

## FISH ANALYSIS METHODS

Fluorescence in situ hybridization (FISH) analysis was performed on 3- $\mu$ m thick formalin-fixed paraffin-embedded tissue section by counting at least 100 tumor cells in an area selected by pathologist. For *TFE3* gene at Xp11.2 and *ALK* gene at 2p23, commercially available *ZytoLight*® SPEC *TFE3* Dual Color Break Apart Probe, for *TFEB* gene at 6p21.1, a bi-color custom probes prepared in the laboratory using the library RP11 localized on 6p21.1 and framing *TFEB* gene, and LSI *ALK* Dual Color Break Apart rearrangement Probe were used according to manufacturer's instructions with appropriate modifications. Tissues section mounted on positively charged slides were heated overnight (56°C) and the day after deparafinized, dewaxed in xylene and treated with an ethanol to water series (100%-85%-70%). Slides were then incubated in TE solution (TRIS 5mM-EDTA 1mM) at 96°C for 15 min, rinsed in distilled water, digested with pepsin (0,4%) in 0.01N HCl at 37°C from 6 to 10 min, and washed again in distilled water. Finally, the slides were dehydrated in ethanol (96%) and air dried. Following slides drying, probe was applied to the marked area and co-denatured with the target DNA in Hybrite (Vysis Inc. Downers Grove IL) at 85° C for 1 min and then incubated overnight at 37°C for complementary sequences pairing. The following day, slides were washed first in 2XSSC/0.3%NP40 for 2 min at 73° $\pm$ 1C then in 2XSSC/0.1%NP40 for 1 min at room temperature, left to dry in the dark at room temperature and counterstained in Vectashield Antifade solution with DAPI (Vector laboratories, Inc. Burlingame CA). The samples were analyzed with a fluorescent microscope (LEICA DM6000B) equipped with appropriate filters, a device camera and the CytoVision FISH imaging and capturing software (Applied Imaging, San Josè, CA).

**Signal patterns interpretation** As the *TFE3* gene is located on the X chromosome, a nucleus without a *TFE3* rearrangement shows 1 or 2 pairs of juxtaposed orange and green signals, forming yellow fusion signal, according to sexual chromosomal pattern of the patient, male or female respectively. A nucleus with *TFE3* translocation shows split apart of the orange-green signal pair resulting in a single orange and a single green signal for the translocated *TFE3* allele in both male and female patients; a female patient shows also one fused yellow single signal corresponding to *TFE3* gene allele not involved in the translocation. Nucleus with *TFEB* rearrangement shows one red, one green and one fusion (yellow) signal, whereas nucleus without *TFEB* rearrangement shows two fusion (yellow) signals. An amplified *TFEB* signal was defined as >10 *TFEB* per nucleus.

A nucleus with ALK translocation shows split apart of the orange-green signal pair resulting in a single orange and a single green signal for the translocated ALK allele and one fused yellow single signal corresponding to *ALK* gene allele not involved in the translocation.