OLIGOPROBES AS PERSONALISED TOOLS FOR TRANSFORMATIVE MEDICINE IN FISH

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Fluorescence *in situ* hybridisation (FISH) is a molecular cytogenetic technique used to detect and localise specific DNA sequences both in metaphase and interphase cells. Due to the high specificity, sensitivity and speed in which this technique can be utilised, FISH is routinely used in diagnostics for a range of disorders from haematological malignancies to solid tumour samples. The process works by utilising fluorescently labelled probes (typically ~100 kb in size) that are designed to be complimentary to the target of interest along chromosomes. Upon binding to the target, samples can be visualised using fluorescence microscopy and abnormalities can be identified.

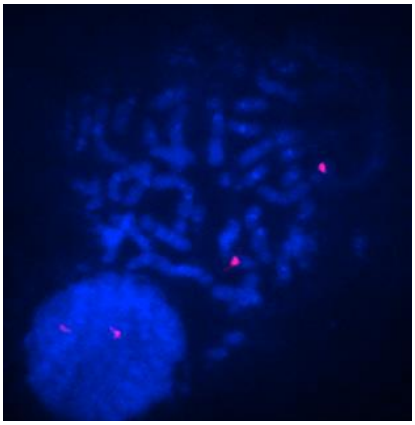

Figure 1: Metaphase and interphase human cells labelled with 17CEN oligoprobes after a 15 minute hybridisation.

Whilst typical hybridisation for commercial probes occur in 16 hours, using short oligoprobes (~ 50 bp), has shown to lead to faster hybridisation kinetics which could allow much quicker diagnosis [1]. These probes are designed synthetically [2], and can therefore be personalised to specific and rare mutations. We have designed oligoprobes for the centromere of chromosome 17, which along with chromosome 1 and 7 can be affected in acute lymphocytic leukaemia (ALL). Our probes have been proven to hybridise to the complementary target in 15 minutes, much quicker than the traditionally used methods [Fig 1].

Our probes have been labelled using methyltransferases, enzymes with specific DNA recognition sites. This technology utilises synthetic cofactors that allow the transfer of fluorophores, as opposed to methyl groups, to the specific DNA sequence [3]. By incorporating methyltransferase recognition sites into the probe design, we can direct fluorophore labelling to the probe. Using methyltransferase directed labelling of FISH probes will allow more control over the labelling density and therefore sensitivity of the probes, as well as increased specificity, and at a fraction of the cost compared to commercial probes.

[1] Aurich-Costa, J., L. Zamechek, et al. "Oligo fluorescence in situ hybridization (oligo-fish), a new strategy for enumerating chromosomes in interphase nuclei." *Fertility and sterility.* **88**: S86. (2007)

[2] Beliveau BJ, et al. “Versatile design and synthesis platform for visualizing genomes with Oligopaint FISH probes” *Proc Natl Acad Sci USA*. **109**(52):21301–21306 (2012).

[3] Neely, R. K., Deen, J. and Hofkens, J., “Optical mapping of DNA: Single-molecule-based methods for mapping genomes.” *Biopolymers***, 95**: 298–311 (2011).