Development of a probe for detection of PPM1D amplification in breast cancer samples

Amplification of *PPM1D* gene located at shromosomal locus 17q23.2 is present in about 10 % breast cancers and less frequently also in other tunors. Recent studies indicate that amplification of PPM1D coding for WIP1 protein phosphatase influence sensitivity to chemotherapy and decreases an effect of doxorubicine. In experimental conditions, inhibition of WIP1 phosphatase leads to improved sensitivity of chemotherapy in p53 positive tumors. Evaluation of PPM1D status is currently not routinelly performed during histopathological analysis mainly because of a small number of clinical studies and also due to high costs for a customized probe production. At IMG ASCR we have optimized a protocol for generation of a probe suitable for detection of PPM1D amplification by FISH.

We used a bacterial artificial chromosome containing a human *PPM1D* gene, the integrity of which was validated by PCR **(Fig. 1).** Amplification of the BAC containing the *PPM1D* using Φ29 polymerase allowed to increase the yield approximately 200x. In the next step we digested BAC DNA by DNAase to generate fragments of average size 500 bp **(Fig. 2).** Next we labeled the fragments by various fluorescent derivatives of nucleotides using DNA polymerase I. After purification and blocking unspecific reaction, the *PPM1D* probe was hybridized with fixed cells containing a known number of copies of *PPM1D* gene and detected by fluorescent microscopy **(Fig. 3).** We optimized mainly different labeled nucleotides, concentrations of enzymes, incubation times and hybridization conditions. As a control probe we used a PCR fragment hybridizing with a satellite region of chromosome 17. In diploid retinal epithelia cells and BJ fibroblast we detected two copies of PPM1D, in aneuploidy A375 and U2OS cells tree and six copies of *PPM1*D and in MCF7 cells derived from breast cancer with known amplification of the *PPM1D* locus about 12-15 copies of *PPM1D*. In all tested cell lines, the probe gave an expected signal corresponding to the number of copies of the *PPM1D*.

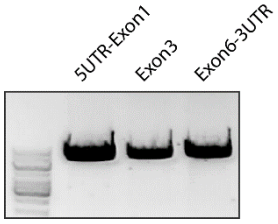
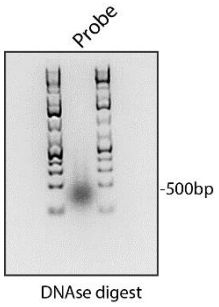


Fig. 1. Validation of BAC sequence using PCR. Exones as well as regulatory sequences of PPM1D are resent on the BAC.

Fig. 2. Fragmentation of the BAC using DNAase. Most fragments migrate at optimal size of 500bp.



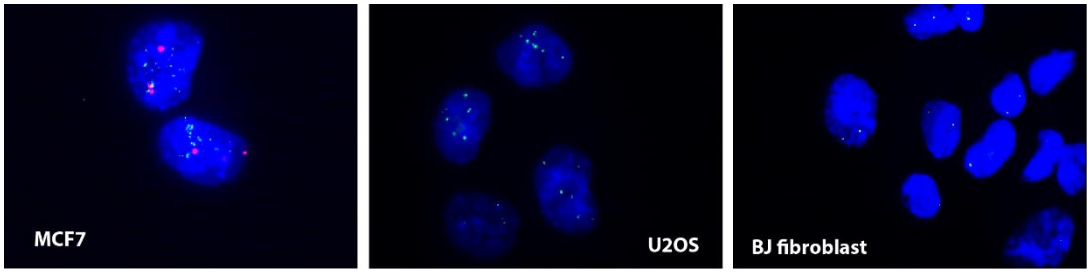


Fig. 3. Representative images of the PPM1D FISH probe in various cell lines (green, ATTO-488). In MCF7 cells, chromosome 17 was labeled in red (Taxas Red). Cell nuclei are labeled blue by DAPI.

Conclusion: Testing of an optimized FISH probe to human *PPM1D* on a panel of cancer cells and control diploid cells confirmed its efficiency in detection of PPM1D amplification of 17q23.2 locus. Additional information or purchasing a nonexclusive license is available upon request at Center for Technology Transfer, IMG ASCR, Vídeňská 1083, 14220 Prgue 4, Tel. 420-241 063 227.