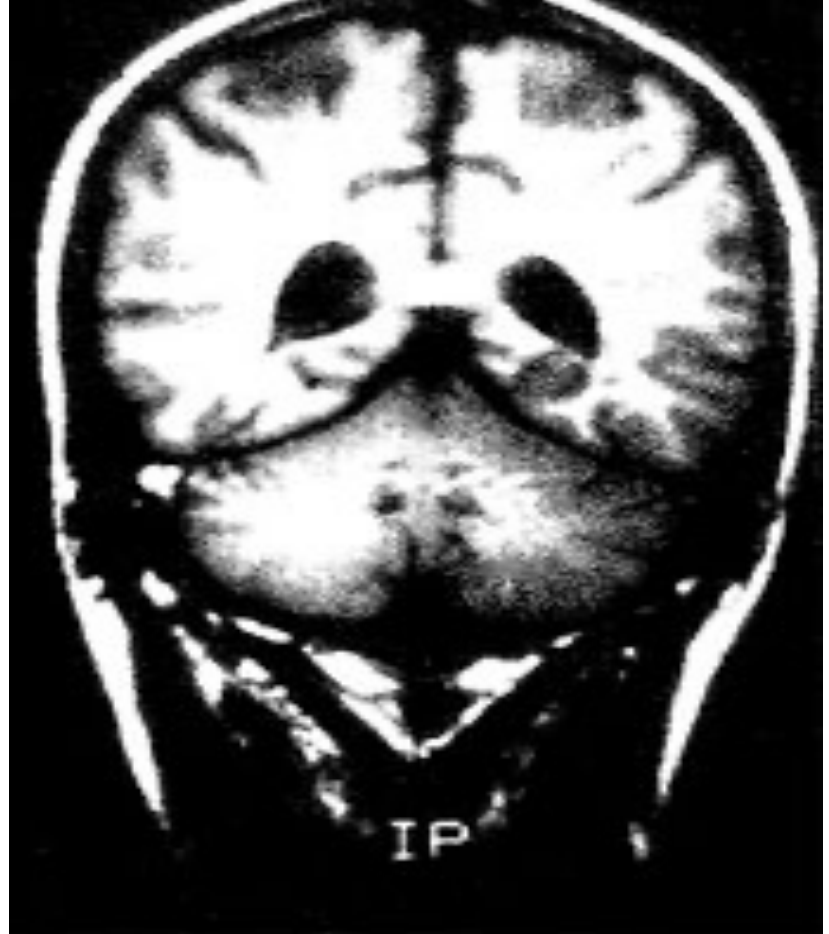


The Temporal Expression Pattern of Neuropsychiatric Risk Gene, Complement Component 4 (C4), During Zebrafish (*Danio Rerio*) Development

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Background:

- Schizophrenia (SZ) is a debilitating psychotic disorder with a global prevalence of 1%. The positive symptoms of SZ include delusions and hallucinations while the negative symptoms include flat affect, anhedonia, etc.
- It has been suggested that the negative symptoms of schizophrenia may be because of excessive synaptic pruning in the brain
- Complement Component 4 has recently been implicated in schizophrenia as studies have shown overexpression in schizophrenic brains post-mortem
- Complement Component 4 plays a role in synaptic pruning. When activated in the pathway by upstream C1q, C4 triggers the action of C3 which then tags synapses for phagocytic activity by macrophages and microglia
- Zebrafish have been an emerging model to study complex brain disorders because of their close homology to humans, and quick development
- The characterization of C4 expression during development in zebrafish has not been determined before.

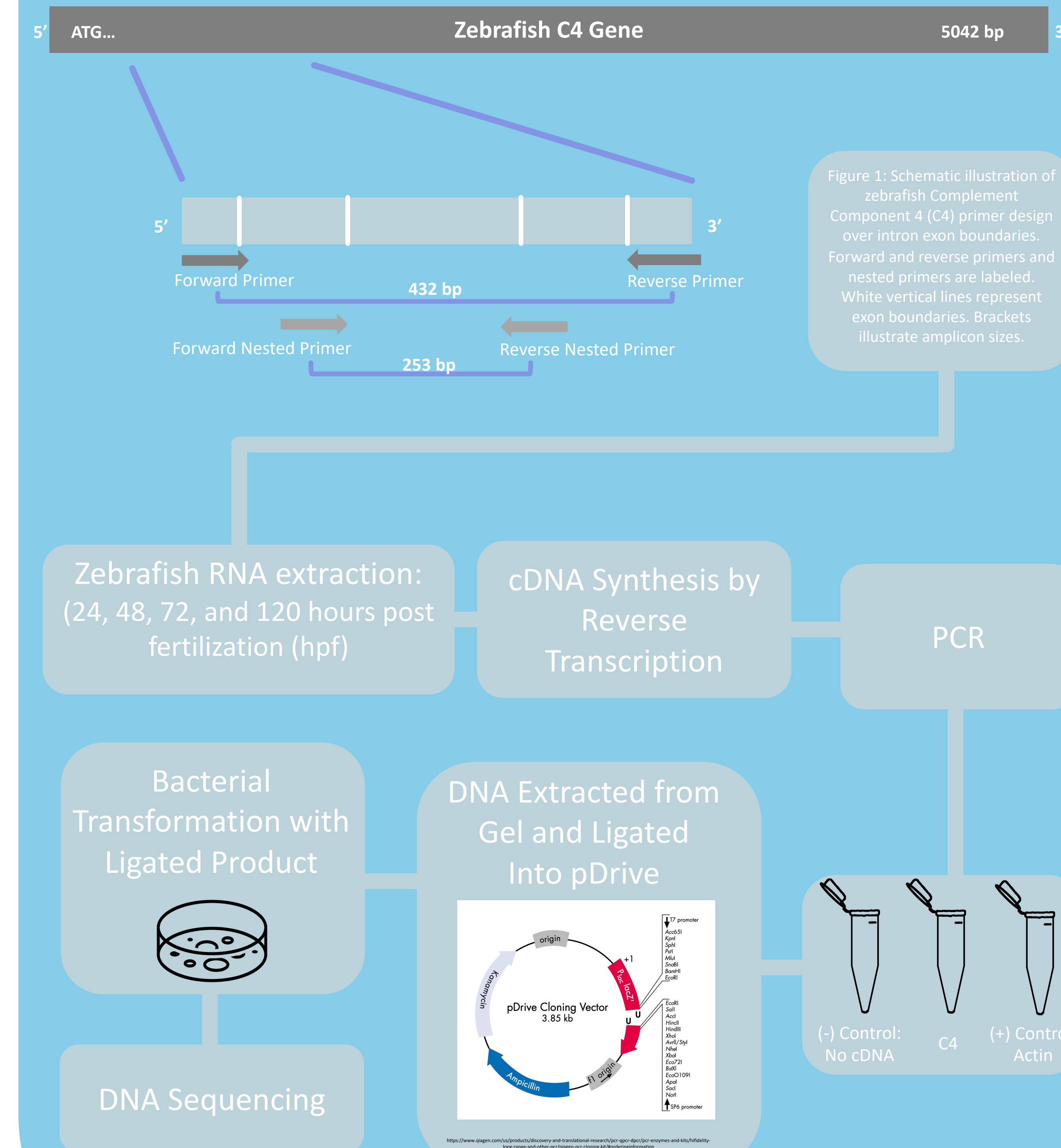
Objective: Determine the Temporal Expression Pattern of C4 during zebrafish development



<http://zebrafishlab.be/media-gallery/detail/6/10>

Methods:

Primer Design:



Zebrafish RNA extraction: (24, 48, 72, and 120 hours post fertilization (hpf))

cDNA Synthesis by Reverse Transcription

PCR

Bacterial Transformation with Ligated Product

DNA Extracted from Gel and Ligated Into pDrive

DNA Sequencing

All research activities in this study were approved by the York College of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

Results cont:

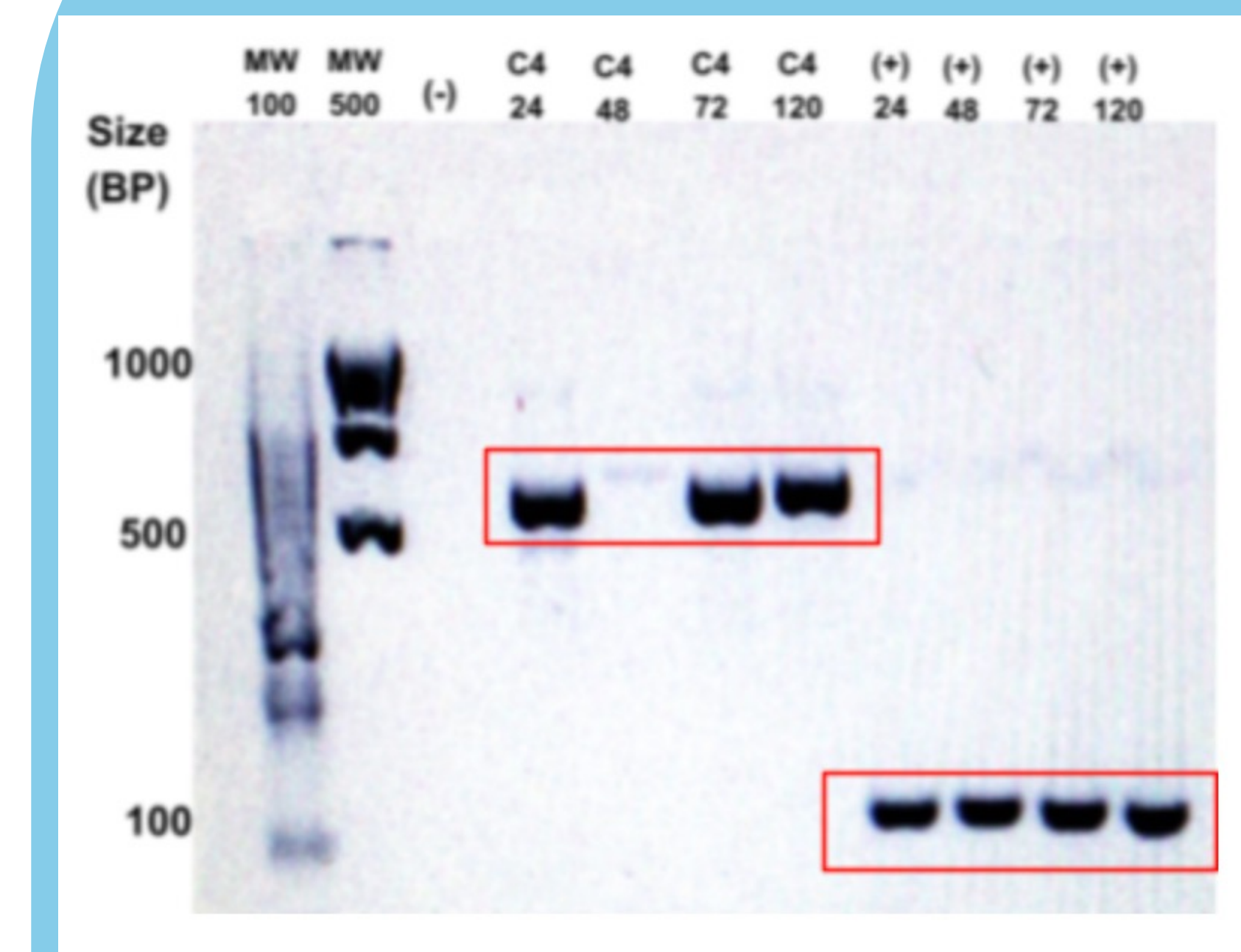
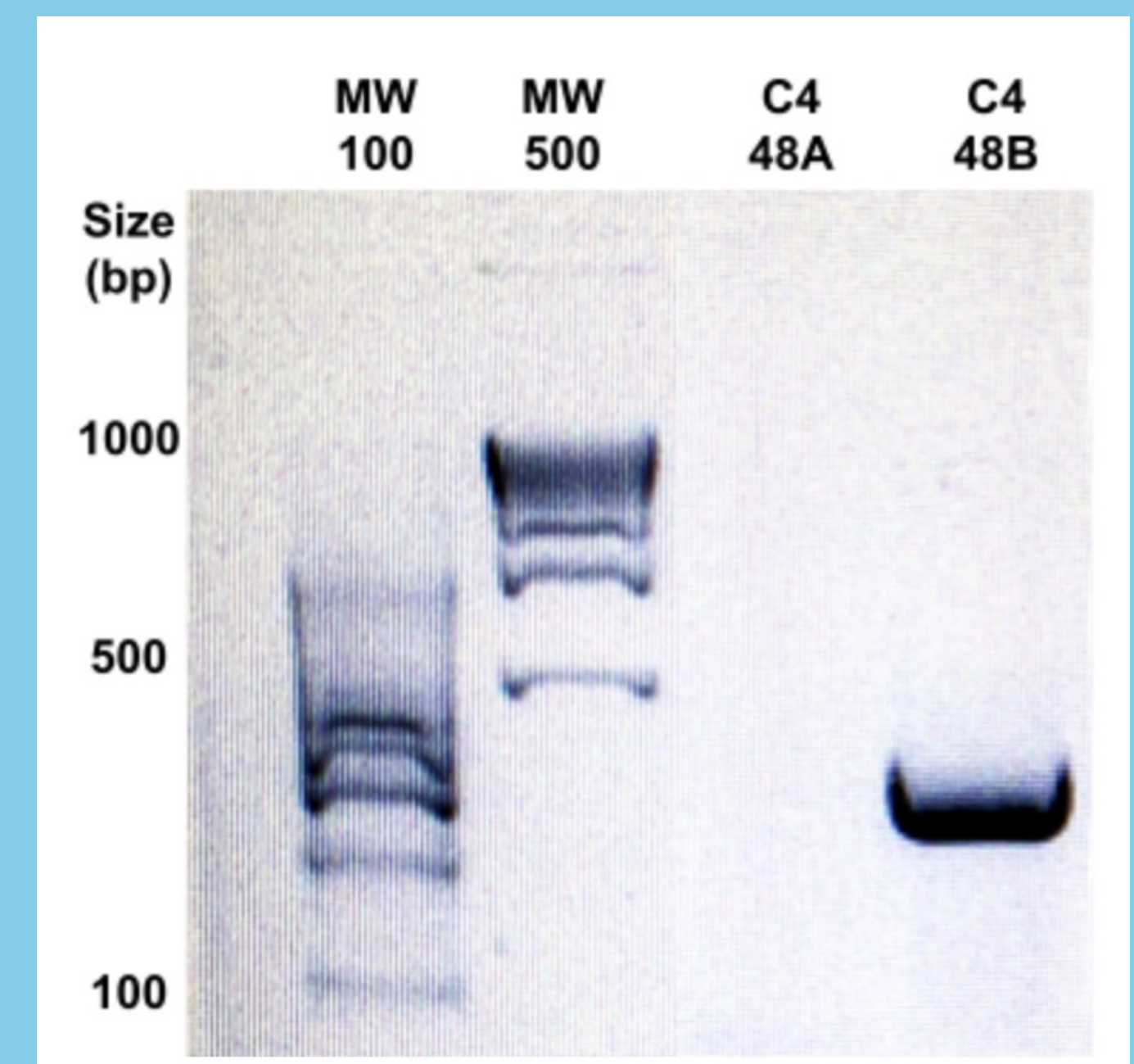


Figure 2: Gel Electrophoresis of Complement Component 4 Gene of *Danio Rerio*, run on 1.5% agarose gel at 180 volts. Lanes 1 and 2 contain ladders (100bp and 500 bp). The following 9 lanes contain the negative control (no cDNA), the C4 gene at different timepoints (24, 48, 72 and 120 hpf) and the positive control, Actin, at the same time points. The red boxes indicate expression at that specific time point tested apart from C4 48 hpf.

Figure 3: Gel Electrophoresis of Complement Component 4 Gene of *Danio Rerio*, run on 1.5% agarose gel at 180 volts. Lanes 1 and 2 contain ladders (100bp and 500 bp). Lanes 3 and 4 contain the C4 gene at 48 hpf with forward and reverse primers (C4 48A) and nested forward and reverse primers (C4 48B).



Results:

	Identities	Similarities
<i>Homo Sapiens</i>	33%	53%
<i>Sus scrofa</i>	33%	53%
<i>Mus musculus</i>	33%	53%
<i>Gallus Gallus</i>	29%	49%

Table 1: Pairwise Comparisons Between Zebrafish and other Vertebrate Complement Component 4 Amino Acid Sequences

Conclusions:

- C4 was expressed at all time points tested based upon expected gel electrophoresis band size with C4 primers and nested primers (Figure 1 and 2)
- Sequencing results proved that the amplicon isolated was determined to be C4
- Future Studies should explore whole mount *in-situ* hybridization to determine where the gene is expressed at key time points during development.

Lit Cited:

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