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Selection of COMBO-FISH Probes for Multi-Purpose Applications

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Studying the cell nucleus and its architecture such as the distribution and structure of chromatin has gained high importance in biological and medical research [1,2]. One fundamental topic in this context is questions about the organisation and organisational parameters of the nanostructure of genes and other genomic elements [2,3,4]. This aimed in an increasing development of new methods for labelling short DNA sequences and detecting their exact localization and size within the nucleus by advanced microscopic methods [4,5].

The common method of fluorescence in situ hybridisation (FISH) is based on labelling DNA by the use of single stranded DNA-probes with appropriate markers binding to the counterpart within the genome [6]. Originally FISH was performed with DNA biochemical amplified either in yeast (Yeast Artificial Chromosome, YAC) or in bacteria (Bacterial Artificial Chromosome, BAC). To achieve DNA hybridisation using these probes is requiring thermal and chemical treatment for denaturation after fixation of the specimen [7,8]. These treatments together with the relatively large amount of probe DNA added, lead to several changes of the three dimensional nuclear structure resulting in a poor reproduction of the chromosomal and chromatin arrangement [9]. Furthermore the creation of those probes does not require any knowledge about the exact sequence of the desired genomic region but only about the specific enzymatic cutting locations within the genome. Thus the length of the labelling probes is determined by this enzymatic and chemical processes rather than by the target sequence specificity of the markers. Nevertheless, FISH is still a standard method [6] used with great success in various fields in research and diagnostics and has fundamental impact on nowadays medical care.

As mentioned, the conventional FISH method has at least two major disadvantages, on the one hand the destruction of the three dimensional structure due to denaturation treatment, which is a general problem for research in the field of genomic alterations and evaluation of the 3D-chromatin structure, and on the other hand the fully or correctly targeting labelling which is already a problem for labelling probe production itself. Both drawbacks can be overcome by the combinatorial design and usage of short oligonucleotide sequences which bind specifically to the desired genomic sequence. The design of such probes has become possible since the human genome has totally been sequenced and since there are methods of automated synthesising pre-determined DNA sequences industrially.

The problem of conserving the original chromosomal structure can be solved by using triplex forming oligonucleotides (TFOs) of a length between 15 and 30 bases, that bind as a third strand into the major groove of the double stranded DNA within the nucleus. The usage of TFOs avoids denaturation of the specimen and in this way conserves the native structure of the chromatin. On the other hand such short probes lack uniqueness within the genome. This problem is solved by the usage of combinatorial sets of short oligonucleotides which only cover the selected region but do not co-localise anywhere else in the genome. This method is called Combinatorial Oligo Fluorescence In Situ Hybridisation (COMBO-FISH) [10]. It can be used as well with the described TFOs as with common double-strand forming probes (Watson-Crick binding probes) both composed of DNA or peptide nucleic acids (PNAs) [10,11,12].

Schmitt et al. have described the algorithms for designing such TFO-probes in ref. [12]. By now this algorithm can also be used to identify and design Watson-Crick-binding probes [13]. In the course of these algorithms one has to find the first and the last nucleotide of the genetic region of interest (ROI) first. With this numbers a set of oligonucleotides binding to this genomic ROI with a minimum of accessory binding sites and no other co-localisation site within the genome is computed. As parameters for this search a minimum and a maximum length of the oligonucleotides, the number of probes determined and the maximum cluster size of probes which are allowed to bind within a sequence of selectable length outside the labelling site are to be defined. The algorithms Schmitt et al. [12] created can be used as well on the genomic sequence database of the human genome as provided by the NCBI [14] as on a self-created database including the TFO-binding sites only.

COMBO-FISH probe sets as well triplex forming as Watson-Crick binding for different genomic regions have been designed and successfully applied to different research aims. It has been shown that two different approaches of probe sets can be extracted from a database: a) combinatorial sets of different probes individually binding and b) unique probes multiply binding to a given target. Typical examples are centromere 9 [15] and 17 [16], 3q telomere [16] and gene regions, e.g., the receptor tyrosine kinase 2 (HER2/NEU) [17,18], growth factor receptor-bound protein 7 (GRB7) [18], breakpoint cluster region (BCR) [19,20], ABL proto-oncogene 1 (ABL) [19,20,21], pseudoautosomal region 1 (PAR1), T-box 1 (TBX-1) [22] and fragile X mental retardation 1 (FMR1) [23] have been designed and used.

Additionally it was possible to identify unique oligonucleotides marking all ALU sequences [16,24] and as such is useful to visualise changes in chromatin structure after irradiation [25]. Using probes binding to a trinucleotide expansion region in the promoter region of the FMR1 gene [23,26] enabled scientists to do a first step towards optical sequencing by the use of COMBO-FISH in combination with localisation microscopy [27,28,29].

Diversity of COMBO-FISH has shown that it is possible to create specific probes for a large amount of chromatin regions [12] in the range of some hundreds base-pairs up to the whole genome. On top studies showed that probes can not only be designed for the human genome but also for many other sequenced genomes [16]. In combination with new and sophisticated microscopy techniques this would open up undreamt-of possibilities in structural and functional biology.

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