

HER^{21655V} Polymorphisms Detection by Non-Invasive Allele-Specific PCR (AS-PCR)

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Abstract

Allele-specific PCR for HER^{21655V} detection using breast cancer patients-obtained frozen tissue origin has been successfully developed. However, impassive approach to collect tissues hampers this method to be used for genetic screening purposes. Therefore, the present study is to test the use of buccal cells as an alternative for genetic material source in detecting HER^{21655V} polymorphism using AS-PCR. Firstly, we prepared standard marker of HER^{21655V} fragment with size of 142 bp for AA genotype and 168 bp for GG genotype using pGEM_HER2 recombinant as source of these fragment. These fragments then used as alternative of DNA ladder to estimate HER^{21655V} amplicon. AS-PCR using buccal cells has successfully performed to detect I655V polymorphism that showed AA genotype for all samples tested. In the next experiment we will conduct AS-PCR using this non-invasive method with more samples collected.

Keywords: Breast cancer; SNP; AS-PCR; HER^{21655V}

Introduction

Currently, cancer becomes world's problem including in Indonesia. Breast cancer is a disease that commonly diagnosed among women after cervical and ovary cancer. South-East Asia, Indonesia places the first rank for breast cancer incident with 16.6 died per 1.000.000 cases [1,2]. Breast cancer is ferocity in breast tissue that can origin from epithelium duct or lobulus. This disease is caused by multiple risk factors and early detection is an easy way to prevent women from this disease such as self-examination of the breast. Many research have focused on breast cancer mainly to find the cause, the drug for the curing, and biomarkers for its diagnosis. The status of breast cancer can be determined by status of molecular marker expressed on its plasma membrane such as Estrogen Receptor (ER), Progesteron Receptor (PR) and Human Epidermal Growth Factor 2 (HER2) [3,4].

HER2/neu is family of EerbB protein or alternative named CD340. This gene located on chromosome 17 (17q11.12) and have function in control breast cells development. This protein was expressed on membrane plasma of epitel cells and in the normal condition this protein regulate the population of breast cells. Defect in this protein can induce abnormality of breast cells proliferation that lead to carcinogenesis [5].

Plenty of evidence reports that breast cancer risk with HER2 polymorphism gene at codon 655, which are conversion amino acid from isoleusin to valine [6,7]. Study of HER2 at codon Ile655Val has been a major topic to be identified by researchers worldwide to recently. Thus, the detection of the HER2 polymorphism gene should use appropriate methods to obtain results with high sensitivity and specificity

HER^{21655V} detection method for breast cancer frozen tissue origin have been successfully developed [8]. However, these methods is still

used for invasive breast cancer samples (making patient ill because of tissue sampling procedures through biopsy). Therefore, this study developed a method to detect of HER^{21655V} using buccal cells as a source of safe and non-painful genetic material in patient. In addition to taking DNA samples from buccal cells it is more commonly used for HER^{21655V} genetic screening program involving multiple samples. Thus, the purpose of this study was to isolate DNA from the buccal cells and to detect the HER^{21655V} SNP using AS-PCR to determine the usefulness of buccal cells as an alternative to non-invasive DNA samples.

Experimental

This research was conducted at the Research Center for Biotechnology, Indonesian Institute of Science (LIPI), Jalan Raya Bogor KM. 46, Cibinong 16911, Indonesia. In February 2017 data were collected with five samples to show the result.

Preparation and purification of gene fragments

Preparation of gene fragments was collected in the Biology Molecular and Diagnosis laboratorium, named pGEM HER2. This preparation aims to obtain AA and GG genotype with optimization size from this laboratory. (Figure 1) [8]. Confirmation of the purity of the gene preparation is also carried out by purification method.

Isolation of DNA from buccal cells

DNA isolation using 5 samples buccal cells in this laboratory. This isolation using Promega Purelink® Lysate Mini kit. Isolation using PBS 400 µl with sterile tube.

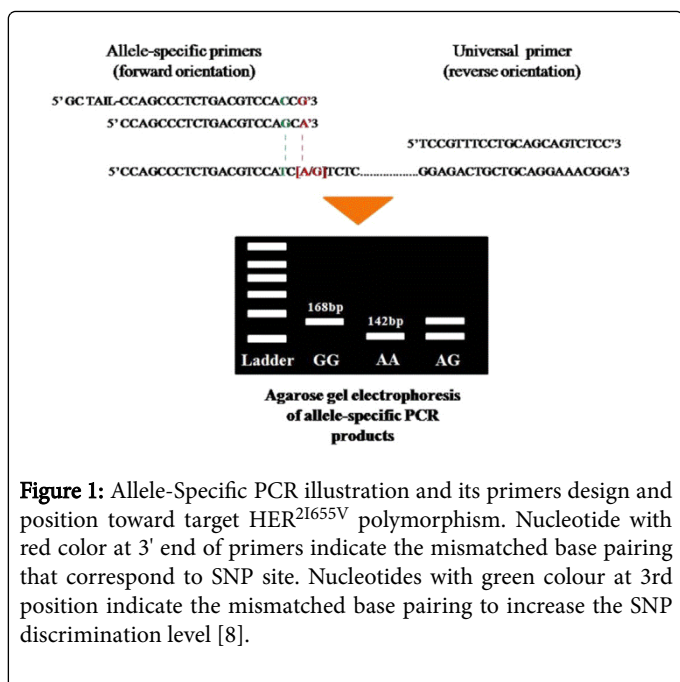


Figure 1: Allele-Specific PCR illustration and its primers design and position toward target HER^{21655V} polymorphism. Nucleotide with red color at 3' end of primers indicate the mismatched base pairing that correspond to SNP site. Nucleotides with green colour at 3rd position indicate the mismatched base pairing to increase the SNP discrimination level [8].

HER^{21655V} detection of buccal cells with AS-PCR

All PCR tube, Pipette tips, distilled water, and pipette are sterilized to UV light for ± 15 minutes prior to use. All treatment and PCR reagent mixing was done under laminar air flow. 5 samples of buccal cells were tested for HER^{21655V} polymorphism using AS-PCR optimized in previous studies. Total volume 12.5 µl was prepared, mixture containing 0.5 µl sample, 11.25 µl buffer mix PCR, 0.125 µl Primer 1 (AA), 0.075 µl Primer 2 (GG), 0.1875 µl Primer 4 (R), and water 0.3625 µl. Samples were evaluated in AS-PCR using 5 minutes at 95°C for Pre Denaturation, 35 cycles of 20 seconds at 95°C, 20 seconds at 54.3°C, 30 seconds at 72°C, and at 72°C for 5 minutes.

Result and Discussion

Preparation of HER^{21655V} gene fragments was aimed to obtain validation of fragment sample that would become a standard marker for AS-PCR isolation samples, so this preparation was important the buccal cells were tested with AS-PCR. To minimize the failure study in the end. Preparation of fragments is performed when a specific target is obtained of 142 bp for genotype AA and 168 bp for genotype GG (Figure 2).

In this figure can be found a different visualization although the samples show the existing genotype. The difference is a thick line above the band caused by several factors such as the amplified DNA sample purity level is still low. The purity of DNA determined by the level of protein contamination by other materials in the solution. DNA is said to be pure if the ratio of two values is between 1.8-2 [9]. Another factor is the emergence of non-specific targets in the band caused by protein contamination as well as RNase or other contaminants. Smear can also be seen on the product. Smear suggests there are other ingredients other than DNA isolate. Smear appears because the template is too much/

high so that one position in the genome (mispriming). If a DNA sample is degraded during the isolation process it can also cause the appearance of a smear on the PCR product. If the DNA amplification is incomplete it will make the PCR product not visible as well. The completeness and purity of DNA affects the PCR amplification product and the primer attachment with the pair which will inhibit the DNA polymerase enzyme activity. After the preparation fragment is complete, the next step is purification.

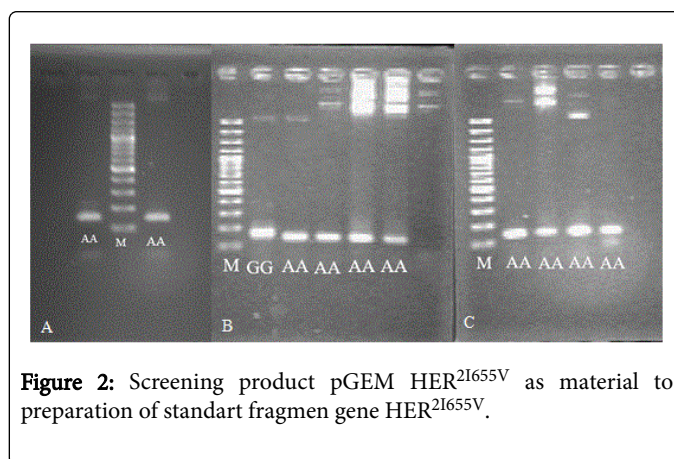


Figure 2: Screening product pGEM HER^{21655V} as material to preparation of standard fragment gene HER^{21655V}.

Purification is a method of making pure fragments of undesirable unspecific targets, proteins, RNAs and other contaminants belonging to isolate (Figure 3).

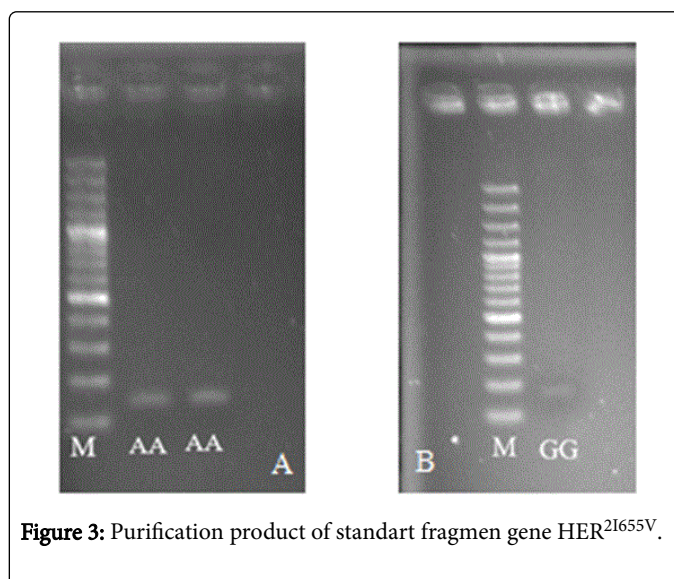


Figure 3: Purification product of standard fragment gene HER^{21655V}.

The thickness of the resulting product is influenced by whether the sample is reinforced or not and completed or not the amplification process. Purification can be successful if done in accordance with the procedures and use of materials with the required functions. The material like membrane binding solution to DNA binding, membrane wash solution to remove the DNA from impurities.

| No. | Code | First concentration | Last concentration (liquided 10 x) | Purity |
|-----|-------------------------------------------|---------------------|------------------------------------|--------|
| 1 | Buccal cells product of right side 3/2/17 | 50 ng | 20 ng | 1,4 |
| 2 | Buccal cells product of right side 3/2/17 | 35 ng | 20 ng | 1,023 |
| 3 | Buccal cells product of right side 3/2/17 | 53 ng | 20 ng | 1,02 |
| 4 | Buccal cells product of right side 3/2/17 | 63 ng | 20 ng | 1,12 |
| 5 | Buccal cells product of right side 3/2/17 | 66 ng | 20 ng | 1,08 |
| 6 | PGEM T HER2. DEV.2.1 Purification result | 37 ng | - | - |
| 7 | PGEM HER2 7/1/15 Isna purification result | 37 ng | - | - |

Table 1: Data of samples.

Visualization of buccal cell isolation showed variation of the results (Figure 4).

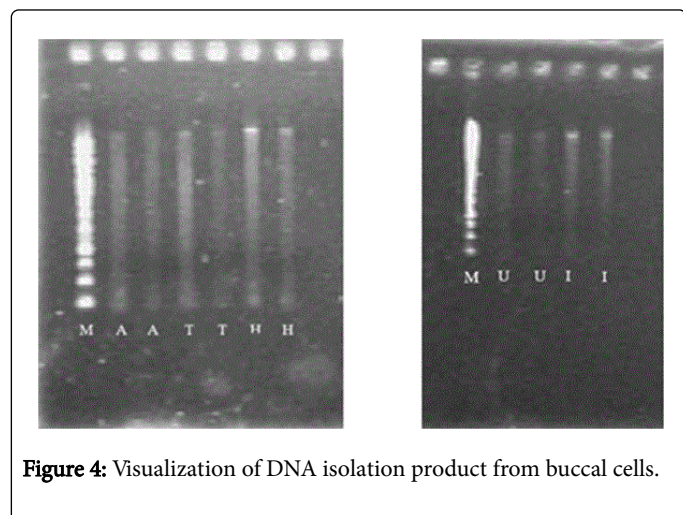


Figure 4: Visualization of DNA isolation product from buccal cells.

Five samples have seen differences in the thickness of the targeted product. Some of the things that can cause differences in these results are the suitability of the methods used when retrieving buccal cells. Visualization of buccal cell isolation indicating these DNA pairs indicates that buccal cell isolation was successful. Buccal cells have purity that is still low because the level is still below 1.8. DNA concentration measurements resulted in a variety of results with a lower product 35 ng and a 66 ng higher product (Table 1).

The confirmation result of marker SNP HER^{21655V} standard indicates that the marker can be used. From Figure 5, observed bands of AA and GG genotype appear to be very clear. So the five samples can be determined with accuracy that they all have AA genotype 142 bp. According to data Budiarto et al. [8] 60-70% Asian societies have AA genotype on the HER^{21655V} gene. While in Europe, ranges between 58-65% of people with AA genotype and 82-88% presence in Africa. In South-East Asia especially Indonesia, women are dominated by AA genotype. Genotypic prevalence indicated from 10 Indonesian women, 8 women had AA genotype and only 2 others had GG and AG. So more samples are tested, it will have opportunity to take GG/AG samples. This study did not show GG/AG genotype because the sample size was too small. The confirmation test using the standard SNP HER^{21655V} marker was successful because the NTC was

not detected by UV light, meaning the study was not exposed to contaminants.

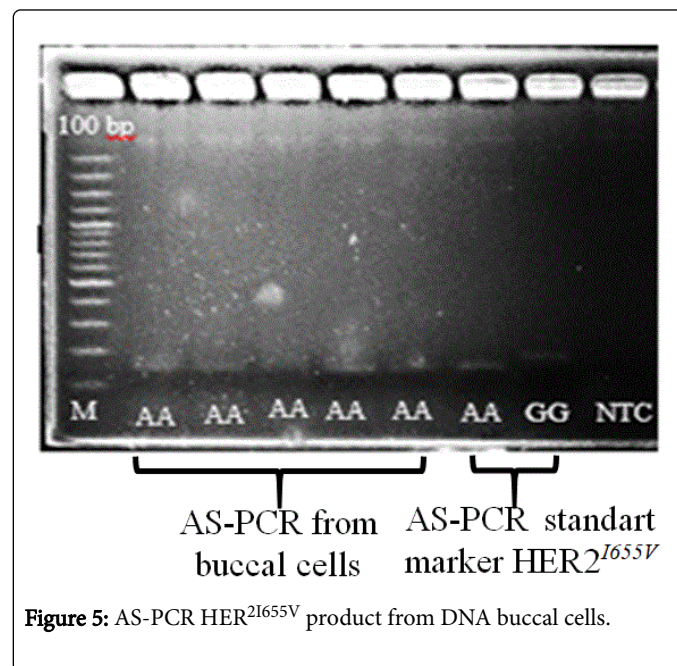


Figure 5: AS-PCR HER^{21655V} product from DNA buccal cells.

Conclusions

HER^{21655V} polymorphisms detection by non-invasive Allele-Specific PCR with five samples from buccal cells gave the best result with AA genotype 142 bp without contamination by Negative Test Control (NTC).

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