Data Article

Dataset reporting detection of breast cancer-related $HER2^{I655V}$ polymorphism using allele-specific polymerase chain reaction

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**Abstract**

The dataset presented in this article is related to the research article entitled “Detection of $HER2$ Gene Polymorphism in Breast Cancer: PCR Optimization Study” (B.R. Budiarto, Desriani, 2016) [1] with some modification in primers used and in PCR optimization strategy to eliminate false-positive result that may occur in $HER2^{I655V}$ polymorphism detection. Combining a new set of primers with PCR gradient, the allele-specific PCR well performs to detect all type of breast cancer-originated $HER2^{I655V}$ genotypes. The validation of this method was done using Sanger DNA sequencing, offering an alternative tool for $HER2^{I655V}$ polymorphism detection in another type of cancer.

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**Specifications Table**

| Subject area                         | Biology                  |
|--------------------------------------|--------------------------|
| More specific subject area           | Molecular Biology, Cancer research |
| Type of data                         | Table and figure         |

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How data was acquired
PCR, DNA sequencing and statistics analysis

Data format
Raw, analyzed

Experimental factors
DNA was extracted from frozen section of breast cancer tissue

Experimental features
Allele-Specific PCR using two specific primer (Forward direction) and one
common primer (reverese direction) to detect HER2<sup>1655V</sup> polymorphism

Data source location
Research Center for Biotechnology, Indonesian Institute of Science (LIPI),
Indonesia

Data accessibility
Data is available with this article

Value of the data
- The data provide a straightforward strategy for clarifying the possibility of a false-positive
amplification generated by allele-specific PCR.
- The data provide the technique to determine the proper dosage of DNA template used in allele-
specific PCR for the success of HER2<sup>1655V</sup> genotyping.
- Data comparison of HER2<sup>1655V</sup> polymorphism between our improved allele-specific PCR with
Sanger DNA sequencing is provided in a way to validate the method easily.
- Allele frequency data for breast cancer-related HER2<sup>1655V</sup> polymorphism can be used as a reference
for researchers who conduct similar experiments for HER2<sup>1655V</sup> polymorphism studies with breast
cancer risk related to specific demography or other races.

1. Data

The detection of breast cancer-related HER2<sup>1655V</sup> polymorphism using allele-specific PCR using
mismatch-specific primers strategy as shown in Fig. 1. The optimum annealing temperature for breast
cancer-related HER2<sup>1655V</sup> genotyping in allele-specific PCR was done using gradient PCR strategy with
SNP-known DNA template (Fig. 2). To test sensitivity of the method, we used two types of genomic
DNA which contain AA genotype or AG genotype in HER2 gene as allele representative with diluted
ranging from 0.019 up to 10 ng of the template at optimized PCR condition (Fig. 3). Then, the reliabil-
ity of allele-specific PCR was compared with data of Sanger DNA sequencing by looking the con-
sistency of HER2<sup>1655V</sup> genotypes data between two methods (Fig. 4 and Table 1). The allele frequency
of breast cancer-originated HER2<sup>1655V</sup> obtained from allele-specific PCR was similar with an allele
frequency data of breast cancer-originated HER2<sup>1655V</sup> from Asian population (Table 2).

2. Experimental design, materials and methods

2.1. Sample collection and genomic DNA extraction

Sixty-one breast cancer tissues from archived biopsies as the frozen section were collected from M.
Djamil Hospital Padang, West Sumatera Province. Genomic DNA was then extracted followed manual
tissue DNA extraction protocol (Pure Link Genomic DNA Mini Kit; Invitrogen). We have also prepared
two types of genomic DNA with SNPs have previously been determined using a Sanger DNA
sequencing method. No.6 tissue-retrieved genomic DNA with HER2 gene carries AG genotype while
DEV (abbreviation of patient’s name) tissue-retrieved genomic DNA with HER2 gene carries AA gen-
otype. All subjects enrolled in this experiment were approved by the local ethics committee, issued by
the Ministry of Health, Republic of Indonesia.

2.2. Allele-specific PCR optimization

The optimum annealing temperature for allele-specific PCR was determined using genomic DNA
with known SNPs. Each of 12.5 μL reaction mixture was contained 11.25 μL of PCR Super Mix
(Invitrogen), 0.1 μM of allele-specific forward primer (5’-CCAGCCCTCTGACGTCCAGCA-3’), 0.06 μM of long allele-specific forward primer (5’-GCGGGCAGGGCGGCGGGGCGGGGCCCCAGCCCTGACGTC- CACCG-3’), 0.15 μM of reverse primer (5’-TCCGTTTCCTGCAGTCTCC-3’), and 0.25 μl (~3–6 ng) of DNA template. The Primers ratio used in this experiment followed the optimized PCR method as suggested by Germer and Higuchi, [2] and Gaundet et al. [3] with minor modification. The PCR amplification profile was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 20 s, gradient temperature annealing from 54.3 °C, 54.9 °C, 57.3 °C, 59.7 °C, 60.8 °C to 62.3 °C for 20 s, and extension at 72 °C for 30 s (Kyratec Super Cycler Thermal Cycler, Australia). All PCR tubes, distilled water, pipette tips, and pipettes were pre-treated by exposing them on UV-light for ± 15-20 minute prior to use. All PCR reagent mixing was done under laminar air flow.

2.3. Sensitivity test of allele-specific PCR

Two genomic DNA with known SNPs (No.6 genomic DNA for AG genotype and DEV genomic DNA for AA genotype) were fixed at 50 ng/μL to make serial dilution ranging from 10 ng declining to
0.019 ng. These diluted DNA then were applied as a template for allele-specific PCR reaction at optimum annealing temperature with PCR reagents and PCR amplification profile followed method mentioned above.

2.4. Validating and genotyping test using allele-specific PCR

Sixty-one samples of breast cancer were tested for HER2^{I655V} polymorphism using optimized allele-specific PCR. The PCR condition and temperature profile were the same as mentioned above except for annealing temperature was fixed at 54.3 °C. The method was validated by direct Sanger DNA sequencing of 61 sample of breast cancer patients by firstly amplified DNA fragment using primer pairs HER2_F (5’-CCAGCCCTCTGACGTCCAT-3’) and HER2_R (5’-TCCGTTTCTGACAGCTCTCC-3’) generating 142 bp PCR product. The PCR amplification profile was as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, an annealing temperature at 60 °C for 30 s, and extension at 72 °C for 30 s. This PCR product was sequenced using HER2_R primer only done by First-Base Asia Ltd.

**Fig. 3.** Sensitivity test of allele-specific PCR in genotyping HER2^{I655V} polymorphism. PCR was performed using optimized PCR condition where annealing temperature was at 54.3 °C. NTC was Non-Template Control. The PCR product was run on 3% agarose under UV illumination.
Fig. 4. Allele-specific PCR of HER2<sub>I655V</sub> polymorphism for 10 of 61 samples of breast cancer patients. (A) Gel electrophoresis data of allele-specific PCR: Yellow arrow head indicated AA genotype with band size of 142 bp; green arrow head indicated GG genotype with band size of 168 bp; red arrows head indicated AA/GG genotype represented two different of PCR band products. The PCR product was run on 3% agarose under UV illumination. M is 100 bp DNA ladder meanwhile NTC (Non-template control) was pointed in lane 11. (B) Sanger DNA sequencing chromatograms of HER2<sub>I655V</sub> polymorphism. Yellow arrow head indicated AA genotype; green arrow head indicated GG genotype; red arrows head indicated AA/GG genotype. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).
2.5. Comparing allele frequency of \( \text{HER2}^{\text{I655V}} \) obtained from our data to published data

Data were tested for statistical significance by using Statistical Package for the Social Sciences software system SPSS-17 statistical software (SPSS, Chicago, IL). Frequency of each \( \text{HER2}^{\text{I655V}} \) allele was calculated using formulation frequency of Ile \( = f(\text{Ile/Ile}) + 0.5f(\text{IleVal}) \), whereas frequency of Val \( = f(\text{Val/Val}) + 0.5f(\text{IleVal}) \). Chi-square test was used to analyze differences in genotype frequencies between our data with published dataset to evaluate any possible deviation from Hardy–Weinberg equilibrium of our genotyping data. Genotype frequencies are interpreted as statistically significant different if only the \( P \) value is less than 0.05 [4].

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.09.033.

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