Technical Evaluation of the Thesis Proposal:
'Population and Mutation Analysis of 14 Y-chromosomal STR loci in the Philippines'
by Sheila Angustia

The candidate proposes to generate a database of 14 Y-chromosomal STR markers for the Philippine population using buccal samples from 350 male volunteers for paternity testing and forensic casework. In addition, the candidate proposes to determine the mutation rate of these markers using 150 father and son pairs. Knowing the effective mutation rates would 'improve forensic probability calculation and the understanding of diversity data'[1].

Overall, there is scientific merit in establishing a local database for Y-chromosomal STR markers since this is one of the most powerful forensic tool for male identification. Knowledge of the mutation rate of Y-STR markers is also important to know the stability of these markers which are solely inherited along the male line. Theoretically, the Y-chromosomal STR profile of a man should be identical to his son, and his grandsons, his paternal cousins and uncles etc. However, if a Y-STR marker has a higher mutation rate, then one has to account for the reason behind a 'mismatch' between the Y-STR DNA profile of a boy and an alleged father. Is the mismatch due to a mutation hence the pair is really a father-son duo; or is the mismatch indicative of non-paternity? In crime scene investigations involving sexual assault charges, the presence of male DNA in a female victim can support the allegation of sexual contact between the alleged perpetrator and the victim. Whether the contact is consensual or not is another question, as long as the supposed victim is not a minor.

However, having evaluated the present proposal, I have the following comments:

- **Review of Related Literature**

The candidate did not provide an exhaustive review of related literature.

•A good proportion of the literature that was cited were sourced from the internet including the websites of commercial manufacturers. Although these sites provide information for the products which are currently available in the market, it would have been better if the candidate cited the actual research papers that were published in the development of the methods and/or selection of STR markers that were subsequently used for commercial production.

• It would benefit the candidate to read more recent literature. Moreover, the citations of some of the articles did not include the year of publication and there are errors in the manner of citing published literature.

• Interestingly, the candidate failed to cite any paper which have been published on Y-STR databasing in Philippine populations as well as the use of these markers for forensic applications in the Philippines. Work on databasing Y-STR markers was initiated by Dr J Miranda of the National Institute of Molecular Biology and Biochemistry of UP Diliman in 2001. Dr Miranda and co-workers already generated a

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Philippine population database of many of the STR markers which were included by the candidate. Papers on Y-STR profiling in Philippine populations are:

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b) Tan, M. M., G. C. Calacal, et al. (2003). "Y chromosome STR allele frequency distribution among Filipino males in the NCR for Forensic Applications." Philippine Journal of Science 132(2): 95-101.

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f) **Salvador, JM,** KA Tabbada, et al. (2008). "Population data of 10 Y-chromosomal STR Loci in Cebu province, Central Visayas (Philippines)." Journal of Forensic Science 53(1): 256-8.

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• The candidate did not include a list of searchable Y-STR databases of Asian populations including Philippine populations that are available online. These sites which are already being used for casework and paternity testing are:

a) http://www.yhrd.org/
b) http://usystrdatabase.org
c) http://www.smgf.org/ychromosome/search.jspx

- **Methodology**

• Preparatory Stage

The candidate did not provide the reason for selecting the 14 Y-STR markers which she has included in her study. In the Review of Related Literature, she cited the recommendation of the Scientific Working Group on DNA Analysis Methods (SWGDAM) that is US-based but failed to trace back the original scientists, mostly European, who were responsible for selecting these Y-STR markers based on their forensic utility. Notably, three of the markers namely DYS321, Y-A71 and Y-A72 were not recommended

by SWGDAM. The candidate did not include any citation for these markers and literature searches made by this evaluator was unsuccessful. This evaluator suspects that Y-A71 and Y-A72 actually refers to Y-GATA-A7.1 (otherwise known as DYS460) and Y-GATA-A7.2 (also known as DYS461) respectively. DYS321 is not included in all the online searchable Y-STR databases which limits its utility in forensic casework. DYS460 and DYS461 are only included in one database (http://www.smgf.org/ychromosome/search.jspx).

The proposed sample size of 350 persons for establishing a population database is acceptable. However, the candidate did not provide information as to how these volunteers were going to be selected, whether informed consent will be obtained and what information will be requested from each volunteer. Moreover, information on who has access to the individual genotype of the volunteers and how this database is to be managed were not included in the proposal.

In the second stage of the study, the issue of selecting father and son pairs is important. How will the candidate screen for 'true fathers and sons'? Will there be additional testing, e.g. autosomal STR testing for cases when mismatches are 'discovered'? How will the candidate handle the information of non-paternity which could be discovered unexpectedly? Is the candidate obliged to reveal this information to the persons concerned?

Moreover, the candidate did not cite papers which have already reported the mutation rates of the common Y-STR markers, many of which are included in the present study. In fact, the US National Institute of Standards and Technology (NIST) has published mutation rates for each of these loci, the values of which are available on their website. For a more exhaustive discussion of Y-STR mutation rates, the paper published by Burgarella, C. and M. Navascues[2], is recommended. In this paper, the authors performed a meta-analysis using pedigree data for 80 loci and individual haplotypes for 110 loci, from 29 and 93 published studies, respectively. The mean predicted Y-STR mutation rate is $2.12 \times 10^{-3} \pm 1.58 \times 10^{3}$.

• Laboratory procedures

Sections of the methodology needs to be revised. The actual method to be used for extraction of DNA from buccal samples, is unclear. The candidate described two procedures namely the Chelex extraction and silica-based DNA extraction methods. Is the candidate proposing to use both methods to extract buccal DNA? What would determine the selection of extraction method? Will the candidate quantitate the amount of DNA in each sample before PCR amplification?

The basis for the PCR reaction parameters described in the proposal, was also not discussed. Did the candidate get the cycling parameters online or from a well-refereed journal? Do all the STR markers have the same cycling parameter?

The candidate also proposes to perform the DNA fragment analysis on either an ALF Express or an ABI3130. Since the ALF Express is one of the older fragment analyzers that can only run single assay samples, further validation of procedures using this machine may not be advisable. To date, only a handful of forensic DNA laboratories actually use ALF Express since this machine is no longer being

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Lastly, one main concern regarding the submitted proposal in its present form, is the issue of novelty of this work. The candidate proposes to generate the Y-STR database of the Philippine population using Y-STR markers, most of which had been analyzed previously. Mutation rate estimation also requires larger datasets and the proposed 150 father and son duos is insufficient to generate this data. More importantly, mutation rates for most of the selected markers included in the proposal, are already available.

- **Evaluation of the thesis project in relation to the Re-Entry Proposal entitled 'Y-chromosomal DNA variation of the Filipino population using Rapidly Mutating (RM) Y-chromosome Specific Short Tandem Repeat (STR) Markers' submitted by Ms. Jazelyn Salvador**

The proposal of Ms. Jazelyn Salvador includes the establishment of a Philippine database using 13 new Y-STR markers that have not been reported previously. These Y-STR markers DYF387S1, DYF399S1, DYF403S1a, DYF404S1, DYS449, DYS518, DYS526b, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627 were first published by Ballantyne and co-workers in 2010[3], and were found to have a 6.5-fold higher mutation rate than the conventional Y-STR markers. Hence these new Y-STRs, known as rapidly mutating Y-STRs (RM-YSTRs) will enable is to differentiate paternally-related males such as fathers and sons, brothers, paternal cousins or uncles[4]. This is important in cases wherein suspects are paternally-related. In addition, RM-YSTR is expected to be very useful when dealing with smaller communities where inbreeding may be more common. RM Y-STR markers are therefore useful in identifying the source of male DNA at the individual level, and would complement the standard set of conventional Y-STR markers.

In fact, Ms. Salvador's project is the Philippine counterpart in a multi-center study of RM-YSTR markers initiated by Dr Manfred Kayser[5]. Dr Kayser is the principal investigator of the work conducted by Ballantyne and co-workers. Hence, the generation of new knowledge on the RM-YSTR markers is expected to increase the utility of DNA typing for human identification, particularly male identification in the Philippines. Notably, Ms. Salvador has a proven track record in DNA typing of Y-STR markers as she has been a co-author in three of the papers on Y-STR typing in the Philippines (see page 3 of this evaluation).

Dr. Maria Corazon A. De Ungria
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[3] Ballantyne, K. N., Goedbloed, M., Fang, R., Schaap, O., Lao, O., Wollstein, A., et al. (2010). Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. *American Journal of Human Genetics, 87*(3), 341-353.

[4] Ballantyne, K. N., V. Keerl, et al. (2011). "A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages." *Forensic Science International Genetics*.(online access)

[5] Dr. Manfred Kayser, Department of Forensic Molecular Biology, Erasmus MC University Medical Center Rotterdam, 3000 CA Rotterdam, The Netherlands.

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