Study on the Interfere Factors of Signal-Noise Ratio during Scanning Gene Chip

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Abstract: This paper study on the interfere factors of signal-noise ratio during scanning gene chip after hybridization basis on analysis of scanning results in different conditions of hybridization solution, blocking solution, hybridization hours, SDS washing and wavelength. Suggestion proper hybridization conditions of genechip. Established better foundation for application of genechip. [The Journal of American Science. 2006;2(1):80-82].

Key words: genechip; signal-noise ratio; hybridization

Introduction

Nucleotide sequence analysis of whole genome of HGP had been accomplished in advance in 2001, and it is coming into functional genome times. That human being is facing even more formidable task is to study the function of genome. Not only find out base sequence of genome but also mastery space-time information which is included. Emergence of genechip technology make that integrate analysis certain life phenomena completely is possible. Along with the HGP is achieved, use genechip technology can ascertain many gene express in cells, search target gene, etc. Genechip technology has high-through character, thousands upon thousands gene hybridization in same conditions, no other than the results of hybridization has reliability and veracity can reflect correctly function express of life information.

This study focus on the genechip which is made by the method of spotting by Robot hand. Study systemically on the interfere factors of signal-noise ratio during scaning gene chip after hybridization. Ascertain proper hybridization conditions of genechip. Providing valuable material for application of genechip.

1. Materials and Methods

1.1 Experimental material:

Gene of hepatitis D was provided by Beijing Military Affairs Medicine Academy of Science.

1.2 Reagent and make up:

UltraHyb hybridization solution and blocking solution ,bought it in Ambion Co., make up hybridization solution and blocking solution by ourself, 1% SDS.

Make up 2X hybridization solution: 10XSSC，0.2%12-alkyl creatine sodium, 0.04% SDS, 500 ug/ml DNA of salmon milt, 10% vitriol sephadex.

Make up blocking solution: 0.25gNaBH4 dissolved in 100ml PBS solution with 20%ethanol.

1.3 Instruments:

Biorobotics Co. MicroGrid TAS Ⅱ Robot, AXON Genechip Scanner, PE Co.9700 PCR Instrument,France BLX-312 UV Crosslinkers, CEL Co.Aldehyde-slide, 384Well, Wet Box, etc.

2. Methods:

2.1 Make up the genechip

(1) 50% DMSO dissolve Oligo nucleotide probe，end concentration 50uM，carry through spotting it with Robot.（If PCR product as spotting DNA，concentration is 0.2 uM ～ 0.5 uM ， spotting DNA need denaturalization）.

(2) Put spotting DNA in designed array, transfer into 384 well，begin to spotting with Robot，Make certain temperature and humidity according to practice circumstances.

(3) After spotting，slides on the condition of dustproof overnight in room temperature.

(4) Put slide in the blocking solution，treated 10 minutes，remove solution from the slides, put in sterile deionization water 30 seconds, take out the slides carefully.

(5) Put treated slides in to the 95℃ water 2 minutes，95% ethanol 5minutes，this slides can be use to hybridization as oligo nucleotide.

2.2 Hybridization and Scanning

(1) Mix 5 ul purified and fluorescence labled PCR product with 5ul sterile deionization water uniformity as

hybridization working solution.

(2) Put genechip on unknited ice, wipe off dust and fibre on the chip, put 20ul hybridization working solution on the centre of genechip, cover with cover slides, eliminate air bubble.

(3) Put genechip in the wet box, hybridization 1.5 hours at 58℃.

(4) Take out genechip after hybridization,put into 0.1% SDS solution, cover slides slide naturally, fulling 2 minutes, fulling with sterile deionization water 2 minutes, 100% ethanol drip on the two sides of genechip, washing again slowly, put on the paper uprightness, airing, scaning.

3. Results and analysis

3.1 Blocking solution influence on signal-noise ratio

Different blocking solution cause different effect of hybridization. From Figure 1 and Figure 2, apart from different of blocking solution, other conditions are same about two genechips, the results of hybridization are difference. Figure 1 use the blocking solution which made by ourselves, Figure 2 uses UltraHyb blocking solution, the effect of blocking solution of making by ourself is not better than UltraHyb bloching solution and easily make false positive. The main composition of blocking solution is NaBH4 which can make aldehyde on the slide deoxidization, moreover not combine with the sample which was labled. The reason that cause the false positive possibly because purity of NaBH4 is not inadequate, cant not make whole aldehyde on the slide deoxidization. The result of scanning is difference use same blocking solution but under different wavelength, from Figure 3 and Figure 4, the definition of scanning is distinct higher under 532 nm wavelength than 635 nm wavelength.

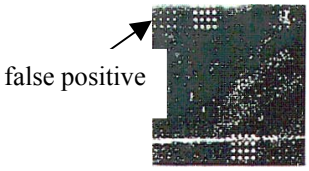

Figure 1. Blocking solution which made by ourselves

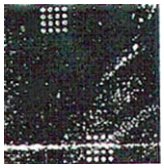

Figure 2. UltraHyb blocking solution

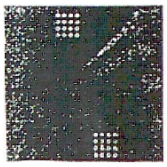

Figure 3. Under 532nm wavelength

Figure 4. Under 635nm wavelength

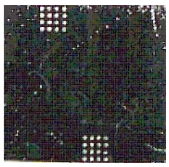

Figure 5. UltraHybhybridization freeze then melt one time

Figure 6. UltraHybhybridization freeze then melt time after time

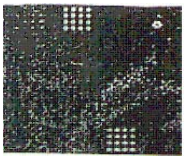

Figure 7. Wash one time with SDS

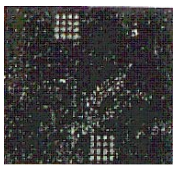

Figure 8. Wash two times with SDS

3.2 Hybridization solution influence on signal-noise ratio

The result of experiment indicate that the hybridization of making by ourself has more difference than UltraHyb hybridization. Moreover UltraHyb hybridization under difference conditions, e.g. freeze then melt time after time has not better result than one time, Figure 5 and Figure 6. Therefore the quality and status of hybridization is key to the hybridization success or not. The times of washing with SDS is affect the result of hybridization, from Figure 7 and Figure 8, the result of wash one time with SDS is better than two times.

4. Discussion

4.1The treatment before spotting on the slides

The sample of spotting on the genechip is the first step of hybridization during hybridization of genechip. If the sample of spotting on the genechip is PCR product,the sample need be denatured before spotting because denatured sample which double strand were opened, make it hybrid easily .If the sample which use to spot is Oligo nucleotide, need not to be denatured. Therefore the sample which is use to spot on the slide should be treated depended on the different situation.

4.2 The circumstance condition of spotting genechip is important

The circumstance condition of spotting genechip before spotting is important condition that determine the hybridization success or not. We should pay more attention that temperature and humidity.based on our many times experiment show that under condition of temperature is 25℃, humidity is 50%, we can get anticipate results. So we need notice that spotting circumstance before hybridization, otherwise the result will be affected.

4.3 The treatment of genechip after spotting and before hybridization

Base on the our experiment, the spotting genechip need to be irradiation about 60 MJ in UV crosslinker, in order to make DNA sample combine with slide fastness.

4.4 Because genechip scanner has intensive sensitivity and differentiate.

The mini-dust or impurity on the genechip can cause brightness background and noise, therefore it should be carry through as clean as condition in process of making, hybridization, washing.

4.5 It should be scanning and determine as soon as possible after hybridization and washing in order to avoid molecular degeneration which was marked by fluorescence.

4.6 Because there are machine transmission device during genechip scaning, the scanner should be put on stable and firmly plat roof, prevent fountainhead shake.

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