## OTR.27 - Screening for rearrangements in *RB1* / 13q14 through real-time PCR

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**Introduction:**

Patients with deletion of chromosomal region 13q that includes the *RB1* gene show retinoblastoma (RB) and variable clinical features. About 5-15% of the patients with RB are heterozygous for a gross deletion that includes the whole or substantial parts of *RB1* gene.
We have designed a method based in real-time PCR for search of deletions / duplications of *RB1* gene. The specificity, sensitivity and clinical utility of the assay were demonstrated in detecting allele-specific copy number variation, and can be useful for analysis relative copy number.

**Objective:**

We have selected, in addition to the *RB1* gene, two other genes (*SUCLA2* and *MED4*) that are adjacently located to *RB1* in chromosomal region 13q14.2.

**Methodology:**

Genomic DNA was isolated from peripheral blood samples. The amplified segments were analyzed by relative quantification, relative copy number method ($2^{-??Ct}$). Each plate contained an internal control (*ALB* gene) and a trisomic sample. The CT's (cycle threshold) values obtained were used to calculate the relative copy number of each sample. All reactions were performed with Sybr Green (Invitrogen®). The ??CT and $2^{-??Ct}$ of each sample were estimated.
Five samples of retinoblastoma patients with partial or total *RB1* deletion detected by MLPA were used for validation. All samples were validated by real-time quantitative PCR.

**Results:**

Nine retinoblastoma patients carried complete deletion of *RB1* gene were identified by the MLPA technique, among 66 retinoblastoma patients tested. These deletions comprise about 10.61% of the mutations identified in retinoblastoma.





**Conclusion:**

Thus, the relative quantification real-time PCR technique to investigate deletions in the *RB1* gene becomes advantageous.

**Keywords: retinoblastoma; Real time PCR; 13q14**