Supplemental Table 1: Preparation of the in-house cell lines distributed in the 2013 and 2014 ESP EQA schemes

Title | Sensitive detection methods are key to identify secondary EGFR c.2369C>T p.(Thr790Met) in non-small cell lung cancer tissue samples.
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Supplemental table 1: Preparation of the in-house cell lines distributed in the 2013 and 2014 ESP EQA schemes

<table>
<thead>
<tr>
<th>Provider</th>
<th>Sample type</th>
<th>Scheme year</th>
<th>Ratio EGFR mutated cell-line/EGFR wild-type cell line</th>
<th>1st EGFR Variant</th>
<th>VAF variant 1 (in %)</th>
<th>2nd EGFR variant</th>
<th>VAF variant 2 (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESP</td>
<td>Cell line</td>
<td>2013</td>
<td>50%/50%</td>
<td>c.2369C&gt;T p.(Thr790Met)</td>
<td>25†</td>
<td>c.2573T&gt;G p.(Leu858Arg)</td>
<td>25†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2014</td>
<td>90%/10%</td>
<td>c.2369C&gt;T p.(Thr790Met)</td>
<td>45†</td>
<td>c.2573T&gt;G p.(Leu858Arg)</td>
<td>45†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50%/50%</td>
<td></td>
<td>25†</td>
<td>c.2573T&gt;G p.(Leu858Arg)</td>
<td>25†</td>
</tr>
</tbody>
</table>

In-house cell lines were created by mixing cell lines with the EGFR mutation with an EGFR-wild-type cell line in a ratio indicated in the respective column. The homogeneous mixed cells were fixed for one hour in neutral-buffered formalin, mixed with warm agar (all cells distributed in 4 tubes) and the agar plugs were embedded in paraffin-blocks conform standard histopathology procedures. Paraffin blocks were cut to sections with a thickness of 4-5 µm, and were provided on glass slides. Refseq EGFR: LRG_304t1 (NM_005228.4). †Variant allele frequency based on the percentage of tumor cells. E.g. cell line of 50% tumor cells in a wild-type background was considered as a VAF of 25%. Abbreviations: EGFR, epidermal growth factor receptor; ESP, European Society of Pathology; LRG, Locus Reference Genomic; VAF, variant allele frequency.