Cloning and Analysis of *Caenorhabditis elegans* Krüppel Like Transcription Factor-1 promoter.

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ABSTRACT

Krüppel Like Transcription Factor is found in many different species including *Homo sapiens*. *Caenorhabditis elegans* is a suitable model organism to study KLF role in mammalian metabolic regulation as the nematode has only 3 *klf* members whereas humans have 17. *Klf-1* influences cellular apoptosis and lipid metabolism and encodes for a Zinc finger protein. Ce-*Klf-1* promoter is 9.5Kb long. A 500 bp sequence is successfully excised at the ATG 5' end from 9.5Kb promoter using PCR and restriction digestion techniques. In this project, we want to excise a 250-300 bp region of Ce-*Klf-1* promoter with the help of PCR and clone that particular segment using cloning vector. The purpose is to introduce the 250-300 bp promoter region into a cloning vector, upstream to a GFP (Green Fluorescent Protein) encoding start site. This project focuses to determine the expression of GFP protein using the 250-300 bp region of Ce-*Klf-1* promoter using an expression vector which will be introduced in *C. elegans* using microinjection.

BACKGROUND

Krüppel Like Transcription Factor

- Klf-1 is conserved between humans and *C. elegans*; 17 members of klf family found in humans versus 3 in worms.
- *C. elegans* Klf-1 shares homology with human klfs-2, 4 and 5.
- *Caenorhabditis elegans* Klf-1 is involved in fat regulation, cell death and phagocytosis.
- Suppression of klf-1 in *C. elegans* results in increased fat deposition in the worm intestine.

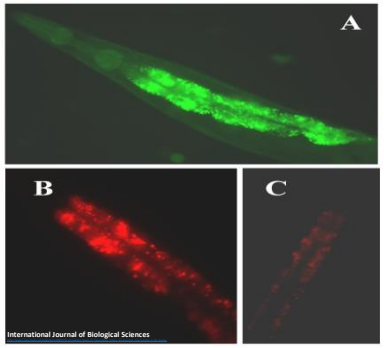

Figure 1: *C. elegans klf 1* in fat regulation (A) The expression of klf-1: gfp. Intense expression of gfp green fluorescent in the intestine of a C. elegans adult hermaphrodite; (B) extensive fat accumulation in klf-1RNAi hermaphrodite; (C) low fat content in wild type adult hermaphrodite.

GOAL

To Determine whether 300 bp region of *C. elegans* klf 1 Promoter is capable of expressing the GFP gene inserted downstream.

RESULTS

Polymerase Chain Reaction Procedure:

- *E.coli* with plasmid pHZ337 were lysed and plasmid extracted and purified using Invitrogen Kit.
- PCR Master mix with necessary components added.
- Thermocycler (95ºC 2 mins, 95 ºC 30 sec, 55 ºC 30 sec, 72 ºC 30 sec, 72 ºC 2 mins)
- Results were run on 1% TAE Agarose gel
- 80 volts

Sample preparation for sequencing:

- *E.coli* with plasmid pHZ337 were lysed and plasmid extracted and purified using Invitrogen Kit.
- Results were run on 1% TAE Agarose gel
- 80 volts
- Results were run on 1% TAE Agarose gel
- Purified plasmid (10µl) sent for sequencing.

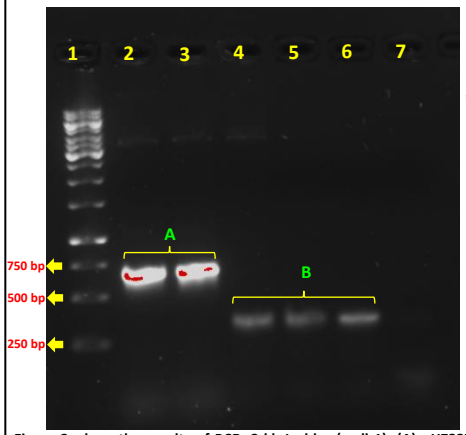

Figure 2: show the results of PCR; 3 kb Ladder (well 1); (A) pHZ337 with GFP primers as control (wells 2 & 3); (B) plasmid pHZ337 with klf p1b primers (wells 4, 5, 6)

pHZ337 Sequence

GNNNNNNNNNNNNNNNNNNNNNNNNNNNATGATCTTACTAACTAACTA
TTCTCATTTAAATTTTCAGAGCTTAAAAATGGCTGAAATCACTCACAAC
GATGGATACGCTAACAACTTGGAAATGAAATAAGCTTGGCCGATTCTG
TGTATCTCTGCGTTTCTGTCTATTTCTTCATTTTCTTATTCCTTTATCAAGT
TTTTTGTTTCGGTTTTTATCTTTTTTTTTTGTTTCGGCTGTGTGTTGAGGAG
GCGATGCATTACCCTCGAAAATGTAAAAGACTACGCCTTTTTTGGAAAT
TTTTGTTGGGGTTTTTAGGGAAAAATCACTTTAAAAAATTTTTTTCATA
GATTGAAAACCAAACTTAAAATCGAAATCTTCAAAAATGAGCAAAAG
ATCGAAAAGCAAAAAATTGAAATTTTTTTCAAAATTTTTTTTTTCAAATT
TTTTCAAAAAATAATTATTTCAAAATTTTCTCAAATTTAGATTTTCTTAT
GTACTTTTTCACAATTTACCCATTTCCCCTCTAAAAAATCTATAAAAATA
TCGATTTTTGCAGATGTTTGGGCCGGAttAGGCTCCGGGTCACTCAGCT
CCTCATCAAGTACACTACAATTCAATTCCACGTCATCCACGATGACGTC
ATCATCGATAAATGCTCTAGAGGATCCCCGGGATTGGCCAAAGGACCC
AAAGGTATGTTTCGAATGATACTAACATAACATAGAACATTTTCAGGA
GGACCCTTGGAGGGTACCGAGCTCAGAAAAAATGACTGCTCCAAAGA
AGAAGCGTAAGGTACCGGTAGAAAAAATGAGTAAAGGAGAAGAACT
TTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAAT
GGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATA
CGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTT
CCATGGGTAAGTTTAAACATATATATACTAACTAACCCTGATTATTTAA
ATTTTCAGCCANCACTTGTCACTACTTTCTGTTATGNGTTCAATGNTNCT
CGAGATACCAGATCATATGAAACGGCATGACTTTTTCANNNNTGCCAT
GCCCNAAGGNNNNGNANAGN

Figure 4: pHZ337 Sequence; Yellow- Forward and reverse *Ce* klf p1b primers; Blue- 300 bp *Ce* klf 1 promoter region

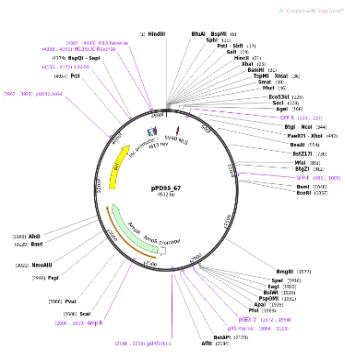

Figure 3: Plasmid Vector pPD95_67

- Plasmid pHZ337 contains a 300 bp sequence of the *C. elegans* klf-1 promoter region which is confirmed by PCR performed with klf p1b primers (Figure 2).
- Plasmid sequence shows the PCR amplified segment (Figure 4).

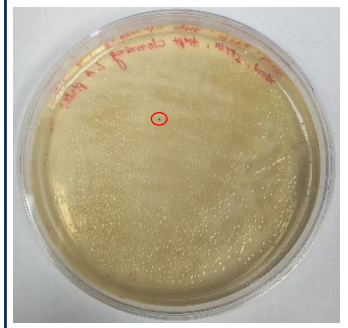

Figure 5: Blue White Screening of competent *E. coli* containing TOPO Vector with 300 bp fragment of *Ce* klf1 promoter; White- transformed colonies with the insert and Blue- transformed colonies without the insert (circled)

DISCUSSION

- Two primer sets- *Ce* **klf p1a** and **klf p1b** were used to amplify the 300 bp klf1 promoter region.
- Klf p1a primer set did not amplify the 300 bp segment due to sequence dissimilarity; however, klf p1b primers worked (Fig. 2).
- Plasmid pHZ337 sequenced (Genewiz) and the primer and 300 bp region identified (Fig. 4).
- The 300 bp amplified segment (using p1b primers) cloned using the TOPO Vector and competent *E. coli* cells on LA agar plates with 50µg/ml ampicillin, 10 mg/ml Xgal and 8mg/ml IPTG. (Fig. 5).
- To confirm the cloned segment, a transformed colony will be further sub-cultured in LB broth with ampicillin and will be amplified using the previously used (klf p1b) primer set.

CONCLUSION

- **Plasmid pHZ337 sequenced.**
- **300 bp region of klf1 promoter using correct primer sets amplified and cloned.**
- **Further confirmation of cloned segment required and will be inserted in expression vector pPD95_67.**

FUTURE DIRECTIONS

After further confirmation of the cloned segment using PCR, the 300 bp region will be inserted in to the expression vector pPD95_67 and then will be micro injected in N2 strain to determine whether the cloned region expresses the GFP gene.

ACKNOWLEDGEMENTS

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REFERENCES

- Brey et al. (2009) Krüppel-like family of transcription factors: an emerging new frontier in fat biology. International Journal of Biological Sciences, 5, 622-636
- Brey, Christopher & Nelder, Mark & Hailemariam, Tiruneh & Gaugler, Randy & Hashmi, Sarwar. (2009). Krüppel-like family of transcription factors: An emerging new frontier in fat biology. International journal of biological sciences. 5. 622-36. 10.7150/ijbs.5.622.
- GENEWIZ- South Plainfield, NJ