NOTCH1 PEST domain mutations adversely affect prognosis in patients with CLL. Since 92\% of these mutations are insertions or deletions and that c.7544_7545delCT comprises 75\% of all mutations, we designed a test that can detect the presence of this mutation specifically and also any insertion or deletion in exon 34.

We used one FAM-labeled forward primer and two reverse primers. One specific for c.7544_7545delCT mutation (product of 356 bp) and another yielding a product of 391 bp.

The test yields three possible outputs: a) A single 391 bp peak: wild type. b) Three peaks (391 bp, 389 bp and 356 bp): heterozygous for c.7544_7545delCT. c) Two peaks (391 bp and another bigger or smaller, depending on the size of insertion / deletion): other insertion or deletion, but not c.7544_7545delCT.

We have studied 63 blood samples from CLL patients, of which 14 had trisomy 12. The frequency of NOTCH1 mutations in our series was: 12\% and 50\% in patients without and with trisomy 12 respectively, in agreement with previous reports. All mutated cases (N=13) had c.7544_7545delCT.

We developed a robust, fast and cost effective assay for identification of NOTCH1 PEST domain mutations that is suitable for implementation in the clinical setting.