CHAPTER V

CONCLUSION

The micro-FISH technique was used to produce the chromosome 21 specific probe in this study. This method was developed and modified from the other research to decrease the microdissection step. We succeeded in yielding the primary DOP-PCR products that derived from only eight copies with the improvement of preparation of microdissected metaphase chromosomes. Furthermore, the secondary DOP-PCR product was achieved by the reamplification step. In spite of the endogenous contamination of previous negative controls usually presented in all reamplification reaction, the hybridization was displayed on target chromosomes. There was unavoidable cross-hybridization between centromeres of chromosome 13 and 21 while using t(21;21) derived-probe or chromosome 21q derived probes. The suitable 21 micro-FISH probes were applied on interphase uncultured amniocytes for prenatal diagnosis. The indication for prenatal diagnosis of all twelve amniotic fluid samples was advance maternal age. The informative FISH results were interpreted from only maximum percentage of signals. These criteria were optimized because our results were not equal to the high percentage of commercial probes. Moreover, the different types of signal and morphology of interphase nuclei were the important factors in scoring dot signals. Most of the uncultured amniocytes only presented cytoplasm around the nuclei that led to failure of hybridization and an insufficient number of scored nuclei. These problems should be solved in further studies.

The FISH results in this study corresponded with conventional karyotyping in eleven cases, whereas 1 case should a false negative of trisomy 21. Many reports indicated that the false negative due to maternal contamination appearance either in hemorrhagic specimens or clear amniotic fluid. This problem could be investigated with detection of the X and Y sex chromosome. In the present group of twelve amniotic fluid samples we found that the indirect FISH with micro-FISH probes was

able to detect trisomy 21 with 100% specificity but only 83.33% sensitivity and the accuracy was 91.66%. This sensitivity should be improved by not only increasing the number (n) of samples but also by making an adjustment of the quality of micro-FISH probes and the uncultured amniocytes preparation. The reliable results of this method have to be determined prior the application in prenatal diagnosis. Although the FISH results from both commercial specific probes and micro-FISH probes are faster than conventional cytogenetic, these techniques were reported as an adjunctive test with the need to weigh the limitation of FISH against cost and speed. Especially, valuable techniques as the micro-FISH help us to study several methods such as conventional cytogenetics, chromosomal microdissection, polymerase chain reaction, agarose gel electrophoresis and fluorescence *in situ* hybridization throughout this research.