Revision of the bachelor thesis of Petr Rathner: IrAM9 – a member of a tioester-containing protein family from the hard tick *Ixodes ricinus*

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Petr Rathner worked out his Bachelors thesis on a tick α-2-macroglobulin superfamily member protein tagged as *Ixodes ricinus* alpha 2 macroglobulin 9 (IrAM9). The aims of this thesis are not stated in the introduction, however, they could be estimated from the concept of his thesis: (i) preparation of cDNA templates from six tissues of *Ixodes ricinus* partially engorged females, (ii) evaluation of tissue specific expression of IrAM9 by RT-PCR profiling over the cDNA templates, (iii) cloning of a partial IrAM9 in to a bacterial expression vector and preparing the recombinant fragment of IrAM9; (iv) preparation and testing of IrAM9 specific antibodies. This is a complex protocol to obtain specific antibodies for further functional study of IrAM9 in the laboratory of Petr Kopáček. Having personal experience and basic knowledge in the field of thioester-group containing proteins I have been pleased to accept this thesis for revision. My comments are following:

A. Formal issues:

Introduction: Even taking in mind this work is a Bc. level thesis there are numerous formal and linguistic mistakes in the introduction to this work:

1.1 - first sentence sounds like ticks would directly cause diseases in hosts. I guess they just transmit the disease causative agents. Second sentence: You use genus and species names for *Borrelia burgdorferi*, you should also follow this concept in the rest of the sentence with *Erlichia* and *Babesia*.
1.2 - protease inhibitors take part in a wide spectrum of biological events from the early evolution of organisms, thus are not evolved especially to defend the host, as I can understand from this subchapter, even they play an important role in the defense mechanisms. Also, lysosomes and lysozymes are phonetically similar words of rather different meanings! English: articles!!! **the** horseshoe crab *L. ployphemus*, **the** *softick O. moubata etc.;* Citation: Saravanan T., 2003 is in wrong format.
1.3 – "...and there are important components..."- I do not understand this formulation.
Methods:
Tables with primers and chemicals could be tagged as table 1. and 2. rather than sub-chapters 2.1.1. and 2.1.2.
2.2.8. – „sonicated " would probably sound better as "sonificated" found in the text. Spacing in between numbers and units should be united. E.c. 13000 rpm X 10min
2.2.11. I miss the used adjuvans for rabbit immunization?
2.2.12 - First sentence does not make sense - species, volumes, boiled in what? I do not like the concept of this sub-chapter - you should more visibly separate the SDS PAGE preparation, electro-blotting to a PVDF membrane and Immunodetection. The pre-incubation of membrane in methanol should be putted inline with the blotting sandwich setup or excluded.

Results and Discussion:
Most of the sub-chapters are again describing performed methods instead of stating clearly what are the results of experiments (results) and putting them in content with the general knowledge (discussion). Figure 7. legend: *Ixodes ricinus/scapularis* should be in normal characters when all the figure legend is in italics. Figure 9: the initial sequence MRGSHHHHHH should be also bold (part of the vector plasmid).
3.7.2. First sentence does not make sense: "...making use the affinity..." did you mean using the affinity?
3.8.- " The result was visualised diamidobenzidine as a substrate..." is definitely not the most fortunate expression
References:
format should be more united, minor mistakes: shortcuts and full names of journals are mixed, with dots an without dots, journal of Innate Immunity – not innate immunity, citation 8 "IBMB 33: 841-851," ... and citation 1: "42, 53-64, 1996", citation in Plos Biol should not be written together and should have year and issue numbering like "PLoS Biol 8(11)" ...etc.

B. Content:
In general the herein presented experimental work has been rigorously performed and is presented clearly with a good general concept. From my subjective point of view, the variety of used methods and the general content accords to the level of top Bc. theses generally defended at the Faculty of Sciences, USB. According to my subjective opinion, also the in-detail method description is very suitable as this thesis serves as the first real resume of laboratory experiments and protocols in student's career.

C. Evaluation: I recommend this work to be defended with a classification mark depending on the oral presentation of this work.

D. Questions to author:

1. 2.2.2. RNA isolation. Was the total isolated RNA really checked on 1% agarose gel in TAE buffer instead of TBE. What is the difference in these buffers?

2. If you will follow with this project in your Ms. degree and you should use your obtained antibody, what would be your future plans. Could you briefly introduce us 2 - 3 next experiments how you could improve its performance and where you can use it?

3. The missing exon in IsAM9 – is this deletion verified by some coding sequence (EST) data or is it just the computational mistake in exon prediction found in the database (Wikel strain genome database of *Ixodes scapularis*?)

In České Budějovice, 12. 6. 2011, worked out by

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