a Reference sequence:

```
CCTTCAACCTCTTCCGCCTCCTCACGCGAGACCTG!
```

Read aligned to reference (deletion in dashes)

```
CCTTCAACCTCTTCCG┤--├CCTCACGCGAGACCTG
```

b Sequence surrounding deletion

```
CCTTCAACCTCTTCCG│CCTCACGCGAGACCTG
```

c Deletion locally repositioned

```
CCTTCAACCTCTT┤--├CCTCCTCACGCGAGACCTG
CCTTCAACCTCTTC┤--├CTCCTCACGCGAGACCTG
CCTTCAACCTCTTCC┤--├TCCTCACGCGAGACCTG
              ←──┤--├──→
CCTTCAACCTCTTCCGC┤--├CTCACGCGAGACCTG
CCTTCAACCTCTTCCGCC┤--├TCACGCGAGACCTG
CCTTCAACCTCTTCCGCCT┤--├CACGCGAGACCTG
```

d Sequences surrounding repositioned deletions

```
CCTTCAACCTCTT│CCT CCTCACGCGAGACCTG
CCTTCAACCTCTTC│CT CCTCACGCGAGACCTG
CCTTCAACCTCTTCC│T CCTCACGCGAGACCTG

CCTTCAACCTCTTCCGC│CTCACGCGAGACCTG  *
CCTTCAACCTCTTCCGCC│TCACGCGAGACCTG  *
CCTTCAACCTCTTCCGCCT│CACGCGAGACCTG  *
```

* Alternate valid deletion placements found.

Ambiguously aligned deletion detection algorithm. Example shows the ambiguous deletion detection algorithm applied to a single short read. (**a**) Reference sequence and aligned read. (**b**) The deletion is "collapsed" and the surrounding sequence stored. (**c**) The deletion is repositioned upstream and downstream of its *Bowtie2*-reported location, up to as many nucleotides as it is long. (**d**) The repositioned deletions are "collapsed" and the surrounding sequences compared to the original surrounding sequence. In any reposition, the absence of mismatches with the original surrounding sequence indicates an alternate valid deletion placement.
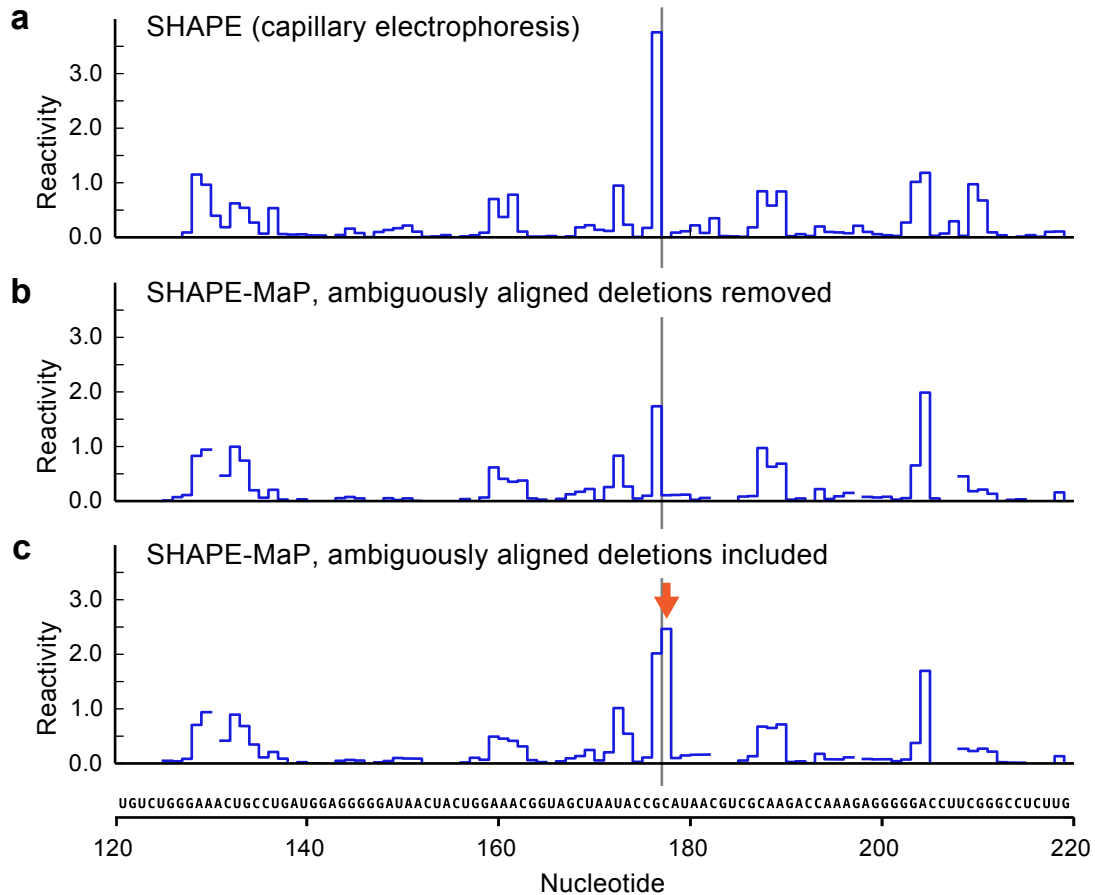
Ambiguously aligned deletion removal. (**a**) SHAPE reactivities from a portion of the bacterial 16S rRNA, identified as stops to primer extension by reverse transcription, read out by capillary electrophoresis using fluorescently-label primers. (**b**) SHAPE-MaP reactivities from the same region of 16S rRNA, obtained using the reverse transcription read-through SHAPE-MaP strategy, including the ambiguous deletion removal algorithm. (**c**) Reactivities from the same experiment, but analyzed omitting ambiguous deletion removal. The orange arrow highlights a nucleotide reactivity inconsistent with the capillary electrophoresis experiment.