

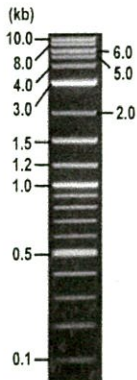


RIKEN DNA BANK

clone name : Yeast mutant CYP51F1m/pBKS-

- Clone ID : RDB _ 16757
- Lot : 16757 _ A8K1
- DNA Concentration : 25 nanogram/microliter
- Volume : 40 microliter
- Form : DNA solution in TE buffer
- Host : DH5alpha
- Culture : LB medium
- Antibiotics : 100 microgram/ml Ampicillin
- Purification : QIAGEN QIAprep Spin Miniprep kit
- Digestion by restriction enzyme

2-Log DNA Ladder
(NEB#N3200L),
250 ng/well



Electrophoresis : 100 nanogram DNA per lane ; 1% agarose gel , 1 x TAE Buffer

Restriction enzyme	Expected size of fragment
<u>BamHI</u>	<u>4.5</u> kbp
<u>BglII</u>	<u>4.5</u> kbp
<u>BamHI+BglII</u>	<u>2.9, 1.6</u> kbp
	kbp
	kbp

● Confirmation of the insertion sequence

Sequence name	Primer name	Sequence name	Primer name
Sequence - A	M13_40	Sequence - E	-
Sequence - B	reverse2	Sequence - F	-
Sequence - C	-	Sequence - G	-
Sequence - D	-	Sequence - H	-

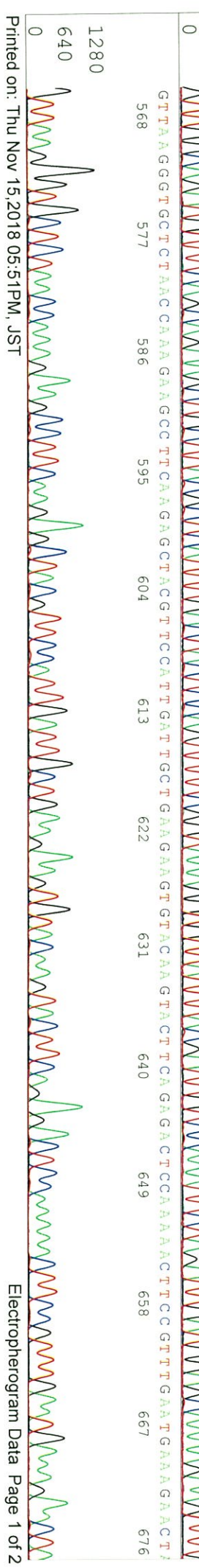
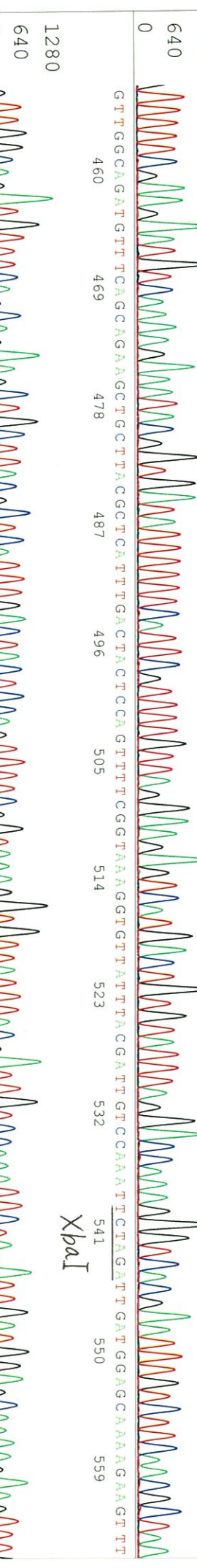
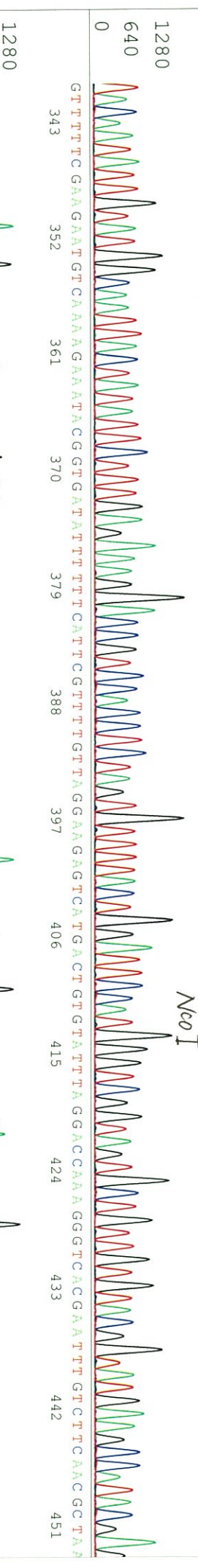
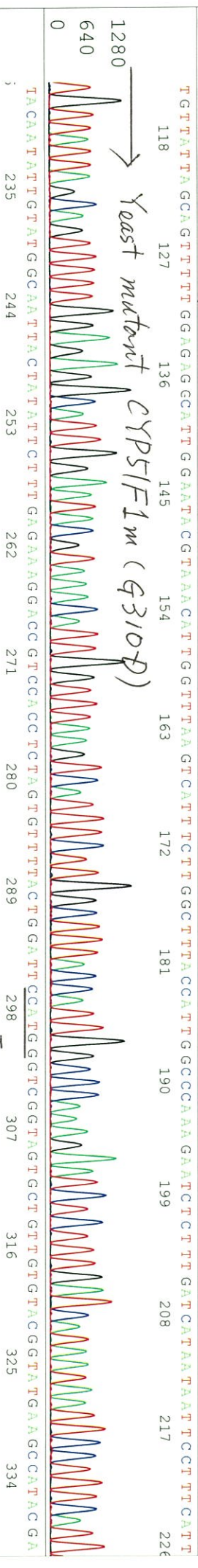
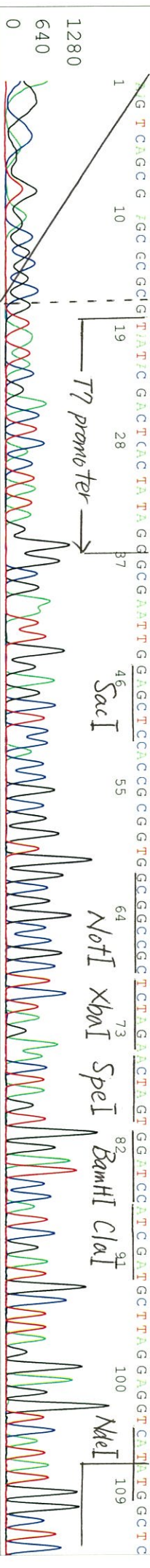


APPROVED BY :

S/N G:154 A:199 T:201 C primer name A : M13_-40

KB.bop 5- GTTTCCAGTCACGACGGTTGTA-3'

KB 1.4.1.8 Cap:17



ACTGGTACTTATTTGACCGTGTGGTTACTTCAACCTGAAATGACTATTTCACCGCTTCAAGATCATTTATGGGTAAGGAAATGAGAGCAAAATTTGGATACCAGATTTCCTTAC

TTGTACAGTGTATTGGATTAAAGGGTTCCACTCCAAATCAACTTCGTC TTCCTTAACCTTACCATTGGAACTATAGAAAGAGAGATCACCGCTCAAAAAGCCTATCTCCGGT

ACTTACATGTC TTTGATTAAAGAAA GAA GAAAGAACAA CGACATTC AAGACAGAGATT TGGATCGATTCC TTGATGAA GAA CTC TACCTAAC GGATCGTTGAA G

TTGACTGATCAA GA ATCGCTAACTTGTAA TTGATGTC TTAATGGG TGG TCAA CATAC TTC TGGC TGCCAC TTC TGCTTGGA ATTTTG TTGCATC T

TGC TGA AAG A CCAG ATGTTCC AACAA CGA TG TACG A GAA CA A TGC TGT TT T EG ATG TTG T A G A A G

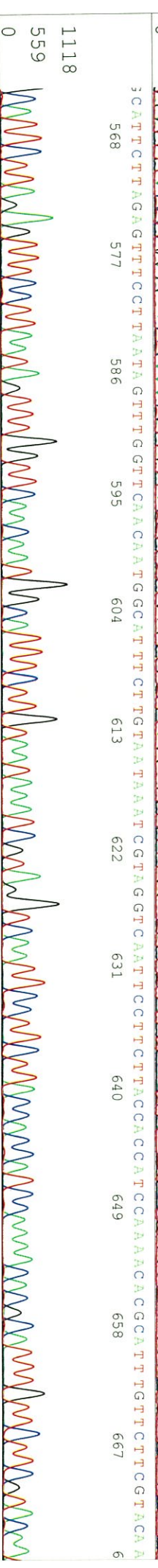
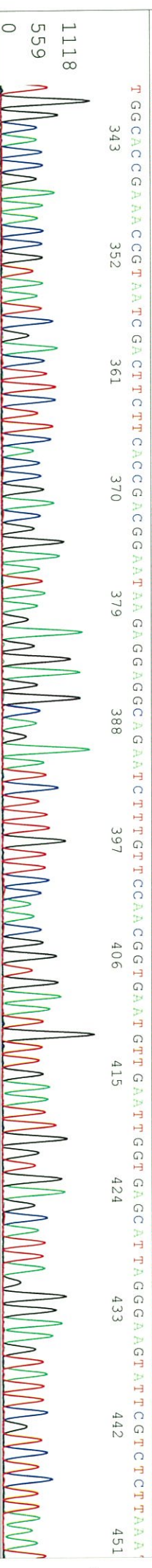
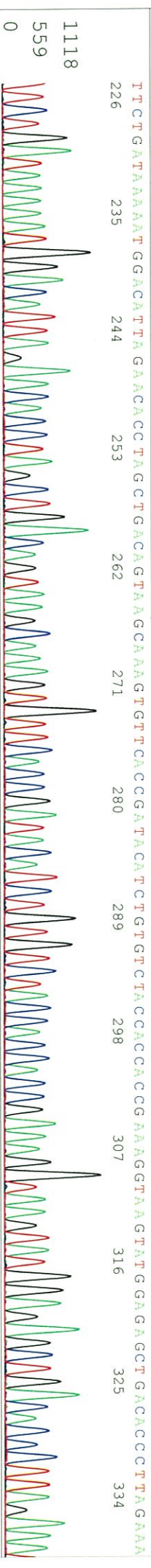
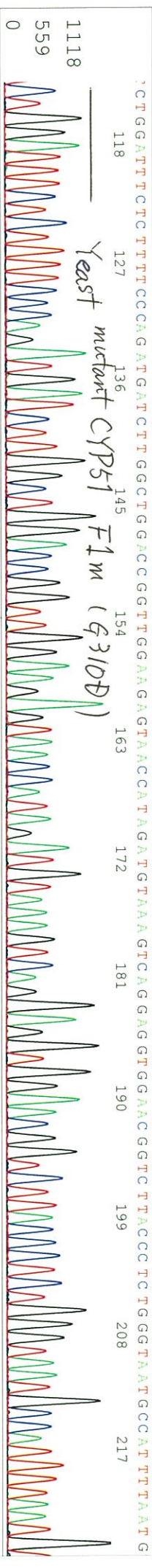
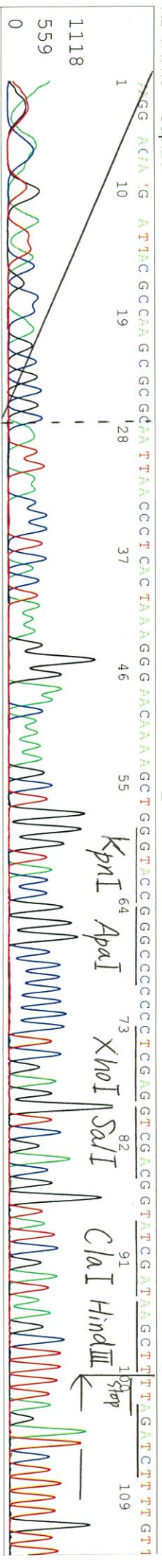
G A T G A C T A C G A T T A C G A T T A C A A C G G T A C T A C A C G G A A A A A T G G T G C C A A A T T G C C

1280 640 0

Yeast mutant CYP51M (G310D)

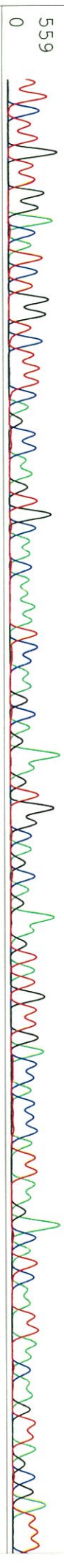


S/N:G:241 A:310 T:270 C primer name B : reverse-2
KB.bop 5-GCCGGATAACAATTTCACACAGG-3'
KB 1.4.1.8 Cap:20

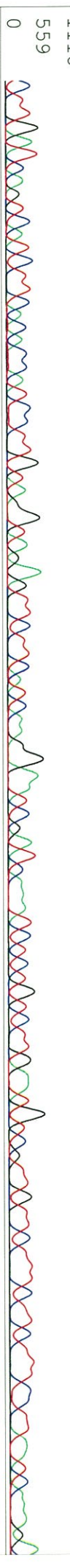


TTCTTGTGGACATCTGGTCTTTCAGCCCAAGTGCACCAAAATCCAAAGAAAGTGGCAAGCAAGAAATGTTGACCAACCATTAAACATCAATTAAACAA GTTAGCGGATTT
76 685 694 703 712 721 730 739 748 757 766 775 784

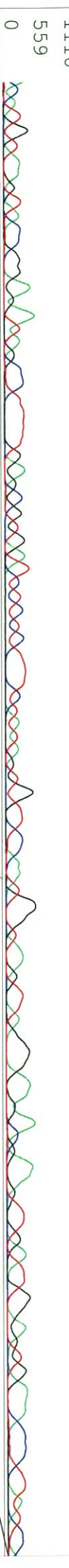
G310D: GGT to GAT (complement)



CTTGATCAAGTCATCTTCAACA CCAATCCTTGTAGGTTCC TTTCAAGAAATCGATCAAAATCTCTGTCCTTGAATGTCGTTGTTCTTCTTCTTCTTAA TCAAA G
793 802 811 820 829 838 847 856 865 874 883 892

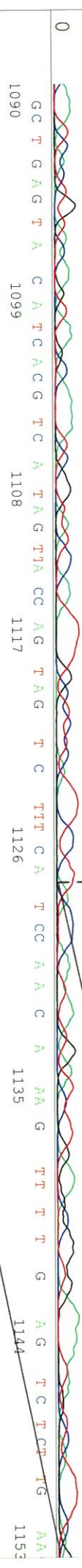


CATGTAAGTACCGAGATAGCCCTTTTGAAGCGTGATCTCTCTTCTATA GTGTTCCAAATGGTTAA GTTA GGGAA GACGAA GTTGATTGG AGTG AAAACCCTTATCC
901 910 919 928 937 946 955 964 973 982 991

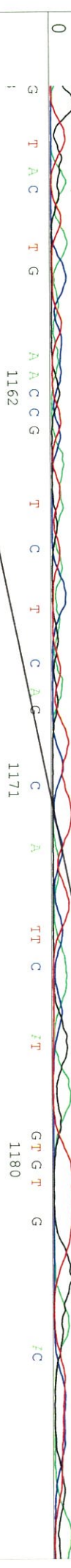


AAATCACCTGTACAA GTAA GCAAAA TCGGTATCCAA TTTTGCCTCTCA TTTCC TTTACCCAA TAA TGATCTG AGCGGTG AAAA TAGTCA TTTCA
1000 1009 1018 1027 1036 1045 1054 1063 1072 1081

Yeast mutant CYP51 F1m (G310D) ←



GC T G A G T A C A T C A C G T C A T A G T T A C C A A G A A C A A C A A A G T T T T A G A G T C T C T C T C T T G A A
1090 1099 1108 1117 1126 1135 1144 1153



G T A C T G A A C C G T C T C A G C A A T T C TT C GTGT G
1162 1171 1180