Development of a digital PCR TERT/TERRA quantification assay in neuroblastoma

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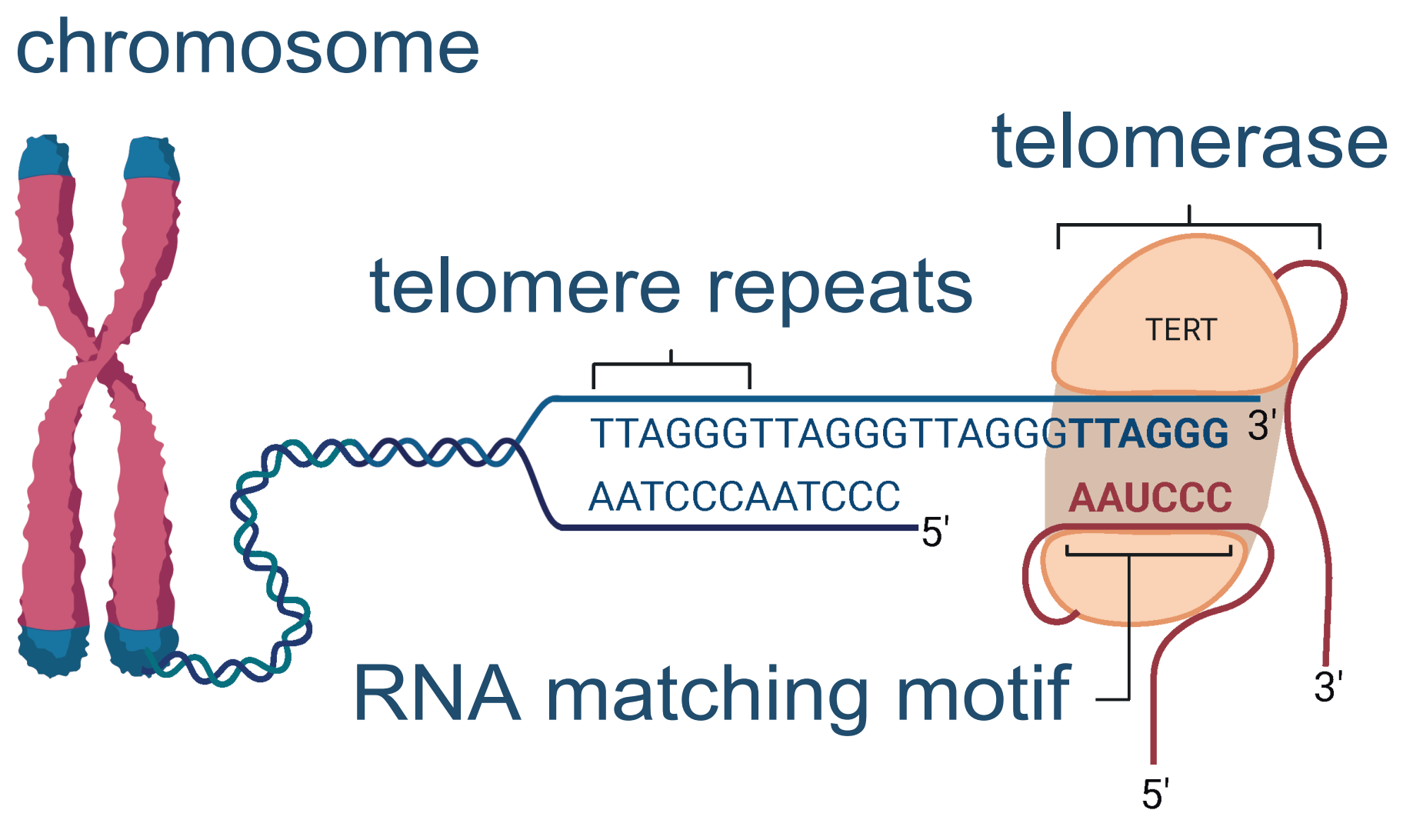
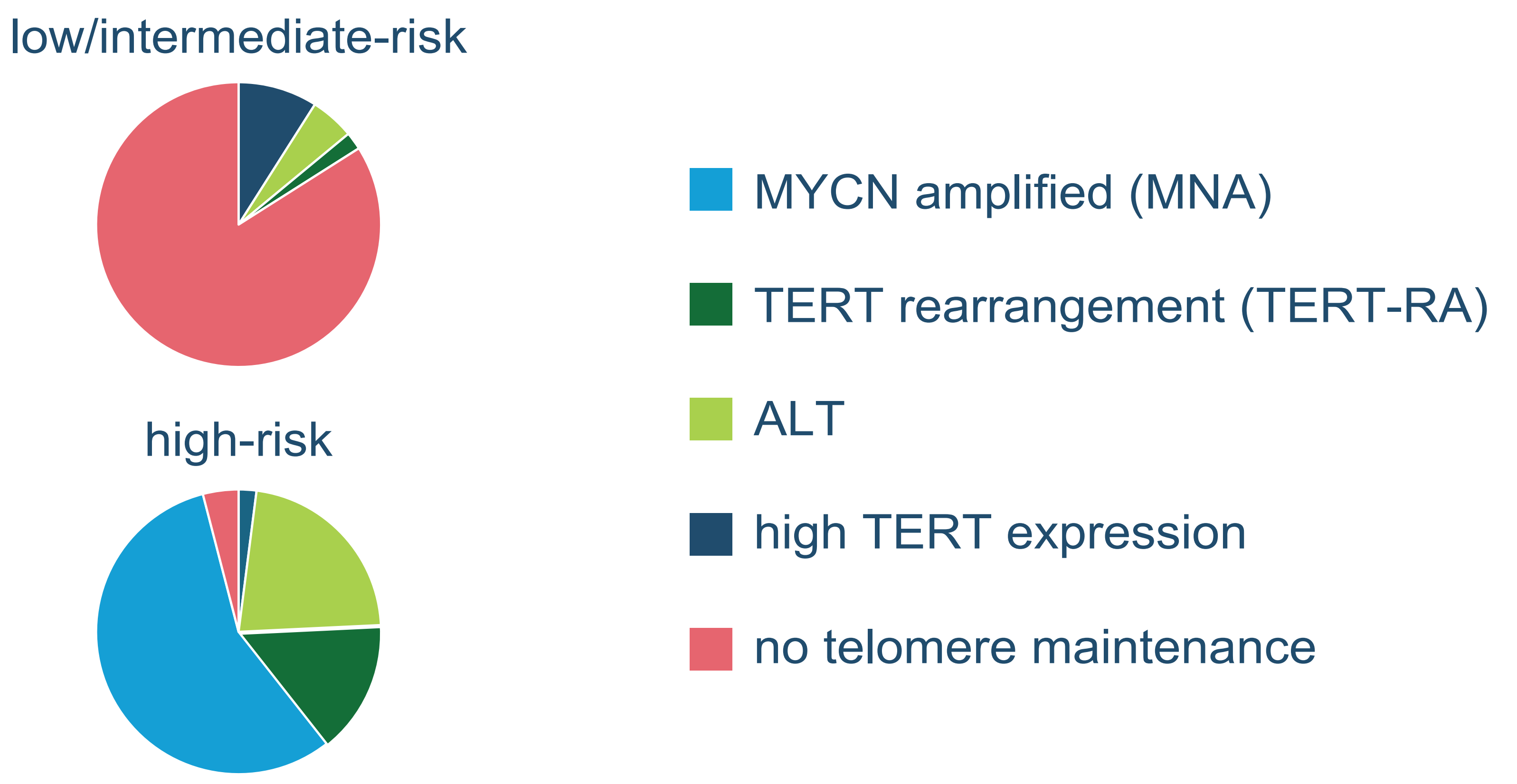
Telomere maintenance mechanisms in neuroblastoma



telomerase activation

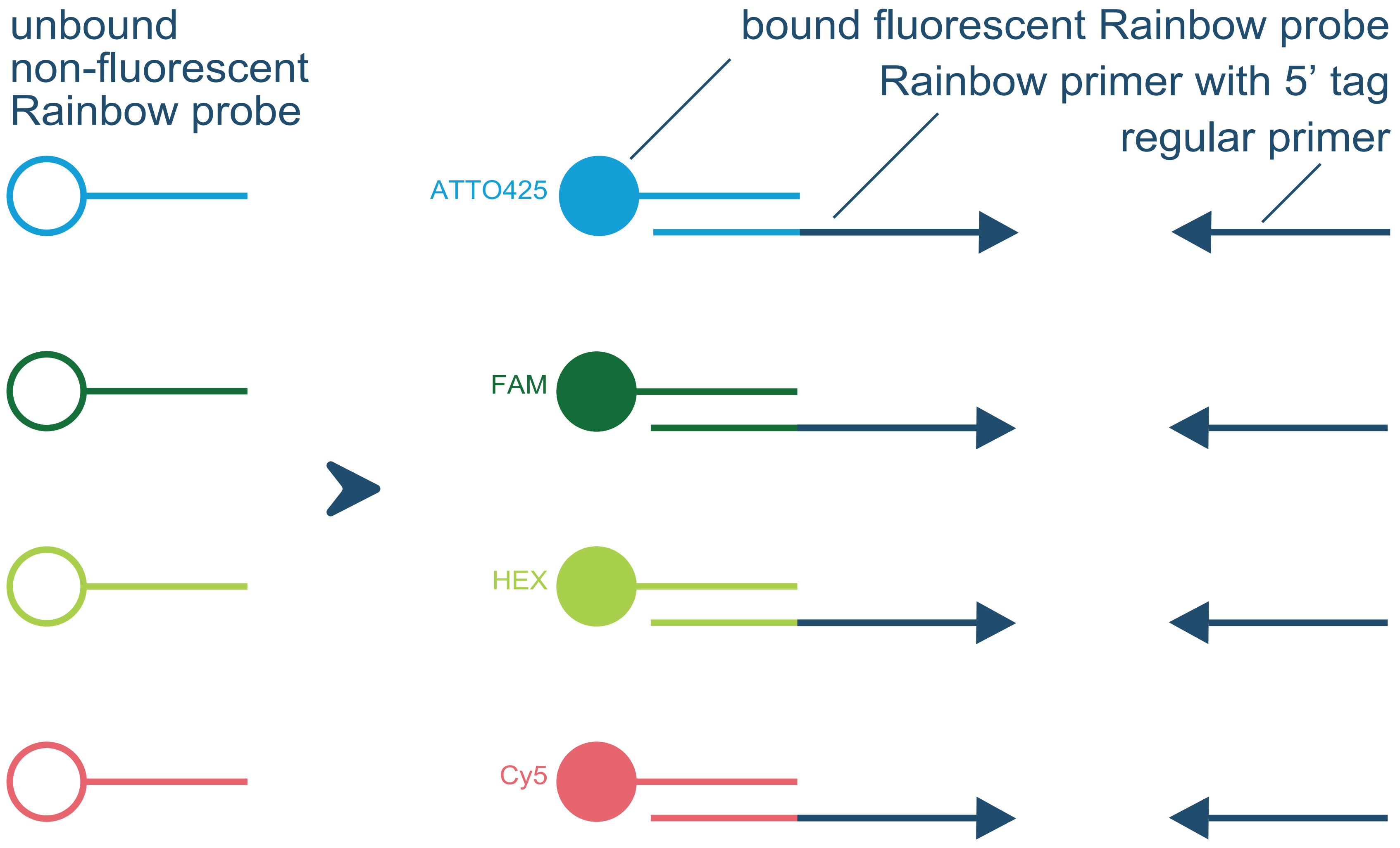
alternative lengthening of telomeres (ALT)

Quantification of TERT and TERRA expression is needed to identify high-risk neuroblastoma[1]



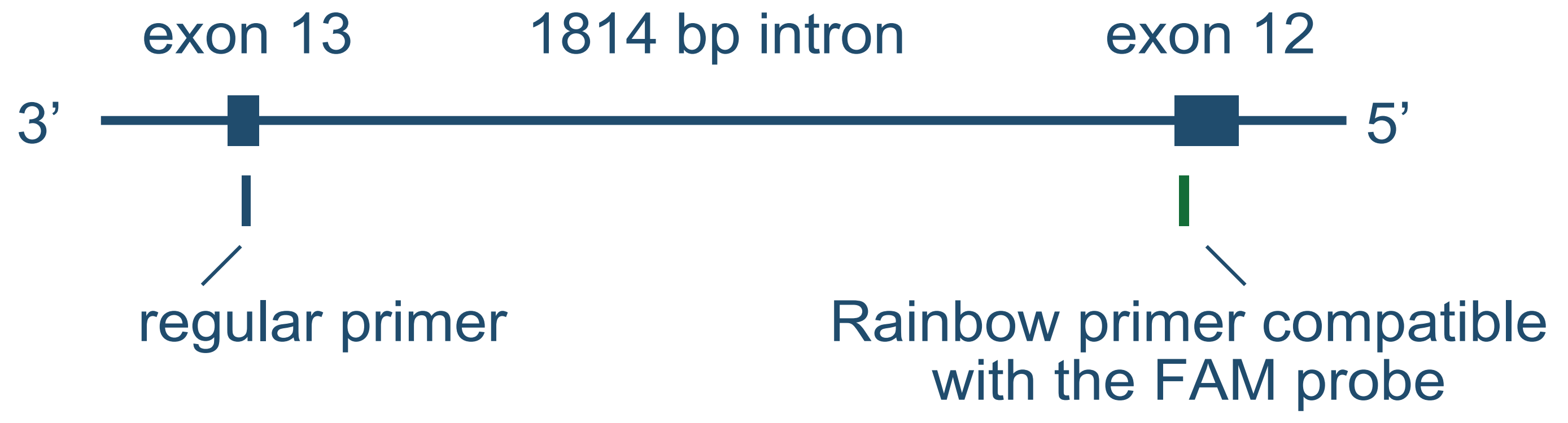
dPCR using Rainbow probes and primers for TERT and TERRA

- One of the primers of a primer pair is modified with a 5’ tag, enabling recognition by the tag-compatible Rainbow probe[2]

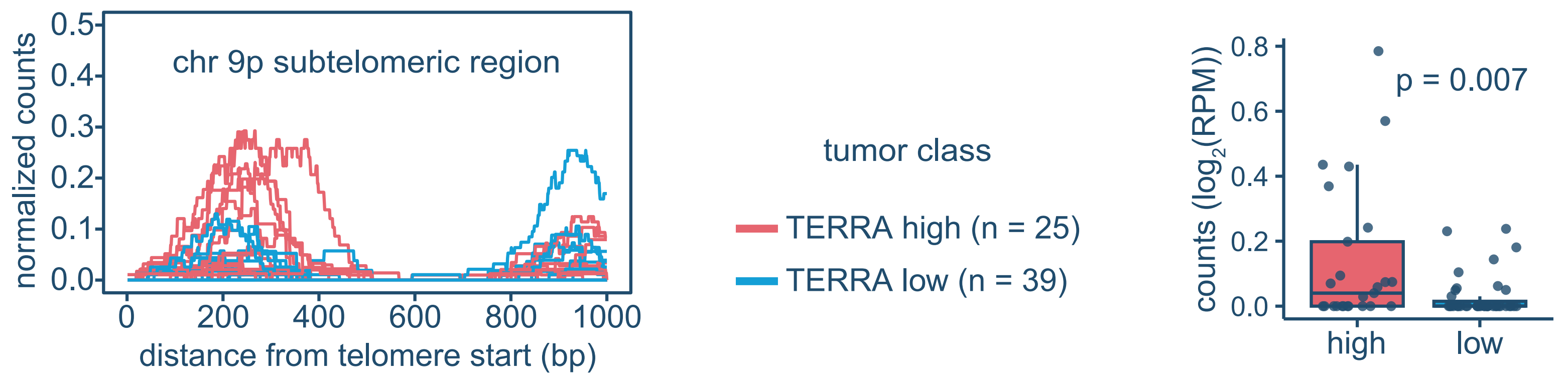


multiplexing on 2-color QX200 Droplet Digital PCR system or 6-color Digital LightCycler system

- TERT exons 12-16 have the highest coverage in tumor RNA sequencing data



- Subtelomeric regions at chromosome arms 1p, 9p and 17q have the highest coverage difference between tumors with high and low TERRA expression

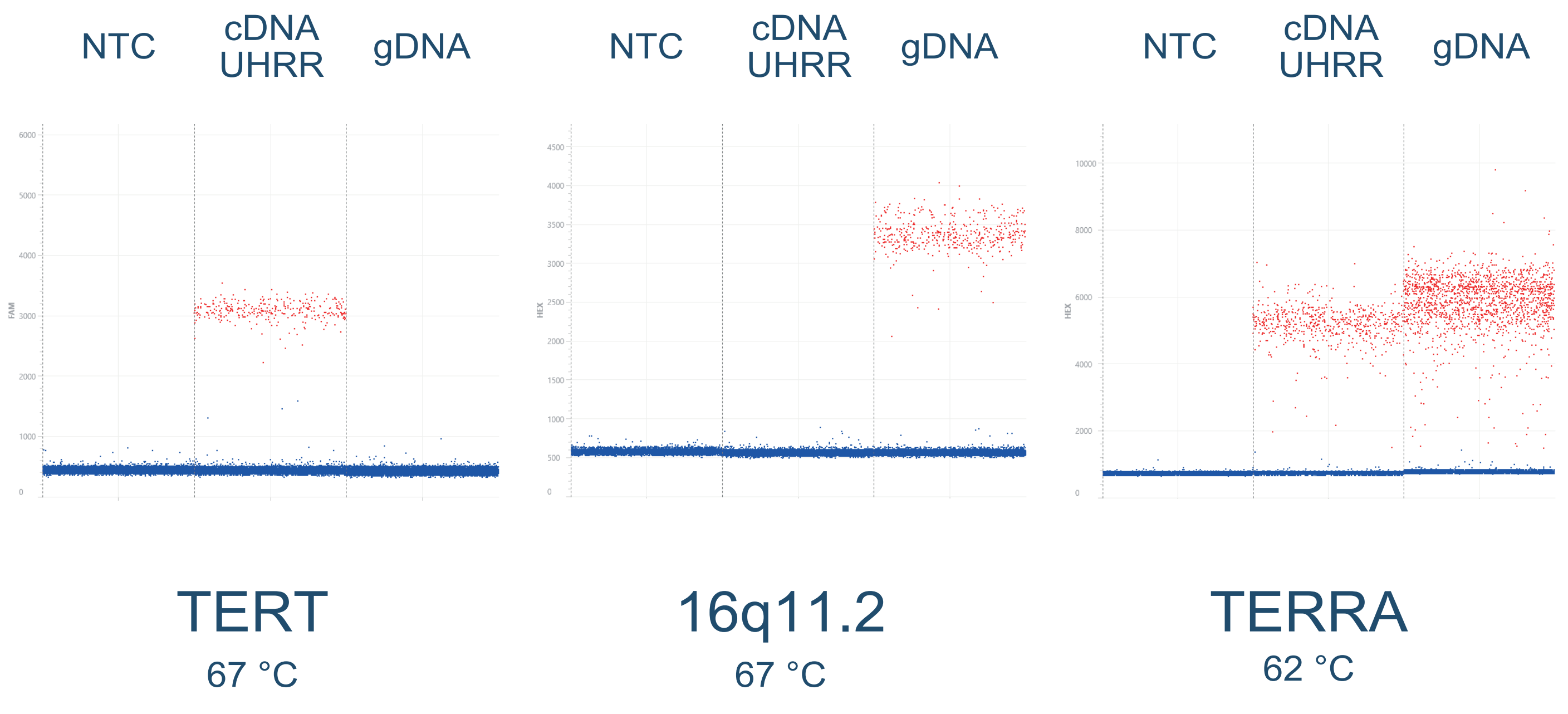


TERRA regular and Rainbow primers compatible with the HEX probe are located in the subtelomeric regions

Data normalization strategies

- for RNA and DNA co-purified samples: 16q11.2 assay[3]
  - enables calculation of transcripts per cell
  - multiplexing for MYCN amplification and ALK (F1174L) mutation detection
  - TERRA quantification requires DNase treatment or tailed reverse transcription for TERRA-specific cDNA synthesis
- for RNA samples: UBC, SDHA, HPRT1, GADPH[4]

Assay validation



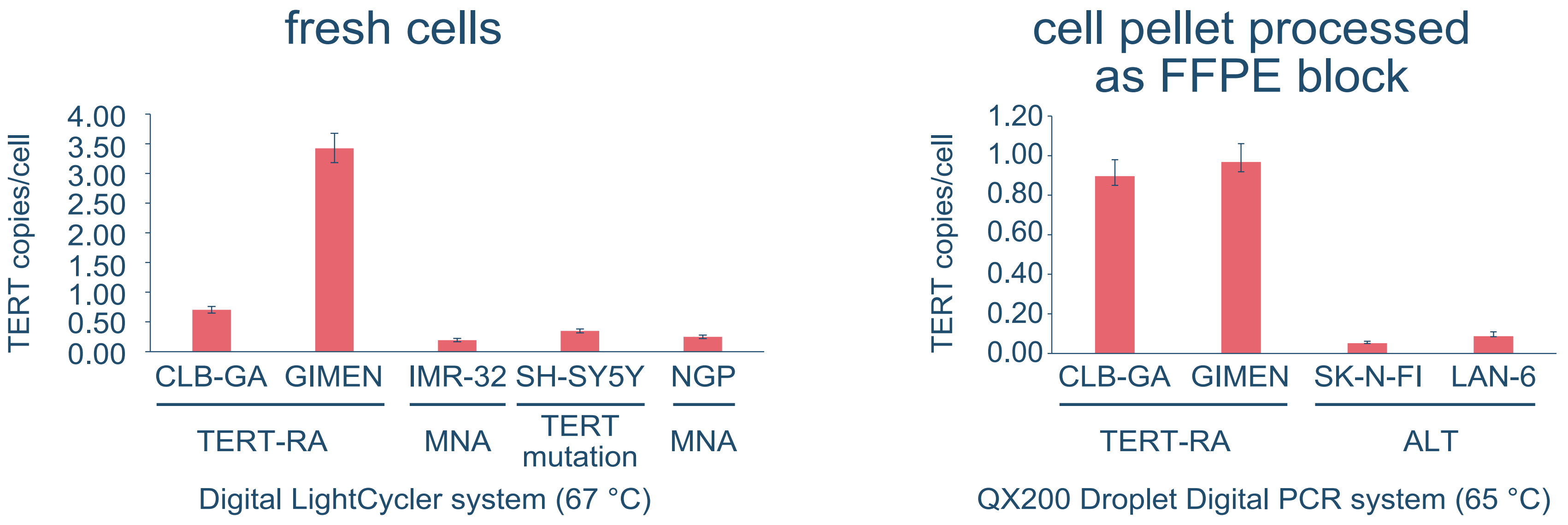
TERT
67 °C

16q11.2
67 °C

TERRA
62 °C

Digital LightCycler system

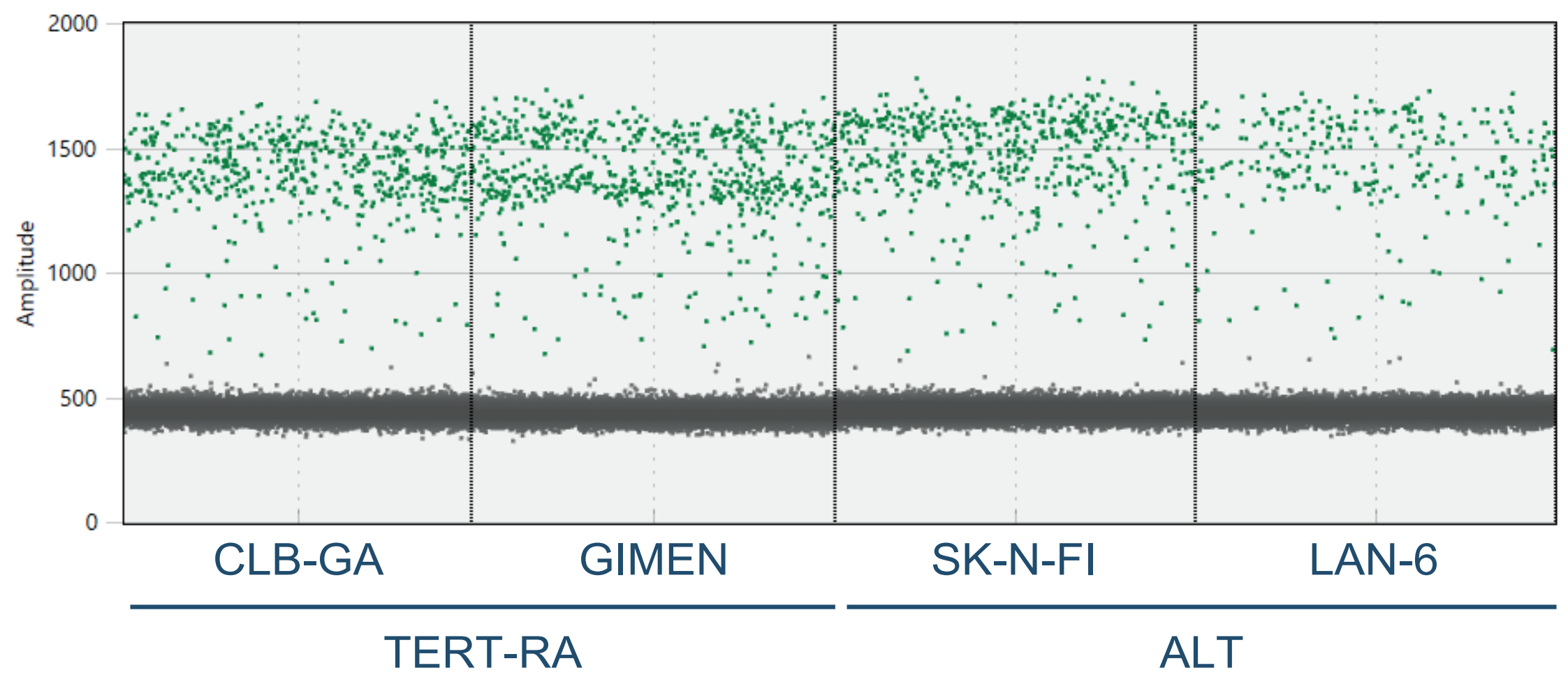
TERT and 16q11.2 testing on neuroblastoma cell lines



TERRA testing on neuroblastoma cell lines

cell pellet processed as FFPE block

cDNA of DNase-treated RNA



Conclusions

We developed a dPCR test compatible with FFPE samples to determine TERT and TERRA transcript quantification. Neuroblastoma cell line data are encouraging, and patient tumor validation data is underway. We are currently expanding the test for ALK mutation detection.

Illustrative figures are created with BioRender.

[1]Werr et al. EJC Pediatric Oncology, 2024. [2]https://www.pxlence.com/index.php. [3]Vandesompele et al., Journal of Clinical Oncology, 2005. [4]Vandesompele et al., Genome Biology, 2002.