

## Appendix

### 1 Materials and Methods

#### 1.1 RNA Isolation and Quantification

**Total RNA isolation from mouse fibroblast cells.** The TRIzol® method (Thermo Fisher Scientific) was used to extract total RNA from mouse fibroblast cells. TRIzol maintains the integrity of RNA while disrupting the cell and cellular components. After treating the mouse fibroblast cells with TRIzol, addition of chloroform followed by centrifuging separates the solution into two visually distinct phases - the aqueous phase and the organic phase. RNA is found exclusively in the aqueous phase which was pipetted out and transferred to another tube. This aqueous phase solution is then first incubated with RNase free DNase I for 20 minutes in a 37°C water bath to remove any contaminating DNA that could have been accidentally pipetted from the interphase. After 20 minutes, RNA is then recovered from the aqueous phase by precipitation with isopropanol for 10 minutes at rtp, then yielding an RNA pellet upon centrifugation for 10 minutes at 4°C. After centrifuging, the supernatant was removed and washed with 1mL of 75% ethanol via vortexing to remove residual salt from the RNA pellet. Centrifuging was performed after 5 minutes. The supernatant is then removed via pipetting and the RNA pellet is air-dried for about 10 minutes before dissolving in 20µl DEPC water for about 10 minutes at 55-60°C on a heat block.

DEPC helps to inactivate ribonucleases through covalent modification of specific amino acids (mostly histidine residues). Wearing gloves is also important to prevent RNase contamination from our fingertips. 0.1% DEPC, not heat, is used to inactivate contaminating RNases, since RNases are stable due to high percentage of cysteine bonds that result in strong disulfide bonds that render RNases to be heat-stable.

**RNA Quantification.** The extracted RNA from above was subsequently quantified via NanoDrop. The NanoDrop was first blanked with DEPC water before taking the readings. The solutions are also maintained at acidic pH as the 2'OH group present on RNA may cause it to undergo alkaline hydrolysis, thereby degrading the mRNA and causing lesser extraction yield.

#### 1.2 RT-PCR (Reverse Transcription - Polymerase Chain Reaction)

**Reverse Transcription.** 2µl of the purified RNA was extracted using a pipette into a new sterile 0.2µl reaction tube. 8µl of RT mix containing 2µl of oligo-dT primers and 6µl of DEPC-treated H<sub>2</sub>O were already present in the reaction tubes given. Incubation of mRNA with the RT mix was at a high temperature of 72 °C for 2 minutes in the reaction tube. This is to break any intra-molecular base-pairings of RNA molecules to ensure that RNA is single-stranded so RT can take place efficiently. The tubes containing oligo-dT primers and mRNA were then gradually cooled to about 42°C for 1h to allow annealing to only mRNA poly-A tail within total RNA.

These tubes were then spun briefly in a microcentrifuge to collect the contents at the base of the reaction tube. Four tubes, each containing 2µl of RNA, underwent incubation with premixed enzyme cocktails. Two tubes, A and C were incubated with the enzyme cocktail(RT+), while tubes B and D were not incubated with the enzyme cocktail, thus acting as negative controls(RT-). The total volume of the mixture of RNA and enzyme cocktail was 20µl (Table A1). The tubes were incubated with the enzyme cocktail for 1 hour at 42 °C. The reaction was then terminated by heating at 70 °C for 10

minutes. All tubes were then placed on ice followed by addition of 80µl of nuclease-free water to prepare for the next PCR step.

**Table A1.** Components of the enzyme cocktail used for reverse transcription.

Volume to be added to respective tube/µl		Enzyme cocktail component
RT(+)	RT(-)	
4	4	5x Reaction Buffer
1	1	RiboLock Ribonuclease Inhibitor (20u/µl)
2	2	10mM dNTPs (10mM each of dATP, dCTP, dGTP, dTTP)
2	0	RevertAid Reverse Transcriptase
1	3	DEPC-treated water

**Polymerase Chain Reaction(PCR).** cDNA from tubes A,B,C and D were re-distributed into new tubes labelled 1,2,3 and 4 respectively. 2.5µl from each original cDNA-containing tube was pipetted into the new tubes. All new tubes 1-4 were already containing a PCR mastermix (Table A2). All contents were then mixed by pipetting, then spun down before storing on ice.

**Table A2.** Contents of PCR Mastermix

Volume/µl	Reagent	Final Concentration
13.50	H2O	
2.50	10x PCR buffer (KOD)	1x
2.50	dNTPs, 2mM of dATP, dCTP, dGTP, dTTP each	0.2mM each
1.00	NcoI-MmLDHA-F, 10µM, forward primer for LDHA	0.4µM
1.00	EcoRI-MmLDHA-R, 10µM, reverse primer for LDHA	0.4µM
1.50	25 mM MgSO <sub>4</sub>	1.5mM
0.50	KOD DNA Polymerase, 1u/µl	0.5 units/25µl
<b>22.50µl total volume (excluding 2.5µl DNA)</b>		

PCR was performed using NDeI-H6-MmLDHA forward primer and EcoRI-MmLDHA as the reverse primer. KOD DNA polymerase is used as the polymerase enzyme to help with the elongation stage of PCR. KOD DNA Polymerase has a speed of 0.5min/kb at 68°C as opposed to Taq DNA polymerase which has a speed of 1min/kb at 72°C. KOD DNA polymerase also has a lower error rate of 1 mutation per 80kb as opposed to Taq DNA polymerase which has an error rate of 1 mutation per kb. Thus, KOD polymerase is chosen as the preferred polymerase for PCR in this experiment due to its

higher speed at a lower temperature and a lower mutation rate as opposed to the commonly used Taq polymerase.

Before 25 cycles of PCR was performed, the cDNA+PCR mix was incubated for 2 minutes at 94°C for DNA strand separation. After which, PCR cycling conditions for the 25 PCR cycles were as follows: denaturation at 94°C for 2 minutes to separate DNA strands of cDNA, annealing at 60°C for 1 minute for primers to anneal to the single stranded cDNA, and DNA synthesis at 68°C for 1 minute. After 25 cycles, the mixture is then incubated for 10 minutes at 68°C to ensure that all DNA is completely synthesised. Then, the RT-PCR product was stored at 4°C to preserve it.

**Agarose Gel Electrophoresis.** Performed on a 1% gel with EtBr/SYBR safe in 1x TAE buffer at 100V for 45 minutes to visualise 5µl of RT-PCR products. 1µl of loading dye was added to 5µl of each sample. Bands were observed under UV-light using a gel documentation system. 1kb of 1µl DNA ladder was mixed with 4µl H<sub>2</sub>O and 1µl of loading dye and was loaded into the leftmost lane.

**RT-PCR Fragment Cleaning.** RT-PCR products from tubes 1 and 3 (both RT+) are purified using binding and washing buffers followed by centrifugation, then quantified using NanoDrop. This is first done by mixing the remaining 20µl of solution in tubes 1 and 3 with 100µl of binding buffer, then both tubes are spun in a centrifuge at 13,000g and r.t.p. for 1 minute. The flow through is then removed and 0.7ml of washing buffer is added to each tube before centrifuging at 13,000g and r.t.p. for 1 minute. The removing of flow through and centrifuging is repeated again. Flow-through is removed and empty spin column spun again in the centrifuged under the same setting. Each spin column is then placed into a new 1.5ml tube whereby 50µl of nuclease-free H<sub>2</sub>O is added directly to the membrane of each spin column and incubated at 37°C for 5 minutes. DNA is then eluted by centrifuging at 13,000g and r.t.p. for 1 minute before taking out the spin columns and capping the tubes. This cleaned cDNA RT-PCR product is then quantified using NanoDrop.

**Restriction Endonuclease (RE) Digestion.** The RT-PCR product was subjected to double-digestion: 20 µl purified RT-PCR product was added to 15µl H<sub>2</sub>O, 4µl RE-Buffer 3.1 (or RE-Buffer 3 + BSA), 0.5µl NcoI, and 0.5µl EcoRI in a 1.5 ml tube. Then, the mixtures were pulsed and incubated at 37°C overnight.

To a separate 1.5ml tube, the vector plasmid was subjected to triple-digestion: 20µl of pET11-H6α vector plasmid was added to 14.5µl H<sub>2</sub>O, 4µl RE-Buffer 3.1, 0.5µl NcoI, 0.5µl EcoRI, and 0.5µl BamHI. This tube was then pulsed down before being incubated at 37°C overnight.

The use of EcoRI, BamHI and EcoRI restriction enzymes allows for the creation of compatible cohesive ends on both the RT-PCR product and the plasmid, facilitating the subsequent ligation of the cDNA fragments into the plasmid vector. This directional cloning ensures that the cDNA fragments are inserted in the correct orientation under the control of the lac-inducible T7 promoter,

### 1.3 Constructing Recombinant Plasmids

**Cleaning-up of cut DNA fragments.** The RT-PCR fragment and pET11-H6α plasmid were subjected to double and triple restriction digestion, respectively, as previously described. The resulting products underwent purification using the same clean-up method as the RT-PCR cleaning performed previously. Both the cut vector and the RT-PCR product were then quantified using NanoDrop after clean-up.

**Ligation of RT-PCR cDNA into pET11-H6 $\alpha$  plasmid.** T4 DNA ligase was added to varying volume ratios of pET11-H6 $\alpha$  and RT-PCR products for ligation to the pET11-H6 $\alpha$  plasmid vector (Table A3). The mixture was then incubated at room temperature for 1 hour.

**Table A3.** The volumes of each mixture component

Vector:Insert Ratio of Reaction Tube	Volume of Mixture Constituent/ $\mu$ l			
	Cut pET11-H6 $\alpha$ Vector	RT-PCR Fragment	Ligation Buffer	T4 DNA Ligase
<b>1:1</b>	4.5	4.5	10.0	1.0
<b>1:2</b>	3.0	6.0	10.0	1.0

**Bacterial Cell Transformation via uptake of Recombinant Plasmid.** Ligated recombinant pET11a plasmids were transformed into DHS $\alpha$  competent E. coli cells (30 $\mu$ l) using the heat shock method: incubating tubes on ice for 30 minutes, followed by heating at 42°C for 90 seconds. 150 $\mu$ l of LB medium was added to each tube, then incubated at 37°C with shaking for 1 hour. The cells were subsequently resuspended by gently pipetting it. 200 $\mu$ l of the suspension was then plated on separate LB plates containing: 100 $\mu$ g/ml ampicillin, 40 $\mu$ g/ml X-Gal and 0.1Mm IPTG and incubated upside-down at 37°C overnight.

DHS $\alpha$  competent E. coli cells are specifically chosen for their ability to efficiently take up foreign DNA when treated with a solution containing a divalent cation (calcium chloride) to increase the permeability of the bacterial cell walls, allowing foreign DNA to be taken up. The use of DHS $\alpha$  competent E. coli cells is essential for the successful introduction of the recombinant plasmids, allowing for the subsequent expression of proteins from the T7 promoter.

**Small-scale Preparation of Recombinant Plasmid DNA.** 4 colonies from the LB plate containing the highest percentage of white colonies is chosen. Each single colony is then transferred into a 15ml tube with 4ml of LB medium containing 50 $\mu$ g/ml ampicillin. Each culture was then incubated overnight on a shaking incubator at 37°C. The cultures are labelled as C1,C2,C3 and C4 respectively to indicate that they originate from different colonies (Table 4).

1.5ml of the bacterial culture is carefully decanted into a 1.5ml microcentrifuge, then centrifuged at 14,000g for 1 minute. The bacterial pellet needs to be firmly attached to the side of the tube after centrifugation. After which, the supernatant is discarded via aspiration and repeat the centrifugation with another 1.5ml of the bacterial culture. The supernatant was then removed via aspiration and the bacterial cell pellet is left to dry by inverting the tube upside-down onto a paper towel for a few minutes until all the residual supernatant has drained out of the tube.

**Table A4.** Constituents and purpose of Solutions A,B,C and D.

Solution	Purpose	Constituents
A	Cell Resuspension	100 $\mu$ g/ml RNaseA + 10mM EDTA + 50mM Tris (pH7.5)
B	Cell Lysis	0.2M NaOH + 1% SDS solution

C	Neutralisation	4.08M guanidine hydrochloride + 0.759M potassium acetate + 2.12M glacial acetic acid
D	Column Wash	60mM potassium acetate + 8.3 mM Tris-HCl + 0.04mM EDTA + 60% ethanol

The bacterial cells were then resuspended using vortex mixing in 250µl of Solution A, followed by gentle mixing (by inverting the tube 5x) with 250µl of Solution B at r.t.p. Vortexing was not done upon addition of Solution B as the chromosomal DNA would be sheared. After which, 10µl of alkaline protease solution was added and the tube is gently mixed. The tube is then incubated for <5 minutes at r.t.p (if incubated for >5 minutes, the plasmid DNA would be nicked). Then, 350µl of Solution C is added to the tube and gently mixed by inverting the tube 5x. The resultant bacterial lysate is then centrifuged for 10 minutes in a microcentrifuge at 14,000g.

While the centrifuge was running, plasmid DNA purification units were prepared by insertion of one Miniprep spin column into a 2ml Collection Tube. After 10 minutes was up, all the cleared cell lysate (supernatant) was micropipetted into the spin column. The spin column containing the supernatant was then centrifuged for 1 minute at 14,000g at r.t.p. The spin column was then removed from the collection tube and the flowthrough was discarded. After discarding flowthrough, the spin column was placed back in to the Collection Tube. Then, 750µl of Solution D was added and the Collection Tube was centrifuged for 1 minute at 14,000g at r.t.p. Flow-through was then discarded and 250µl of Solution D was added into the spin column and the Collection Tube was centrifuged for 14,000g for 2 minutes at r.t.p.

Each spin column is then transferred into a sterile, new 1.5ml microcentrifuge tube. The plasmid DNA in the spin column was eluted by adding 50µl of nuclease-free water, and incubated at 2 minutes at r.t.p. The spin column was then centrifuged for 1 minute at 14,000g at r.t.p. The plasmid DNA was then quantitated via NanoDrop before storing at -20°C for long-term storage.

**Plasmid DNA Digestion.** From each colony taken from the Small-scale Preparation of Recombinant Plasmid DNA step, pipette 5µl of each colony sample into 3 new reaction tubes, resulting in 15 tubes in total. The last 3 tubes were positive controls containing a control plasmid. Refer to Table A5 for clearer breakdown of the reaction tubes used:

**Table A5.** Breakdown of the tubes that were prepared before conducting RE digest. 5µl of each colony (from the Small-scale Preparation of Recombinant Plasmid DNA step) was pipetted into each of the 3 tubes, A total of 15 tubes was thus used.

Reaction Tube Content Source	Colony 1	Colony 2	Colony 3	Colony 4	Positive Control Plasmid
(Quantity) x (Volume) of reaction tubes	3 tubes x 5µl each	3 tubes x 5µl each	3 tubes x 5µl each	3 tubes x 5µl each	3 tubes x 5µl each

To each set of 3 tubes per colony, each tube was subjected to reaction with a different reaction each (Table A6). Each tube was then subjected to the relevant treatment (Table A7) before pipetting the relevant amounts into each reaction tube. The reactions were then incubated at 37°C for 1 hour.

**Table A6.** Treatment subjected to the 3 tubes respectively.

Reaction Tube (out of the 3 tubes)	Treatment
1st	Uncut (i.e. no treatment)
2nd	Eco-RI digested plasmid
3rd	Eco-RI/NcoI double-digested plasmid

**Table A7.** Constituents for each treatment per tube.

Treatment	Constituent (μl)					
	Plasmid	H2O	Buffer	EcoRI	NcoI	Total
Uncut plasmid	5.0	4.0	1.0	-	-	10.0
EcoRI-digested plasmid	5.0	3.5	1.0	0.5	-	10.0
EcoRI/NcoI double-digested plasmid	5.0	3.5	1.0	0.5	0.5	10.0

**Agarose Gel Electrophoresis.** Performed on a 1% agarose gel with EtBr Safe in 1x TAE buffer at 100V for 45 minutes. 2μl of loading dye was added to 10μl mixture present in each tube. Each 12μl of each tube was then loaded into individual wells. 1μl of 1kb DNA ladder was mixed with 2μl of loading dye and loaded into an individual well. The resulting bands were visualised under UV light and photographed. Based on this gel electrophoresis results, the plasmid that was most cleanly digested was selected for sequencing.

#### 1.4 Sequencing of DNA

**Mastermix Preparation.** 5μl big dye + 5μl sequencing buffer + 15μl template is mixed together to form 5 portions of the mastermix, whereby one portion is to account for pipetting error. To 4 separate MicroAmp™ PCR tubes, add 5μl of the mastermix. Then to each of the 4 tubes, 5μl of each primer (pET11aF, LDHA 430-450, pET11aR, and LDHA 570-550) was added into a separate PCR tube respectively and mixed i.e. one primer per tube.

**PCR.** PCR was conducted with the following parameters: 96°C for 30 seconds, 50°C for 15 seconds, and 60°C for 2 minutes, repeated for 25 cycles. The PCR machine was then set to hold at 4°C.

**Ethanol Precipitation.** To each MicroAmp™ PCR tube, add 80μl of prepared ETOH/sodium acetate (consisting 3.0μl 3M sodium acetate, pH4.6, 62.2μl non-denatured 95% ethanol and 14.5μl deionised water) and mix. Then, transfer each mixture to a separate 1.5ml Eppendorf tube and briefly vortex. The tubes were then left on ice for 15minutes for the ethanol products to precipitate. The Eppendorf tubes were subsequently spun for 20 minutes at maximum speed at r.t.p. After which, the supernatant was carefully aspirated with a pipette and discarded. The resulting pellet was barely visible to the eye.

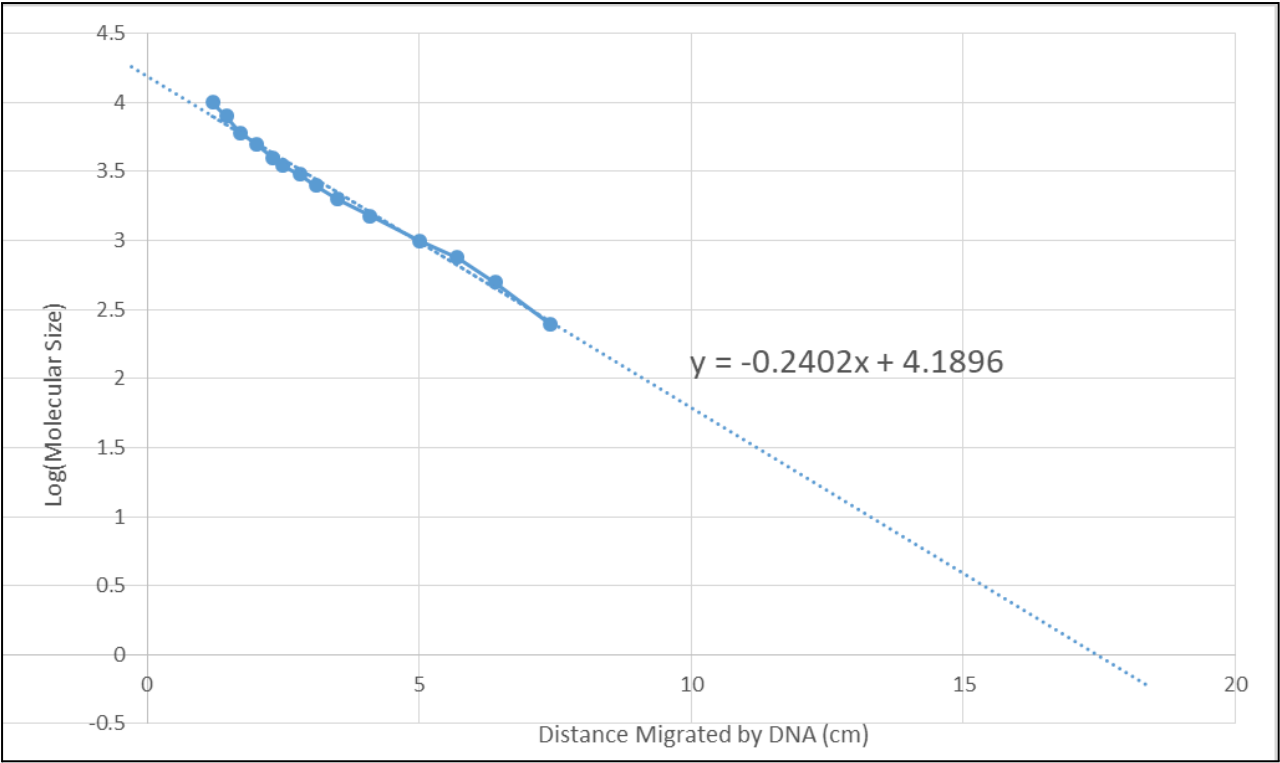
To rinse the pellet, 500µl of 75% ethanol was added to the pellet and was subsequently vortexed. The ethanol-pellet mixture was then microcentrifuged at maximum speed before aspirating and discarding the supernatant. As much as possible of the ethanol was removed (as ethanol will impact sequencing) before the pellet was dried at 50°C on a heat block for 10 minutes. The pellet was monitored to ensure it did not over-dry. Subsequent steps were performed by an external lab to be sequenced by professionals who used the ABI PRISM 3100 to sequence the pellets.

**Using BLAST to perform Sequence Analysis.** Sequencing results were conducted by an external laboratory. These results were then aligned using BLAST on the NCBI website to generate a comprehensive plasmid sequence, aiding in identifying discrepancies and distinguishing between sequencing errors and mutations.

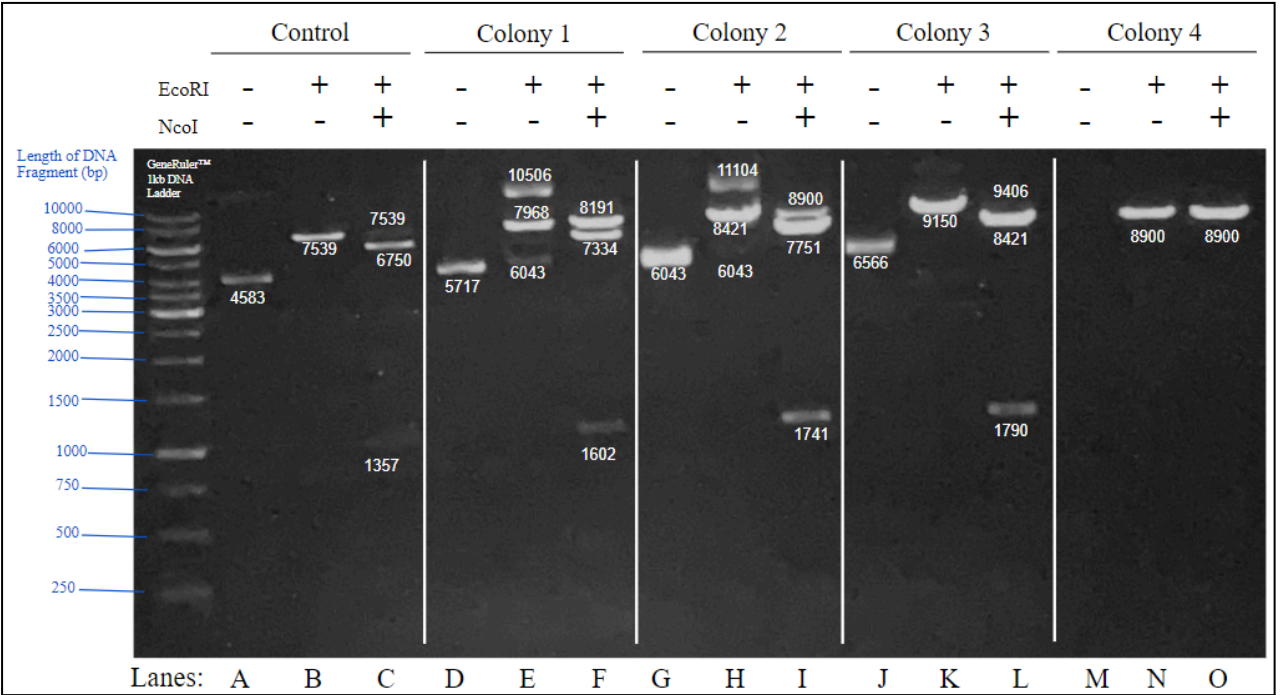
## 2 Tables of Migration Distances and Standard Curves

**Table A8.** Calculated migration distance for each bp.

<b>Molecular Size (bp)</b>	<b>Log(Molecular Size)</b>	<b>Distance Migrated by DNA (cm)</b>
10000	4	1.2
8000	3.903089987	1.45
6000	3.77815125	1.7
5000	3.698970004	2
4000	3.602059991	2.3
3500	3.544068044	2.5
3000	3.477121255	2.8
2500	3.397940009	3.1
2000	3.301029996	3.5
1500	3.176091259	4.1
1000	3	5
750	2.875061263	5.7
500	2.698970004	6.4
250	2.397940009	7.4



**Fig. A1** Graph plot of Log(molecular size) against distance migrated by DNA(cm). Standard Curve Equation is:  $\log(\text{mol. size}) = k * \text{migration} + c$



**Fig A2.** Estimated sizes of the sample bands based on the standard curve equation in Fig. A1.



### 3 BLAST Sequence Alignments

Score 1664 bits(901)	Expect 0.0	Identities 938/955(98%)	Gaps 8/955(0%)	Strand Plus/Plus
CDS: Putative 1 Query	1 77	G T L K D Q L I V N L L K E E Q A P Q N GGGAACCTCAAGGACAGCTGATTGTGAATCTTCTTAAGGAAGAGCAGGCTCCCCAGAA		136
Sbjct CDS:L-lactate dehydr	141 2	GGCAACCTCAAGGACAGCTGATTGTGAATCTTCTTAAGGAAGAGCAGGCTCCCCAGAA A T L K D Q L I V N L L K E E Q A P Q N		200
CDS: Putative 1 Query	21 137	K I T V V G V G A V G M A C A I S I L M CAAGATTACAGTTGTTGGGGTTGGTGCTGTTGGCATGGCTTGCCATCAGTATCTTAAT		196
Sbjct CDS:L-lactate dehydr	201 22	CAAGATTACAGTTGTTGGGGTTGGTGCTGTTGGCATGGCTTGCCATCAGTATCTTAAT K I T V V G V G A V G M A C A I S I L M		260
CDS: Putative 1 Query	41 197	K D L A D E L A L V D V M E D K L K G E GAAGGACTTGGCGGATGAGCTTGCCCTTGTGACGTCTGGAAGACAACTCAAGGGCGA		256
Sbjct CDS:L-lactate dehydr	261 42	GAAGGACTTGGCGGATGAGCTTGCCCTTGTGACGTCTGGAAGACAACTCAAGGGCGA K D L A D E L A L V D V M E D K L K G E		320
CDS: Putative 1 Query	61 257	M M D L Q H G S L F L K T P K I V S S K GATGATGGATCTCCAGCATGGCAGCTCTTCCCTTAAACACCAAAATGTCTCCAGCAA		316
Sbjct CDS:L-lactate dehydr	321 62	GATGATGGATCTCCAGCATGGCAGCTCTTCCCTTAAACACCAAAATGTCTCCAGCAA M M D L Q H G S L F L K T P K I V S S K		380
CDS: Putative 1 Query	81 317	D Y C V T A N S K L V I I T A G A R Q Q AGACTACTGTGTAAGTGCAGAACTCCAAGCTGGTCATTATCACCGCGGGGCGCTCAGCA		376
Sbjct CDS:L-lactate dehydr	381 82	AGACTACTGTGTAAGTGCAGAACTCCAAGCTGGTCATTATCACCGCGGGGCGCTCAGCA D Y C V T A N S K L V I I T A G A R Q Q		440
CDS: Putative 1 Query	101 377	E G E S R L N L V Q R N V N I F K F I I AGAGGGGGAGAGCGGCTCAACCTGGTCCAGCGAAACGTGAACATCTTCAAGTTTCATCAT		436
Sbjct CDS:L-lactate dehydr	441 102	AGAGGGGGAGAGCGGCTCAACCTGGTCCAGCGAAACGTGAACATCTTCAAGTTTCATCAT E G E S R L N L V Q R N V N I F K F I I		500
CDS: Putative 1 Query	121 437	P N I V K Y S P H C K L L I V S N P V D TCCCAACATTGTCAAGTACAGTCCACACTGCAAGCTGCTGATCGTCTCCAATCCAGTGGGA		496
Sbjct CDS:L-lactate dehydr	501 122	TCCCAACATTGTCAAGTACAGTCCACACTGCAAGCTGCTGATCGTCTCCAATCCAGTGGGA P N I V K Y S P H C K L L I V S N P V D		560
CDS: Putative 1 Query	141 497	I L T Y V A W K I S G F P K N R V I G S TATCTTGACCTACGTGGCTTGAAAAATCAGTGGCTTTCCAAAAACCGAGTAATTGGGAAG		556
Sbjct CDS:L-lactate dehydr	561 142	TATCTTGACCTACGTGGCTTGAAAAATCAGTGGCTTTCCAAAAACCGAGTAATTGGGAAG I L T Y V A W K I S G F P K N R V I G S		620
CDS: Putative 1 Query	161 557	G C N L D S A R F R Y L M G E R L G V H TGTTTGCAATCTGGATTACGCGCGTTCCGTACCTGATGGGAGAGAGGCTGGGGGTCA		616
Sbjct CDS:L-lactate dehydr	621 162	TGTTTGCAATCTGGATTACGCGCGTTCCGTACCTGATGGGAGAGAGGCTGGGGGTCA G C N L D S A R F R Y L M G E R L G V H		680
CDS: Putative 1 Query	181 617	A L S C H G W V L G E H G D S S V P V W CGCGCTGAGCTGTACGGCTGGGTCTGGGAGAACATGGCGACTCCAGTGTGCTGTGTG		676
Sbjct CDS:L-lactate dehydr	681 182	CGCGCTGAGCTGTACGGCTGGGTCTGGGAGAACATGGCGACTCCAGTGTGCTGTGTG A L S C H G W V L G E H G D S S V P V W		740
CDS: Putative 1 Query	201 677	S G V N V A G V S L K S L N P E L G T D GAGTGGTGTGAATGTTGCCGGCTCTCCCTGAAGTCTTTAAACCAGAACTGGGCACTCA		736
Sbjct CDS:L-lactate dehydr	741 202	GAGTGGTGTGAATGTTGCCGGCTCTCCCTGAAGTCTTTAAACCAGAACTGGGCACTCA S G V N V A G V S L K S L N P E L G T D		800
CDS: Putative 1 Query	221 737	A D K E Q W K E V H K Q V V D S A Y E V CGCAGACAAGGAGCAGTGGAGGAGGTTTCAACAGCAGGTGGTGACAGTGCCTACGAGGT		796
Sbjct CDS:L-lactate dehydr	801 222	CGCAGACAAGGAGCAGTGGAGGAGGTTTCAACAGCAGGTGGTGACAGTGCCTACGAGGT A D K E Q W K E V H K Q V V D S A Y E V		860
CDS: Putative 1 Query	241 797	I K L K G Y T S W A I G L S V A D L A E GATCAAGCTGAAGGTTACACATCTTGGGCCATTGGCTCTCTGTGGCAGACTTGGCTGA		856
Sbjct CDS:L-lactate dehydr	861 242	GATCAAGCTGAAGGTTACACATCTTGGGCCATTGGCTCTCTGTGGCAGACTTGGCTGA I K L K G Y T S W A I G L S V A D L A E		920
CDS: Putative 1 Query	261 857	S I M K N L R R V H P I S P * L R G S GAGCATAATGAAGAACCTTAGGCGGGTGATCCCATTTCT-CCATGATTAAAGGGGCTCTA		915
Sbjct CDS:L-lactate dehydr	921 262	GAGCATAATGAAGAACCTTAGGCGGGTGATCCCATTTCCACCATGATTAAAGGGTCTCTA S I M K N L R R V H P I S T M I K G L Y		980
CDS: Putative 1 Query	279 916	M E S M R M S S S G P W I L G Q K E S TGGAATCAATGAGGATGTCTTCTCAG-G-CCCATGGATCTGGGACAAAA-GGAATCTC		972
Sbjct CDS:L-lactate dehydr	981 282	TGGAATCAATGAGGATGTCTTCTCAGTGTCCCATGTATCTCTGGGACAAAAATGGAATCTC G I N E D V F L S V P C I L G Q N G I S		1040
CDS: Putative 1 Query	298 973	R C C E G X L T P E K K P P E K K -GATGTTGTGAAGG-GANACTGACTCTGAGaaaaag-ccc-ctgaaaaaaagc		1023
Sbjct CDS:L-lactate dehydr	1041 302	GGATGTTGTGAAGGTGACACTGACTCTGAGGAAGAGGCCCGCTGAAGAAAGAGC D V V K V T L T P E E E A R L K K S		1095

**Fig A3.** Pairwise alignment of pET11aF against NM\_010699.2. pET11aF had a 2% amino acid mismatch as seen by the significantly larger number of pink alphabets (which indicate amino acid mismatches)from position 895 onwards. (nucleotide on left, CDS on the right)

Score 1714 bits(928)	Expect 0.0	Identities 976/999(98%)	Gaps 10/999(1%)	Strand Plus/Minus
Query 51	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	110
Sbjct 1137	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	1078
Query 111	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	170
Sbjct 1077	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	1018
Query 171	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	230
Sbjct 1017	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	958
Query 231	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	290
Sbjct 957	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	898
Query 291	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	350
Sbjct 897	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	838
Query 351	CTGCTTGTGAACCTCTTCACTGCTCTT	CTGCTTGTGAACCTCTTCACTGCTCTT	CTGCTTGTGAACCTCTTCACTGCTCTT	410
Sbjct 837	CTGCTTGTGAACCTCTTCACTGCTCTT	CTGCTTGTGAACCTCTTCACTGCTCTT	CTGCTTGTGAACCTCTTCACTGCTCTT	778
Query 411	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	470
Sbjct 777	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	718
Query 471	ATGTTCTCCAGGACCGCGCTGACAGCT	ATGTTCTCCAGGACCGCGCTGACAGCT	ATGTTCTCCAGGACCGCGCTGACAGCT	530
Sbjct 717	ATGTTCTCCAGGACCGCGCTGACAGCT	ATGTTCTCCAGGACCGCGCTGACAGCT	ATGTTCTCCAGGACCGCGCTGACAGCT	658
Query 531	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	590
Sbjct 657	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	598
Query 591	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	650
Sbjct 597	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	538
Query 651	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	710
Sbjct 537	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	478
Query 711	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	770
Sbjct 477	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	418
Query 771	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	830
Sbjct 417	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	358
Query 831	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	890
Sbjct 357	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	298
Query 891	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	949
Sbjct 297	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	238
Query 950	CCTGTCAACAG-ACCAACCCCAA-AACT	CCTGTCAACAG-ACCAACCCCAA-AACT	CCTGTCAACAG-ACCAACCCCAA-AACT	1006
Sbjct 237	CATGCCAACGACCAACCCCAACACTGT	CATGCCAACGACCAACCCCAACACTGT	CATGCCAACGACCAACCCCAACACTGT	178
Query 1007	aaaaa-aT-C-CAACAG-TGGCCCT-GAG	aaaaa-aT-C-CAACAG-TGGCCCT-GAG	aaaaa-aT-C-CAACAG-TGGCCCT-GAG	1039
Sbjct 177	AAGAAGATTCACAATCAGCTGGTCTTG	AAGAAGATTCACAATCAGCTGGTCTTG	AAGAAGATTCACAATCAGCTGGTCTTG	139

Score 1714 bits(928)	Expect 0.0	Identities 976/999(98%)	Gaps 10/999(1%)	Strand Plus/Minus
CDS: Putative 1	307	S S C S R R S E G S P T Q A R R * A P		
Query	51	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	110
Sbjct	1137	TTAGAACTGCAGCTCCTCTCGGATTC	TTAGAACTGCAGCTCCTCTCGGATTC	1078
CDS: Putative 1	289	R K R L L * H * R * L M R S E M K D W S		
Query	111	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	170
Sbjct	1077	CTCTTCTCAGGAGTCAGTGTACCTTCA	CTCTTCTCAGGAGTCAGTGTACCTTCA	1018
CDS: Putative 1	272	V H S V S S S M R M S E M S V R L * P P		
Query	171	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	230
Sbjct	1017	ACATGGGACACTGAGGAAGACATCCTCA	ACATGGGACACTGAGGAAGACATCCTCA	958
CDS: Putative 1	253	F P I C G G L T R * * A R L W T Q W L S		
Query	231	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	290
Sbjct	957	AATGGGATGCACCGCTTAAGGTTCTTCA	AATGGGATGCACCGCTTAAGGTTCTTCA	898
CDS: Putative 1	235	A L P G P H T V K * S S * R T P V T W W		
Query	291	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	350
Sbjct	897	GCCAATGGCCAGGATGTGTAACCTTCA	GCCAATGGCCAGGATGTGTAACCTTCA	838
CDS: Putative 1	217	R S T F R R G S S R T Q T L A W N Q T L		
Query	351	CTGCTTGTGAACCTCTTCACTGCTCTTGT	CTGCTTGTGAACCTCTTCACTGCTCTTGT	410
Sbjct	837	CTGCTTGTGAACCTCTTCACTGCTCTTGT	CTGCTTGTGAACCTCTTCACTGCTCTTGT	778
CDS: Putative 1	197	L S * P S A P L M * V V G C L C V P T A		
Query	411	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	470
Sbjct	777	AGACTTCAGGAGACGCGGCAACATTCAC	AGACTTCAGGAGACGCGGCAACATTCAC	718
CDS: Putative 1	179	M N E W S G A T V A * R T F G W G R E W		
Query	471	ATGTTCTCCAGGACCGCGTGAACCTTCA	ATGTTCTCCAGGACCGCGTGAACCTTCA	530
Sbjct	717	ATGTTCTCCAGGACCGCGTGAACCTTCA	ATGTTCTCCAGGACCGCGTGAACCTTCA	658
CDS: Putative 1	160	* T V S G R Q I W I A V V E L * E T K P		
Query	531	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	590
Sbjct	657	CAGGTAACGGAACCGCGTGAATTCAGAT	CAGGTAACGGAACCGCGTGAATTCAGAT	598
CDS: Putative 1	142	F A V S K G L W T P * S I W Q I P S S *		
Query	591	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	650
Sbjct	597	AAAGCCACTGATTTTCAAGCCAGTGGT	AAAGCCACTGATTTTCAAGCCAGTGGT	538
CDS: Putative 1	124	C S A T H V T S S L T P F S S S S S T *		
Query	651	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	710
Sbjct	537	CAGCTTGCAGTGTGAGCTGTACTTGACA	CAGCTTGCAGTGTGAGCTGTACTTGACA	478
CDS: Putative 1	105	T E S S W T S G A R G R K S V P X R P S		
Query	711	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	770
Sbjct	477	GTTTCGCTGGACAGGTTGAGCGGCTCT	GTTTCGCTGGACAGGTTGAGCGGCTCT	418
CDS: Putative 1	85	L S W S P T R L * V T T K A P S L K Q P		
Query	771	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	830
Sbjct	417	AATGACAGCTTGGAGTTGCGAGTTACAC	AATGACAGCTTGGAGTTGCGAGTTACAC	358
CDS: Putative 1	66	K L S S A A M S S I W * R A R S N T K W		
Query	831	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	890
Sbjct	357	TTTAAGGAAGAGGCTGCCATGCTGAGAT	TTTAAGGAAGAGGCTGCCATGCTGAGAT	298
CDS: Putative 1	47	S T L L P L S K R W S R * * S V S P V A		
Query	891	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	949
Sbjct	297	GACGTCAACAAAGGCAAGCTCTCCGCA	GACGTCAACAAAGGCAAGCTCTCCGCA	238
CDS: Putative 1	29	R D V S W G W F Q L R T R P L Q E E *		
Query	950	CCTGTCAACAG-ACCAACCCCAA-AACT	CCTGTCAACAG-ACCAACCCCAA-AACT	1006
Sbjct	237	CATGCCAACGACCAACCCCAACACTGT	CATGCCAACGACCAACCCCAACACTGT	178
CDS: Putative 1	11	F F G V L P G S P A M		
Query	1007	aaaaa-aT-C-CAACAG-TGGCCCT-GAG	aaaaa-aT-C-CAACAG-TGGCCCT-GAG	1039
Sbjct	177	AAGAAGATTCACAATCAGCTGGTCTTG	AAGAAGATTCACAATCAGCTGGTCTTG	139
CDS: Putative 1	13	L L N V I L Q D K L T A M		

**Fig A4.** Pairwise alignment of pET11aR sequence against NM\_010699.2 (nucleotide on left, CDS on the right)

Score 979 bits(530)	Expect 0.0	Identities 534/536(99%)	Gaps 1/536(0%)	Strand Plus/Plus
Query 9	AAACCTGAGTAATTGGAAGTGGTTGCAATCTGGATTACGCGCGGTTCCGTTACCTGATGG	68		
Sbjct 603	AAACC-GAGTAATTGGAAGTGGTTGCAATCTGGATTACGCGCGGTTCCGTTACCTGATGG	661		
Query 69	GAGAGAGGCTGGGGGTTACGCGCTGAGCTGTACGGCTGGGTTCCGGGAACATGGCG	128		
Sbjct 662	GAGAGAGGCTGGGGGTTACGCGCTGAGCTGTACGGCTGGGTTCCGGGAACATGGCG	721		
Query 129	ACTCCAGTGTGCTGTGTGGAGTGGTGAATGTTGCCGGCTCTCCCTGAAGTCTCTTA	188		
Sbjct 722	ACTCCAGTGTGCTGTGTGGAGTGGTGAATGTTGCCGGCTCTCCCTGAAGTCTCTTA	781		
Query 189	ACCCAGAACTGGGCACTGACGACAGGAGCAGTGAAGAGGTTACAGCAGGTTGG	248		
Sbjct 782	ACCCAGAACTGGGCACTGACGACAGGAGCAGTGAAGAGGTTACAGCAGGTTGG	841		
Query 249	TGGACAGTGCCTACGAGGTGATCAAGCTGAAAGGTTACACATCTCGGCCATTGGCTCT	308		
Sbjct 842	TGGACAGTGCCTACGAGGTGATCAAGCTGAAAGGTTACACATCTCGGCCATTGGCTCT	901		
Query 309	CTGTGGCAGACTTGGCTGAGAGCATAATGAAGAACCTTAGCGGGTGATCCCATTTCCA	368		
Sbjct 902	CTGTGGCAGACTTGGCTGAGAGCATAATGAAGAACCTTAGCGGGTGATCCCATTTCCA	961		
Query 369	CCATGATTAAGGGTCTCTATGGAATCAATGAGGATGCTTCTCAGTGCCCATGTATCC	428		
Sbjct 962	CCATGATTAAGGGTCTCTATGGAATCAATGAGGATGCTTCTCAGTGCCCATGTATCC	1021		
Query 429	TGGGACAAAATGGAATCTCGGATGTTGGAAGTGACACTGACTCTGAGGAAGAGGCC	488		
Sbjct 1022	TGGGACAAAATGGAATCTCGGATGTTGGAAGTGACACTGACTCTGAGGAAGAGGCC	1081		
Query 489	GCCTGAAGAAGAGCGACAGACCCCTCTGGGAATCCAGAAGGAGCTGCAGTTCTAA	544		
Sbjct 1082	GCCTGAAGAAGAGCGACAGACCCCTCTGGGAATCCAGAAGGAGCTGCAGTTCTAA	1137		

Score 979 bits(530)	Expect 0.0	Identities 534/536(99%)	Gaps 1/536(0%)	Strand Plus/Plus
CDS: Putative 1	1	N L S N W K W L Q S G F S A V P L P D G		
Query	9	AAACCTGAGTAATTGGAAGTGGTTGCAATCTGGATTACGCGCGGTTCCGTTACCTGATGG	68	
Sbjct	603	AAACC-GAGTAATTGGAAGTGGTTGCAATCTGGATTACGCGCGGTTCCGTTACCTGATGG	661	
CDS:L-lactate dehydr	156	H R V I G S G C N L D S A R F R Y L Y		
CDS: Putative 1	21	R E A G G S R A E L S R L G P G R T W R		
Query	69	GAGAGAGGCTGGGGGTTACGCGCTGAGCTGTACGGCTGGGTTCCGGGAACATGGCG	128	
Sbjct	662	GAGAGAGGCTGGGGGTTACGCGCTGAGCTGTACGGCTGGGTTCCGGGAACATGGCG	721	
CDS:L-lactate dehydr	175	G E R L G V H A L S C H G W V L G E H G		
CDS: Putative 1	41	L Q C A C V E W C E C C R R L P E V S *		
Query	129	ACTCCAGTGTGCTGTGTGGAGTGGTGAATGTTGCCGGCTCTCCCTGAAGTCTCTTA	188	
Sbjct	722	ACTCCAGTGTGCTGTGTGGAGTGGTGAATGTTGCCGGCTCTCCCTGAAGTCTCTTA	781	
CDS:L-lactate dehydr	195	D S S V P V W S G V N V A G V S L K S L		
CDS: Putative 1	60	P R T G H * R R Q G A V E G G S Q A G G		
Query	189	ACCCAGAACTGGGCACTGACGACAGGAGCAGTGAAGAGGTTACAGCAGGTTGG	248	
Sbjct	782	ACCCAGAACTGGGCACTGACGACAGGAGCAGTGAAGAGGTTACAGCAGGTTGG	841	
CDS:L-lactate dehydr	215	N P E L G T D A D K E Q W K E V H K Q V		
CDS: Putative 1	79	G Q C L R G D Q A E R L H I L G H W P L		
Query	249	TGGACAGTGCCTACGAGGTGATCAAGCTGAAAGGTTACACATCTCGGCCATTGGCTCT	308	
Sbjct	842	TGGACAGTGCCTACGAGGTGATCAAGCTGAAAGGTTACACATCTCGGCCATTGGCTCT	901	
CDS:L-lactate dehydr	235	V D S A Y E V I K L K G Y T S W A I G L		
CDS: Putative 1	99	C G R L G * E H N E E P * A G A S H F H		
Query	309	CTGTGGCAGACTTGGCTGAGAGCATAATGAAGAACCTTAGCGGGTGATCCCATTTCCA	368	
Sbjct	902	CTGTGGCAGACTTGGCTGAGAGCATAATGAAGAACCTTAGCGGGTGATCCCATTTCCA	961	
CDS:L-lactate dehydr	255	S V A D L A E S I M K N L R V H P I S		
CDS: Putative 1	117	H D * G S L W N Q * G C L P Q C P M Y P		
Query	369	CCATGATTAAGGGTCTCTATGGAATCAATGAGGATGCTTCTCAGTGCCCATGTATCC	428	
Sbjct	962	CCATGATTAAGGGTCTCTATGGAATCAATGAGGATGCTTCTCAGTGCCCATGTATCC	1021	
CDS:L-lactate dehydr	275	T M I K G L Y G I N E D V F L S V P C I		
CDS: Putative 1	135	G T K W N L G C C E G D T D S * G R G P		
Query	429	TGGGACAAAATGGAATCTCGGATGTTGGAAGTGACACTGACTCTGAGGAAGAGGCC	488	
Sbjct	1022	TGGGACAAAATGGAATCTCGGATGTTGGAAGTGACACTGACTCTGAGGAAGAGGCC	1081	
CDS:L-lactate dehydr	295	L G Q N G I S D V V K V T L T P E E E A		
CDS: Putative 1	154	P E E E R X H P L G N P E G A A V L		
Query	489	GCCTGAAGAAGAGCGACAGACCCCTCTGGGAATCCAGAAGGAGCTGCAGTTCTAA	544	
Sbjct	1082	GCCTGAAGAAGAGCGACAGACCCCTCTGGGAATCCAGAAGGAGCTGCAGTTCTAA	1137	
CDS:L-lactate dehydr	315	R L K K S A D T L W G I Q K E L Q F		

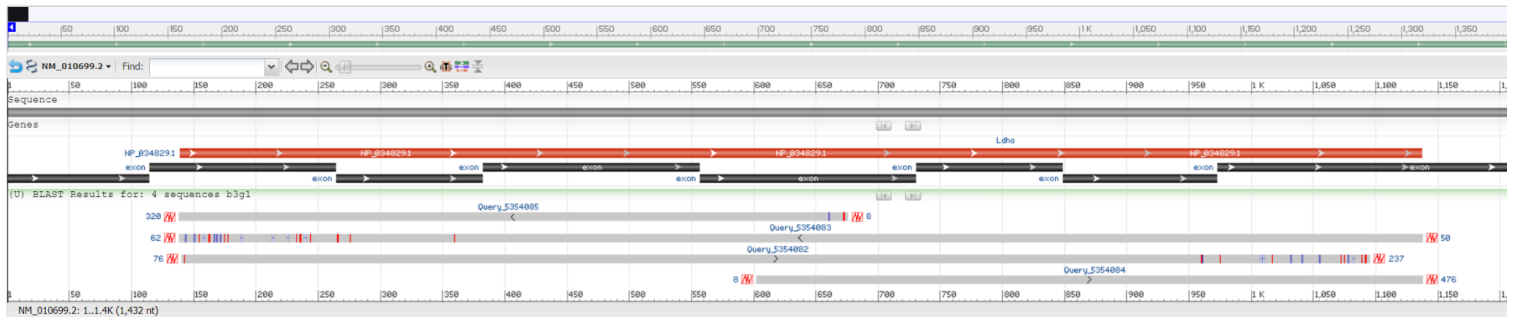
**Fig A5.** Pairwise alignment of LDHA430-450 cut fragment against NM\_010699.2. Only one mismatch was found at query position 15. (nucleotide on left, CDS on the right)

Score 979 bits(530)	Expect 0.0	Identities 535/537(99%)	Gaps 1/537(0%)	Strand Plus/Minus
Query 9	CCCAAGCTCTCTCC-ATCAGGTAAACGGAACGCGCTGAATCCAGATTGCAACCACTTCC	67		
Sbjct 675	CCCCAGCTCTCTCCATCAGGTAAACGGAACGCGCTGAATCCAGATTGCAACCACTTCC	616		
Query 68	AATTACTCGGGTTTTTGGGAAGGCACCTGATTTTCAAGCCACGTAGGTCAAGATATCCAC	127		
Sbjct 615	AATTACTCGGGTTTTTGGGAAGGCACCTGATTTTCAAGCCACGTAGGTCAAGATATCCAC	556		
Query 128	TGGATTGGAGACGATCAGCAGCTTGCAGTGTGGACTGTACTTGACAATGTTGGGAATGAT	187		
Sbjct 555	TGGATTGGAGACGATCAGCAGCTTGCAGTGTGGACTGTACTTGACAATGTTGGGAATGAT	496		
Query 188	GAACCTGAAGATGTTACGTTTCTGCTGGACAGGTTGAGCGGGCTCTCCCCCTTGTCTG	247		
Sbjct 495	GAACCTGAAGATGTTACGTTTCTGCTGGACAGGTTGAGCGGGCTCTCCCCCTTGTCTG	436		
Query 248	ACGGGCCCCGGGTGATAATGACAGCTTGGAGTTCGAGTTACACAGTAGTCTTTGCT	307		
Sbjct 435	ACGGGCCCCGGGTGATAATGACAGCTTGGAGTTCGAGTTACACAGTAGTCTTTGCT	376		
Query 308	GGAGACAATTTTGGTGTTTAAGGAAGAGGCTGCCATGCTGGAGATCCATCATCTCGCC	367		
Sbjct 375	GGAGACAATTTTGGTGTTTAAGGAAGAGGCTGCCATGCTGGAGATCCATCATCTCGCC	316		
Query 368	CTTGAGTTTGTCTTCCATGACGTCAACAAGGGCAAGCTATCCGCCAAGTCTTATTAA	427		
Sbjct 315	CTTGAGTTTGTCTTCCATGACGTCAACAAGGGCAAGCTATCCGCCAAGTCTTATTAA	256		
Query 428	GATACTGATGGCACAAGCCATGCCAACAGCAACCAACCAACTGTAATCTTGTCTG	487		
Sbjct 255	GATACTGATGGCACAAGCCATGCCAACAGCAACCAACCAACTGTAATCTTGTCTG	196		
Query 488	GGGAGCCTGCTCTTCTTAAGAAGATTACAATCAGCTGCTTGGAGGTTGCCAT	544		
Sbjct 195	GGGAGCCTGCTCTTCTTAAGAAGATTACAATCAGCTGCTTGGAGGTTGCCAT	139		

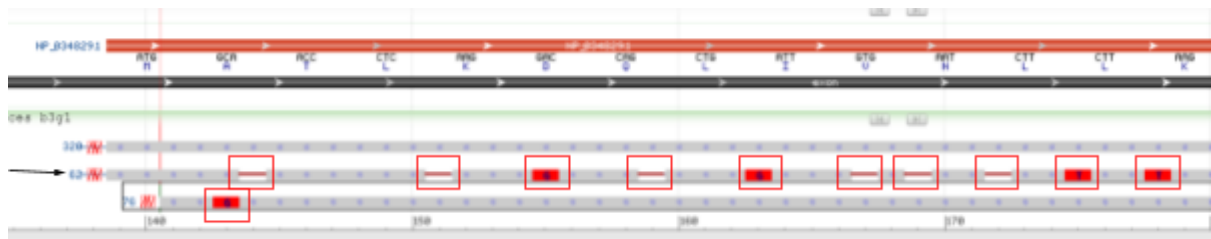
Score 979 bits(530)	Expect 0.0	Identities 535/537(99%)	Gaps 1/537(0%)	Strand Plus/Minus
CDS: Putative 1	178	L G R E M L Y R F R A S D L N C G S G		
Query	9	CCCAAGCTCTCTCC-ATCAGGTAAACGGAACGCGCTGAATCCAGATTGCAACCACTTCC	67	
Sbjct	675	CCCCAGCTCTCTCCATCAGGTAAACGGAACGCGCTGAATCCAGATTGCAACCACTTCC	616	
CDS:L-lactate dehydr	179	G L R E G M L Y R F R A S D L N C G S G		
CDS: Putative 1	159	I V R N K P F G S I K W A V Y T L I D V		
Query	68	AATTACTCGGGTTTTTGGGAAGGCACCTGATTTTCAAGCCACGTAGGTCAAGATATCCAC	127	
Sbjct	615	AATTACTCGGGTTTTTGGGAAGGCACCTGATTTTCAAGCCACGTAGGTCAAGATATCCAC	556	
CDS:L-lactate dehydr	159	I V R N K P F G S I K W A V Y T L I D V		
CDS: Putative 1	139	P N S V I L L K C H P S Y V K I N P I I		
Query	128	TGGATTGGAGACGATCAGCAGCTTGCAGTGTGGACTGTACTTGACAATGTTGGGAATGAT	187	
Sbjct	555	TGGATTGGAGACGATCAGCAGCTTGCAGTGTGGACTGTACTTGACAATGTTGGGAATGAT	496	
CDS:L-lactate dehydr	139	P N S V I L L K C H P S Y V K I N P I I		
CDS: Putative 1	119	F K F I N V N R Q V L N L R S E G E Q Q		
Query	188	GAACCTGAAGATGTTACGTTTCTGCTGGACAGGTTGAGCGGGCTCTCCCCCTTGTCTG	247	
Sbjct	495	GAACCTGAAGATGTTACGTTTCTGCTGGACAGGTTGAGCGGGCTCTCCCCCTTGTCTG	436	
CDS:L-lactate dehydr	119	F K F I N V N R Q V L N L R S E G E Q Q		
CDS: Putative 1	99	R A G A T I I V L K S N A T V C Y D K S		
Query	248	ACGGGCCCCGGGTGATAATGACAGCTTGGAGTTCGAGTTACACAGTAGTCTTTGCT	307	
Sbjct	435	ACGGGCCCCGGGTGATAATGACAGCTTGGAGTTCGAGTTACACAGTAGTCTTTGCT	376	
CDS:L-lactate dehydr	99	R A G A T I I V L K S N A T V C Y D K S		
CDS: Putative 1	79	S V I K P T K L F L S G H Q L D M M E G		
Query	308	GGAGACAATTTTGGTGTTTAAGGAAGAGGCTGCCATGCTGGAGATCCATCATCTCGCC	367	
Sbjct	375	GGAGACAATTTTGGTGTTTAAGGAAGAGGCTGCCATGCTGGAGATCCATCATCTCGCC	316	
CDS:L-lactate dehydr	79	S V I K P T K L F L S G H Q L D M M E G		
CDS: Putative 1	59	K L K D E M V D V L A L E D A L D K M L		
Query	368	CTTGAGTTTGTCTTCCATGACGTCAACAAGGGCAAGCTATCCGCCAAGTCTTATTAA	427	
Sbjct	315	CTTGAGTTTGTCTTCCATGACGTCAACAAGGGCAAGCTATCCGCCAAGTCTTATTAA	256	
CDS:L-lactate dehydr	59	K L K D E M V D V L A L E D A L D K M L		
CDS: Putative 1	39	I S I A C A M G V A G V G V T I K N Q		
Query	428	GATACTGATGGCACAAGCCATGCCAACAGCAACCAACCAACTGTAATCTTGTCTG	487	
Sbjct	255	GATACTGATGGCACAAGCCATGCCAACAGCAACCAACCAACTGTAATCTTGTCTG	196	
CDS:L-lactate dehydr	39	I S I A C A M G V A G V G V T I K N Q		
CDS: Putative 1	19	P A Q E E K L L N V I L Q D K L T A M		
Query	488	GGGAGCCTGCTCTTCTTAAGAAGATTACAATCAGCTGCTTGGAGGTTGCCAT	544	
Sbjct	195	GGGAGCCTGCTCTTCTTAAGAAGATTACAATCAGCTGCTTGGAGGTTGCCAT	139	
CDS:L-lactate dehydr	19	P A Q E E K L L N V I L Q D K L T A M		

**Fig A6.** Pairwise alignment of LDHA570-550 cut fragment against NM\_010699.2. Only one mismatch was found at query position 25. (nucleotide on left, CDS on the right)

## 4 BLAST Analysis



**Fig A7. Graphical Representation:** alignment of (from top to bottom) LDHA 570-550, pET11aR, pET11aF and LDHA 430-450 sequences against NM\_010699.2. From the diagram, there are no aligned red lines common in all the sequences, showing that no mutation was present. For a mismatch to be characterised as a mutation, the mismatch needs to appear in the same location in more than one sequence.



**Fig A8. Close-up of mismatching.** The red boxes indicate either a sequencing error or a mutation. The middle sequence as indicated by the arrow (belonging to the pET11aR sequence) had many red boxes at its end, so it is plausible that these are sequencing errors and not mutations since these mismatches are localised at the 5' end of the gene and machines tend to have a higher error rate when sequencing the ends of sequences, supported by the many gaps seen. Given that none of the other sequences shared the same mismatches as the pET11aR sequence, it can be confirmed that the mismatches are due to sequencing error and not mutation.

### Discussion about Impact of Mutations on LDHA Enzymatic Activity

Although no mutations were observed in B3G1's sequences, presence of mutations would have disrupted the enzymatic function of the LDHA, especially if the mutation is located at a critical location such as the reaction site of LDHA, thereby reducing its ability to convert pyruvate to lactate. Alteration in enzymatic activity can disrupt cellular metabolism, leading to metabolic disorders or contributing to the progression of diseases such as cancer (Nguyen et al., 2001).

Mutations also have the potential to modify the substrate specificity of LDHA, thereby impacting the efficiency of lactate production. These alterations in substrate specificity could disrupt the equilibrium between aerobic and anaerobic metabolism, consequently influencing cellular energy generation and maintaining redox homeostasis. (Goldberg et al., 2001). Additionally, alterations in the LDHA enzyme structure due to mutations may compromise its stability, folding, or interactions with cofactors and substrates. Such changes can result in diminished enzyme function, protein misfolding, aggregation, or degradation. These structural disruptions have the potential to negatively impact cellular functions relying on LDHA activity, thereby playing a role in the development of diseases. (Le et al., 2010)

## References

Nguyen, A. T., & Hegele, R. A. (2001). Lactate dehydrogenase A: From glycolysis to the Warburg effect. *Trends in Endocrinology & Metabolism*, 23(4), 168-175.

Goldberg, E., & Witt, A. (2001). Lactate Dehydrogenase. *Encyclopedia of Life Sciences*.  
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Le, A., Cooper, C. R., Gouw, A. M., Dinavahi, R., Maitra, A., Deck, L. M., ... & Dang, C. V. (2010). Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proceedings of the National Academy of Sciences*, 107(5), 2037-2042.