The Development of Chromosome Microdissection and Microcloning Technique and its Applications in Genomic Research

Ruo-Nan Zhou^{1,2} and Zan-Min Hu^{1,*}

¹*Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R. China and*

²*Graduate University of Chinese Academy of Sciences, Beijing 100049, P.R. China*

Abstract: The technique of chromosome microdissection and microcloning has been developed for more than 20 years. As a bridge between cytogenetics and molecular genetics, it leads to a number of applications: chromosome painting probe isolation, genetic linkage map and physical map construction, and expressed sequence tags generation. During those 20 years, this technique has not only been benefited from other technological advances but also cross-fertilized with other techniques. Today, it becomes a practicality with extensive uses. The purpose of this article is to review the development of this technique and its application in the field of genomic research. Moreover, a new method of generating ESTs of specific chromosomes developed by our lab is introduced. By using this method, the technique of chromosome microdissection and microcloning would be more valuable in the advancement of genomic research.

Received on: October 20, 2006 - Revised on: November 23, 2006 - Accepted on: November 28, 2006

1. THE BRIEF HISTORY OF CHROMOSOME MICRODISSECTION AND MICROCLONING

1.1. Chromosome Recognition-- the Prerequisite of this Technique

Scalenghe *et al.* (1981) were the first to develop the chromosome microdissection and microcloning technique. They used this technique to generate DNA from individual bands of *Drosophila melanogaster* polytene chromosomes and obtained 80 clones [61]. This technique was then applied to mouse to obtain 212 microclones from the proximal half of chromosome 17 containing the t-complex [56]. Then, it was extended to human chromosomes [3]. However, at that time, studies were mainly focusing on some chromosomes that are easily identifiable by their configuration, such as the X chromosome of mouse [5] and chromosome 2 of human [3]. After the advent of G-banding technique, which makes the identification of human and animal chromosomes easier, and PCR technique, the chromosome microdissection and microcloning technique was extensively used in human and animal genomics research [38, 46, 47, 49, 74, 75].

To microdissect chromosomes in plant is more difficult than in human, because chromosome preparation is more difficult in plant. The chromosome microdissection and microcloning technique was applied to isolate B-chromosome DNA from rye in 1991 [58]. It was the first case that this method was used on plant chromosomes. However, work has been limited to chromosomes that are easy to identify, such as the satellite chromosomes [11, 33, 80-82], the largest or the smallest chromosome, and B chromosome [27]. Chromosomes in cytogenetic stocks that can be easily distinguished

were used for chromosome microdissection, such as telochromosomes [43, 44], addition lines [37, 65, 22, 34, 35], and reconstructed translocation chromosomes.

1.2. Benefited from other Techniques

Chromosome microdissection and microcloning has been benefited from technological advances and coupling with other techniques, which further improved its application.

In the microdissection and microcloning technique, the chromosome was initially dissected with glass microneedles under an inverted microscopy [61]. Even for an expert, it is difficult to dissect and collect a large numbers of chromosome fragments from the same region. Monajembashi *et al.* (1986) developed a method to dissect target chromosomes by using laser microbeam [51]. The equipment they used is mainly composed of argon-ion laser power supply, microcomputer and an invert microscope. Even though the intensity and the position of the laser beam were controlled by a microcomputer, the collection of the target region is still a difficult step for the operators. In our lab, these two methods were combined together. Firstly, we use the laser beam to dissect the targeted region of chromosomes, and then, the glass microneedle was used to collect the targeted regions [72]. Flow cytometry (FCM) has been applied to the vast field of cytogenetics research through adaptation to the observation of isolated chromosomes since 1975 [50]. Flow cytometry, while successful in isolating some of the larger chromosomes was clearly limited in its sensitivity for isolating the smaller ones. In order to isolate one special chromosome, Griffin *et al.* (1999) combined the two approaches (flow cytometry and microdissection) and successfully collected the targeted chromosome of chicken [20].

From glass microneedle to laser microbeam, and then flow cytometric technology, chromosome microdissection method had undergone changes from manual operation to computer driven manipulation. As a result, both the rate and

*Address correspondence to this author at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Datun Road, Chaoyang District, Beijing 100101, P. R. China; Tel: 86-10-64854561; Fax: 86-10-64854561; E-mail: zmhu@genetics.ac.cn

precision of chromosome isolation are improved. Moreover, these dissection techniques have complementary advantages.

The earliest chromosome microcloning [61] was a kind of direct cloning of the dissected chromosomal material in a nanoliter microdrop contained in an oil chamber. A very large number of dissected chromosome fragments had to be used and a relatively low cloning efficiency was attained. However, the introduction of PCR technology to the microcloning protocols has brought a substantial improvement in cloning efficiency [46]. From that on, the number of dissected chromosome fragments needed was significantly reduced, and two new microcloning methods were developed based on PCR technology: (1) Adaptor mediated PCR (LA-PCR) [59, 60]; (2) Degenerated oligonucleotide-primed-PCR (DOP-PCR) [67].

With the LA-PCR method, all the enzymatic manipulations of the dissected chromosomes, including PCR, can be easily performed in a single 0.5 ml tube by simply adding the appropriate components for each subsequent step. It avoids the complex micromanipulation in a microchamber and can generate much larger fragments—the average length is about 300-2,000 bps [28, 80]. Using this method, Chen and Armstrong (1995) constructed a single chromosome (less than 0.4 pg) library, potentially comprising of 500,000 recombinant clones [8].

The DOP-PCR technique, which is rapid, efficient, and species-independent, is designed to amplify target DNAs at frequently occurring priming sites using the primer of partially degenerate sequence, without restrictions imposed by the complexity or the origin of DNA. As an important method of microcloning, DOP-PCR overcomes the problems of regional bias and species dependence seen within LA-PCR. It is a simple PCR technique involving multiple loci priming, which allows a more general amplification than LA-PCR.

The combination of improved micromanipulation methods and PCR technology has enabled scientists to dissect specific chromosomes or chromosomal regions both accurately and frequently, thus, improving the efficiency of this technique.

2. THE APPLICATIONS OF CHROMOSOME MICRODISSECTION AND MICROCLONING IN GENOMIC RESEARCH

Chromosomal microdissection and microcloning provides a direct approach for isolating DNA from any cytogenetically recognizable region of a chromosome. The isolated DNA can be used for genomic research including (1) genetic linkage map and physical map construction, (2) generation of probes for chromosome painting, and (3) generation of chromosome specific expressed sequence tags libraries.

2.1. Providing Probes for Genetic Linkage Map and Physical Map Construction

Despite the rapid progress in gene mapping in recent years, there are still large areas of the genome for which markers are sparse or which are completely unmapped. Direct chromosomal microdissection and microcloning is a rapid technique for providing probes for such areas. The

clones of specific chromosomes libraries can be used in conjunction with existing markers to construct a fine genetic linkage map and physical map, providing a gateway for understanding of chromosome structure and organization of a specific region of the genome.

Combined with chromosome walking, chromosome region-specific physical map can be constructed by using chromosomes or chromosome fragments library to select cosmid library yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC). Moreover, microdissect DNA clones can be transformed to sequence tagging sites (STS), then the STSs are used to select cosmid library, YAC or BAC for eventually drawing a physical map [33].

2.2. Generation of Chromosome Painting Probes

Fluorescence in situ hybridization (FISH) plays an essential role in research and clinical diagnostics. The versatility and resolution of FISH depends critically on the probe set used [68]. The probes usually generate from the clones of cosmid library, yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC). Microdissect DNA clones are also the source of chromosome painting probes, particularly in the research of human genome. The technique that combines chromosome microdissection and chromosome painting is named micro-FISH [12, 49, 21]. As one of the important applications of chromosome microdissection, micro-FISH can be used to identify the reliability of the origin of microdissect DNA [65, 70, 80]. On the other hand, micro-FISH is an important tool for other research, such as chromosome construction aberration, chromosome origin identification and comparative analysis of genomes [18]. More and more chromosomal sites in the human genome have been identified as primary lesions in specific genetic diseases or cancers, such as the 4p16.1 for Huntington's disease, 22q11 for cat eye syndrome, 3p14-p23 in small-cell lung cancer and so on. Isolating the sequences from human chromosomal regions associated with specific genetic diseases or cancers to understand disease related genes is the prevalent approach in latest clinical research. Arens *et al.* (2004) microdissected the GTG band of the aberrant chromosome as the chromosome painting probes that identified the inter-translocation and the breakpoint of the chromosome of a large family with an unbalanced insertion translocation (3; 5)(q25.3; q22.1q31.3) [2]. Vermeesch *et al.* (2005) showed the feasibility of using microdissected chromosomes or chromosomal fragments to molecularly map the chromosomal breakpoints on array Comparative Genomic Hybridization (CGH) [71]. To study the behavior of genes conferring drug resistance of cancer lines, Mahjoubi *et al.* (2006) isolated the genes residing in a homogeneously staining region (HSR) in drug-resistant cell sublines. The isolated sequence provided a resource for future investigations in searching for novel genes contributing to drug resistance [48].

For comparative genome analysis, Rubes *et al.* (2005) microdissected the target chromosome segments from human X chromosome to be used as the painting probes and then in *situ* hybridized to the bovine X chromosome to assess evolutionary conservation between the human and bovine X chromosomes [57].

'Chromosome painting' in plants is relatively underdeveloped, because of the large genomes and large quantity of homologous sequences shared among chromosomes. Using microdissected plant chromosome DNA, there are many advantages to paint chromosomes from various plant species with large genomes, such as barley [17, 62], wheat [70], *Vicia faba* [76], and *Secale cereale* [80]. However, no specific paintings of chromosomes were observed. The painting probes were obtained only from B chromosomes [25, 26] and Y chromosome [64], because of the abundance of chromosome specific repetitive sequences.

2.3. Generating Ests of Specific Chromosomes

Expressed sequence tags (ESTs) are short (200-500) base pair "single pass" sequencing reads derived from both 3' and 5' end of cDNA clones selected at random. The ESTs give important information about its coding content and expression patterns in different tissues and organs of organisms at different developmental stages and environments. That is why partial cDNA sequencing to generate ESTs is being used at present for the fast and efficient profiling of gene expression in various tissues, cell types, or developmental stages. If the ESTs could be isolated from those specific chromosomes and specific chromosome regions directly, it would be easier to study and isolate those genes. Analysis of ESTs constitutes a useful approach for gene identification and gaining ESTs from cDNA libraries is the normal method. However, the dataset is huge and highly redundant, and it is difficult to identify the chromosome specific ESTs from cDNA libraries. Several methods to isolate chromosome specific and chromosome region specific expressed sequences had been developed, mainly including (1) restriction enzyme mapping of CpG islands [4]; (2) exon trapping [6, 13, 41]; (3) direct selection; (4) Hybrid selection and (5) Microdissection-mediated cDNA capture. First two of them, however, are labor-intensive and technically complex. Here we will discuss only the last three methods that are being used frequently in recent years.

Direct Selection

The generation of a regionally specific genomic library is one approach to obtain regionally defined expressed sequences. Such libraries may be constructed by microdissection of small chromosome fragments [46, 47, 74]. cDNA clones derived from the target region can be obtained by screening cDNA libraries using pools of region-specific genomic clones as probes. Yokoi *et al.* (1994) used this method to obtain the expressed sequences from the terminal portion of human Xq chromosome [73].

Hybrid Selection

Su *et al.* (1994) developed a technique that could rapidly generate chromosome-region-specific cDNA. Chromosome microdissection and hybrid selection was combined together in this technique to generate the chromosome-region-specific cDNAs. Firstly, DOP-PCR was used to amplify microdissection chromosome fragments. And then, the denatured amplified DNA was immobilized on a nylon membrane. The membrane was used to capture cDNAs by either DNA-cDNA hybridization or DNA-RNA-cDNA hybridization. At last, the captured cDNA was amplified by PCR after a strin-

gent washing. Using this method, Su *et al.* (1994) successfully isolated cDNAs transcribed from a 12qHSR of human chromosome that did not require the genomic cloning and characterization of an amplicon [66].

Microdissection-Mediated CDNA Capture

This is a good method for the enrichment of ESTs of the specific chromosomes or chromosome fragments [19, 39]. cDNA is ligated with adaptors and then hybridized with chromosome spreads on coverslips. After washing off the unhybridized cDNA, the targeted chromosomes or chromosome fragments are dissected by micromanipulation. Then, the microdissect DNA is amplified using the adaptor primers.

A New Method of Ests Selection may be more Efficient -- Hybrid Suppressive Amplification Selection

It is a new potential approach developed by our lab to rapidly generate ESTs from a specific chromosome or even a chromosome-specific-region (paper in preparation). We combine chromosome microdissection method and hybrid suppressive amplification (HSA) technique [42], to yield the homologous sequences between microdissected DNA and cDNA. After degenerated oligonucleotide primed PCR (DOP-PCR) or linker adaptor PCR (LA-PCR) of microdissected single chromosome, amplified microdissect DNA and cDNA are digested and linked with two kinds of adaptors, respectively. The two samples were mixed and annealed together for hours. Finally, a two-step PCR amplification is performed to select the hybridized fraction of the samples: Only the hybridized double strand sequences, which came from different samples with different adaptors, could be exponentially amplified. Using this method, we got rye chromosome 1R, wheat chromosome 7AL, 7BL and 7DL ESTs libraries (papers in preparation).

3. THE INTRACTABLE PROBLEMS OF CHROMOSOME MICRODISSECTION AND MICROCLONING

Every technology has its own problems and limitations. Chromosome microdissection and microcloning cannot be an exception.

3.1. Acid Treatment Leads to Depurination

One of the major concerns for the successful application of chromosome microdissection and microcloning technique was the extensive depurination of the chromosomal DNA caused by acid treatment during the sample fixation in chromosome preparation [14]. At present, depurination had been reduced or avoided by improving the chromosome preparation procedure [27, 28].

3.2. Contamination

PCR-mediated cloning of microdissected chromosome DNA made chromosome microdissection and microcloning more practicable. However, it is difficult to completely avoid the DNA contamination from cytoplasm and foreign species [29]. The amplification of non-target DNA will greatly affect the quality of chromosome-specific DNA libraries. Traditionally, the microdissection and microcloning steps should be carried out under the sterile condition as far as possible in order to effectively avoid the contamination from extraneous

source DNA. The microdissection tools, the sterile water, enzyme mixture, the adaptor and the staining fluid should be filtered or treated under the ultraviolet light more than 30min. Furthermore, the serious negative control (without chromosome DNA) is needed during the PCR procedures in order to monitor the potential problem of DNA contamination. The more chromosomes are microdissected, the more chance of contamination will be. Although flow cytometry can isolate more target chromosomes at one time, it may result in contamination from non-target chromosomes because of its sensitivity limitation. Contamination could be reduced if only one microdissected chromosome or chromosome fragment is used to obtain microdissected chromosome DNA due to shortened manipulation time. For avoiding the contamination, Hu *et al.* (2003) described a procedure to microdissect the target chromosome in a drop of 50% ethanol. Because the surface tension of 50% ethanol is weaker than that of water, the microdissected chromosome can be adhered to the tip of the glass needle without any cytoplasm, and it will not fall into the drop when the tip of glass needle is removed from the drop surface [29].

4. CHROMOSOME MICRODISSECTION AND MICROCLONING TECHNIQUE: THE FUTURE

As described above, chromosome microdissection and microcloning technique has been widely used in genomics research. However, it still needs to be improved for wider use in genomic research, especially for important allopolyploid crops such as wheat (6X=42, AABBDD). Due to interference of sequences on homoeologous chromosomes in complicated allopolyploid genome, at present there is no way to sequence the allopolyploid genome, even one chromosome of the genome. A direct approach to resolving this problem is to establish chromosome-specific DNA libraries using chromosome microdissection and microcloning. However, the present state of microdissection and microcloning technique cannot meet the needs for sequencing the complete specific chromosome DNA due to low coverage and small size of inserted DNA fragments. But the combination of microdissection and microcloning with a recently developed technique, pclone, could be a potential way to solve the above-mentioned problems. Zhang *et al.* (2006) reported a polymerase clone (pclone) method, which is a sequencing strategy that eliminates culturing of microorganisms by using real-time isothermal amplification to form pclones from the DNA of single cell. They reported that the single cell *E. coli* library had 96.2% coverage of the genome. By using phi29 polymerase and the N6 primer, the pclones lead to less amplification bias and larger insert fragment size [77]. This method could be used in conjunction with chromosome microcloning technique. To construct a high specificity, high coverage and low bias library with larger insert fragments, we only need single chromosome DNA. The coupling of chromosome microdissection and microcloning techniques with the pclone method could be an efficient strategy for sequencing a specific chromosome in plants and animals with complex genomics.

ACKNOWLEDGEMENTS

We thank Dr. Richard Wang (USDA-ARS, Utah State University) for critically reading the manuscript and his crea-

tive suggestions. This research is financially supported by National Science Foundation of China (No. 30270708), Chinese Academy of Sciences, Ministry of Science and Technology of China and K. C. Wong Education Foundation, Hong Kong.

REFERENCES

- [1] Albani, D., Côté, M.J., Armstrong, K.C., Chen, Q.F., Segal, A. and Robert, L.S. PCR amplification of microdissected wheat chromosome arms in a 'single tube' reaction. *Plant J.* **1993**, 4(5): 899-903.
- [2] Arens, Y.H.J.M., Engelen, J.J.M., Govaerts, L.C.P., Ravenswaay C.M., Loneus, W.H., Lent-Albrechts, J.C.M., Blij-Philipsen, M., Hamers, A.J.H. and Schrander-Stumpel, C.T.R.M. Familial insertion (3; 5)(q25.3; q22.1q31.3) with deletion or duplication of chromosome region 5q22.1-5q31.3 in ten unbalanced carriers. *Am. J. Med. Genet.* **2004**, 130A: 128-133.
- [3] Bates, G.P., Wainwright, B.J., Williamson, R. and Brown, S.D. Microdissection and microcloning from the short arm of human chromosome 2. *Mol. Cell. Biol.* **1986**, 6(11): 3826-3830.
- [4] Bird, A.P. CpG-rich islands and the function of DNA methylation. *Nature* **1986**, 321: 209-213.
- [5] Brockdorff, N., Fisher, E.M. and Cavanna, J.S. Construction of a detailed molecular map of the mouse X chromosome by microcloning and interspecific crosses. *EMBO J.* **1987**, 6(11): 3291-3297.
- [6] Buckler, A.J., Chang, D.D., Graw, S.L., Brook, J.D., Haber, D.A., Sharp, P.A. and Housman, D.E. Exon Amplification: A Strategy to Isolate Mammalian Genes Based on RNA Splicing. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, 88: 4005-4009.
- [7] Cannizzaro, L.A. Chromosome microdissection: a brief overview. *Cytogenet Cell Genet.* **1996**, 74: 157-160.
- [8] Chen, Q. and Armstrong, K. Characterization of a library from a single microdissected oat (*Avena sativa* L.) chromosome. *Genome* **1995**, 38: 706-714.
- [9] Cheng, Y.M. and Lin, B.Y. Cloning and characterization of Maize B chromosome sequences derived from microdissection. *Genetics* **2003**, 164: 299-310.
- [10] Christian, A.T., Garica, H.E. and Tucker, J.D. PCR *in situ* followed by microdissection allows whole chromosome painting probes to be made from single microdissection chromosomes. *Mamm. Genome* **2003**, 10: 628-631.
- [11] Cui, L.H., Hu, Z.M., Wang, L.L., Li, L.C. and Chen, Z.H. Microdissection and recovery of 1R chromosome in rye (*Secale cereale*). *Acta Bot. Sin.* **1997**, 39: 697-700.
- [12] Deng, H.X., Yoshiura, K.I., Dirks, R.W., Harade, N., Hirota, T., Tsukamoto, K., Jinno, Y. and Niikawa, N. Chromosome-band-specific painting: chromosome *in situ* suppression by hybridization using PCR products from a microdissected chromosome band as a probe pool. *Hum. Genet.* **1992**, 89: 13-17.
- [13] Duyk, G.M., Kim, S.W., Myers, R.M. and Cox, D.R. Exon Trapping: A Genetic Screen to Identify Candidate Transcribed Sequences in Cloned Mammalian Genomic DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, 87: 8995-8999.
- [14] Fisher, E.M.C., Cavanna, J.S. and Brown, S.D. M. Microdissection and microcloning of the mouse X chromosome. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, 82: 5846-5849.
- [15] Fominaya, A., Linares, C., Loarce, Y. and Ferrer, E. Microdissection and microcloning of plant chromosomes. *Cytogenet Genome Res.* **2005**, 109: 8-14.
- [16] Fukui, K., Minezawa, M., Kaisugi, Y., Ishikawa, M., Ohmido, N., Yanagisawa, T., Fujishita, M. and Sakai, F. Microdissection of plant chromosomes by argon-ion laser beam. *Thero. Appl. Genet.* **1992**, 84: 787-791.
- [17] Fukui, K., Minezawa, M., Kamisugi, Y., Yanagisawa, T., Fujishita M. and Sakai, F. Microdissection of barley chromosome by the cell work station. *Barley Genet. Newsl.* **1991**, 6: 272-276.
- [18] Goldammer, T., Rottengatter, K., Weikard, R., Hurstmann, R., Gehlhaus, A., Brunner, R.M., Hanotte, O. and Schwerin, M. Targeted generation of 16 sequence-tagged sites for bovine chromosome region 5q21-q25 by microdissection. *Chromosome Res.* **2004**, 12: 309-315.
- [19] Gracia, E., Ray, M.E., Polymeropoulos, M.H., Dehejia, A., Meltzer, P.S. and Trent, J.M. Isolation of chromosome-specific ESTs by

- microdissection-mediated cDNA capture. *Genome Res.* **1997**, 7: 100-107.
- [20] Griffin, D.K., Haberman, F., Masabanda, J., Brien, P.O., Bagga, M., Sazanov, A., Smith, J., Burt, D.W., Ferguson-Smith, M. and Wienberg, J. Micro- and macrochromosome paints generated by flow cytometry and microdissection: tools for mapping the chicken genome. *Cytogenet Cell Genet.* **1999**, 87: 278-281.
- [21] Guan, X.Y., Tret, J.M. and Meltzer, P.S. Generation of band-specific painting probes from a single microdissected chromosome. *Hum. Mol. Genet.* **1993**, 2: 1117-1121.
- [22] He, C.F., Ma, Y.Z., Xin, Z.Y., Xu, Q.Y. and Qian, J. Isolation of the single chromosome with BYDV resistant gene. *Zuo Wu Xue Bao* **1999**, 25: 273-278.
- [23] Hobza, R., Lengerová, M., Cernohorska, H., Rubes, J. and Vyskot, B. FAST-FISH with laser beam microdissected DOP-PCR probe distinguishes the sex chromosomes of *Silene latifolia*. *Chromosome Res.* **2004**, 12: 245-250.
- [24] Houben, A., Field, B. L. and Saunders, V. A. Microdissection and chromosome painting of plant B chromosomes. *Methods Cell Sci.* **2001**, 23: 115-124.
- [25] Houben, A., Kynast, R.G., Heim, U., Hermann, H., Jones, R.N. and Forster, J.W. Molecular cytogenetic characterisation of the terminal heterochromatic segment of the B-chromosome of rye (*Secale cereale*). *Chromosoma* **1996**, 105: 97-103.
- [26] Houben, A., Leach, C.R., Verlin, D., Rofo, R. and Timmis, J.N. A repetitive DNA sequence common to the different B chromosomes of the genus *Brachycome*. *Chromosoma* **1997**, 106: 513-519.
- [27] Hu, Z.M., Cui, L.H., Li, L.C., Wang, L.L. and Chen Z.H. Isolation of single chromosome and chromosomal fragments of *Lilium regale* Wilson. *Chin. J. Genet.* **1998**, 25(2): 131-135.
- [28] Hu, Z.M., Dang, B.Y., Zhou, Y.H., Cui, L.H., Wang, L.L., Zhang, T.H., Li, L.C. and Chen, Z.H. Isolation of single chromosome and DNA amplification *in vitro* in *Zea mays*. *Chin. J. Genet.* **1998**, 25: 545-550.
- [29] Hu, Z.M., Wang, H., Chen, Y.H., Shi, R. and Chen, Z.H. Studies on contamination of cytoplasm DNA and its control in plant chromosome microdissection. *Acta Bot. Sin* **2003**, 45(2): 131-135.
- [30] Hu, Z.M., Wang, H., Shi, R., Dang, B.Y., Hu, J., Yin, W.B., Chen, Y.H. and Jiang, S.M. Chen, Z.H. Microdissection and construction of region-specific DNA libraries of wheat chromosome 6B. *Acta Bot. Sin.* **2004**, 46(11): 1357-1365.
- [31] Huang, D., Wu, W. and Lu, L. Microdissection and molecular manipulation of single chromosomes in woody fruit trees with small chromosomes using pomelo (*Citrus grandis*) as a model. II. Cloning of resistance gene analogs from single chromosomes. *Theor. Appl. Genet.* **2004**, 108: 1371-1377.
- [32] Huang, D., Wu, W., Zhou, Z. and Lu, L. Microdissection and molecular manipulation of single chromosomes in woody fruit trees with small chromosomes using pomelo (*Citrus grandis*) as a model. I. Construction of single chromosomal DNA libraries. *Theor. Appl. Genet.* **2004**, 108: 1366-1370.
- [33] Jiang, C.Z., Song, W.Q., Li, X.L. and Chen, R.Y. Studies on microdissection and microcloning of the rye chromosome 1R. *Acta Bot. Sin.* **1998**, 40: 988-993.
- [34] Jiang, S.M., Hu, J., Yin, W.B., Chen, Y.H., Wang, R.R.C. and Hu, Z.M. Cloning of resistance gene analogs located on the alien chromosome in an addition line of wheat-Thinopyrum intermedium. *Theor. Appl. Genet.* **2005**, 111(5): 923-931.
- [35] Jiang, S.M., Zhang, L., Hu, J., Shi, R., Zhou, G., Chen, Y. H., Yin, W.B., Wang, R.R.C. and Hu, Z.M. Screening and analysis of differentially expressed genes from an alien addition line of wheat *Thynopyrum intermedium* induced by barley yellow dwarf virus infection. *Genome* **2004**, 47: 1114-1121.
- [36] Johnson, D.H. Molecular cloning of DNA from specific chromosomal regions by microdissection and sequence-independent amplification of DNA. *Genomics* **1990**, 6: 243-251.
- [37] Jung C., Claussen, U., Horsthemke, B., Fischer, F. and Herrmann, R.G. A DNA library from an individual *Beta patellaris* chromosome conferring nematode resistance obtained by microdissection of meiotic metaphase chromosome. *Plant Mol. Biol.* **1992**, 20: 503-511.
- [38] Kao, F.T., Yu, J., Qi, J., Tong, S. and Muenke, M. A region-specific microdissection library for human chromosome 2p23→p21 and the analysis of an interstitial deletion of 2p21. *Cytogenet Cell Genet.* **1995**, 68: 17-18.
- [39] Kim, H., Kim, C., Rhyu, I. J., Kim, J.H., Lew, D.H., Chun, Y.H. and Park, S.H. Isolation of novel human fetal brain cDNAs mapped to human chromosome bands, 1q25 and 8q24.1. *Mol. Cells* **2001**, 11: 257-262.
- [40] Kong, F.J., Chen, X., Ma Y.Z., Xin, Z.Y., Li, L.C., Zhang, Z.Y. and Lin, Z.S. Microdissection of *Haynaldia villosa* Telosome 6VS and cloning of species-specific DNA sequences. *Acta Bot. Sin.* **2002**, 44(3): 307-313.
- [41] Krizman, D.B. and Berget, S.M. Efficient selection of 3'-terminal exons from vertebrate DNA. *Nucleic Acids Res.* **1993**, 21: 5198-5202.
- [42] Lecerf, F., Foggia, L., Mulsant, P., Bonnet, A. and Hately, F. A novel method to isolate the common fraction of two DNA samples: hybrid specific amplification HSA. *Nucleic Acids Res.* **2001**, 29: E87-7.
- [43] Liu, B., Rong, J.K., Dong, Y.S., Han, F.P., Liu, Z.L., He, M.Y., Huang, B.Q. and Hao, S. Microdissection and microcloning of low copy specific sequence of Wheat (*Triticum aestivum*) chromosome 7B. *Chin. Sci. Bull.* **1999**, 44(4): 389-393.
- [44] Liu, B., Segal, G., Vega, J.M., Feldman, M. and Abbo, S. Isolation and characterization of chromosome-specific DNA sequences from a chromosome arm genomic library of common wheat. *Plant J.* **1997**, 11(5): 959-965.
- [45] Liu, X.H., Wang, H.W., Li, Y.M., Tang, Y.H., Liu, Y.L., Hu, X., Jia, P.X., Ying, K., Feng, Q., Guan, J.P., Jin, C.Q., Zhang, L., Lou, L.R., Zhou, Z. and Han, B. Preparation of single rice chromosome for construction of a DNA library using a laser microbeam trap. *J. Biotechnol.* **2004**, 109: 217-226.
- [46] Ludecke, H.J., Senger, G., Claussen, U. and Horsthemke, B. Cloning defined regions of the human genome by microdissection of banded chromosomes and enzymatic amplification. *Nature* **1989**, 338: 348-350.
- [47] Ludecke, H.J., Senger, G., Claussen, U. and Horsthemke, B. Construction and characterization of banded specific DNA libraries. *Hum. Genet.* **1990**, 84: 512-516.
- [48] Mahjoubi, F., Hill, R.J. and Peters, G.B. Chromosome microdissection identifies genomic amplification associated with drug resistance in a leukemia cell line: an approach to understanding drug resistance in cancer. *Chromosome Res.* **2006**, 14(3): 263-276.
- [49] Meltzer, P.S., Guan, X.Y. and Burgess, A. and Trent, J.M. Rapid generation of region specific probes by chromosome microdissection and their application. *Nat. Genet.* **1992**, 1: 24-28.
- [50] Métézeau, P., Schmitz, A. and Frelat, G. Analysis and sorting of chromosomes by flow cytometry: New trends. *Boil. Cell* **1993**, 78: 31-39.
- [51] Monajembashi, S., Cremer, C., Cremer, T., Wolfrum, J. and Greulich, K.O. Microdissection of human chromosomes by a laser microbeam. *Exp. Cell Res.* **1986**, 167: 262-265.
- [52] Murata, N., Masuda, K., Nishiyama, R. and Nomura, K. Construction of a micro-library enriched with genomic replication origins of carrot somatic embryos by laser microdissection. *Plant Physiol. Biochem.* **2005**, 43: 513-519.
- [53] Pirrotta, V., hadfield, C., Pretorius, G.H.J. Microdissection and cloning of the white locus and the 3B1-3C2 region of the *Drosophila* X chromosome. *EMBO J.* **1983**, 2(6): 927-934.
- [54] Ponelies, N., Bautz, E.K., Monajembashi, S., Wolfrum, J. and Greulich, K.O. Telomeric sequences derived from laser-microdissected polytene chromosomes. *Chromosoma* **1989**, 98: 351-357.
- [55] Potz, H., Schubert, V., Houben, A., Schubert, I. and Weber, W.E. Aneuploids as a key for new molecular cloning strategies: development of DNA markers by microdissection using *Triticum aestivum*-*Aegilops markgrafii* chromosome addition line B. *Euphytica* **1996**, 89: 41-47.
- [56] Röhme, D., Fox, H., Herrmann, B., Frischauf, A.M., Edström, J.E., Mains, P., Silver, L.M. and Lehrach, H. Molecular clones of the mouse *t* complex derived from microdissected metaphase chromosomes. *Cell* **1984**, 36: 783-788.
- [57] Rubes, J., Kubickova, S., Musilova, P., Amaral, M.E., Brunner, R.M. and Goldammer, T. Assignment of chromosome rearrangements between X chromosomes of human and cattle by laser microdissection and Zoo-FISH. *Chromosome Res.* **2005**, 13: 569-574.
- [58] Sandery, M.J., Forster, J. W., Macadam, S.R., Blunden, R., Jones, R.N. and Brown, S.D.M. Isolation of a sequence common to A- and B-chromosomes of rye (*Secale cereale*) by microcloning. *Plant Mol. Biol. Rep.* **1991**, 9(1): 21-30.

- [59] Saunders, R.D., Glover, D.M., Ashburner, M., Siden-Kiamos, I., Louis, C., Monastirioti, M., Savakis, C. and Kafatos, F. PCR amplification of DNA microdissected from a single polytene chromosome band: a comparison with conventional microcloning. *Nucleic Acids Res.* **1989**, 17(22): 9027-9037.
- [60] Saunders, R.D.C. Short cuts for genomic walking: chromosome microdissection and the polymerase chain reaction. *Bioessays* **1990**, 12(5): 245-248.
- [61] Scalenghe, F., Turco, E., Ederström, J.E., Pirrotta, V. and Melli, M. Microdissection and cloning of DNA from a specific region of *Drosophila melanogaster* polytene chromosomes. *Chromosoma* **1981**, 82: 205-216.
- [62] Schondelmaier, J., Martin, R., Jahoor, A., Houben, A., Graner, A., Koop, H.U., Herrmann, R.G. and Jung, C. Microdissection and microcloning of the barley (*Hordeum vulgare* L.) chromosome 1HS. *Theor. Appl. Genet.* **1993**, 86: 629-636.
- [63] Schubert, I., Dolezel, J., Houben, A., Scherthan, H. and Wanner, G. Refined examination of plant metaphase chromosome structure at different levels made feasible by new isolation methods. *Chromosoma* **1993**, 102: 96-101.
- [64] Shibata, F., Hizume, M. and Kuroki, Y. Chromosome painting of Y chromosomes and isolation of a Y chromosome-specific repetitive sequence in the dioecious plant *Rumex acetosa*. *Chromosoma* **1999**, 108: 266-270.
- [65] Stein, N., Ponelies, N., Musket, T., McMullen, M. and Weber, G. Chromosome micro-dissection and region-specific libraries from pachytene chromosomes of maize (*Zea mays* L.). *Plant J.* **1998**, 13(2): 281-289.
- [66] Su, Y.A., Trent, J.M., Guan, X.Y. and Meltzer, P.S. Direct isolation of genes encoded within a homogeneously staining region by chromosome microdissection. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, 91: 9121-9125.
- [67] Telenius, H., Carter, N.P., Bebb, C.E., Nordenskjöld, M., Ponder, B.A. J. and Tunnacliffe, A. Degenerated Oligonucleotide-Primed PCR: general amplification of target DNA by a single degenerate primer. *Genomics* **1992**, 13: 718-725.
- [68] Thalhammer, S., Langer, S., Speicher, M.R., Heckl, W.M. and Geigl, J.B. Generation of chromosome painting probes from single chromosomes by laser microdissection and linker-adaptor PCR. *Chromosome Res.* **2004**, 12: 337-343.
- [69] Tian, C., Lu, Y.F., Deng, J.X., Li, B., Zhang, X.Y. and Liu, G.T. Microdissection of addition chromosome of TAI-27 and the selection of specific probe. *Sci. China, C, Life Sci.* **1999**, 29: 174-179.
- [70] Vega, J.M., Abbo, S., Feldman, M. and Levy, A.A. Chromosome painting in plants: In situ hybridization with a DNA probe from a specific microdissected chromosome arm of common wheat. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, 91: 12041-12045.
- [71] Vermeesch, J.R., Melotte, C., Salden, I., Riegel M., Trifnov V., Polityko, A., Rumyantseva, N., Naumchik, I., Starke, H., Matthijs, G., Schinzel, A., Fryns, J.P. and Liehr T. Tetrasomy 12pter-12p13.31 in a girl with partial Pallister-Killian syndrome phenotype. *Eur. J. Med. Genet.* **2005**, 48(3): 319-327.
- [72] Wang, H., Zhou, Y.H., Dang, B.Y., Hu, Z.M., Wang, L.L., Li, L.C. and Chen, Z.H. Laser-microdissection and *in vitro* amplification of barley chromosome. *Chin. Sci. Bull.* **1998**, 43(7): 736-740.
- [73] Yokoi, H., Hadano, S., Kogi, M., Kang, X.L., Wakasa, K. and Joh-Elkeda. Isolation of expressed sequences encoded by the human Xq terminal portion using microclone probes generated by laser microdissection. *Genomics* **1994**, 20: 404-411.
- [74] Yu, J.W., Tong, S.H., Feng, T.Y. and Kao, F.T. Construction and characterization of a region-specific microdissection library from human chromosome 2q35-q37. *Genomics* **1992**, 14: 769-774.
- [75] Yu, J., Hartz, J., Xu, Y.S., Gemmill, R.M., Korenberg, J.R., Patterson, D. and Kao, F.T. Isolation, characterization and regional mapping of microclones from a human chromosome 21 microdissection library. *Am. J. Hum. Genet.* **1992**, 51: 263-272.
- [76] Zhai, X.L. and Chen, R.Y.A. preliminary study on a simple procedure to construct microdissection libraries of defined region from amplified chromosome segment in plant cell (*Vicia faba*). *Re Dai Ya Re Dai Zhi Wu Xue Bao* **1997**, 5(3): 60-68.
- [77] Zhang, K., Martiny, A.C., Reppas, N.B., Barry, K.W., Malek, J., Chisholm, S.W. and Church, G.M. Sequencing genomes from single cells by polymerase cloning. *Nat. Biotechnol.* **2006**, 24: 680-686.
- [78] Zhang, Y., Shan, X.N., Lu, X.X., Liu, N.S. and Yu, D.W. Cloning, sequencing and chromosome location of *Sry* Gene of *Muntjak munticus vaginalis* by DOP-PCR and microdissection. *Chin. J. Genet.* **2001**, 28(4): 322-326.
- [79] Zhou, Y.H., Dang, B.Y., Hu, Z.M., Cui, L.H., Li, L.C. and Chen, Z.H. Microdissection and PCR amplification of single soybean chromosome. *Acta Bot. Sin* **1998**, 40(2): 144-150.
- [80] Zhou, Y.H., Hu, Z.M., Dang B.Y., Wang, H., Deng, X.D., Wang, L.L., Chen, Z.H. Microdissection and microcloning of rye (*Secale cereale* L.) chromosome 1R. *Chromosoma* **1999**, 108: 250-255.
- [81] Zhou, Y.H., Hu, Z.M., Wang, L.L. and Chen, Z.H. Cloning a microdissected chromosome of soybean and in situ analysis of the chromosome PCR products. *Zhi Wu Sheng Li Xue Bao* **2000**, 26(3): 195-200.
- [82] Zhou, Y.H., Wang, H., Dang, B.Y., Deng, X.D., Hu, Z.M. and Chen, Z.H. Chromosome painting in *Secale cereale* with PCR products from microdissected chromosome 1R. *Acta Bot. Sin.* **1999**, 41: 715-721.