Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ248	**Date of Submission:**	11/7/04
Lexicon Contract Name:	DNA042	**Mutation Type:**	✓ Standard Knock out
LexVision Name:	MEM651N1		☐ Conditional
Reference accessions:	NM_133211.2, IPI00009812	**Is this gene X-linked?**	**Yes**

Required Materials:
- ✓ pKOS clone DNA(s) __pKOS26_____
- ✓ Target Vector DNA __pKOS26 FTV13_____
- ✓ Targeted ES Cell DNA__1F1 _____
- ✓ Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' Internal**
Name of Probe:	**20/21**	**Puro C/D**
Restriction Enzyme for Genomic Digest:	**EcoRI**	**NheI**
Predicted Wild-type Band (kb):	**11.0 kb**	**---**
Predicted Mutant Band (kb):	**9.4 kb**	**15.8 kb**
Probe Size:	**382 bp**	**949 bp**

PCR Strategies:
For standard knockouts, give wildtype and mutant-specific strategies
For conditionals, give 5' loxP and cre-excision strategies

Wild type-specific (absent in targeted allele)		**Mutation-specific product (absent in wt)**	
5' Primer Name:	DNA042-3	5' Primer Name:	DNA042-16
3' Primer Name:	DNA042-11	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	324 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	633 bp

5' loxP strategy		**Distinguish Cre-excised and wt**	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:

Southern probes
DNA042-20 5' – GTTCATTTCCTGGCCATTGG
DNA042-21 5' – CCCTATCATGGTCACCAAAC
PuroC 5'-GCGCCAGCTTACCATGACCGAGTA
PuroD 5'-CAGCTGGTTCTTTCCGCCTCAGA

PCR Genotyping
DNA042-3 5' – GGGCAGACCTTAGACTTAAG
DNA042-11 5' –CATGAGTTTGTCCAGAAGCC
DNA042-16 5' –GACGGAGGTGCTGTTTACAC
GT IRES-2 5' –GGCCGCTATGGCCTCTAGAGCG

Genomic Sequence Deleted:

AAATCCCTGAGGGCATTCCCACTAACACCACCAATCTTACCCTTACCATCAACCACATACCAAGCATCTCTCCAGATTCCTTCCGTAGGCTGAACCATC
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TGTCATGGCTCAGATTGGTGGGAAATCATCAATATATG

Genomic Locus: *(the deleted sequence represents nt10114 – 13226 in the sequence below. KOS26 used to generate the TV represents nt 8846 – 19487 in the sequence below.)*

TTTCACAAGTTCAGTGCATGTCTTCCTCTCACATCCTGTTTGCAAGAGCTGGGTCACATGGTCACATTTATTGGCAACAGAGCATGGGAATGCAAGCAT
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Selection Cassette:

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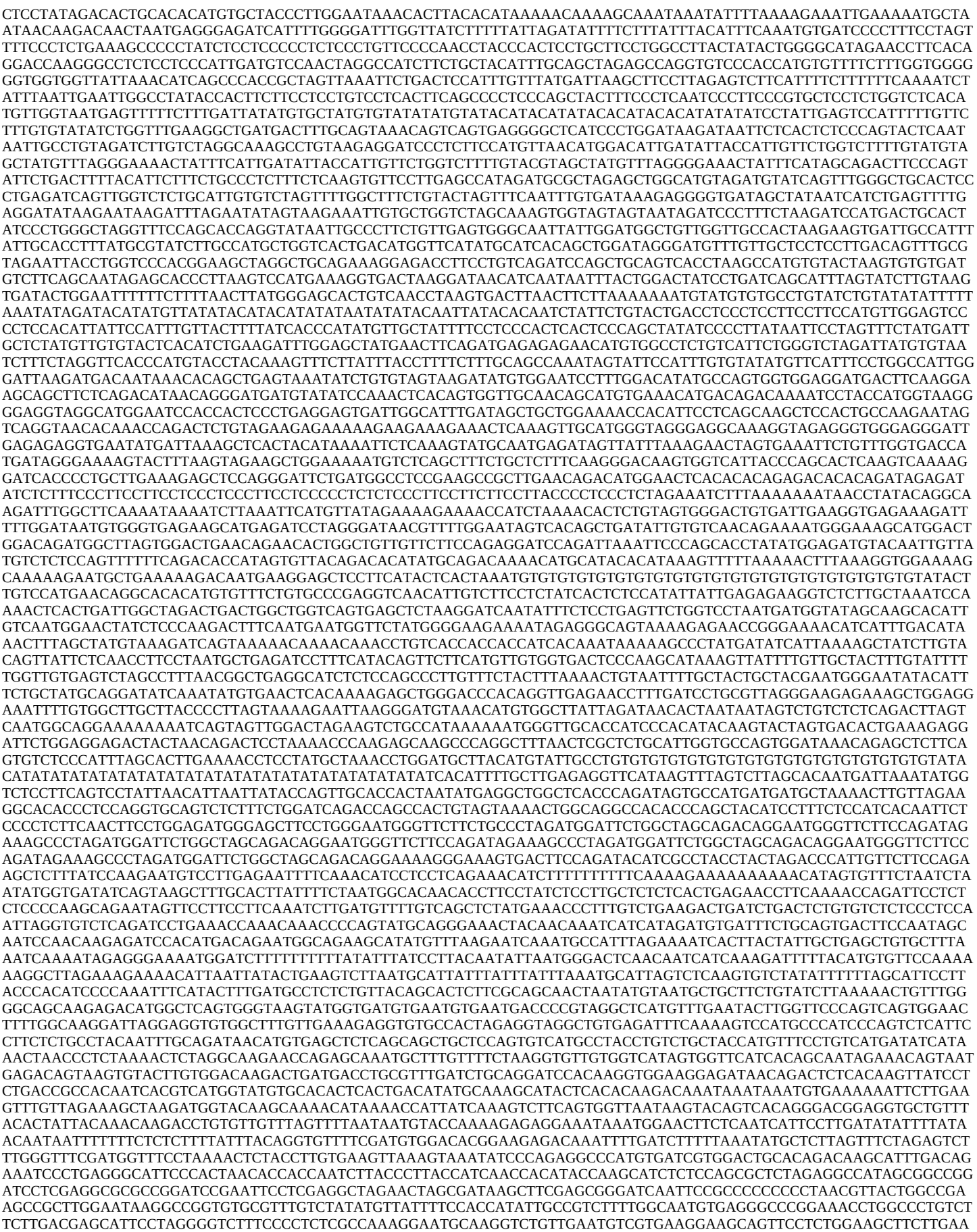

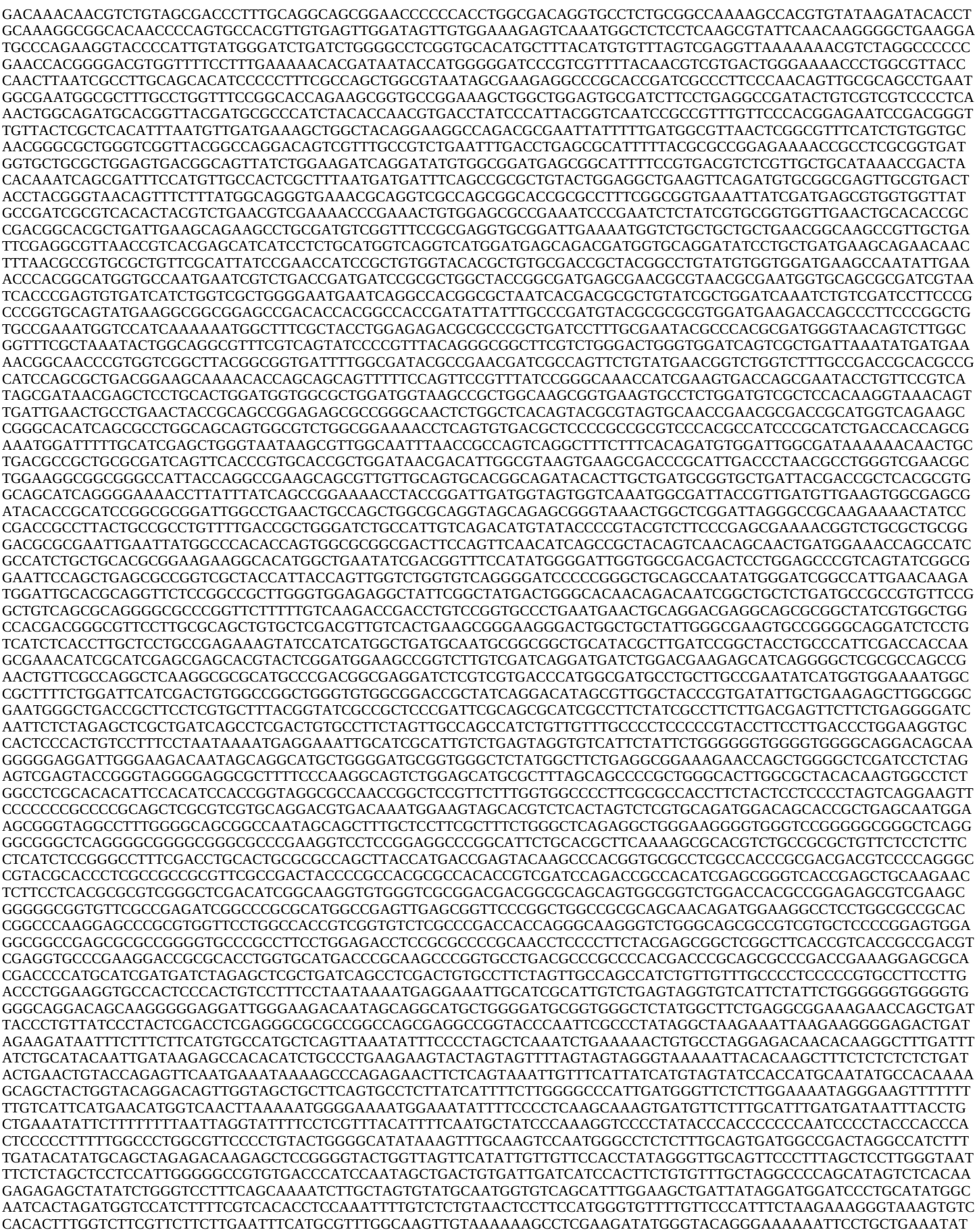

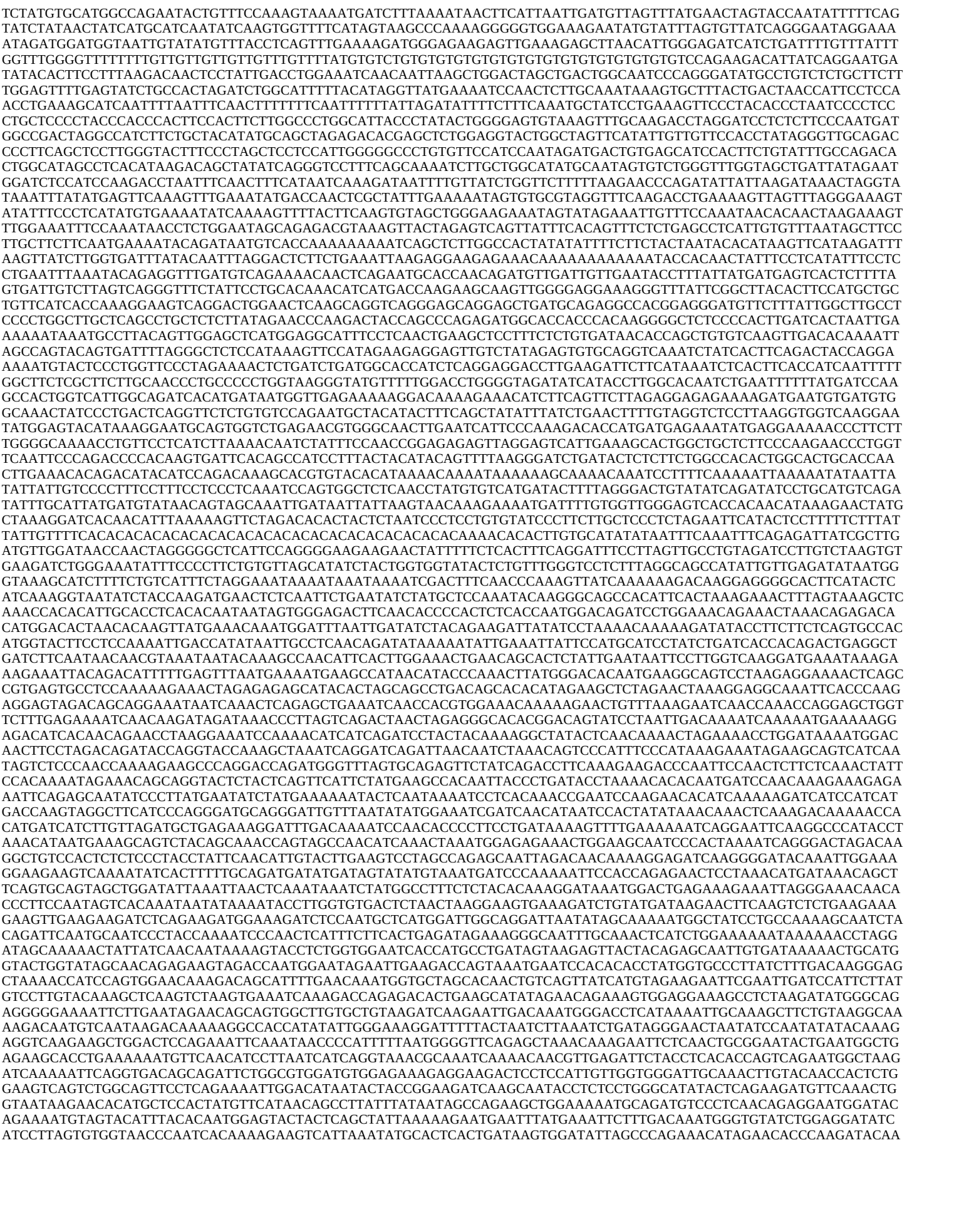

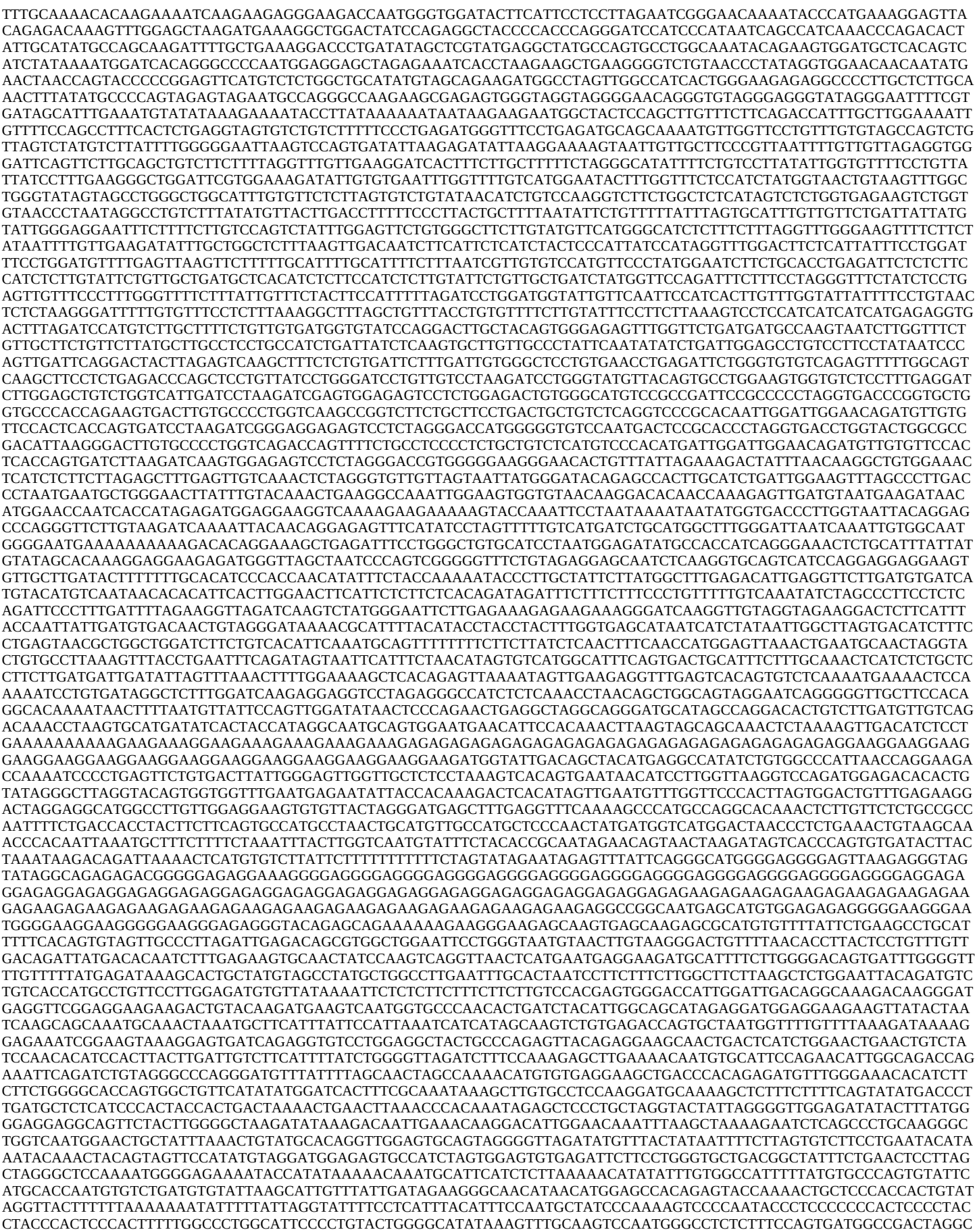

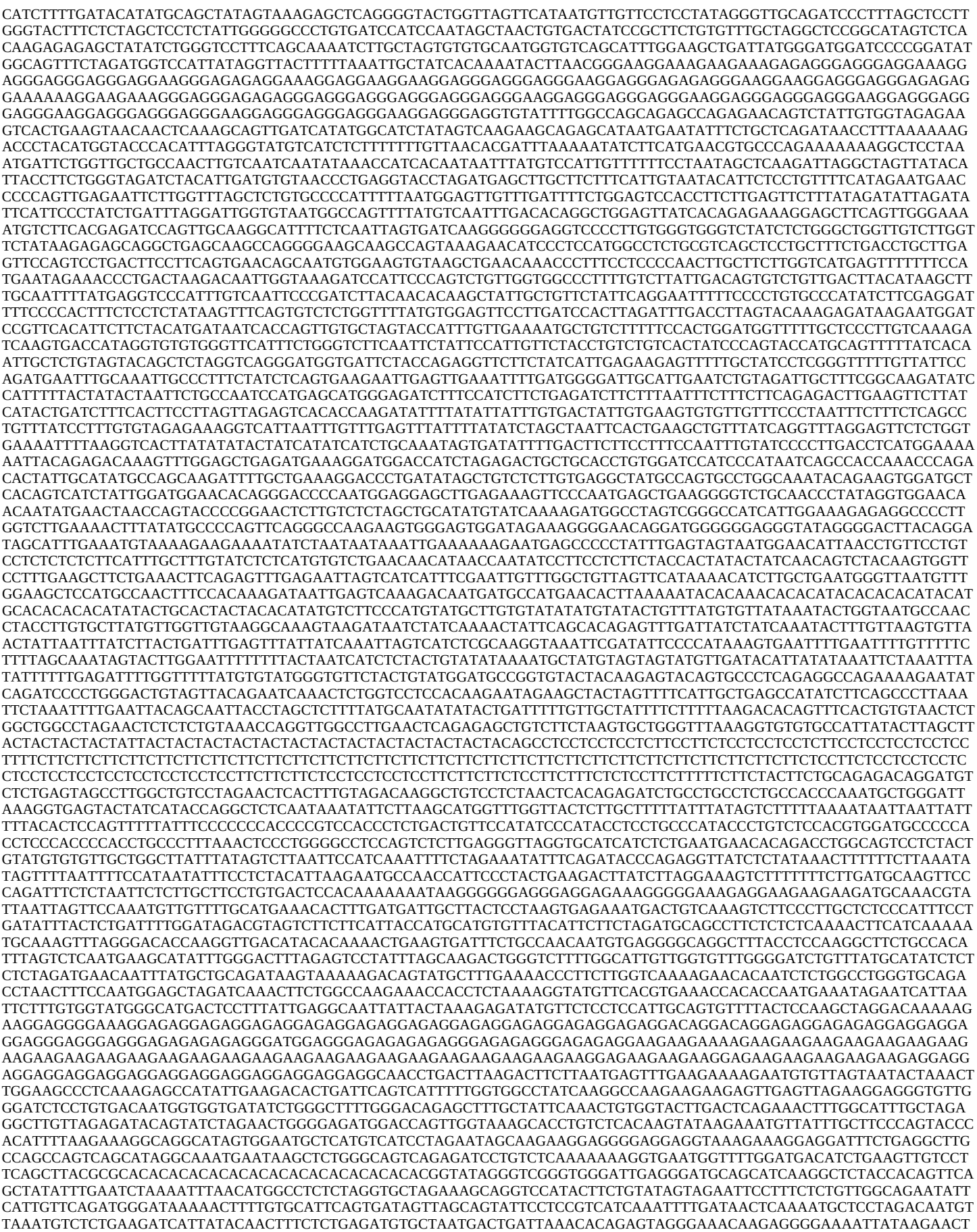

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