

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Patocs A, Platzer P, Eng C. Breast-cancer stromal cells with *TP53* mutations. N Engl J Med 2008;358:1634-6.

Table 1. Routine quality control (QC) protocol and outcome

Quality Control Steps	Results of QC Testing for Patocs et al. 2007 Study	Theoretical Results If QC Fails
Analysis of archivally-derived germline DNA for <i>TP53</i> mutations when <i>TP53</i> mutation/variant found in somatic DNA (either epithelium and/or stroma)	No <i>TP53</i> mutation in corresponding germline DNA for all samples with somatic mutations**. Only known polymorphisms of <i>TP53</i> noted in germline DNA	>5% with random <i>TP53</i> mutation and variation
Random selection of 10-20% of tested samples with mutations. Blinded repeat mutation analysis from corresponding LCM-procured frozen samples	100% Concordance for mutations and LOH between archived template and corresponding frozen template	<90% Concordance for mutations and LOH between archived template and corresponding frozen template
Random selection of another 10-20% of study samples and subject to mutation analysis of <i>SDHB</i> *	No <i>SDHB</i> mutations found in germline, epithelium and stromal DNA from all tested samples (except for known polymorphisms)	≥10% with <i>SDHB</i> mutations and variants not previously described
Select subset (in this study, 14 samples) with stromal <i>TP53</i> mutations subjected to re-LCM and DNA extraction by third party for blinded mutation analysis	100% Concordance	<90% Concordance
Two PCR-based technologies for mutation detection	100% Concordance: denaturing gradient gel electrophoresis and direct sequencing	<90% Concordance
Non-PCR-based technology	Immunohistochemistry – see Patocs et al. 2007 Chromosome in-situ hybridization <sup>+</sup>	N/A

\**SDHB* has 8 exons (to match the 6 exons examined in *TP53*) but not known to play a somatic role in breast cancer

\*\* Literature suggests that there may be a <2% likelihood of finding an unexpected germline *TP53* mutation (ie occult Li-Fraumeni syndrome) in series of all comers with breast cancers

<sup>+</sup>Chromosome in-situ hybridization only detects deletions of a certain minimal length