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High variability in melting profiles from gel pad arrays

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We would like to point out an incorrect statement crediting our previous work in the recent paper of Siripong and colleagues (2006). The authors claim that 'Recent work has suggested that the data-analysis tools used to assess melting profiles on gel-based microarrays can result in poor-quality melting profiles (Pozhitkov *et al.*, 2005), but work by our group (e.g. Urakawa *et al.*, 2002; 2003; El Fantroussi *et al.*, 2003; Kelly *et al.*, 2005) has demonstrated good melting-profile replication and has not shown any of the problematic data types described by Pozhitkov and colleagues (2005).' In truth, P.A. Noble analysed the data of Urakawa and colleagues (2002; 2003) and El Fantroussi and colleagues (2003) and co-authored these papers, as well as Kelly and colleagues (2005). The reason why their statement is incorrect and misleading is because the method used for image processing in the Urakawa and colleagues (2002; 2003) and El Fantroussi and colleagues (2003), and Kelly and colleagues (2005) studies (which were co-authored by P.A. Noble) produced biased results as clearly demonstrated in the paper by Pozhitkov and colleagues (2005).

Despite of the fact that the standard deviations of each data point in the Siripong and colleagues (2006) study (and previous studies) were relatively small (within-array reproducibility), there is an inconsistent systematic error associated with the grid placement that is not reflected by standard deviation. Therefore, any comparison between arrays is biased, with the results of a comparison significantly dependent upon the exact placement of the grid, as well as other factors discussed in Pozhitkov and colleagues (2005) and Pozhitkov and Noble (2007).

It is impossible for other members of the Urakawa and colleagues (2002; 2003), El Fantroussi and colleagues (2003) and Kelly and colleagues (2005) group to demonstrate 'good melting profile replication' because the original images were not stored; therefore, they could not perform re-analysis of the raw images to determine reproducibility of the data – contrary of what was stated in the article by Siripong and colleagues (2006). This is why the statement under consideration, especially made in retrospective terms, is not accurate and very misleading to the readers.

We would like to suggest that the authors correct themselves, make their raw data publicly available, and provide an explicit description of the method used for image processing in the Siripong and colleagues (2006) paper. It has been 5 years since a MIAME (minimum information about a microarray experiment) standard was proposed for all microarray studies (Brazma *et al.*, 2001). All world-class journals adhere to this minimum standard. The standard requires disclosing the entire array data set to the readers. The Pozhitkov and colleagues (2005) paper and all subsequent papers by our group followed this standard.

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