

Evolution of Developmental Genes

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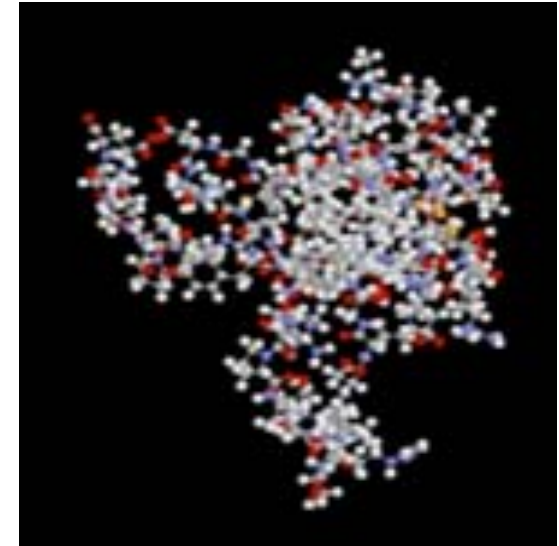
Supervisor: Dr. Kay Nolan

Genomic Imprinting

- Differential expression of a gene depending on parent of origin
- Imprinted genes are functionally haploid
- Gene is vulnerable if mutation occurs
- So why did imprinting evolve?
- IGF2 and Neuronatin are both imprinted developmental genes

IGF2

- Insulin-like Growth Factor 2
- Powerful growth promoter
- Fetal development
- Regulated by IGF2R
- IGF2 gene is imprinted
- Paternally derived allele expressed



IGF2 and the IGF2 receptor

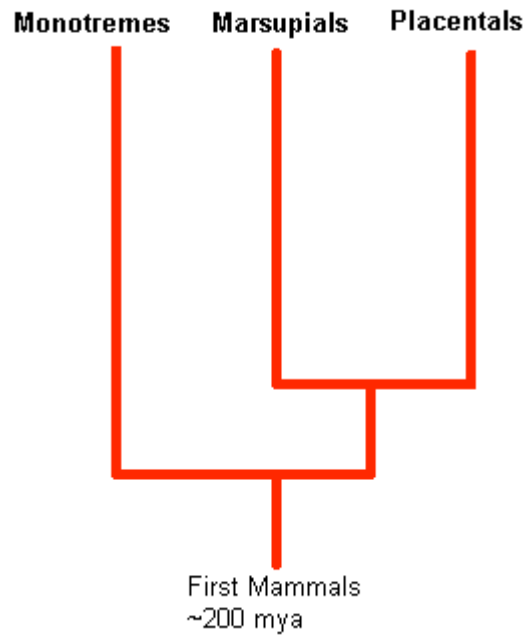
- Collaborative researchers in Bristol working on evolutionary changes in the binding affinity of IGF2 to the IGF2 receptor
- human IGF2 binds to human IGF2 receptor with high affinity
- human IGF2 binds opossum IGF2 receptor with low affinity
- Does opossum IGF2 bind to opossum IGF2 receptor with high or low affinity

Aims of Project

- Create plasmid vector containing Opossum IGF2 insert
- Vector used to produce recombinant opossum IGF2 protein
- Study evolution of IGF2 and the IGF2 receptor

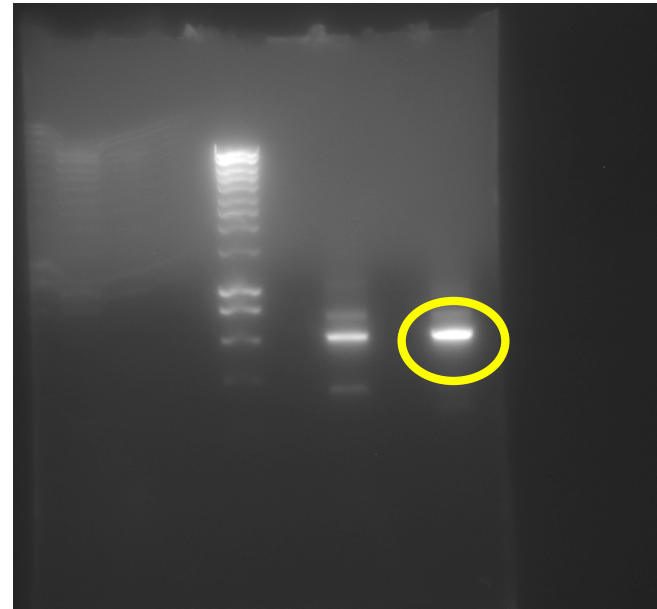
The Opossum

- Why opossum?
- Marsupial
- Phylogenetic position



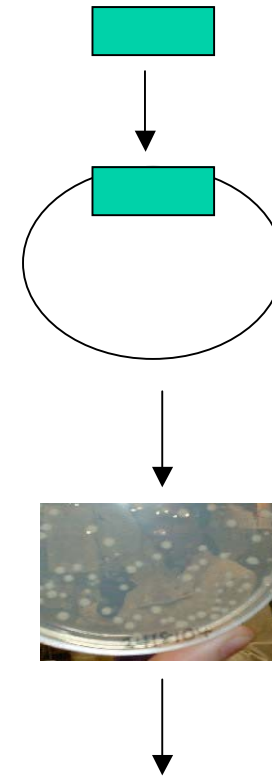
PCR for IGF-2

- PCR used to amplify opossum IGF2 cDNA
- Annealing Temperature of PCR varied
- Expected ~600 bp fragment was obtained



Cloning of PCR Fragment

- Fragment cut out of gel
- Ligated into Vector
- *E. coli* transformed
- Four colonies chosen
- Plasmids Purified
- Sent for Sequencing



ATGCTGAATGCCAT

Sequencing Results

- Cloning of opossum IGF2 was successful
- 3 of 4 sequences were identical to each other and to opossum IGF-2

```
AY552325      CTGCTTCCGCAGCTGCGATCTGGCCCTGCTGGAGACCCTACTGTGCCACCCTCCGC CAA GTC 420
Seq 2        CTGCTTCCGCAGCTGCGATCTGGCCCTGCTGGAGACCCTACTGTGCCACCCTCCGC CAA GTC 329
Seq 3        CTGCTTCCGCAGCTGCGATCTGGCCCTGCTGGAGACCCTACTGTGCCACCCTCCGC CAA GTC 329
Seq 4        CTGCTTCCGCAGCTGCGATCTGGCCCTGCTGGAGACCCTACTGTGCCACCCTCCGC CAA GTC 329
Seq 5        CTGCTTCCGCAGCTGCGATCTGGCTCTGCTGGAGACCCTACTGTGCCACCCTCCGC CAA GTC 329
*****
```

- 4th sequence: one bp mismatch at position 293
- C replaced with T in DNA sequence
- Causes the conserved substitution of Valine for Alanine in deduced Amino Acid Sequence

Conclusion

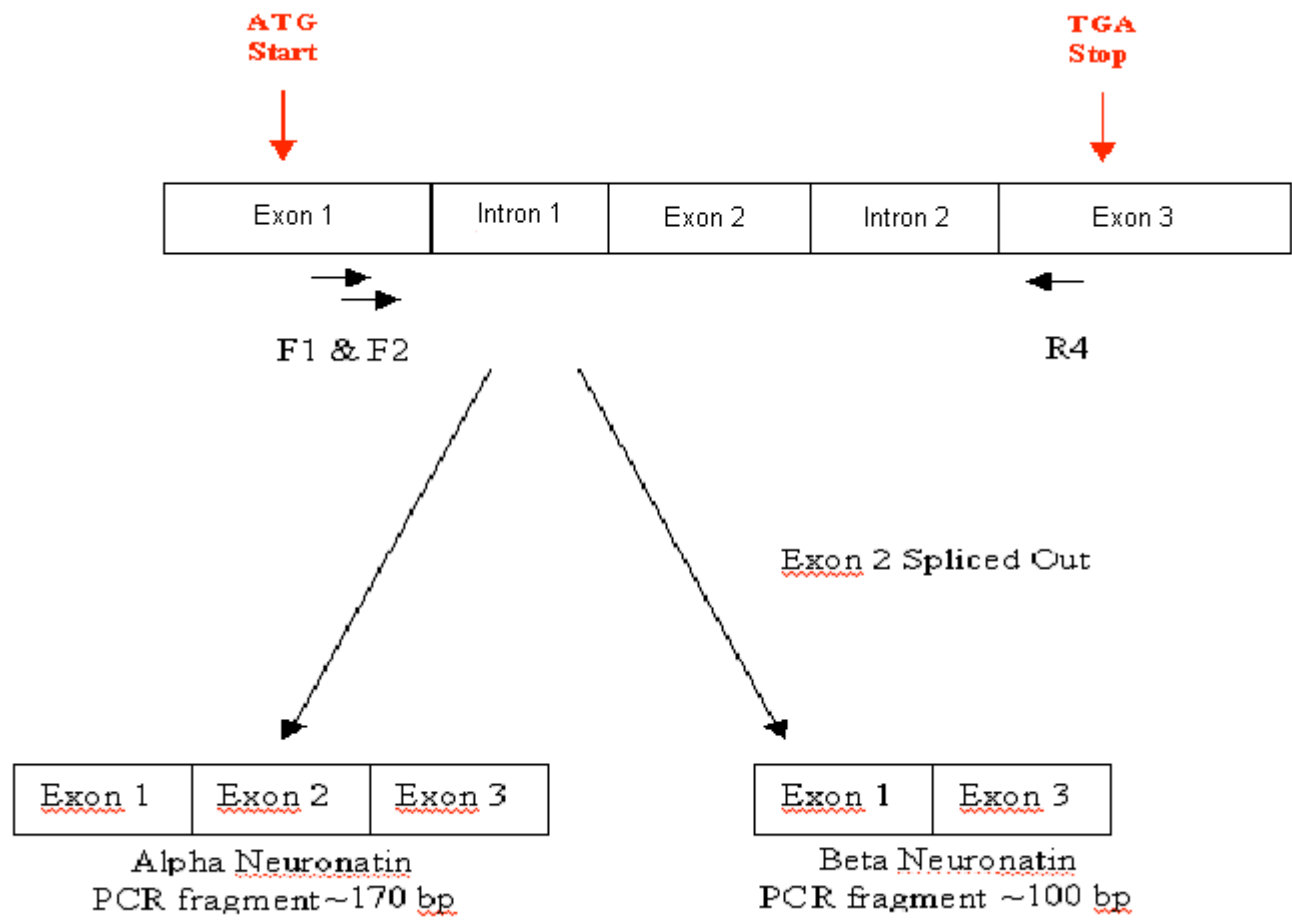
- Successfully cloned IGF-2 from opossum
- Mismatch may be due to error during PCR
- OR
- Could be actual single nucleotide polymorphism
- cDNA library may be from heterozygous individual

Cloning and Sequencing of Labrador Neuronatin Gene

Neuronatin

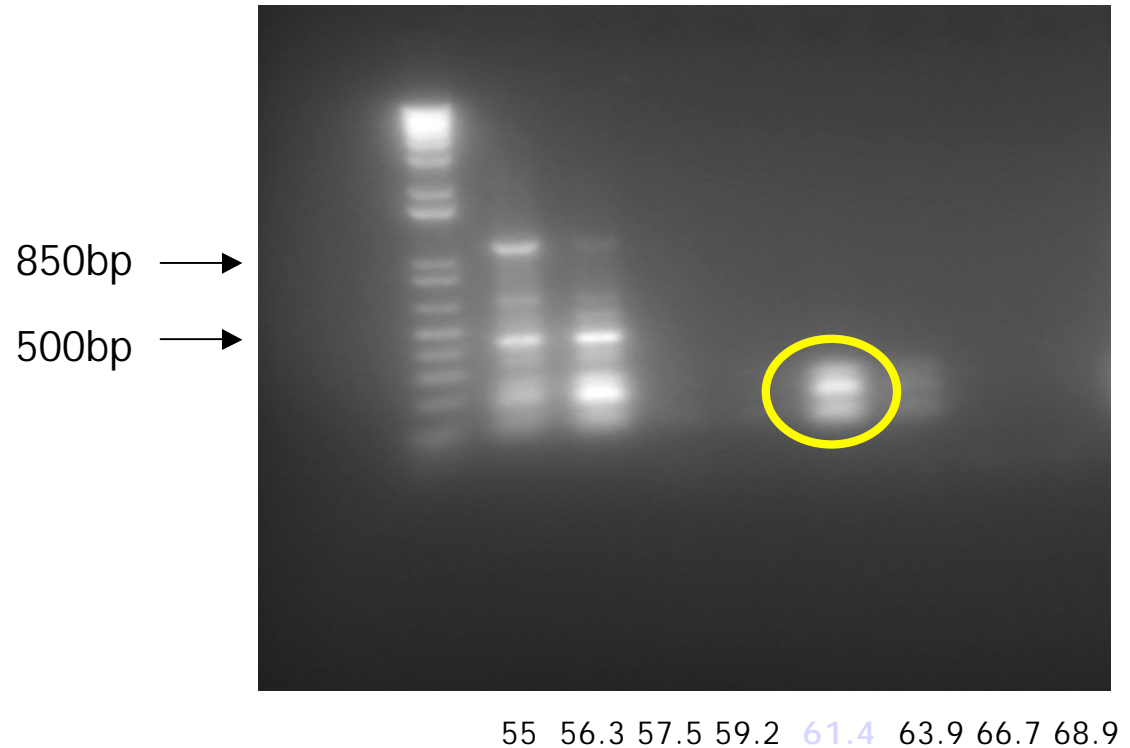


- First identified in rat brain by Joseph *et al.* (1994)
- High levels expressed in Fetal Nervous System
- Imprinted: Expressed from paternal allele only
- 2 mRNA species – a & b – alternative splicing



PCR Products with Primers F1

- Primers F1 & R4
- Temperature varied
- Optimal Temperature
61.4°C

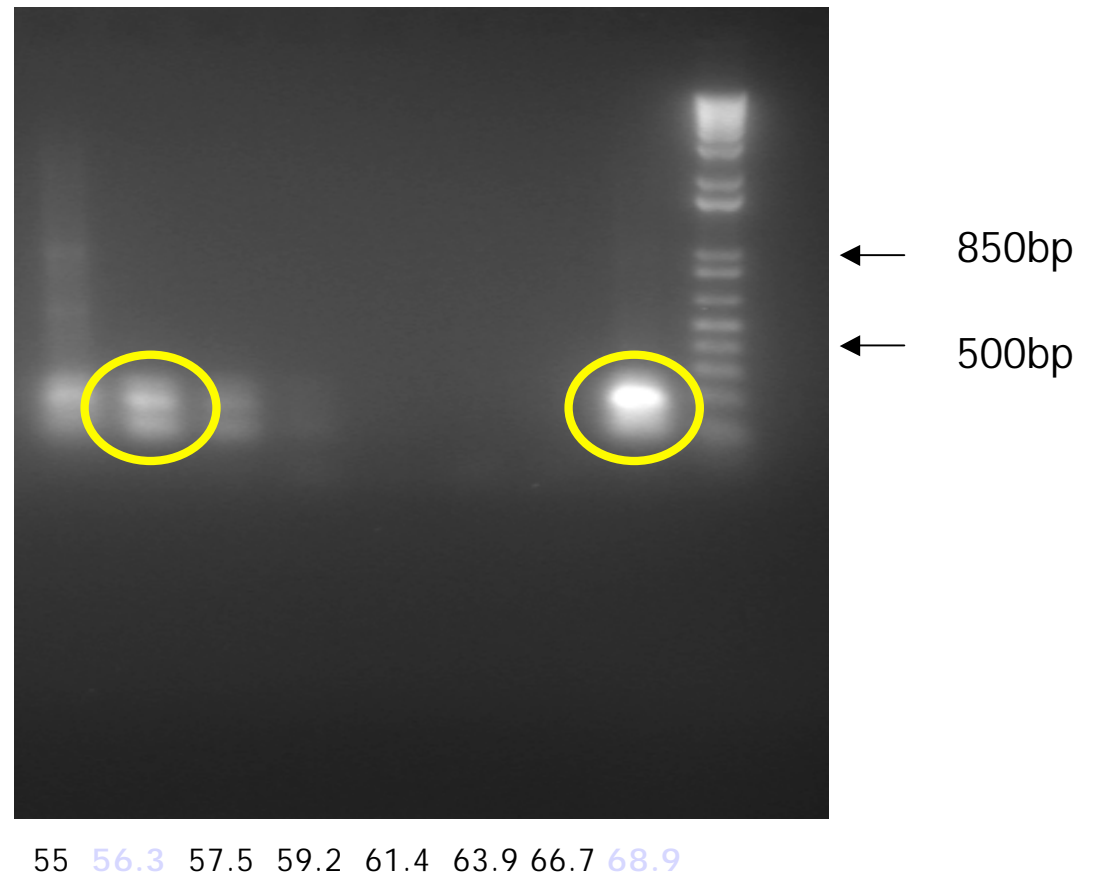


PCR Products with Primers F2

- Primers F2 & R4
- Temperature varied
- Optimal Temperature

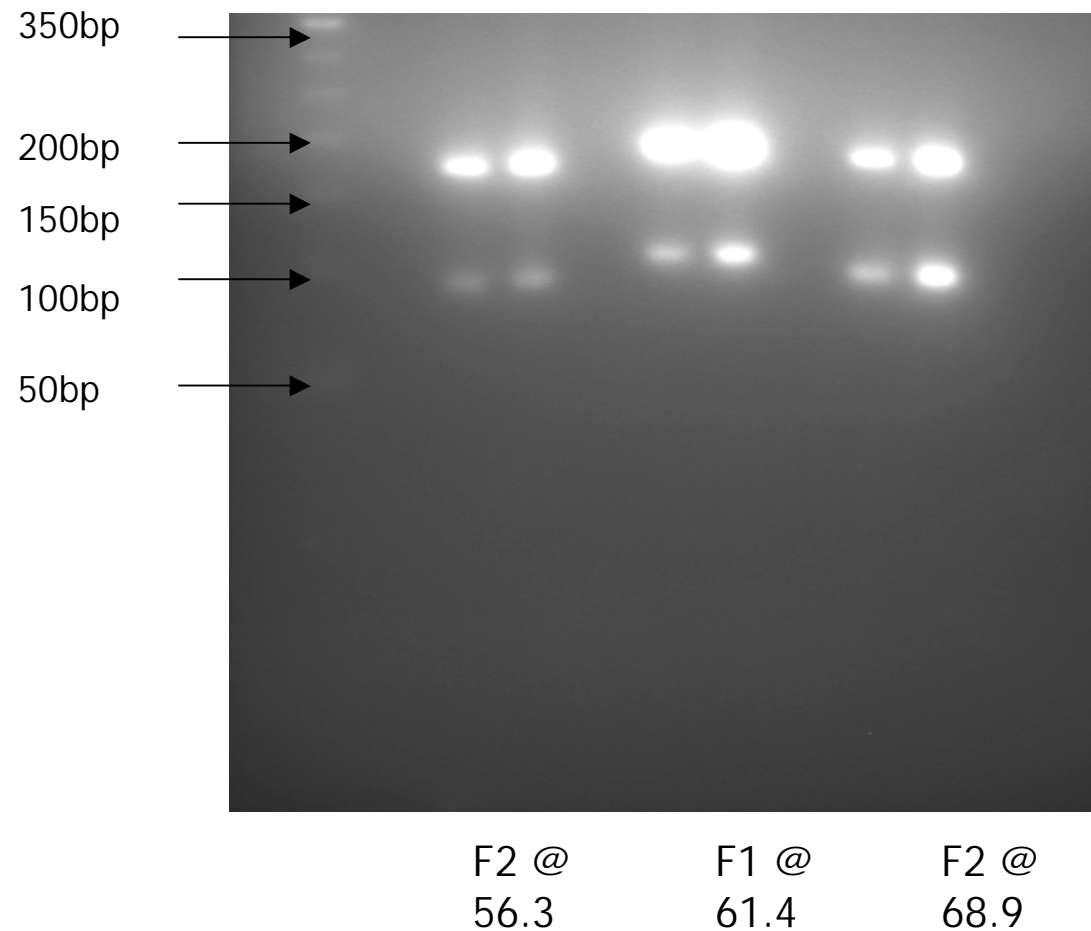
56.3oC

68.9oC



High Resolution Gel of PCR products

- F2 @ 56.3
 - ~90bp
 - ~170bp
- F1 @ 61.4
 - ~100bp
 - ~180bp
- F2 @ 68.9
 - ~90bp
 - ~170bp



Sequence Results

Clustal Alignment for alpha-Neuronatin

```
XM847444      TGGTACATATTCCGTGTGCTGCTGCAGGTGTTTCCTGGAATGCTGCATTTACTGGGTAGGA 180
1c            TGGTACATATTCCGTGTGCTGCTGCAGGTGTTTCCTGGAATGCTGCATTTACTGGGTAGGA 75
*****

XM847444      TTCGCTTTTCGAAATCCTCCAGGGACACAGCCCATTGCGAGAAGTGAGGTGTTTCAGGTAC 240
1c            TTCGCTTTTCGAAATCCTCCAGGGACACAGCCCATTGCGAGAAGTGAGGTGTTTCAGGTAC 135
*****

XM847444      TCCCTGCAGAAGCTGGCATAACGGTGTCGAGGACCGGGCGGCAGGTGTTGGGAGAGCGC 300
1c            TCCCTGCAGAAGCTGGCATAACGGTGTCGAG----- 167
*****
```

Sequence Results

Clustal Alignment for beta-neuronatin

```
XM_860793      GGAACCATGGCGGCAGTAGCGGCAGCCTCGGCTGAGCTGCTCATCATCGGCTGGTACATA 120
6b             -----CTGCTCATCATCGGCTGGTACATA 24
                *****

XM_860793      TTCCGTGTGCTGCTGCAGGTGTTTCAGGTA CTCCCTGCAGAAGCTGGCATAACACGGTGTCG 180
6b             TTCCGTGTGCTGCTGCAGGTGTTTCAGGTA CTCCCTGCAGAAGCTGGCATAACACGGTGTCG 84
                *****

XM_860793      AGGACCGGGCGGCAGGTGTTGGGAGAGCGCCGGCAGCGAGCCCCCAACTGAGGCCCCAGC 240
6b             AG----- 86
                **
```

Conclusion



- Both alpha and beta Neuronatin were successfully cloned
- Alpha and beta neuronatin were both shown to be expressed in the cDNA library studied
- This cDNA library can now be used for further research on the imprinting status of the Neuronatin gene in Dogs

Acknowledgements

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