**A novel enzymatic color reaction based biochip detection assay for miRNA biomarkers detection in HCC**

To develope an enzymatic color reaction-based miRNA array for early detection of miRNA biomarkers of liver hepatocellular carcinoma in Chinese population.

技术说明：

There are different molecular methods currently available for the detection of RNA expression level. These can generally be categorized as either real-time fluorescence-based quantitative PCR methods, solid phase hybridization assays, and sequencing. These assays still face limitations such as limited amount of covered loci, high costs and sample preparation.

Among the molecular diagnostic methods, real-time PCR techniques are widely used. A variety of probes have been used for real-time monitoring, including the TaqMan probes, FRET probes, molecular beacons and bioprobes. Real-time PCR based assays have the advantages of speed (testing time of 1.5–2 h) and avoiding cross-contamination. Although different kinds of probes are available, assays based on real-time PCR are still limited to a relatively small amount of testing genes and loci, and require sophisticated instrumentation with high associated costs.

DNA microarrays (biochips) immobilize a large number of oligonucleotides at a precisely defined spot on a carrier surface, which can then be used for hybridization to fluorescence-labeled samples. Fluorescence-based microarray assay requires expensive equipment and reagents, which are significant factors in clinical application. The significant progress in recent years in research on transcriptome of cancer has generated a larger number of miRNA biomarkers that could potentially be used in the biochip testing assays. Solid phase hybridization assays are based on interactions between labeled miRNA samples with miRNA probes bound to a solid support. Linear probe assays and DNA microarrays (DNA biochips) are frequently used of these assays.

Enzymatic color reaction is a common detection method that is widely used in immunology inspection. We first established the enzymatic-color-microarray (ECM) technique ten years ago, ECT is the combination of the enzyme-based color reaction and microarray (biochip) technology. Briefly, the ECM technique is carried out as follows. Primers with one terminus modified with biotin are used to amplify the target sequences and the resulting PCR products are therefore end labeled with biotin. Probes specific for the target loci are immobilized on the surface of a microarray according to the classical mode of modification with amidocyanogen. The amplified and biotin-labeled target sequences are then hybridized to the corresponding probes immobilized on the surface of the microarray. The microarray is then incubated with streptavidin-alkaline phosphatase conjugates, which undergo affinity binding to the hybridization products mediated by the biotin-streptavidin interaction. The chromogenic substrate of alkaline phsophatase, 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP), is used in conjunction with an oxidant, nitro blue tetrazolium (NBT), to produce a deep or lyons blue color.

We successfully applied this technique to develop serious genotyping products for personalized medicine diagnosis, We also developed related instruments for automated operation and detection, including automated hybridization and washing machine, image machine, which is very cheap. The instrument and diagnosis products have been got approval from the government.

In this study we report an enzymatic color reaction-based biochip detection assay used for the identification of miRNA biomarkers of HCC. The process includes sample preparation method, biochip hybridization, and an enzymatic color reaction. Specific software is used for data acquisition and automated interpretation. The whole testing process is semi-automatic and can be finished in four and a half hours. This assay significantly reduces the time required and the cost involved.

Our **enzymatic color reaction-based biochip detection assay** has several advantages compared with the assays mentioned above because it solves the problems of testing time, flux, expense and sample preparation. In addition, the detection system realized a high level of automation, as the monitoring and measurement of the hybridization and color reaction operates automatically, while specific software is used to interpret data from the scanner and to generate test reports. This automation of detection and data analysis reduces variability in the process. The biochip system has good scalability and new probes specific for other loci and genes can easily be added. The assay is rapid, and the whole testing progress can be finished in four and a half hours. The final advantage of our system is that it is based on detection of a color reaction, which means that it has less stringent requirements in terms of instrumentation, reagents and cost.

In summarize, the biochip-based system developed is shown to be suitable for miRNA expression detection. This system is simple to use, rapid and automatic，have high validity (sensitivity and pecificity), efficacy, and cost-effectiveness, and should prove to be a reliable new tool for the clinical diagnosis of cancer.

研究内容：

In this project, we choose previou reported miRNA candidate biomarkers(more than 20 miRNAs) in HCC to produce microarray, Serum or Plasma samples are chosen for detection. The study contents include:

1. miRNA Probe design and optimization.
2. miRNA preparation from plasma, preparation protocol should be simplified and time limited.
3. miRNA labeling, we will try two approaches: one is directly labeling without amplification, another is PCR-based labeling.
4. Optimization of hybridization, color reaction and imagine
5. Quantitative reverse-transcriptase polymerase chain reaction assay will be also applied to evaluate the expression of selected microRNAs.
6. validate their analytical and clinical performance

A logistic regression model will be constructed using a training cohort (n>1000) and then validated using another two independent test cohorts(n>2000). Area under the receiver operating characteristic curve (AUC) was used to evaluate diagnostic accuracy.

7）Apply for the approval license from Chinese Government.

Goals：

1. the detection system realized a high level of automation, as the monitoring and measurement of the hybridization and color reaction operates automatically, while specific software is used to interpret data from the scanner and to generate test reports.
2. The total process will be finished within 5 hrs.
3. Total plasma needed will be within 1 mL
4. The instruments need including: PCR machine, hybridization and washing machine, imagine system and computer, the overall price will be about 80,000RMB

上海百傲科技股份有限公司简介

About Baiao

Shanghai Baiao is a biotech company founded on Nov, 2000. Baiao’s headquarter is located to Shanghai Xuhui District, and the manufacture base is located to Shanghai Songjiang District. Jiaxing Huidong biotech Ltd. is a wholly-owned subsidiary of Baiao. The R&D, manufacture, and Marketing Management of Baiao are strictly carried out according to ISO13485.

Baiao aims to develop the diagnosis product of personalized medicine, and created a unique, completely chromogenic reaction based gene microarray technology. Baiao has 8 invention patents, 5 utility model patents, 1 computer software copyright, 12 know-hows. Baiao achieved a series of renovations in genetic testing, and many products in personalized medicine fill the gap in the field in China. Four of the company’s products had won the III class in-vitro diagnostic reagent certificate. There are CYP2C19 gene testing kit, ALDH2（Glu504Lys）gene testing kit, MTHFR（C677T）gene testing kit, CYP2C19 and VKROC1 gene polymorphism testing kit. Baiao also won 1 II class Medical Devices certificate and 2 I class in-vitro diagnostic reagent certificates. The diagnostic products of Baiao had been used in over a hundred hospitals and medical examination center with good feedbacks.

Baiao has successfully completed over 10 industrialization projects cover from national to local government leve Baiao has taken an active part in providing genetic diagnostic scientific research cooperation with clinical centers, universities, and research institutions which has domestic reputation.

Baiao is being a key player in the personalized medicine of China. On Nov 13th, 2013, Baiao officially goes public on the list of stock Exchange in the “new third board” (an over-the-counter market for growth enterprises). Stock abbreviation: baiao tech; stock code: 430353. Baiao is dedicated to facilitate the applications in Healthcare to serve the people for a better life.

商业计划：

Shanghai baiao technology Co. Ltd. aim to the R&D of diagnosis genetic testing products in personalized medicine since 2001. The company has instruments for molecular biology and microarray research, which including microarrayer, microarray scanner, fully-automatic microarray hybridization instrument, real-time qPCR [Amplifier](http://dict.youdao.com/w/pcramplifier/), regular PCR [Amplifier](http://dict.youdao.com/w/pcramplifier/), ultraviolet spectrophotometer, Fluorescence confocal scanner, regular electrophoresis apparatus, capillary electrophoresis apparatus, Autoclave, superclean bench, drying cabinet, High precision electronic balance, supercentrifuge, etc. The company has the most integrated biochip product supply chain, which cover from raw material, reagents, consumptive material to corollary equipment, software. 95% of the supplies are home-made. Baiao’s headquarter is located to Shanghai Xuhui District, and the manufacture base is located to Shanghai Songjiang District. The manufacture base in Songjiang district is GMP standard, which possess 2000 square meter area including a 100000 degree purification workshop.

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Below is the business plan for the project:

1、R&D：about 6 month，contents：Laboratory Scale Test, Pilot Scale Test, draft of the manual, [quality](http://dict.youdao.com/w/quality/) [criterion](http://dict.youdao.com/w/criterion/), design the Packaging of the kit, draft the production processes documents etc.

2、clinical research：about 6 month，contents：examine no less than 1000 cases in three hospitals， write clinical research report, monitor its clinical value，.

3、authorities test：about 6 month，contents：random sampling no less than 3 lots, examine according to the quality criterion, inspection reports should be issued.

4、Approval of the product：about 8 months, content: draft all the materials for the need of approval, submit it to the China Food and Drug Administration.

5、market warmup：about 1 year, content：draft propaganda materials,Investment invitation，launch the access to no less than 100 hospitals through distributors, declear the value of the product

6、marketing program：built network of the specilists, attract publicity by academic and hospital meetings, free access of the related instruments. Annual increase of the hospital is 100.

7、customer service：utilize the customer service team of Baiao, strengthen the regular maintenance and periodic calibration of the instruments ，trouble shooting in customer’s examine in time, etc.