GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: B6;129S5-Ms4a4btm1Lex/Mmucd **MMRRC:** 032478-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	15

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	

Primers:

Name	Nucleotide Sequence (5' - 3')
1. DNA444-16	AGCCCCAACCTTACAAATCC
2. neo3a	GCAGCGCATCGCCTTCTATC
3. DNA444-25	GTAGTCAGTGTGGCTGTGGG

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	338	mutant
1 & 3	242	wildtype

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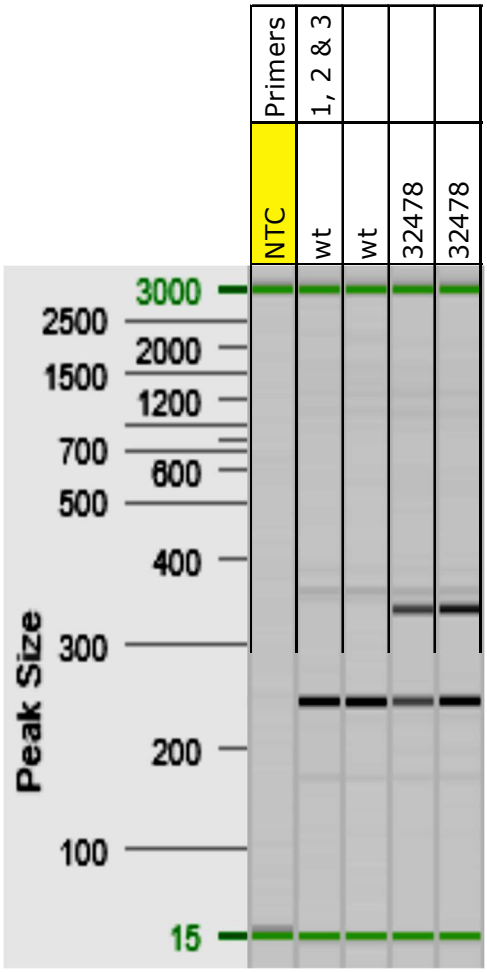

Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ5518	**Date of Submission:**	1/13/05
Lexicon Contract Name:	DNA446	**Mutation Type:**	✓ Standard Knock out
LexVision Name:	MEM773N1		☐ Conditional
Reference accessions:	NM_021718; NM_029499	**Is this gene X-linked?**	No

Required Materials:
- ✓ pKOS clone DNA(s) __pKOS65_____
- ✓ Target Vector DNA __pKOS65-FTV2_____
- ✓ Targeted ES Cell DNA __2C4_____
- ✓ Genomic Map

Southern Blot Analysis:
External/Internal Probe Strategies

	5' External	**3' External**	**3' Internal**
Name of Probe:	**21 + 22**	**23 + 24**	**Neo 2/5**
Restriction Enzyme for Genomic Digest:	**XmnI**	**ApaLI**	**XbaI**
Predicted Wild-type Band (kb):	**11.1 kb**	**13.1 kb**	**-------**
Predicted Mutant Band (kb):	**7.4 kb**	**7.4 kb**	**10.1**
Probe Size:	**332 bp**	**253 bp**	**609 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:	DNA444-25	5' Primer Name:	Neo3a
3' Primer Name:	DNA444-16	3' Primer Name:	DNA444-16
Predicted Wild-type Band (bp):	242 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	338 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
DNA444-21 5' – ACAAATCAAAGTGTAGAGGG
DNA444-22 5' – GGGAATTTCAGTATTACTGC
DNA444-23 5' – GCCTTCTAGTTGAATGTTCAC
DNA444-24 5' – TGGAACATAGCAATACCTGC
NEO-2 5' – CCTCAGAAGAACTCGTCAAG
NEO-5 5' - GGCAGCGCGGCTATCGTG

PCR Genotyping
DNA444-25 5' – GTAGTCAGTGTGGCTGTGGG
DNA444-16 5' – AGCCCCAACCTTACAAATCC
Neo3a 5' – GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

CCGGATCCCTGTCCATTGCAGCAGGAGTGACACCTACAAAATGCCTGGTATGTAATTTGGTATGTTTTGCTATGGAAAAGGAGTAATTTCATCAACTAT
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TTC

Genomic Locus: (*The deleted sequence represents nt 10047 – 11530 in the sequence below. KOS65 used to generate the TV represents nt 5443 – 15755 in the sequence below.)*

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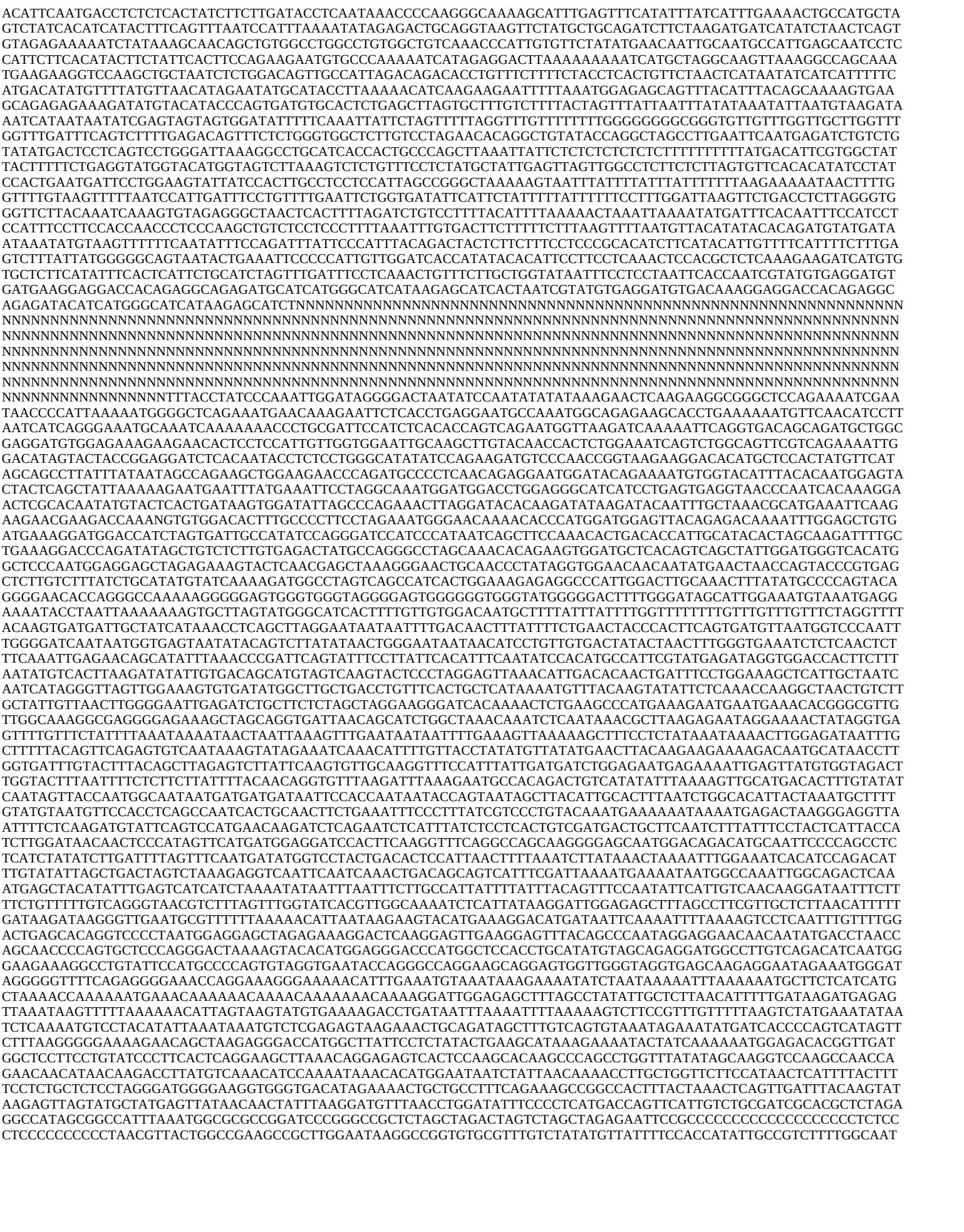

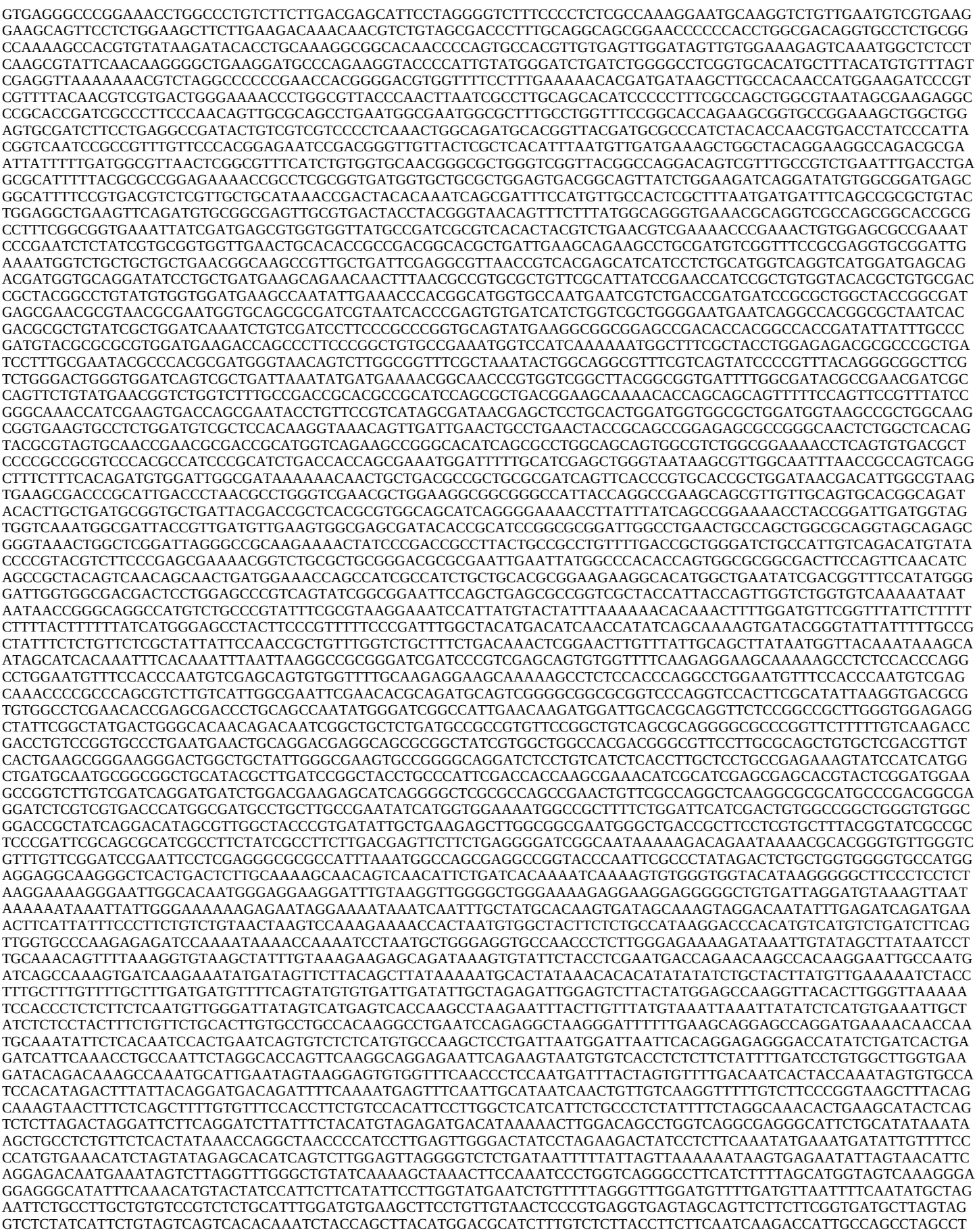

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