**Abstract**

In this study, a Y639F mutation was introduced in T7 RNA Polymerase by substituting an adenine for a thymine on position 1916. The goal was to determine if this point mutation would increase, decreased or have no significant effect on the enzymatic activity of T7 RNAP. This was achieved by first triggering protein expression in pTAC-MAT-Tag-1/T7 positive transformant cells by Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction, isolating and purifying T7RNAP from the lysate of such cells by Immobilized Metal Affinity Chromatography (IMAC), and finally conducting a comparative assessment on the enzymatic activity of the wild and mutant T7 RNAP by real-time polymerase chain reaction (qPCR). The hypothesis was that the Y639F mutation would increase the enzymatic activity of T7RNAP due to an increase in hydrophobicity at the core of enzyme activity site. Based on the assumption that the enzymatic activity was proportional to the change in relative fluorescence unit (RFU) over time, this hypothesis was supported; the RFU of the T7RNAP wild and mutant type was found to be 104.5 RFU/min and 479.7 RFU/min respectively.

**Introduction**

The induced point mutations of T7 RNAP do not only make it possible to enzymatically assay and compare the mutant from the wild T7 RNAP version, but it also opens room for further investigation regarding properties of amino acid and their respective effect on the activity of T7 RNAP. This studies aims at assessing the enzymatic effect of inducing a Y639F mutation in T7 RNAP. That is to determine whether this missense mutation will increase, decrease, or have no effect on the catalytic activity of T7 RNAP. The mutant T7 RNAP obtained from this study was chimeric as it was fused with a MAT-Tag (metal affinity tag) sequence on its N-terminus sequence due to the sub-cloning of the gene T7 RNAP into a pTAC-MAT-Tag-1 (5358 bp) [1] destination plasmid.

T7 RNAP is a 99KD, single unit, polypeptide chain. It contains 883 amino acid residues whose function is to catalyze the 5′ → 3′ synthesis of its own RNA. Its structure consists of a N-terminal domain [1-325 bp], the open right hand domain [326-879 bp] and the C-terminal end [880-883 bp] [2]. The open right hand domain, which makes up the DNA binding site as well as the RNA

corresponds to the uninduced pTAC-MAT-Tag-1/T7 lysate differ from lane 2 mainly by the intensity of the bands which appear to be duller. Apart from the intensity, they share seem to share the exact same protein at the exact same position (exact same molecular weight). Lane 4, which correspond to water induced pTAC-MAT-Tag-1/T7 lysate and it is very similar to lane 3. The unnoticeable difference between lane 3 and 4 is that on lane 4, the bands appear to have a slightly improved intensity. Lane 5, which correspond to the uninduced pTAC-MAT-Tag-1/T7 lysate is very similar to lane 2 and far more intense than lane 3. Lane 6, which corresponds to the IPTG induced pTAC-MAT-Tag-1 lysate, similar to lane 5 with a slightly reduced intensity. Lane 7 which correspond to the uninduced pTAC-MAT-Tag-1 lysate has a large that runs from the bottom up to the top. The protein band cannot be differentiated as a result. In all cases except lane 7, most if not protein bands could be traced at the same molecular weight level. The few bands that appear to be absence seem to simply have a lost their intensity. It was speculated that T7RNAP was observed at approximately 102 kDa. However, there was not much evidence behind that claim yet since T7RNAP was not isolated. The band at 102 kDa could as well correspond to a different protein whose molecular weight can be approximated to 102 kDa.

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CLUSTAL O(1.2.3) multiple sequence alignment – T7 RNAP

Wild        MNTINIAKNDFSDIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLK
Mutant      -NTINIAKNDFSDIELAAIPFNTLADHYGERLAREQLALEHESYEMGEARFRKMFERQLK
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Wild        AGEVADNAAAKPLITTLLPKMIARINDWFEEVKAKRGKRPTAFQFLQEIKPEAVAYITIK
Mutant      AGEVADNAAAKPLITTLLPKMIARINDWFEEVKAKRGKRPTAFQFLQEIKPEAVAYITIK
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Wild        TTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAKHFKKNVEEQLNKRVGHVYKK
Mutant      TTLACLTSADNTTVQAVASAIGRAIEDEARFGRIRDLEAKHFKKNVEEQLNKRVGHVYKK
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Wild        AFMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCIEMLIESTGMVSLHRQNAGVVGQD
Mutant      AFMQVVEADMLSKGLLGGEAWSSWHKEDSIHVGVRCIEMLIESTGMVSLHRQNAGVVGQD
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Wild        SETIELAPEYAEAIATRAGALAGISPMFQPCVVPPKPWTGITGGGYWANGRRPLALVRTH
Mutant      SETIELAPEYAEAIATRAGALAGISPMFQPCVVPPKPWTGITGGGYWANGRRPLALVRTH
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Wild        SKKALMRYEDVYMPEVYKAINIAQNTAWKINKKVLAVANVITKWKHCPVEDIPAIEREEL
Mutant      SKKALMRYEDVYMPEVYKAINIAQNTAWKINKKVLAVANVITKWKHCPVEDIPAIEREEL
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Wild        PMKPEDIDMNPEALTAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIWFPYNM
Mutant      PMKPEDIDMNPEALTAWKRAAAAVYRKDKARKSRRISLEFMLEQANKFANHKAIWFPYNM
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Wild        DWRGRVYAVSMFNPQGNDMTKGLLTLAKGKPIGKEGYYWLKIHGANCAGVDKVPFPERIK
Mutant      DWRGRVYAVSMFNPQGNDMTKGLLTLAKGKPIGKEGYYWLKIHGANCAGVDKVPFPERIK
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Wild        FIEENHENIMACAKSPLENTWWAEQDSPFCFLAFCFEYAGVQHHGLSYNCSLPLAFDGSC
Mutant      FIEENHENIMACAKSPLENTWWAEQDSPFCFLAFCFEYAGVQHHGLSYNCSLPLAFDGSC
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Wild        SGIQHFSAMLRDEVGGRAVNLLPSETVQDIYGIVAKKVNEILQADAINGTDNEVVTVTDE
Mutant      SGIQHFSAMLRDEVGGRAVNLLPSETVQDIYGIVAKKVNEILQADAINGTDNEVVTVTDE
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Wild        NTGEISEKVKLGTKALAGQWLAYGVTRSVTKRSVMTLAYGSKEFGFRQQVLEDTIQPAID
Mutant      NTGEISEKVKLGTKALAGQWLAYGVTRSVTKRSVMTLAFGSKEFGFRQQVLEDTIQPAID
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Wild        SGKGLMFTQPNQAAGYMAKLIWESVSVTVVAAVEAMNWLKSAAKLLAAEVKDKKTGEILR
Mutant      SGKGLMFTQPNQAAGYMAKLIWESVSVTVVAAVEAMNWLKSAAKLLAAEVKDKKTGEILR
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Wild        KRCAVHWVTPDGFPVWQEYKKPIQTRLNLMFLGQFRLQPTINTNKDSEIDAHKQESGIAP
Mutant      KRCAVHWVTPDGFPVWQEYKKPIQTRLNLMFLGQFRLQPTINTNKDSEIDAHKQESGIAP
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Wild        NFVHSQDGSHLRKTVVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMVDTYESCD
Mutant      NFVHSQDGSHLRKTVVWAHEKYGIESFALIHDSFGTIPADAANLFKAVRETMVDTYESCD
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Wild        VLADFYDQFADQLHESQLDKMPALPAKGNLNLRDILESDFAFA
Mutant      VLADFYDQFADQLHESQLDKMPALPAKGNLNLRDILESDFAFA
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**Figure A: Protein sequence of the wild and mutant type T7RNAP**. In yellow is highlighted the point where the mutation takes place.