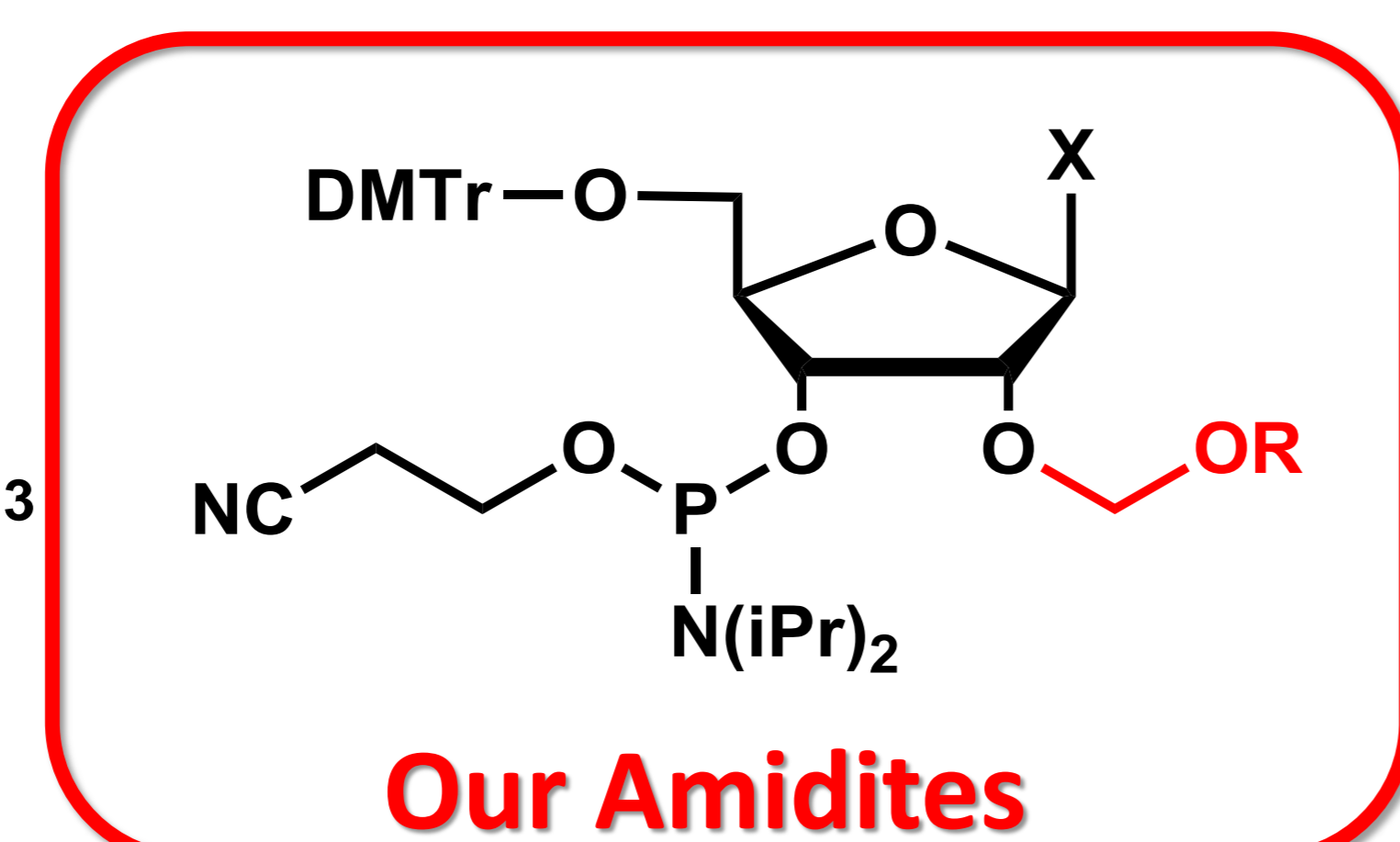
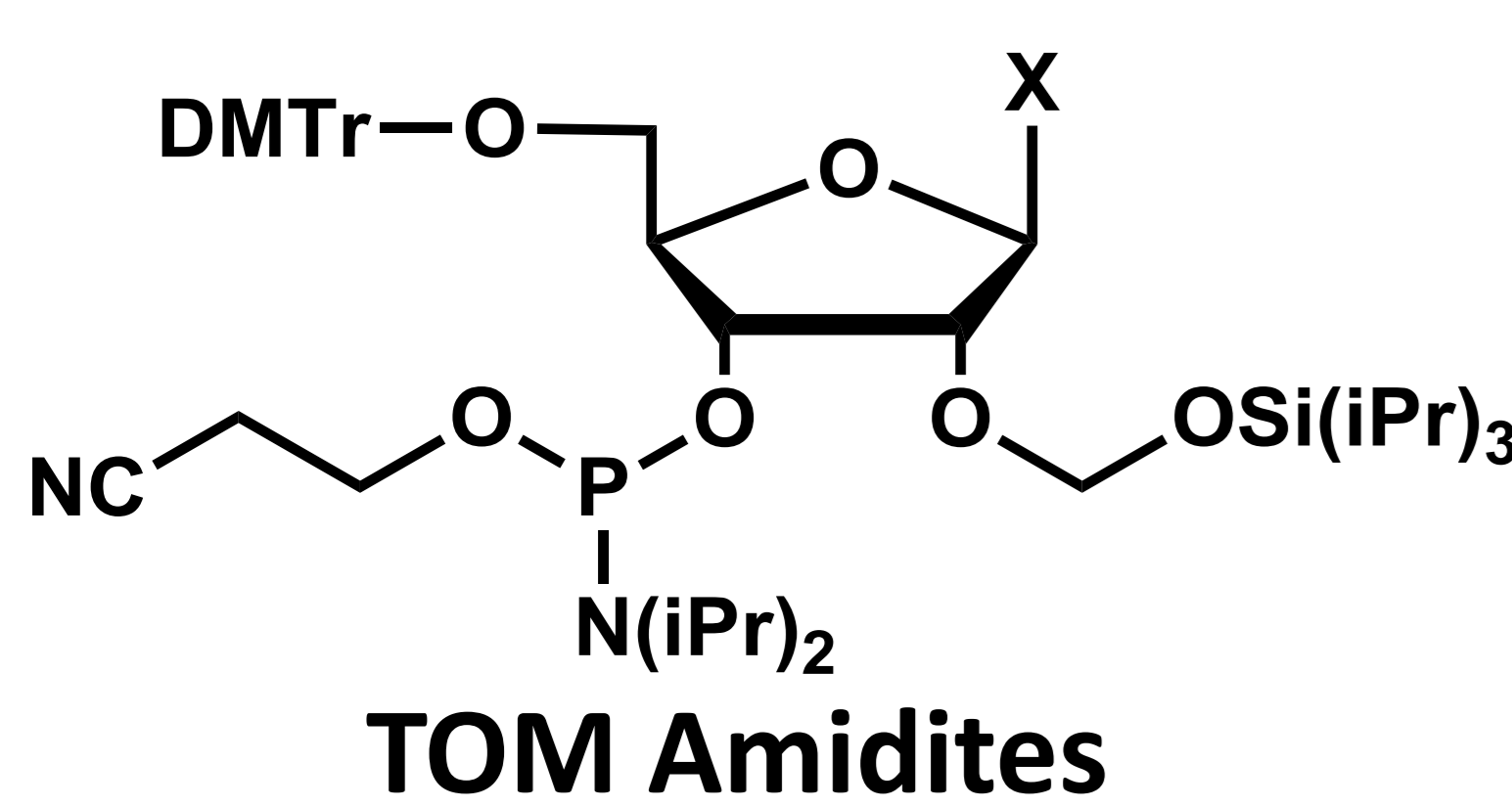
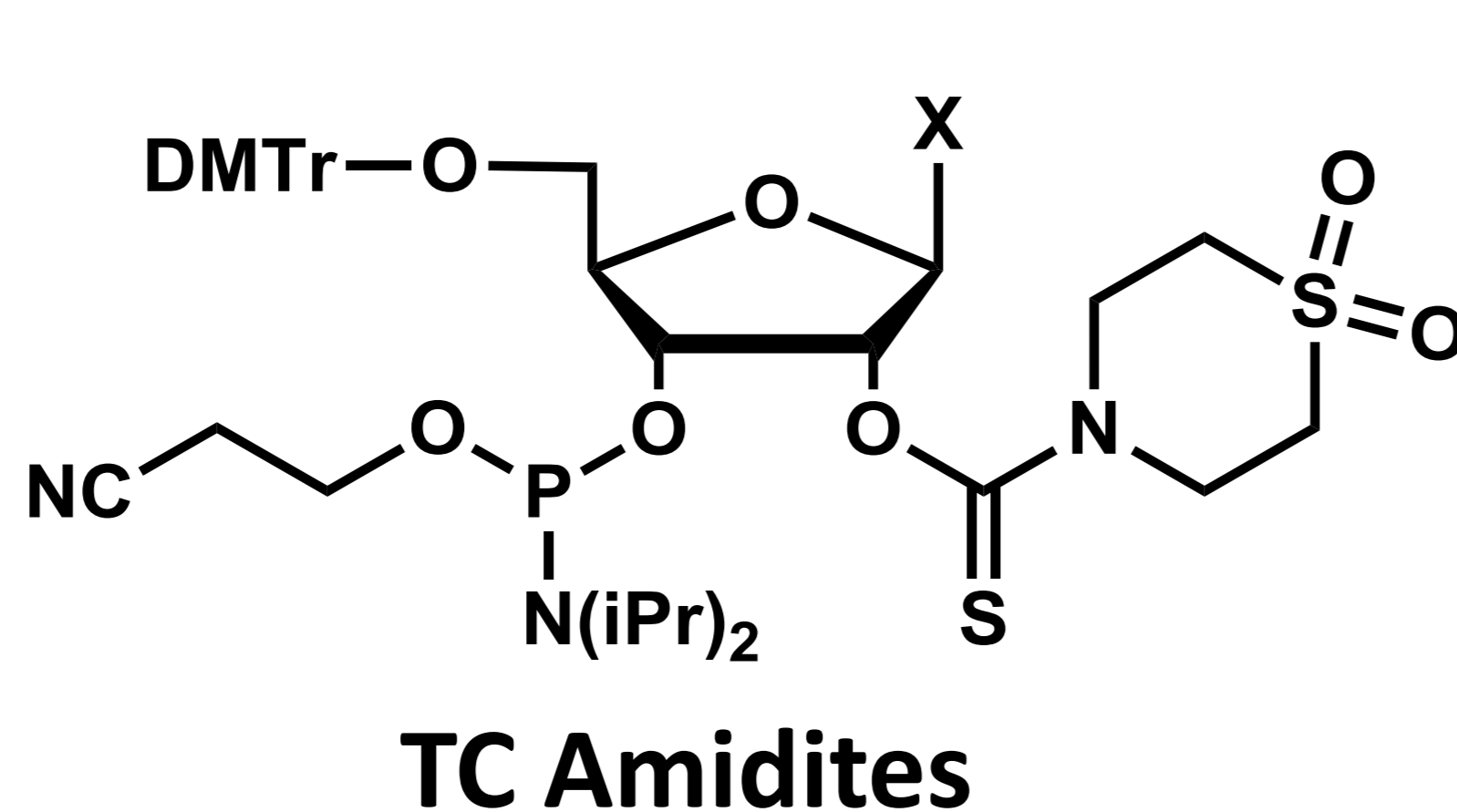
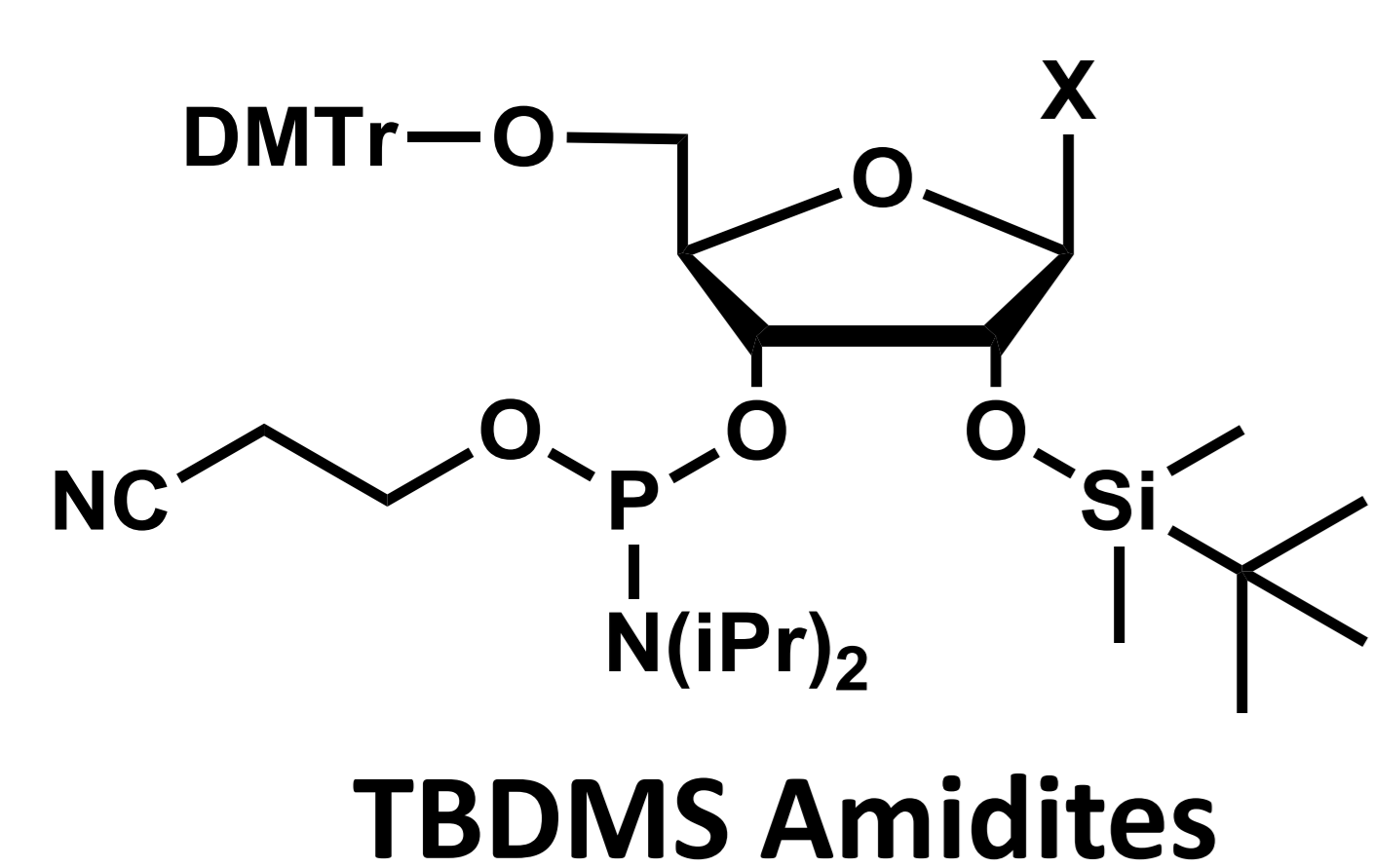
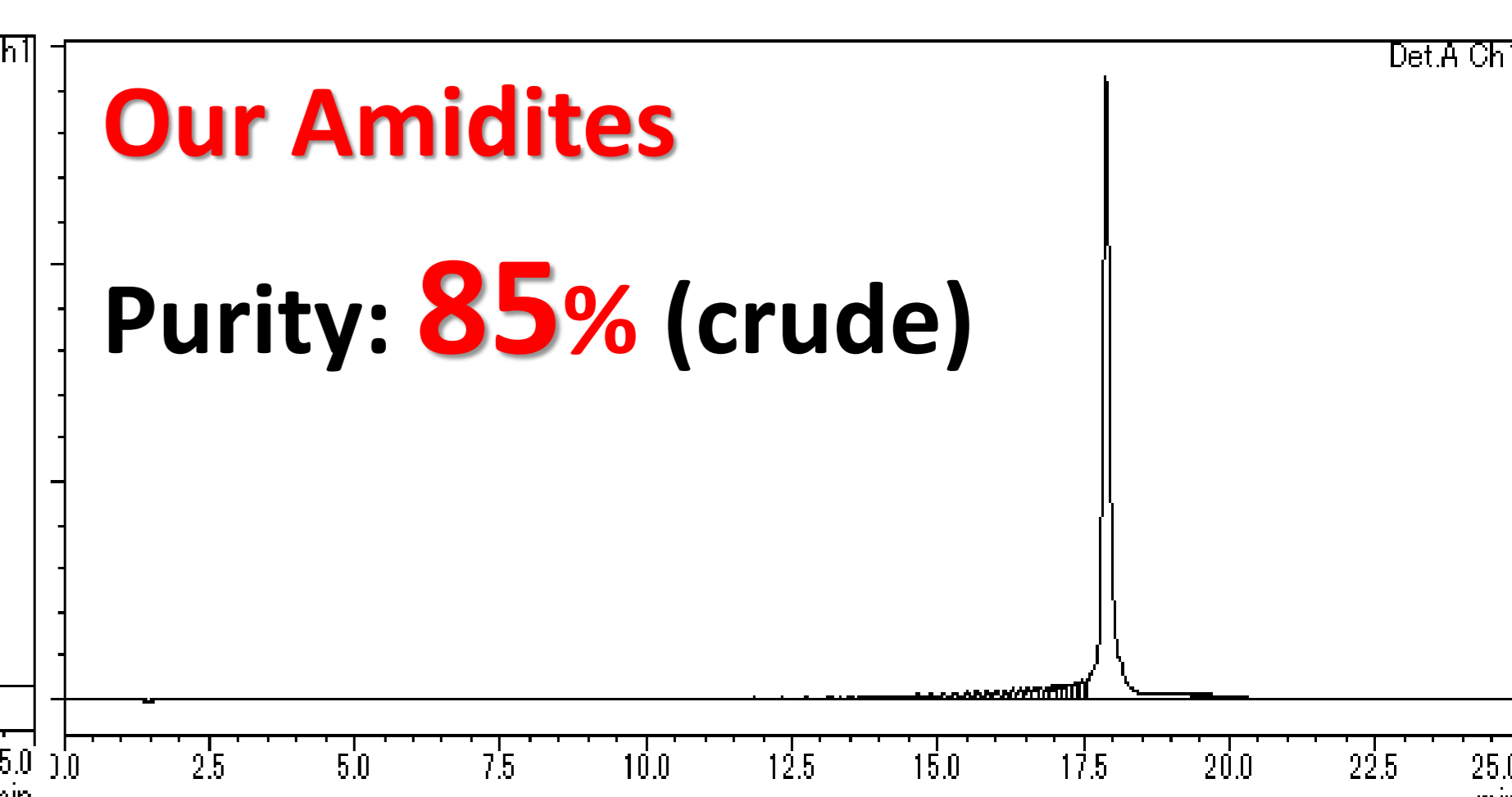
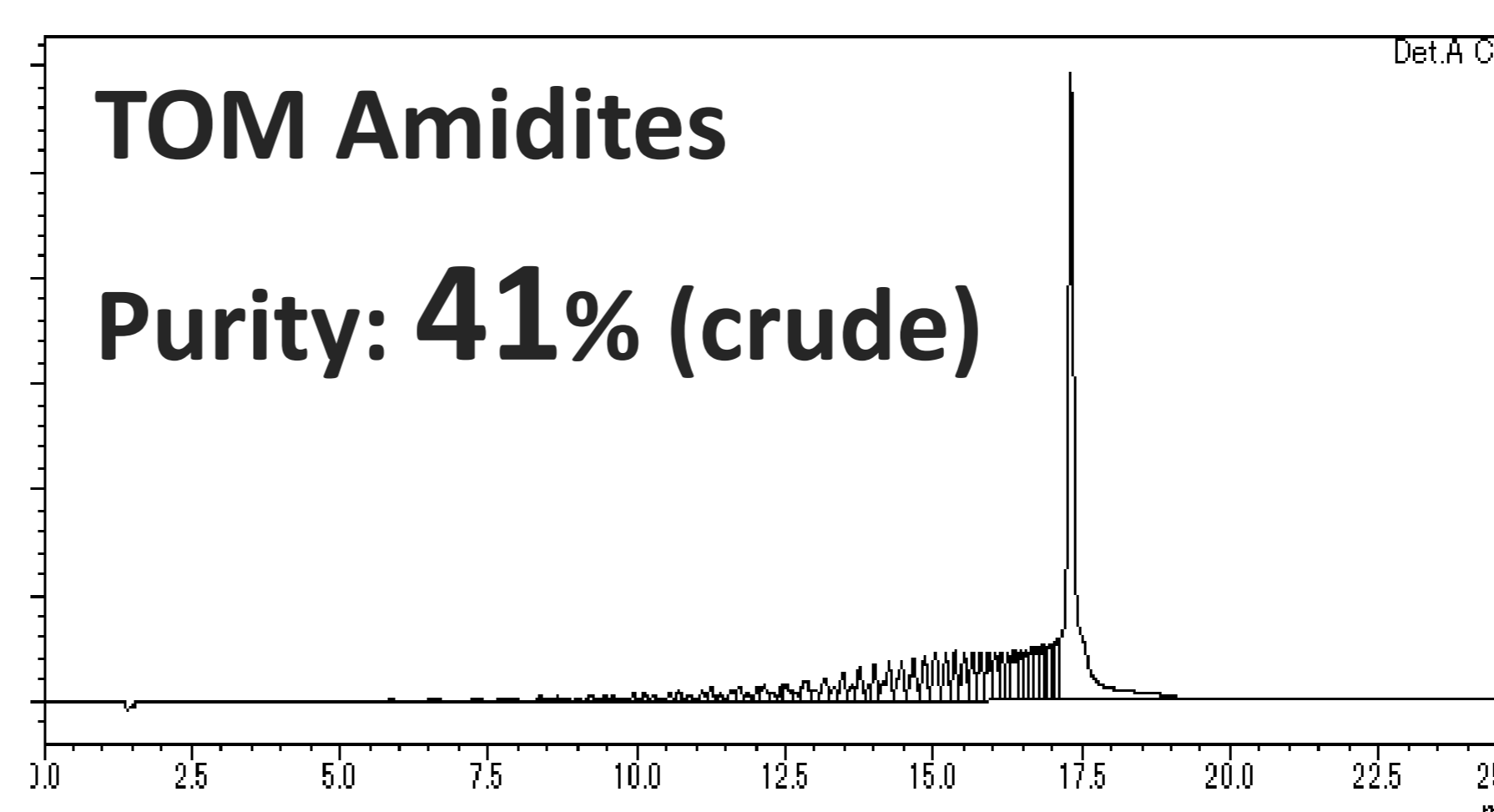
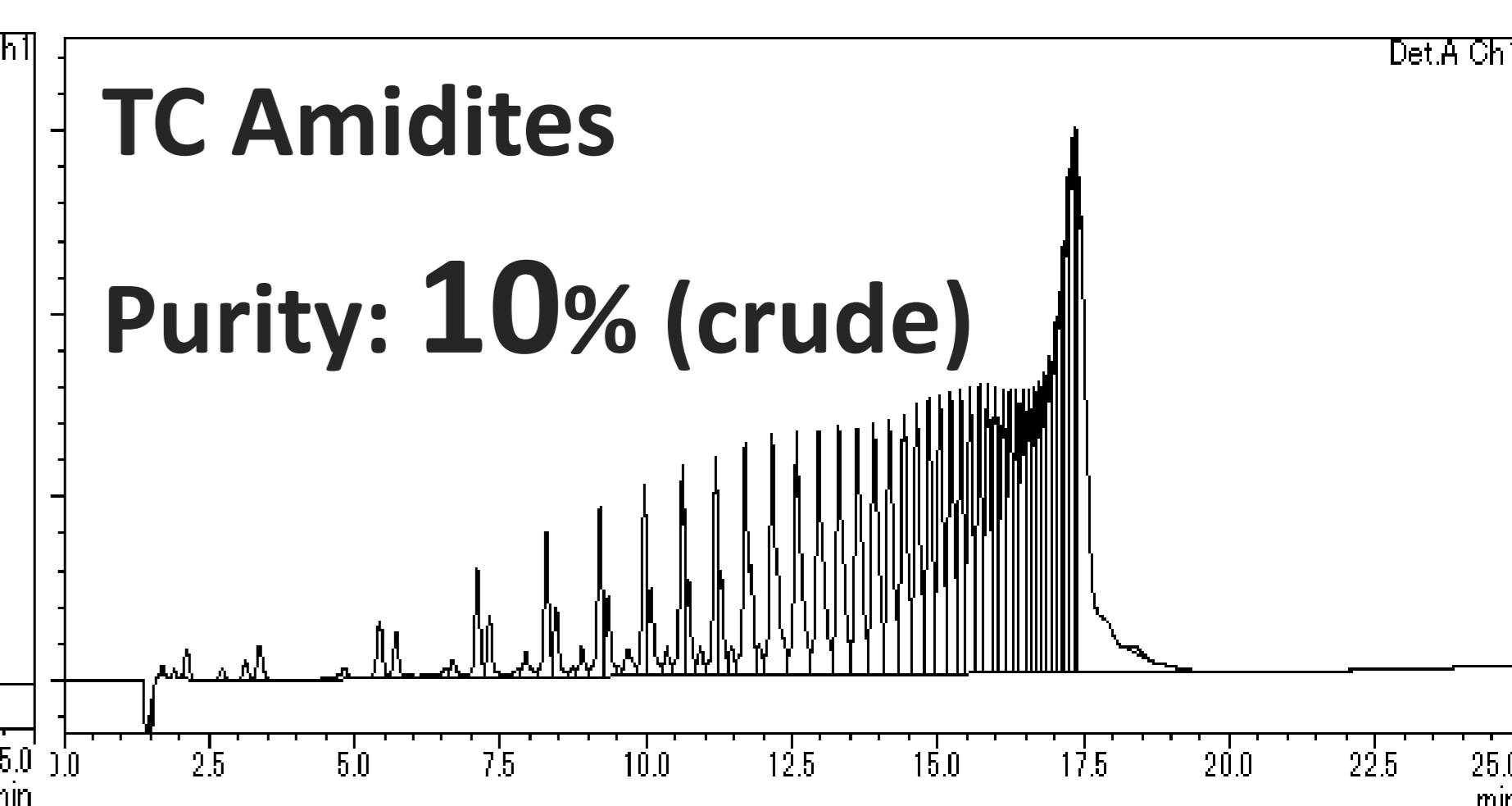
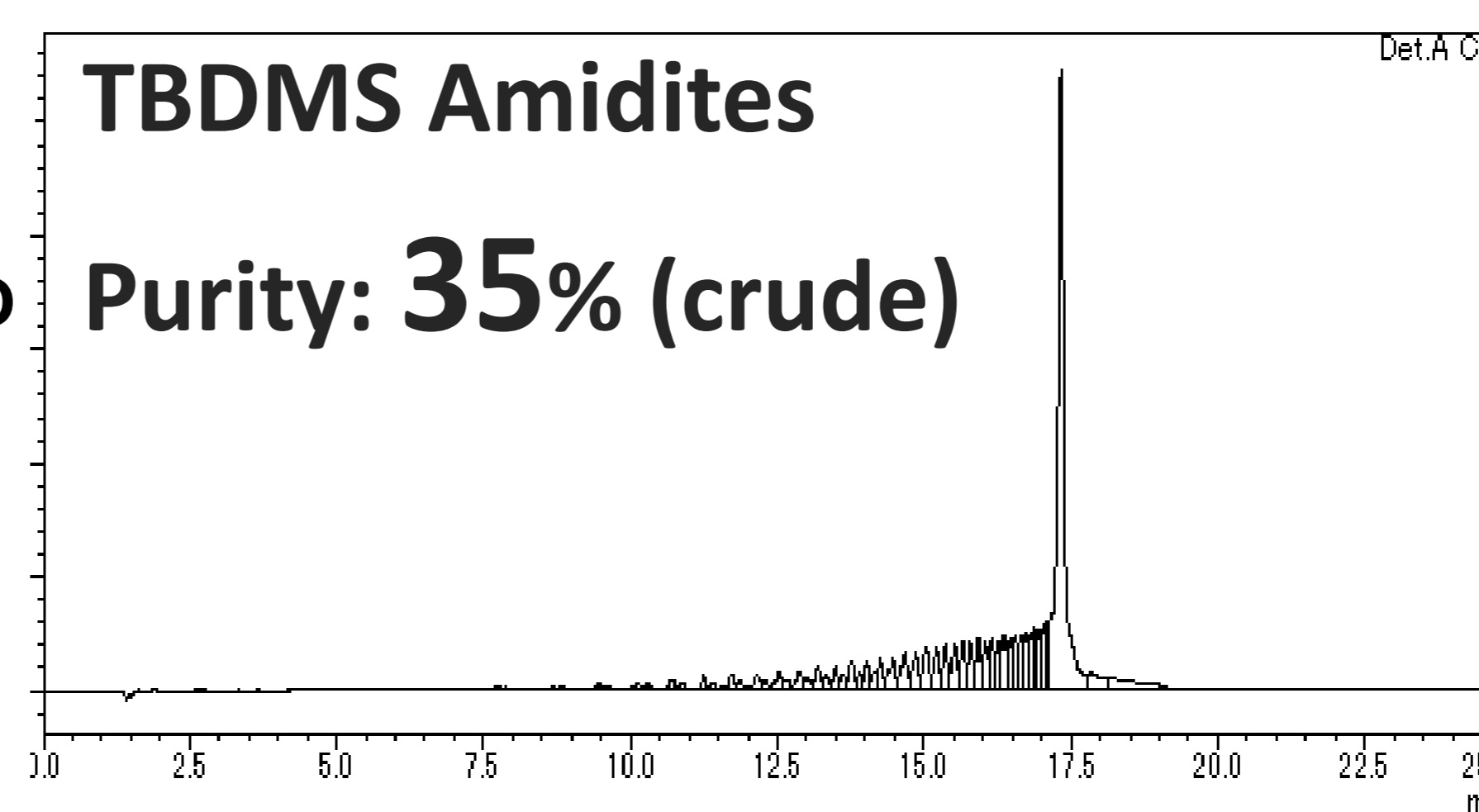
Abstract

In recent years, numerous studies have actively used long RNAs, such as pre-miRNA and CRISPR RNA. However, it is difficult to synthesize long RNA oligonucleotides on a large-scale. We have achieved large-scale syntheses of long RNA oligonucleotides (> 50mer), using our technologies and already manufactured ca. 1 kg as non-GMP and ca 500 g as GMP. In this study, we introduce one of our technologies for long RNA syntheses: **AMIDITES**.

Materials and Methods

 Structures of **Our Amidites** and Conventional Amidites

Comparison of Synthetic Capabilities, Synthesizing Poly-U (50mer)


 *(X=A^{PG}, G^{PG}, C^{PG}, U)


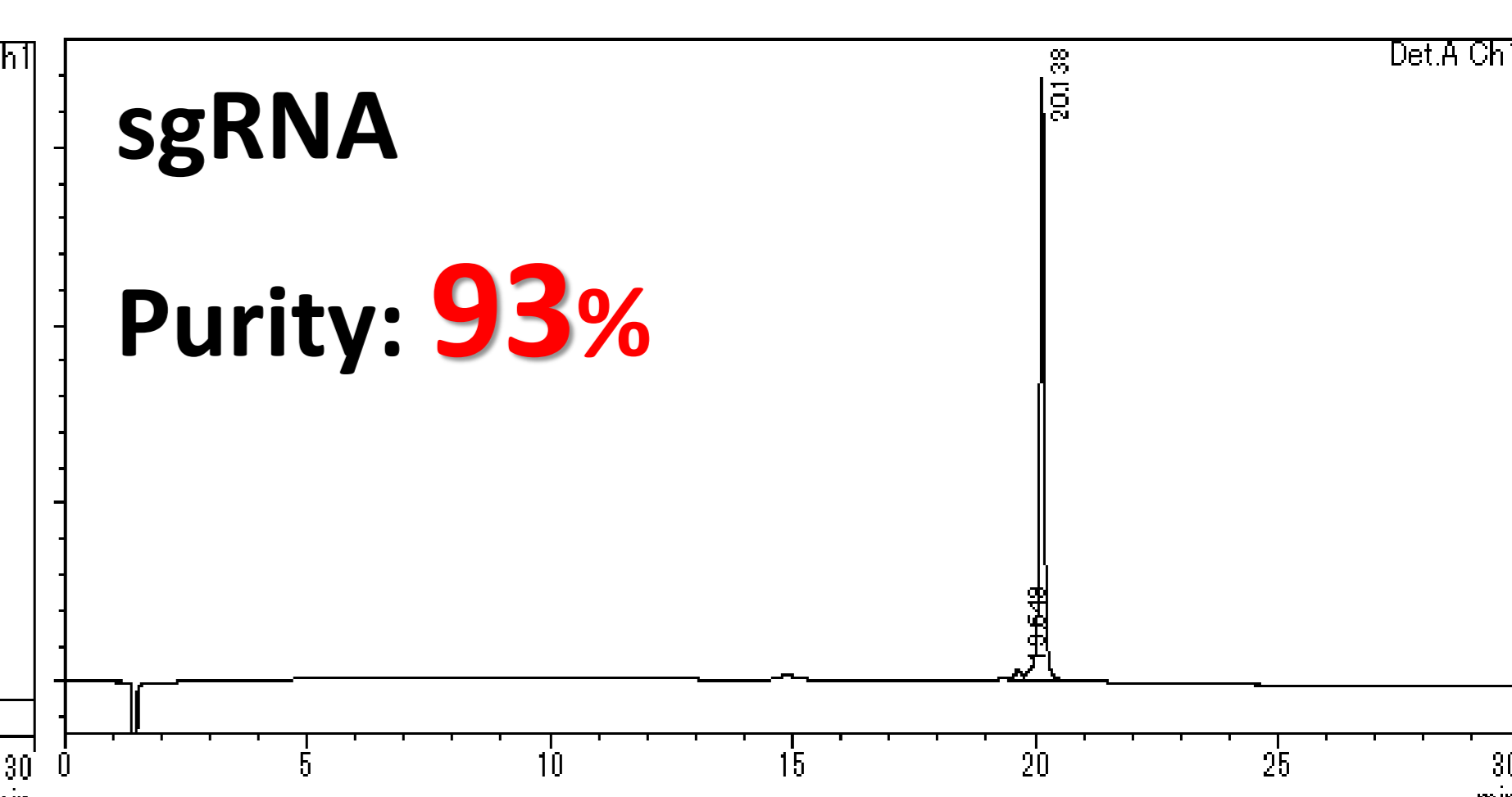
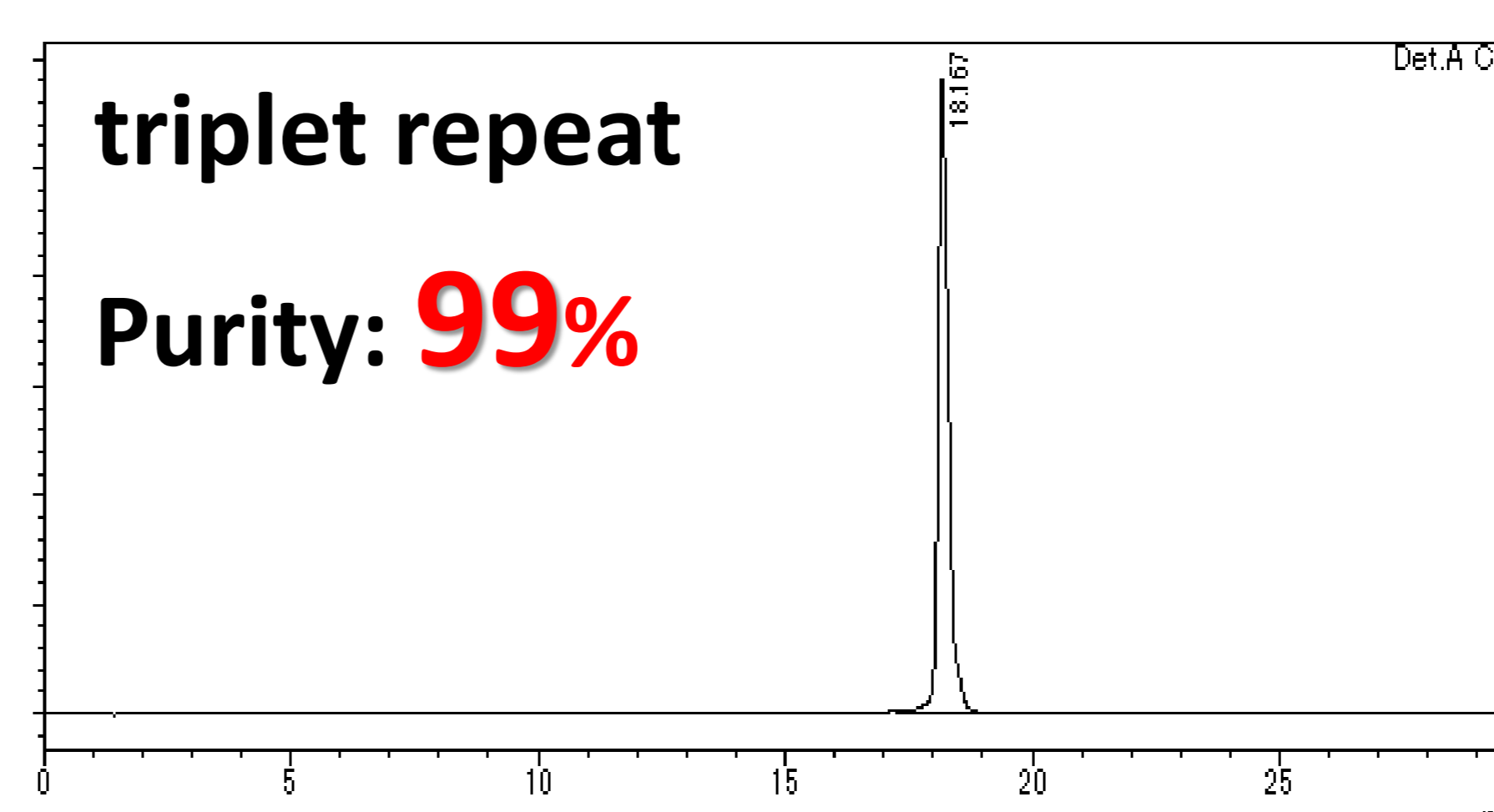
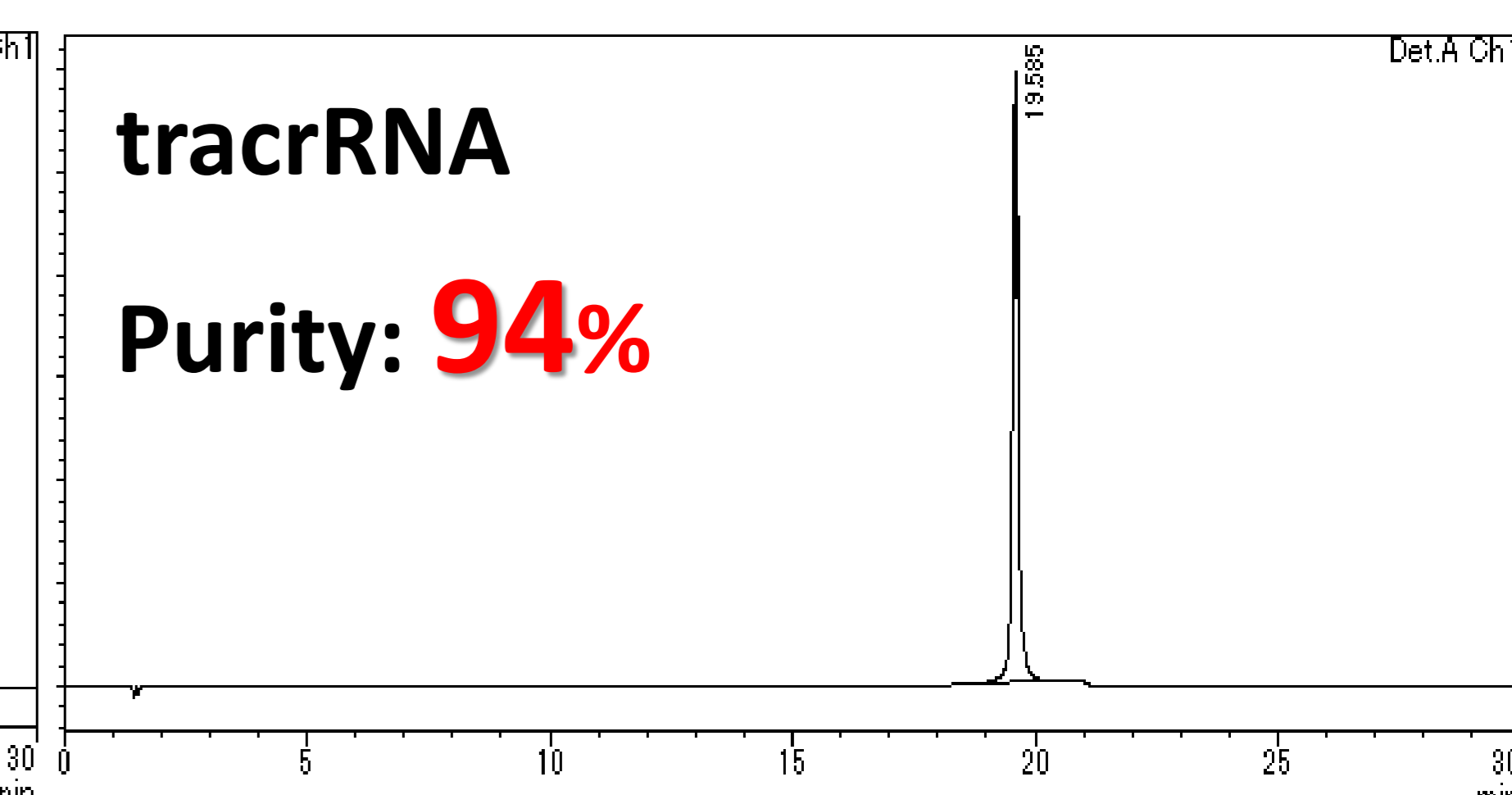
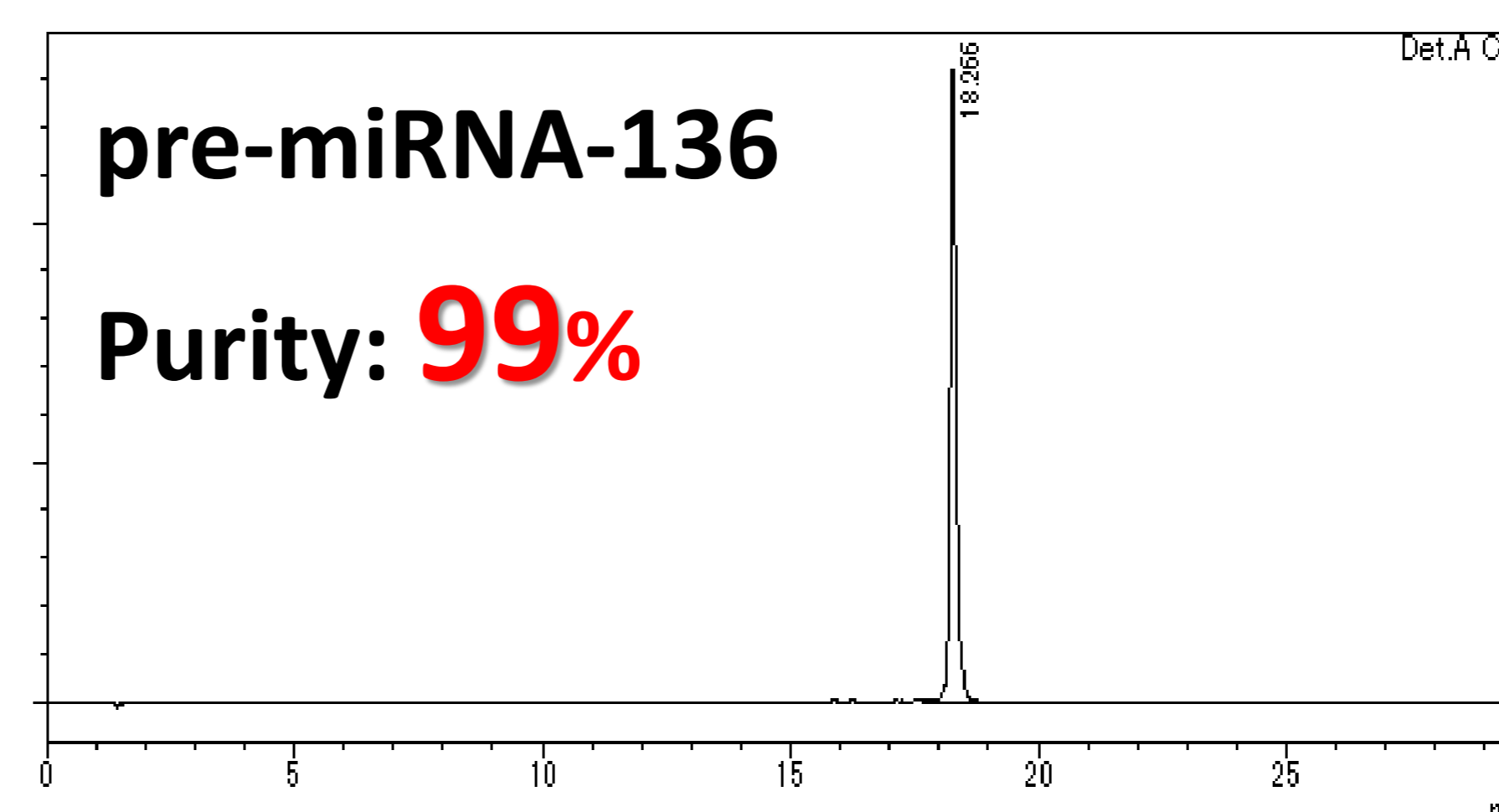
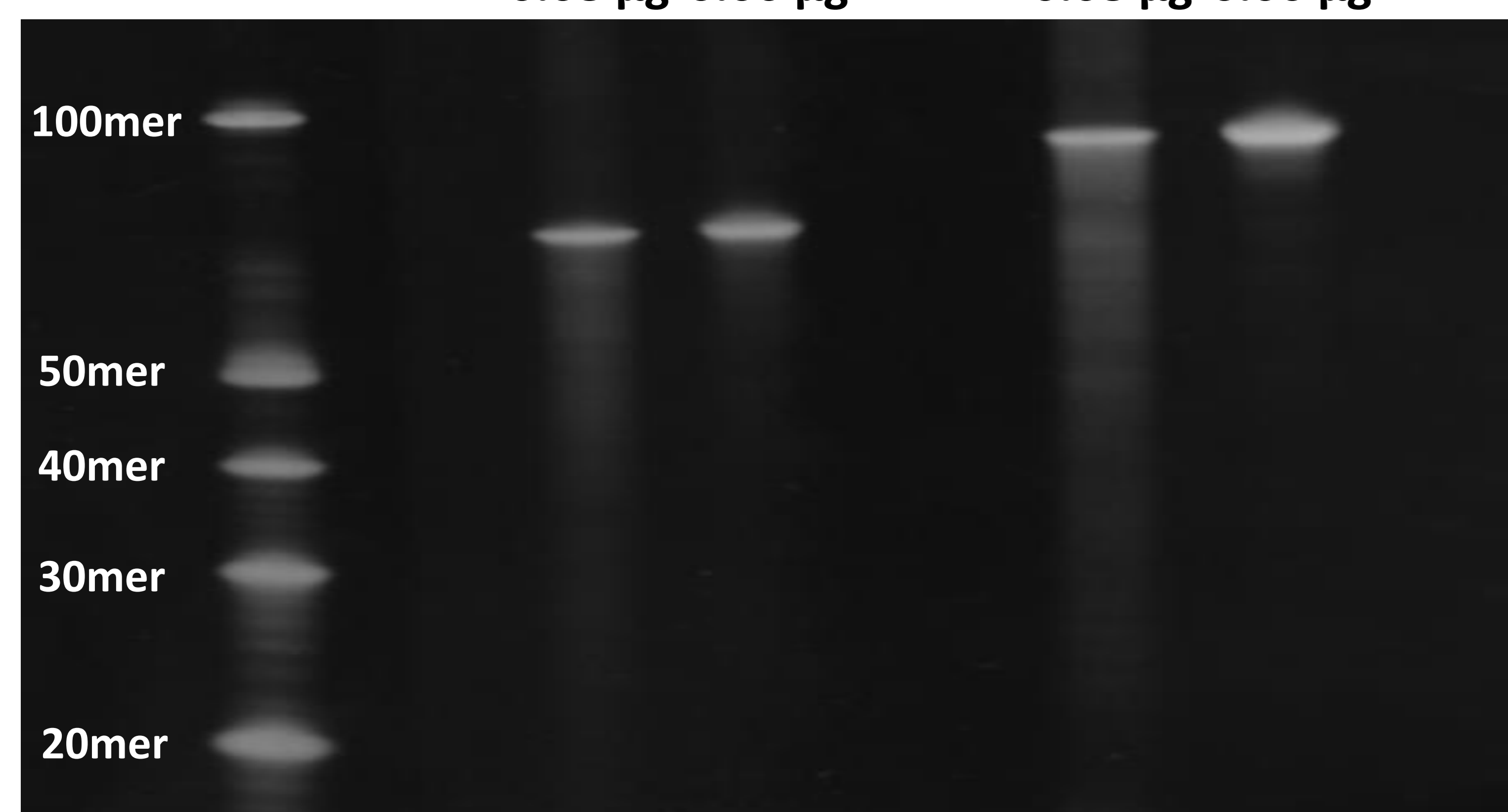
Results

 Results of **Long RNA Oligonucleotides** Synthesis

Name	RNA sequence(5'>3')	Length	Purity
pre-miRNA-136	ACUCCAUUUUGUUUUGAUGAUGGGAUUC UUAUGCUCUCAUCGUCUCAAUGAG UCU	56mer	99%
triplet repeat	CUGCUGCUGCUGCUGCUGCUGCUGCUGC CUGCUGCUGCUGCUGCUGCUGCUGCUGC CUGCUGC	60mer	99%
tracrRNA	GGAACCAUUCAAAACAGCAUAGCAAGU UAAAAUAAGGCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAGUCGGUGCU	80mer	94%
sgRNA	AUAACUCAAUUUGUAAAAAAGUUUUAG AGCUAGAAAUAAGCAAGUUAAAAUAAGG CUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUUUUU	103mer	93%

*MS results are same as theoretical value in all cases

DynaMarker Small RNA II	tracrRNA 80mer		sgRNA 103mer	
	Crude	Purified	Crude	Purified
	0.08 µg	0.06 µg	0.08 µg	0.06 µg


**Manufacturing Results of
Long RNA Oligonucleotides (> 50mer)**

non-GMP

ca. 1000g

GMP

ca. 500g

Conclusion

1. We found that our amidites are very useful for synthesizing long RNA oligos and using those amidites facilitates easy synthesis of over 100mer RNA, such as sgRNA for CRISPR.
2. We have completed long RNA oligo manufacturing with excellent purities by using our amidites.