Comparative Evaluation of Heat Shock and Electroporation Transformation of Canine Homlogue of HER2 Gene Cloned in a PUC57 Plasmid Construct

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1. Abstract

Human epidermal growth factor receptor 2 (HER2) is an important prognostic marker in the case of breast cancer and other tumors. Canines remain one of the most important models for comparative oncology with humans due to the great similarity in the spontaneous presentation and development of cancer, and in the high homology in the amino acid sequence within the HER2 gene-encoded polypeptide. In this study, the canine homolog of the HER2 DNA sequence constructed in PUC57 plasmid was transformed in DH5α *E.coli* by heat-shock and electroporation. The plasmid was extracted from DH5α by Miniprep protocol and the HER2 gene 3738 bp was successfully amplified from PUC57 plasmid by polymerase chain reaction (PCR). Both heat shock and electroporation were efficient and resulted in the suspected amplicon. However, electroporation gave a higher transformation rate. HER2 DNA sequence will be used for the production of the recombinant HER2 protein in the near future.

Keywords: *Recombinant HER2, Breast cancer, Electroporation, Heat shock, Transformation.*

2. Introduction

Human Epidermal growth factor (HER2) protein, a member of the human epidermal growth factor receptors (EGFR), plays a significant role within the pathogenesis of several mammalian cancers like breast, ovary, uterine, cervix, stomach, colon, lung, bladder, head, neck and esophagus cancers [1, 2].

The overexpression of HER2 results in cell proliferation and tumorigenesis and occurs approximately in 20-30% of breast cancer cases. HER2 positive tumors are generally correlated with a quick rate of tumor progress and a poor prognosis [3].

HER2 is overexpressed also in canines and is related to poor clinical diagnosis. Canines are the foremost available model for comparative oncology with humans due to the great similarity within the spontaneous cancer presentation and development [4].

Recombinant DNA technology has enabled scientists to supply an enormous number of diverse proteins in large quantities in micro-organisms, that was previously unavailable and somewhat expensive [5].

The recombinant protein expression in cells in which they do not occur naturally is termed heterologous Protein production. Bacterial expression systems are widely used for the preparation of heterologous eukaryotic as well as prokaryotic gene products [6].

E.coli is the preferable model for cloning and expression for several reasons viz: *E. coli* cells have a fast growth rate and their generation time in convenient environmental conditions is about 20 minutes [7]. Moreover, high

cell density cultures (about 200-gram dry cell weight/liter) can be obtained [8,9]. The availability of plenty of host strains and vectors developed for boosting expression rate and the presence of huge data about the physiology and genetics of *E.coli* have added more advantages to the host. Another reason, the transformation of *E.coli* with DNA is a rapid, straightforward process and cost-effective [10,11].

Transformation of *E. coli* is performed by Electroporation and heat shock method [12,13].

The heat shock method depends on the exposure of competent *E. coli* cells to temperature shock from 0˚C to 42˚ C which facilitates exogenous DNA entry [14].

Electroporation relies on increasing cell membrane permeability due to exposure to an electric field of a certain amplitude. The increased membrane permeability enables molecules that cannot enter cells in a normal state to get in and out of the cell [15].

The present investigation was directed to clone HER2 DNA sequence in *E.coli* cells by the aforementioned two main methods of transformation. In the next segment of the investigation series, the HER2 DNA sequence will be used for the expression of the recombinant HER2 protein.

3. Materials and Methods

3. 1 Transformation of PUC57-HER2 into chemically competent DH5α *E. coli* cells:

HER2 gene insert of the dog (*Canis lupus familiaris*), (accession number EU239091) constructed in PUC57 cloning vector (Biomatik, USA) was employed in this study. The plasmid was supplied as a modified version to be expressible in prokaryotes in high yield. Lyophilized PUC 57 plasmid containing HER2 gene insert was reconstituted with nuclease-free water to produce a working concentration of 10 ng/µl.

The reconstituted plasmid was transformed in *E. coli* DH5α chemically competent cells (Takara Corp., Japan). Heat shock was carried out by mixing one microliter of PUC57 plasmid with 100µl of DH5alpha cells followed by incubation on ice for 30 minutes. The proper heat-shock step was done at 42˚C for 30secounds and consequent incubation on ice for 2 minutes.

SOC (Super optimal broth for catabolite repression) medium was added to the transformed cells to a final volume of 1 ml. The transformed cell suspension was incubated at 37˚C for 1hr with shaking at 200 RPM on an orbital shaker. One hundred microliters were spread on LB agar plate (Serva, Germany) using a sterile glass spreader [16] followed by incubation of the inoculated plates at 37˚C for overnight.

2. 2. Transformation of PUC57-HER2 gene into electrically competent DH5α *E.coli* cells:

One microliter of PUC57- HER2 was added to 50 µl DH5α electrically competent cells (Takara corp., Japan) into a pre-chilled electroporation cuvette (Invitrogen™, USA). The cuvette was placed into the electroporator (BTX, USA) adjusted to 2.5KiloVolt electric current, with the capacitance of 25 microfarads and resistance of 200 ohms [17]. After few milliseconds, 1 ml SOC medium was immediately added into the

electroporation cuvette, incubated for 1 hour at 37˚C with shaking 200 RPM and plated on LB agar plate then incubated overnight at 37˚C.

3. 3. Miniprep recovery of HER2-PUC57 plasmid from *E.coli* cells:

PUC57 cloning plasmid was extracted from *E.coli* cells by Genejet plasmid miniprep kit (Thermofisher, USA) using alkaline lysis method [18]. Briefly, a colony was picked up, inoculated in 5 ml LB broth and incubated overnight with shaking at 37˚C and 200 RPM. The bacterial cell suspension was centrifuged and the pellet was resuspended in 250 µl solution containing RNase then lysed using 250 µl lysis buffer. After thorough mixing of cells with lysis buffer, neutralization solution was added followed by centrifugation at 6000 xg for 5 minutes. The supernatant was collected carefully and added to a silica-based spin column. After centrifugation, the column was washed twice with 500 µl washing buffer and eluted in 100 µl Elution buffer (10 mM Tris-HCl, pH 8.5). The concentration of the eluted plasmid DNA was measured using a Nanodrop spectrophotometer.

3. 4. The polymerase chain reaction of HER2 coding sequence:

The coding sequence of the HER2 gene (3738 bp) was amplified from PUC57 plasmid by polymerase chain reaction (PCR) using a specific forward primer 5`GACTGCAGGAGCTCGCCCTGTT ACCC-3` and reverse primer 5`TAGCGGCCGCGTCGACCCCCAA GTACTCTGGGT-3` using the high fidelity prime star polymerase (Takara, Japan). The PCR mixture contained: 1X prime star GXL buffer, 200 µM dNTPs, 1 µM of each primer, 4 ng of DNA, 1.25 units of prime star polymerase and nuclease-free water up to 50µl total. PCR was performed using the 3 PCR steps cycle according to the manufacturer instructions (denaturation 98˚C for 10 seconds, annealing 60˚C for 15 seconds, and extension 68˚C for 3 minutes) for 30 cycles. The PCR product was visualized using electrophoresis in 0.7% agarose against 1 kb DNA ladder (Serva, Germany).

4. Results

4. 1. Transformation of PUC57-HER2 into *E. coli* competent cells:

Seventy-six colonies grew on the LB agar plate after overnight incubation at 37˚C in the case of chemically competent cells. On the other hand, 266 colonies were encountered in the case of electro-competent cells (figure 1). Transformation efficiency was calculated by the following equation: (Number of bacteria colonies × dilution ratio × original transformation volume (µl)) ÷ (plated volume (µl) × transformed DNA (µg)) [19]. The transformation efficiency in the case of electrocompetent cells was 5.2×10^{6} cfu/µg against 7.6×10^{5}cfu/µg in the case of the chemically competent cells (table 1). Miniprep of plasmid yielded 200 ng DNA/µl with an absorbance of 1.88 at 260/280 nm wavelength. Concerning the DNA concentration and purity of the mini preparation product following the electroporation transformation, the concentration was 176.9 ng/µl and the OD value at 260/280 nm wavelength was 1.9.

4. 2. The polymerase chain reaction of HER2 coding sequence:

A specific HER2 gene product (3738 bp) was detected via agarose gel

electrophoresis against Serva 1 Kb ladder as shown in figure (2). No differences were detected between the PCR products using plasmid DNA harvested from either electroporated or heat-shocked competent cells.

5. Discussion

The current study targeted transformation trials followed by confirmatory PCR of a synthesized Canine HER2 gene homologue PUC57 plasmid construct. This study represents a segment in a research project for investigating the canine breast cancer receptors and encoding genes as a model simulating the human counterpart. Competent DH5α *E.coli* cells were utilized as the host cells. The transformation was conducted by heat shock and electroporation methods as a preliminary step in the expression of the recombinant HER2 protein.

The cloned HER2 DNA sequence was modified by the construct supplier (Biomatik, USA) to exclude codon bias as prokaryotes show certain codon preferences different from Eukaryotes. This modification ensures efficient cloning and expression in *E.coli* in high amounts [10].

The Transformation efficiency is the number of colonies (transformants) per microgram of DNA used. The PUC57-HER2 plasmid construct was supplied with DNA concentration of 10 ng/µl. To be optimized for ideal heat shock and electroporation protocols, the DNA was diluted to a total volume of 1000 µl with SOC medium, of which 100 µl were plated. Therefore an equivalent of 1 ng of plasmid DNA was plated on each agar plate.

The obtained results showed that the Electroporation gave higher transformation rates than the classic heat shock method (table 1). This finding came in agreement with similar studies in which electroporation was found to be superior to the heat-shock transformation when higher transformation efficiency, as well as plasmid DNA harvest, are considered. Moreover, electroporation is faster and relatively effortless. However, electroporation requires well-purified DNA and even low concentrations of residual salts from DNA preparations may interfere with the electrical current and causes arcing, resulting in excessive cell death and lower transformation efficiency [20]. Therefore, some researchers prefer the heat shock method of chemically competent cells because of its easy cost-effective procedure, consistent results, and absence of special devices [21].

Considering the PCR products from minipreps of plasmid harvests obtained from heat-shocked or electroporated cells, both transformation protocols resulted in almost similar products.

6. Conclusion

From this study, it was concluded that the HER2-PUC57 plasmid construct can be transformed efficiently and that selection of the transformation method can rely on the laboratory facilities rather than the product quality. The amplified HER2 PCR product will be employed for expression to obtain HER2 protein that can be used as an immunogen for the preparation of nanobodies. This is running and awaiting results that will be announced and published in a separate article.

7. Acknowledgment

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8. Conflict of interest

No conflict of interest to be declared.

9. Ethical approval: No ethical approval present because we didn't use laboratory animals.

10. References

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Table 1: Transformation efficiency after heat shock and electroporation of puc57 plasmid in DH5α *E. coli*

Comparison issue	**Heat shock method**	**Electroporation**
Number of bacteria colonies/plate	76	266
Dilution ratio	10	20
Transformation volume µl	1000	1000
Plated volume µl	100	100
Transformed DNA µg	0.01	0.01
Transformation efficiency cfu/µg	7.6×10^6	5.34×10^6

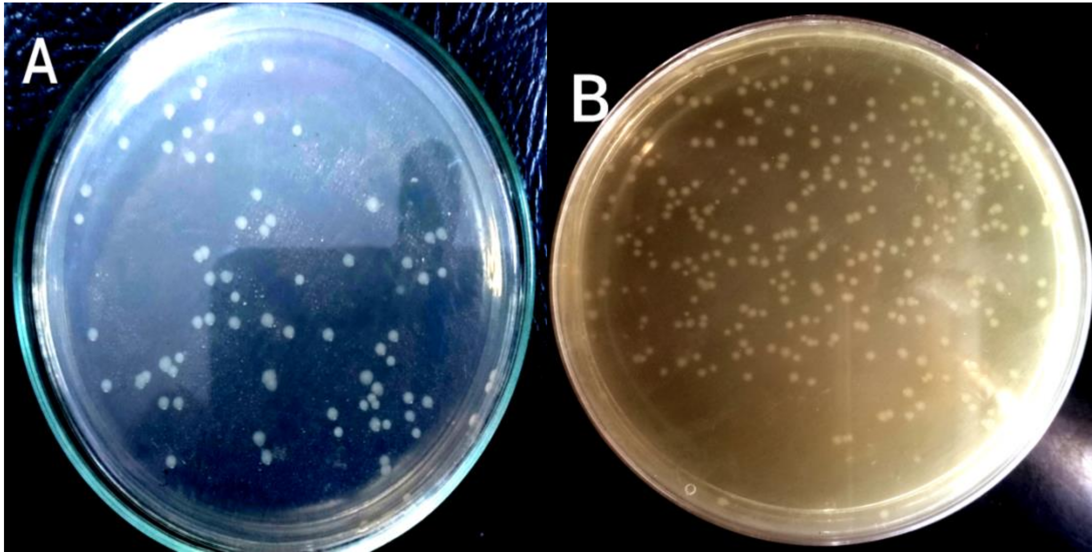

Fig. 1: LB agar plates of DH5α *E.coli* transformed with the PUC57 plasmid construct.

A: Heat shock transformed cells.

B: Electroporation transformed cells.

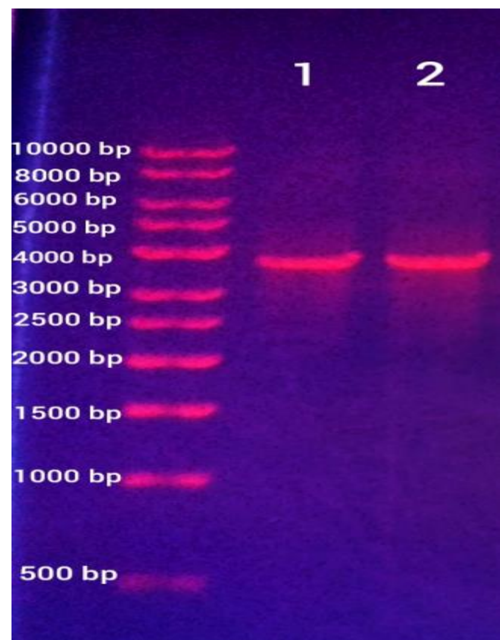

Fig. 2: Agarose Gel electrophoresis of HER2 gene in PUC57 plasmid construct. DNA size marker (1kBp Serva ladder, Germany): sizes of the different fractions of the ladder are illustrated. Lane 1: specific HER2 gene band (3738 bp) of the electroporated cell harvest. lane2: specific HER2 gene band (3738 bp) of the heat-shocked cell harvest.