T7 RNA Polymerase Nucleotide Sequence

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GCTGGTGAGG TTGCGGATAA CGCTGCCGCC AAGCCTCTCA TCACTACCCT ACTCCCTAAG
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ACCACTCTGG CTTGCCTAAC CAGTGCTGAC AATACAACCG TTCAGGCTGT AGCAAGCGCA
ATCGGTCGGG CCATTGAGGA CGAGGCTCGC TTCGGTCGTA TCCGTGACCT TGAAGCTAAG
CACTTCAAGA AAAACGTTGA GGAACAACTC AACAAGCGCG TAGGGCACGT CTACAAGAAA
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TGGTCTTCGT GGCATAAGGA AGACTCTATT CATGTAGGAG TACGCTGCAT CGAGATGCTC
ATTGAGTCAA CCGGAATGGT TAGCTTACAC CGCCAAAATG CTGGCGTAGT AGGTCAAGAC
TCTGAGACTA TCGAACTCGC ACCTGAATAC GCTGAGGCTA TCGCAACCCG TGCAGGTGCG
CTGGCTGGCA TCTCTCCGAT GTTCCAACCT TGCGTAGTTC CTCCTAAGCC GTGGACTGGC
ATTACTGGTG GTGGCTATTG GGCTAACGGT CGTCGTCCTC TGGCGCTGGT GCGTACTCAC
AGTAAGAAAG CACTGATGCG CTACGAAGAC GTTTACATGC CTGAGGTGTA CAAAGCGATT
AACATTGCGC AAAACACCGC ATGGAAAATC AACAAGAAAG TCCTAGCGGT CGCCAACGTA
ATCACCAAGT GGAAGCATTG TCCGGTCGAG GACATCCCTG CGATTGAGCG TGAAGAACTC
CCGATGAAAC CGGAAGACAT CGACATGAAT CCTGAGGCTC TCACCGCGTG GAAACGTGCT
GCCGCTGCTG TGTACCGCAA GGACAAGGCT CGCAAGTCTC GCCGTATCAG CCTTGAGTTC
ATGCTTGAGC AAGCCAATAA GTTTGCTAAC CATAAGGCCA TCTGGTTCCC TTACAACATG
GACTGGCGCG GTCGTGTTTA CGCTGTGTCA ATGTTCAACC CGCAAGGTAA CGATATGACC
AAAGGACTGC TTACGCTGGC GAAAGGTAAA CCAATCGGTA AGGAAGGTTA CTACTGGCTG
AAAATCCACG GTGCAAACTG TGCGGGTGTC GATAAGGTTC CGTTCCCTGA GCGCATCAAG
TTCATTGAGG AAAACCACGA GAACATCATG GCTTGCGCTA AGTCTCCACT GGAGAACACT
TGGTGGGCTG AGCAAGATTC TCCGTTCTGC TTCCTTGCGT TCTGCTTTGA GTACGCTGGG
GTACAGCACC ACGGCCTGAG CTATAACTGC TCCCTTCCGC TGGCGTTTGA CGGGTCTTGC
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GTACTGGCTG ATTTCTACGA CCAGTTCGCT GACCAGTTGC ACGAGTCTCA ATTGGACAAA
ATGCCAGCAC TTCCGGCTAA AGGTAACTTG AACCTCCGTG ACATCTTAGA GTCGGACTTC
GCGTTCGCGT AA
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A to G

A to G results in amino acid change Asn823 to Asp. Amino acid 823 is on surface of protein and is not found in any mutagenesis studies of T7 RNAP

Protein Sequence 883 AA 98855 MW (translated from above)

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MNTINIAKND FSDIELAAIP FNTLADHYGE RLAREQLALE HESYEMGEAR FRKMFERQLK
AGEVADNAAA KPLITTLLPK MIARINDWFE EVKAKRGKRP TAFQFLQEIK PEAVAYITIK
TTLACLTSAD NTTVQAVASA IGRAIEDEAR FGRIRDLEAK HFKKNVEEQL NKRVGHVYKK
AFMQVVEADM LSKGLLGGEA WSSWHKEDSI HVGVRCIEML IESTGMVSLH RQNAGVVGQD
SETIELAPEY AEAIATRAGA LAGISPMFQP CVVPPKPWTG ITGGGYWANG RRPLALVRTH
SKKALMRYED VYMPEVYKAI NIAQNTAWKI NKKVLAVANV ITKWKHCPVE DIPAIEREEL
PMKPEDIDMN PEALTAWKRA AAAVYRKDKA RKSRRISLEF MLEQANKFAN HKAIWFPYNM
DWRGRVYAVS MFNPQGNDMT KGLLTLAKGK PIGKEGYYWL KIHGANCAGV DKVPFPERIK
FIEENHENIM ACAKSPLENT WWAEQDSPFC FLAFCFEYAG VQHHGLSYNC SLPLAFDGSC
SGIQHFSAML RDEVGGRAVN LLPSETVQDI YGIVAKKVNE ILQADAINGT DNEVVTVTDE
NTGEISEKVK LGTKALAGQW LAYGVTRSVT KRSVMTLAYG SKEFGFRQQV LEDTIQPAID
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VLADFYDQFA DQLHESQLDK MPALPAKGNL NLRDILESDF AFA
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T7 RNA Polymerase with flanking sequences

ctttgctatgc catagcattt ttatccataa gattagcgga tcctacctga cgctttttat cgcaactctc
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Sac 1 site

ATGAACACGA TTAACATCGC TAAGAACGAC TTCTCTGACA TCGAACTGGC TGCTATCCCG
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CACTTCAAGA AAAACGTTGA GGAACAACTC AACAAGCGCG TAGGGCACGT CTACAAGAAA
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AAGCGTTGCG CTGTGCATTG GGTAACTCCT GATGGTTTCC CTGTGTGGCA GGAATACAAG
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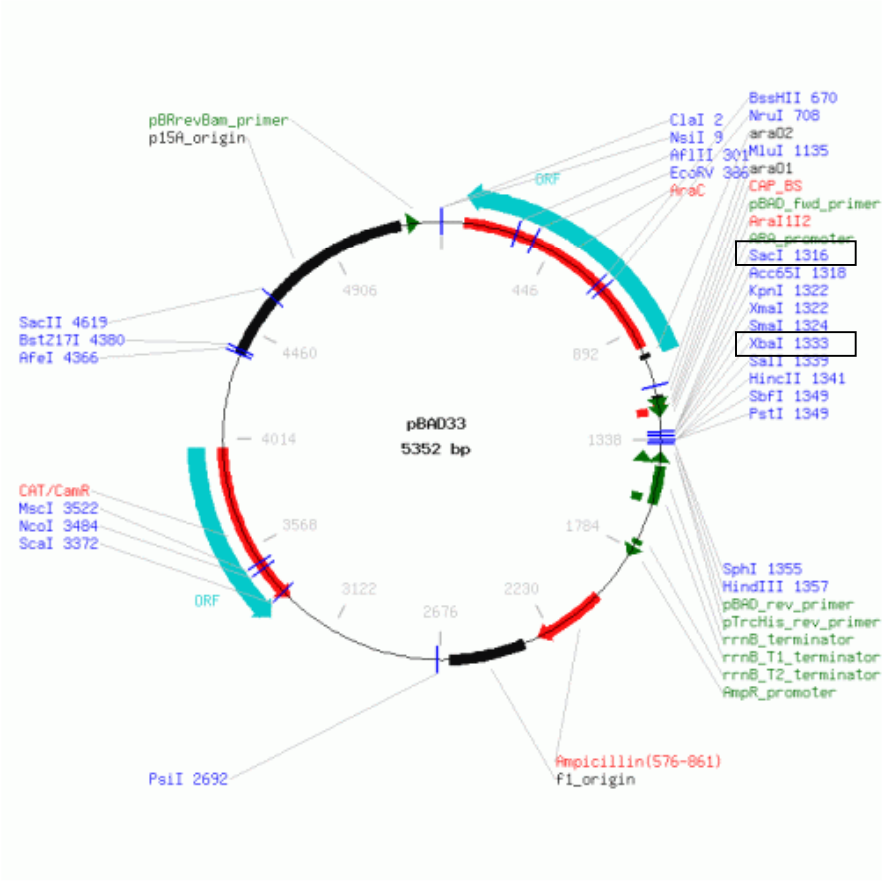
GTACTGGCTG ATTTCTACGA CCAGTTCGCT GACCAGTTGC ACGAGTCTCA ATTGGACAAA
ATGCCAGCAC TTCCGGCTAA AGGTAACTTG AACCTCCGTG ACATCTTAGA GTCGGACTTC
GCGTTCGCGT AA….

….tctagagtcg
XbaI site

pBAD
Sequence can be found at
https://www.lablife.org/g?a=seqa&id=vdb%5fg2%2ejPxWBK9aksPdtGbxXRrF06TxA%2e4%2d%5fsequence%5f549a5591ef126b8ee61fb291e3498cd6c3147325%5f10

Plasmid Name	pBAD33
Source/Vendor	Beckwith Lab
Plasmid Type	Bacterial Expression
Plasmid Size	5352
Sequencing Primer	5' ctgtttctccatacccgtt 3' ctcatccgccaaaacag
Bacterial Resistance	Chloramphenicol
Notes	Vector backbone: pACYC-184 Cloning site 5': SacI Cloning site 3': HindIII Article: Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. Guzman LM et al. (J Bacteriol. 1995 Jul . 177(14):4121-30. Pubmed)
Plasmid Sequence	View Sequence



T7RNAP sequence was amplified with PCR from pAR1217 with SacI and XbaI sites at the ends and cloned into pPAD33 to create pTARA, with T7 RNA polymerase under the control of AraPBAD promoter and therefore AraC/arabinose control.

Schematic for pTARA construction