GENERATION A TRANSGENIC ZEBRAFISH MODEL OF PARKINSON DISEASE

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ABSTRACT

WIRENI AYUNINGTYAS. J2B 004 096. **. Generation A Transgenic Zebrafish Model Of Parkinson Disease.** Under the guidance of Herma van der Linde, Dr. Rob Willemsen and Dr. Annemiek Wilmienk.

The aim of this study was to clarify the mechanism of human Leucine- Rich Repeat Kinase 2 (hLRRK2) gene with point mutation of amino acid substitution from glycine to serine at residue 2019 (G2019S) in parkinson disease (PD). This is done by cloning the hLRRK2 G2019S gene into a vector with GFP (green fluorescence protein) under neuron-specific zebrafish Tyrosine Hydroxylase (zfTH) promoter. The promoter drives over-expression of a fusion protein between reporter gene-green fluorescent protein (GFP) - and LRRK2 gene holding a missense mutation (G2019S) driving in dopaminergic neuron. This research utilizing two methods of cloning, the first method was cloning the LRRK2 G2019S to $pTGL_{wt}$ by digestion and ligation. The second method was cloning of LRRK2 G2019S by site directed mutagenesis. This study also observing the behaviour of the wild type zebra fish using a locomotor activity test as a control for the transgenic zebrafish in further studies. Under the method of digestion and ligation, $pTGL_{wt}$ and $pCGL_{G2019S}$ were isolated (mini and maxi preparation). Subsequently, the sequencing process is applied to the whole construct of $pTGL_{wt}$ and $pCGL_{G2019S}$ prior to digestion of both constructs. Purification of $pTGL_{wt}$ and $LRRK2_{G2019S}$ is done using TAE agarose gels with Nucleospin Extract II Kit. Afterwards, transformation ligation mix is done from pTGL and $LRRK2_{G2019S}$ into *E.coli* competent cell, which the gene inserted is checked later using colony PCR before applying another sequencing.

By site directed mutagenesis, PD- associated mutants (LRRK2 G2019S) were introduced in $pTGL_{wt}$ with primers that contain point mutation during PCR processing. This study is succeeded in resulting a construct of $pTGL_{G2019S}$ which will be useful in studying the transgenic zebrafish as the model of PD.

Key word : clone, LRRK2 gene, Parkinson disease, zebrafish

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ABBREVIATIONS

bp	base pairs
DNA	Deoxyribo Nucleid Acid
dNTP	deoxyribonucleotide triphosphate
dpf	day after fertilization
Exo	Exonuclease
GFP	Green Fluorescence Protein
G2019S	amino acid substitution from glycine to serine at residue 2019
	$pTGL_{wt}$
hLRRK2	human Leucine-rich repeat kinase 2
$hLRRK2_{G2019S}$	human Leucine-rich repeat kinase 2 with point mutation G2019S
kb	kilo base pairs
LB	Lactose Broth
LBs	Lewy Bodies
LRRK2	Leucine-Rich Repeat Kinase 2
MgCl	Magnesium Chloride
pCGL	plasmid with construct CMVpromoter, GFP, and LRRK2
$pCGL_{G2019S}$	plasmid with construct CMVpromoter, GFP, and LRRK2 with point mutation G2019S
PCR	Polymerase Chain Reaction
PD	Parkinson Disease
pTG	plasmid with construct TH promoter and GFP
pTGLwt	plasmid with construct TH promoter, GFP and wild type LRRK2 gene
$pTGL_{G2019S}$	plasmid with construct TH promoter, GFP and LRRK2 gene with G2019Smutation
Sap	Shrimp Alkaline Phosphate
SDM	Site Directed Mutagenesis
SDM pTGL	pTGL construct from site directed mutagenesis
SDM $pCGL_{G2019S}$	$pCGL_{G2019S}$ construct from site directed mutagenesis
TH	Tyrosine Hydroxylase
wt	wild type

zf	zebrafish
zLRRK2	zebrafish Leucine-rich repeat kinase 2
zf TH	zebrafish Tyrosine Hydroxylase