Reviewer's report

Title: Multiplex pyrosequencing assay using AdvISER-MH-PYRO algorithm: a case for rapid and cost-effective genotyping analysis of prostate cancer risk-associated SNPs

Version: 1 Date: 9 March 2015

Reviewer: Tomas Kirchhoff

Reviewer's report:

The study test the applicability of multiplex pyrosequencing assay to be used for a genotyping of a subset of SNPs previously associated with prostate cancer risk. This study suggests that due to the ability of multiplex testing of 9 SNPs this can be a robust alternative method with potential utilization in clinical setting. The authors claim that the method is feasible, cheap and with fast turnaround. This is a well-written study.

Major Compulsory Revisions

A. General points:

The main concern is the study's clinical relevance. While MGRSs have been assessed in prostate cancer risk prediction, the clinical impact of such analyses is modest at most. It is not completely clear whether 9 SNPs tested are clinically important (this is mainly based on 1 study that needs to be validated). Also, the observed significance of previously published findings on 9 SNPs is very borderline, as published AUC improvement by 1% compared to clinical surrogates alone is very modest for a meaningful clinical applicability. One novelty would be in the application of the multiplex platform. But again, relatively cumbersome design and low-plexing level makes pyrosequencing not very suitable for the purpose tested in the study. Especially when the current research and clinical setting often demands the genotyping of dozens to hundreds of variants.

B. Specific points:

1. The method uses huge amount of input DNA, 500 ng of genomic DNA per 1 reaction (1 multiplex). Assuming 2 reactions, this would be about 1 microgram of DNA input per patient (on the side note: this would be enough for 2-3 whole-genome sequencing reactions). This should have been commented and discussed in the paper, as this is a serious limitation: 1 microgram of DNA for getting the information from 9 SNPs is neither practical nor realistic.

2. While concordance on pyrosequencing between uniplex and multiplex reactions showed 100%, the question is whether the genotypes can be verified by alternative method (outside of pyrosequencing), e.g. Sanger sequencing. Such additional validation would be helpful to see the real reproducibility of
pyrosequencing method.

3. Authors should comment of comparison to other (more efficient) methods for this purpose, e.g. Sequenom that allows much higher plexing level with much less cumbersome tuning and much lower DNA input needed (often in nanograms).

Minor Essential Revisions

1. Description of methodology using AdvISER-MH-PYRO software is tedious and should be shortened (adding instead the manufacture’s protocol for a reference). From the lengthy description it almost seems that the authors have developed the methods themselves while they only used the manufacture’s product.

2. Table legends are missing (only titles are displayed), hence it is difficult to navigate through the data presented in Tables.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests