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Specific Primer Design to Detect Connexin-36 Gene in *Rattus Norvegicus Brain*

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ABSTRACT

Connexin-36 (Cx36) is the intercellular channel proteins which most commonly found in the brain. The study aims to obtain the best primary design for analyzing Cx36 expression in the brain of mice using the Quantitative Real Time PCR (qRT-PCR) method. This type of research is a true experimental study using mRNA from four mice brains as the sample. In silico was analyzed using Primer 3 and OlygoAnalizerTM by IDT tools using gen database from NCBI. Three primes with the most optimal criteria were then evaluated using the qRT-PCR method. The results showed that all of the primers was able to amplify the Cx36 gene using qRT -PCR. The best primer, showed a single melt curve, is the primes no.5 with the range Tm 80-81°C. Comparison results of previously published in silico Cx36 primers showed that primer no. 5 also had an optimal value on each criterion. So the primary design result that has the optimum specificity and effectiveness to amplify the Cx36 gene in the brain of Rattus norvegicus rats is primer no.5 (F: 5'-ATTTCCCGCTTCTACATCATCCAAG-3' and R: 5'-CACAGCAAACACCAGAAAG-3').

Keywords*: Primer, Cx36, qRT-PCR, Rattus norvegicus.*

INTRODUCTION

Connexin-36 (Cx36) is a protein that forms a gap junction channel in granule cell axons located in the dentate gyrus. It is also the main connexin isoform that forms electrical synapses in the brain of adult mammals (Rimkute *et al.*, 2018). In addition, the gap junction channel played an important role in intercellular communication of central nervous system(Dong, Liu and Li, 2018). This protein is encoded by Cx36 gene located on chromosome 2 with a transcriptional start in exon I (Teubner *et al.*, 2000). The expression of Cx36 increases during the first two weeks of postnatal development (Deepthi Thomas and Joanne Mm Senecal, 2020).

The qRT-PCR method is reliable for determining the expression of Cx36 at the gene level. One of the main components that determine the success of qRT-PCR method is the highly specific and sensitive

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primer(Zhu et al., 2020). The primer acts as a marker to amplify the specific target of DNA fragments (Cahyadi *et al.*, 2018). Specific primers should anneal precisely at desired gene. If the chosen primer pair do not efficiently anneal to the sample RNA or anneal to other parts inhibiting to PCR reaction, the PCR result will be affected as a little or no product yield. Moreover, if the primer anneals to non-targeted region, then the PCR reaction will amplify undesired regions of RNA (Dong, Liu and Li, 2018). Therefore, the highly specific primer urgently needs to accurately amplify Cx36 gen using qRT-PCR.

Several requirements are needed to be fulfilled by a PCR primer to obtain a successful PCR reaction. The requirements are (1) primer length is around 18 to bases, (2) melting temperature (Tm) of the primer fall between 60-67°C, (3) GC content is about 40-60% and relatively similar between forward and reverse primer, (4) less probability to form secondary structures such as hairpins, dimers, heterodimers within pairs, and self-complement (Stujanna *et al.*, 2022).

Secondary structure is important factor to consider when designing a primer because the primer may anneal to each other much more readily than to the template(Cahyadi *et al.*, 2018). Primer may anneal to each other, called primer dimer or anneal to itself, to form primer hairpins (Cahyadi *et al.*, 2018). In silico study using some commonly used and free web-based design tools may prevent dimerization of the primers (Cahyadi *et al.*, 2018). Some widely used tools are Primer3Plus, OligoAnalyzer™ by IDT, and Primer-BLAST.

Study related to the primer design for the Cx36 was limited. Therefore, the purpose of this study is to design a specific primer that could be used to amplify the Cx36 gene of rat brains by the qRT-PCR method.

METHOD

1. In silico Primer Design

In silico primer design was carried out using free web-based bioinformatic tools, namely Primer3Plus (Primer3Plus (bioinformatics.nl), OligoAnalyzer™ by Integrated DNA Technologies (IDT) (OligoAnalyzer Tool - primer analysis | IDT (idtdna.com) (IDT), and Primer-BLAST at the National Center for Biotechnology Information (NCBI) (National Center for Biotechnology Information (nih.gov)), and Molecular Evolutionary Genetic Analysis ver11 (MEGA11).

The first step was obtaining the Cx36 gene sequences at the NCBI site in the "Gene" sub-search tool. Then, "RefSeq"

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and "Rattus norvegicus" was selected in sequence content and organism option. Next, coding sequences of the well-annotated gene were downloaded in FASTA format and used in the subsequent steps.The gene sequence collected may have several isoforms. Hence, multiple sequence alignment was done to the genes using MEGA 11 tools to obtain the conserved region of the gene used in primer design. The conserved sequence was divided into several groups of 300 and 200 base lengths. This step aimed to obtain pairs of primer with proper amplicon lengths to produce specific sequences used as the template.

The first tool used in designing primer during this study was Primer3Plus. It was started on the "General and Advance Setting" menu for the desired primary criteria. Primer length was set no more than 15-30bp, product size was around 150-250bp, Tm was approximately 55-65°C, GC content percentage of 40-60 %. Primers which close to optimum value in each parameter were then selected for further analysis. Further tests with OlygoAnalyzer™ by IDT were carried out to assess the possibility of the primers forming secondary structures, such as hairpins, self-dimers, and heterodimers. The primers were then analysed with Primer-BLAST to determine the specificity of the primer to the target gene. Then the 3 best primers were selected and synthesized by IDT inc.

2. RNA Isolation of Rat's brain

The organs used in this study were brain of rats stored in -80°C freezer from the previous study. The brains were collected from 4 female *Rattus norvegicus* rats with 6 weeks old age and body weight of 100-150 grams. The brain was homogenized, washed, and purified by Promega SV Total RNA Isolation System kit manual procedure (Promega, 2004). The concentration and purity of the isolated RNA were measured by using absorbance in 260/280 nm using the Thermo Scientific Varioskan-LUX multimode reader.

3. Detection of Cx36 gene using qRT-PCR method

The isolated mRNA was used as a template for qRT-PCR test. This study used two-step qRT-PCR. The first step was using Toyobo ReverTra Ace™ qPCR RT Master Mix kit with gDNA remover (Toyobo, 2004a) to produce cDNA by reverse transcription reaction(Toyobo, 2004b). The process was done in an Applied Biosystem Veriti 96-well Thermal Cycler instrument. The second step of PCR was quantitative PCR which was done using Toyobo THUNDERBIRD™ SYBR™ qPCR Mix (No and Co, 2004).

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The process was done in Applied Biosystem 7500 Fast Real-Time PCR System machine.

4. In silico study of pre-existing primer

The primer reference was obtained from a relevant article in PubMed conducted on June 28, 2022. The pre-existing primer was collected from published articles tested using the same in silico tool and then compared to the latest primer obtained in this study.

RESULT

1. Cx36 in silico Primer Design

The Cx36 gene of *Rattus norvegicus* was found in NCBI as Gjd2 gene (Gap junction protein delta 2) with RefSeq gene ID 50564. Coding sequences with the accession number NC_051338.1 were referred to by the RefSeq page of the gene and then used as the template for the next step. This gene has two isoforms aligned using the MEGA 11 tools to gain the conserved sequence. The results of the primer design performed using Primer3Plus and further analyzed with OlygoAnalyzer™ by IDT and Primer-BLAST are summarized in Table 1. Based on the data analysis, primer numbers 5, 8, and 7 showed the most optimum value on all the criteria

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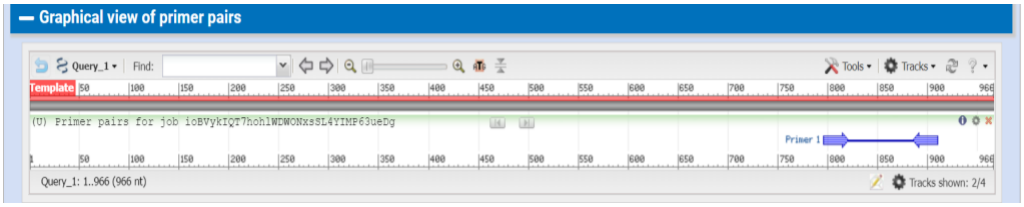
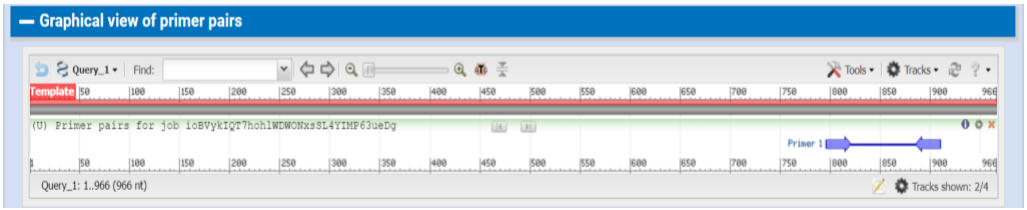
Table 1. Cx36 Primer Design Results

No	Sequence Position		Length of sequences	Base	Start	Product	Tm	GC	Hairpins		Dimers		Heterodimers		Self-complementarity	Self-3' complementarity	Blast
									Sum	ΔG	Sum	ΔG	Sum	ΔG			
1	601-900	F	TTCTATATGGCTTCAGTGTCCCAGG	25	56	234	65,5	48	2	-0.49 – 0.4	3	-5,02	5	-6.47	3.00	0.00	>XM_039105729.1
		R	TCTCATAGACTGACTTCCTCTTGGC	25	289		65,1	48	6	-0,71 - 0,24	3	-3,53			4.00	0.00	
2	667-966	F	GTGCTCAATCTGGCTGAACTTAACC	25	130	115	65,5	48	4	-0,65 - 0,34	4	-4,85	3	-5,09	4.00	3.00	>XM_039105729.1
		R	TTGGCAGGTCTTTGTTACGAATCTC	25	244		65	44	6	0,45-1,37	2	-3,61			5.00	2.00	
3	667-966	F	GCTCAATCTGGCTGAACTTAACCATC	26	132	113	65,5	46,2	5	-0,49 - 0,41	3	-5,02	3	-5,02	5.00	1.00	>XM_039105729.1
		R	TTGGCAGGTCTTTGTTACGAATCTC	25	244		65	44	6	0,45 - 1,37	2	-3,61			5.00	2.00	
4	567-866	F	GGTATTTCCCGCTTCTACATCATCC	25	14	201	64,6	48	2	-0,16 - 0,06	2	-3,61	4	-5,46	3.00	0.00	>XM_039105729.1
		R	CACAGCAAACATGAACACCAGAAAG	25	214		64,8	44	3	-0,08 - 0,32	4	-5,38			4.00	0.00	
5	567-866	F	ATTTCCCGCTTCTACATCATCCAAG	25	17	198	64,8	44	2	0,73 - 1,45	2	-3,61	4	-5,46	3.00	0.00	>XM_039105729.1
		R	CACAGCAAACATGAACACCAGAAAG	25	214		64,8	44	3	-0,08 - 0,32	4	-5,38			4.00	0.00	
6	601-800	F	TTCTATATGGCTTCAGTGTCCCAGG	25	56	119	65,5	48	2	-0,49 - 0,4	3	-5,02	4	-5,47	4.00	3.00	>XM_039105729.1
		R	AAACATGAACACCAGAAAGACCGTC	25	174		65,4	44	1	0,32	4	-5,38			5.00	5.00	
7	601-800	F	TTCTATATGGCTTCAGTGTCCCAGG	25	56	122	65,5	48	2	-0,49 - 0,4	3	-5,02	4	-5,47	4.00	3.00	>XM_039105729.1
		R	AGCAAACATGAACACCAGAAAGACC	25	177		65,7	44	2	0,31 - 0,32	4	-5,38			4.00	0.00	
8	767-966	F	GTGCTCAATCTGGCTGAACTTAACC	25	30	115	65,5	48	4	-0,65 - -0,34	4	-4,85	3	-5,09	4.00	3.00	>XM_039105729.1
		R	TTGGCAGGTCTTTGTTACGAATCTC	25	144		65	44	6	0,45 - 1,37	2	-3,61			5.00	2.00	
9	101-300	F	GGTGTACGATGATGAGCAGACC	22	32	96	64,2	54,5	3	0,24 - 0,71	3	-4,41	2	-3,61	4.00	3.00	>XM_039105729.1
		R	TATATGGGAGATGGGAAAGGCGCG	23	128		67,7	54,2	2	0,57 - 0,4	4	-10,36			4.00	4.00	
10	101-300	F	GGTGTACGATGATGAGCAGACC	22	32	101	64,2	54,4	3	0,24 - 0,71	3	-4,41	4	-5,91	4.00	3.00	>XM_039105729.1
		R	AACGTATATGGGAGATGGGAAAGGC	25	132		66	48	3	0,22 - 0,57	4	-6,3			4.00	2.00	
11	201-400	F	CCTGCTGAGTCTATAGGGGGAC	22	58	78	64,7	59,1	5	-0,76 - -0,12	6	-6,59	3	-5,09	6.00	1.00	>XM_039105729.1
		R	TGCAACTTCTTATCTTCTCGCTTGC	25	137		65,4	44	1	-1,33	4	-7,05			4.00	2.00	
12	601-800	F	TGCATCAAGGAGGTAGAATGTTATGTG	27	6	114	64,9	40,7	5	-0,02 - 0,98	4	-7,05	4	-5,47	4.00	0.00	>XM_039105729.1
		R	GTTAAGTTCAGCCAGATTGAGCACC	25	119		65,5	48	4	-0.68 - 0,1	4	-4,85			4.00	0.00	
13	601-800	F	TGCATCAAGGAGGTAGAATGTTATGTG	27	6	193	64,9	40,7	5	-0,02 - 0,98	4	-7,05	3	-4,64	4.00	0.00	>XM_039105729.1
		R	GTTAAGTTCAGCCAGATTGAGCACC	27	198		63,9	48	2	-0,87 - -0,21	2	-3,61			4.00	0.00	

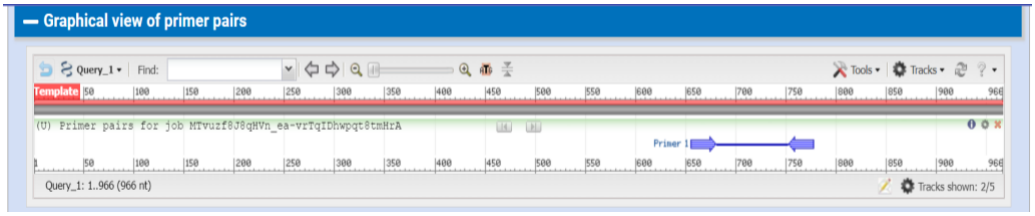
Accuracy of the primer position also dramatically affects the results. The following is an overview of the primer position on the Cx36 gene sequence:



Primer no. 5



Primer no. 8



Primer no. 7

Figure 1. Overview of Primer Position of Cx36 gene

Septiasari, A., Ningsih, Sri S., Stujanna, Endin N., Avissa R., Ujianti I. 2024. **Specific Primer Design to Detect Connexin-36 Gene in *Rattus Norvegicus* Brain**. Surabaya : *The Journal of Muhamadiyah Medical Laboratory Technologist*. Vol: 7, No.1 (14-24). DOI : 10.30651/jmlt.v7i1.17985

2. Isolation of mRNA

The results of total RNA extracted from the rat brain were measured for its concentration and purity. The isolated RNA concentration and its purity are shown in table 2. The data showed that all samples have appropriate purity and concentration.

Table 2. mRNA results and tissue purity

Organ	mRNA concentration (ng/µl)	Purity 260/280
Rat Brain 1	91.51	1.7564
Rat Brain 2	102.1	1.7867
Rat Brain 3	56.49	1.6713
Rat Brain 4	89.93	1.7439

3. qRT-PCR Cx36 gene

mRNA samples were employed as templates for qRT-PCR test. The best three primers (primer number 5, 8, and 7) were performed as primers to amplify Cx36 gene. Table 3 resumed the results of the CT and melted temperature of the 3 best pairs of primers:

Table 3. PCR results of Cx36 gene

No	Sample	CT βactin	CT Cx36	Tm_1	Tm_2	NTC
Primer 5	Rat 1	19,185	28,967	81,776	-	*undetermined*
	Rat 2	18,065	30,527	79,282	-	
	Rat3	18,983	30,484	82,321	-	
	Rat 4	18,950	31,777	79,282	-	
Primer 8	Rat 1	19,185	28,151	81,776	-	36,938
	Rat 2	18,065	27,394	79,282	-	
	Rat 3	18,983	29,342	82,321	-	
	Rat 4	18,950	30,592	79,282	-	
Primer 7	Rat 1	19,185	25,600	74,138	79,984	25,674
	Rat 2	18,065	24,226	74,761	-	
	Rat 3	18,983	25,460	74,839	80,607	
	Rat 4	18,950	25,794	74,761	80,607	

Septiasari, A., Ningsih, Sri S., Stujanna, Endin N., Avissa R., Ujianti I. 2024. **Specific Primer Design to Detect Connexin-36 Gene in *Rattus Norvegicus* Brain**. Surabaya : *The Journal of Muhamadiyah Medical Laboratory Technologist*. Vol: 7, No.1 (14-24). DOI : 10.30651/jmlt.v7i1.17985

Based on the data above, the average β-actin CT value was approximately 18-19, while the CT value for Cx36 was 24-31. Primer number 5 and 8 showed single melting temperature which around 79-82°C. Whereas primer number 7 showed double melting temperature around 74-80°C. Melt curve graphs represented the primary specificity in amplifying the target gene (Figure 5). The single and stream peak was performed by β-actin and primer 5. Non-Template Control (NTC) was performed in this reaction as negative control. The NTC results were not detected only from primer no. 5

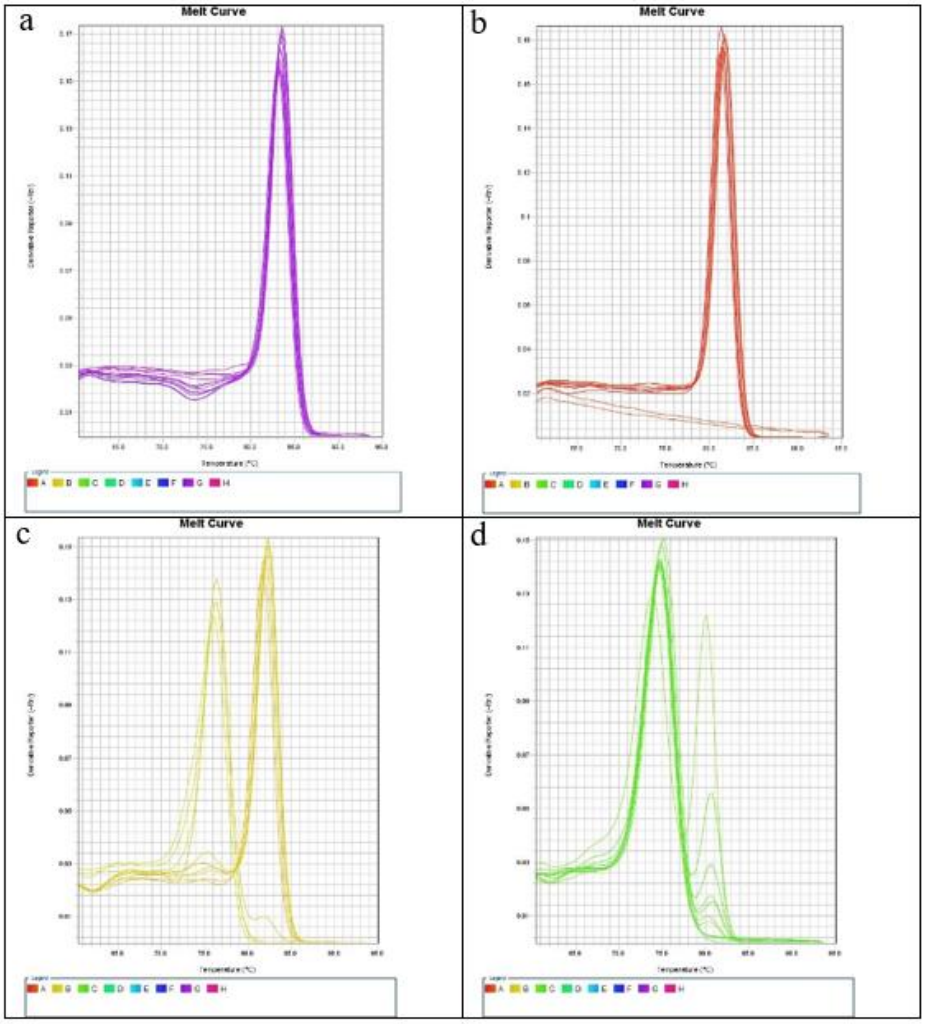


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Figure 5: Overview of melt curve (a) ß-actin; (b) primary no. 5; (c) primary no. 8; (d) primary no. 7

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4. In silico test of pre-existing primer

Reference examined at PubMed on 28th June 2022. The study obtained seven relevant articles, and then the 3 most relevant articles were chosen because they list the complete sequences of the Cx36. Next, the pre-existing primer was compared with primer no.5 in silico. Table 4 presents the results of in silico analysis between pre-existing primer and primer no.5. In silico test results showed that the primer no.5 (d) has fairly close to the optimum value for all of the criteria.

Table 4. Cx36 Primary Design Reference

	References		Length of sequences	Base	Start	Product	Tm	GC	Hairpins		Dimers		Heterodimers		Self-complementarity	Self-3' complementarity	BLAST
									Sum	ΔG	Sum	ΔG	Sum	ΔG			
a	(Beheshti, Zeinali and Esmaeili, 2017)	F	GCCGGTACTCGACTGTCTTC	20	320	107	63,7	60	2	-1,55 - -0,67	9	-9,75	12	-5,19	5.00	0.00	>XM_039105729.1
		R	ATCTTCTCGCTTGCTCCCAC	20	426		64,1	55	2	1,06 - 1,24	6	-3,61			2.00	0.00	
b	(Calabrese *et al.*, 2003)	F	CACAGCGATGGGGGAATGGA	20	400	149	66,3	60	1	-1,56	7	-3,61	19	-6,14	4.00	0.00	>XM_039105729.1
		R	TGCCCTTTCACACATAGGCA	20	548		64,1	50	2	-2,46 - -2,25	9	-8,16			4.00	1.00	
c	(Nicholson *et al.*, 2001)	F	GGCTGCTGGAAGCCGCGGTGC	21	26	897	73,9	76,2	2	-3,07 - -2,52	16	-16,5	18	-9,82	6.00	4.00	>XM_039105729.1
		R	GAAGACAGTAGAGTACCGGCGTT	23	922		65,3	52,2	4	-0,09 – 0,46	11	-9,75			4.00	3.00	
d	567-866 (the best primer)	F	ATTTCCCGCTTCTACATCATCCAAG	25	17	198	64,8	44	2	0,73 - 1,45	2	-3,61	4	-5,46	3.00	0.00	>XM_039105729.1
		R	CACAGCAAACATGAACACCAGAAAG	25	214		64,8	44	3	-0,08 - 0,32	4	-5,38			4.00	0.00	

DISCUSSION

In silico primer design of this study produced 13 pairs of primers (Table 1). All primer pairs have nearly close to optimum primer and amplicon length (Praja and Rosalina, 2021). Optimum primer and amplicon length are closely related to lack of probability of mispriming risk. Correspondingly, the Tm number of all primer pairs showed quite close to optimal value. It means that the primer has proper capability to produce a good PCR product (Cahyadi *et al.*, 2018). On the other hand, GC content of all primer pairs most likely different between forward and reverse primer. Different GC content potentially reduced the effectiveness of primer annealing process on the qRT-PCR (Dong, Liu and Li, 2018). The three best primers were selected based on the closest to the optimal value on all criteria and the least possibility of conforming to secondary structures. The best chosen primers are primer no. 5, primer no. 8 and primer no. 7. These three primers would be further evaluated using qRT-PCR.

All total extracted RNA from Rat's brains were used as the sample templates for qRT-PCR reaction. The recommended optimum value of RNA purity is 1.7-2.0 260/280 absorbance value (Lusian, 2021). Table 2 shows that all the extracted RNA in this study has sufficient concentration and is free of protein contaminants as well. Therefore, it could be used for subsequent experiments.

Based on qRT-PCR result, all the samples presented amplification plots on the Cx36 and ß-actin genes (data not shown). Table 3 shows the CT values of ß-

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actin and Cx36 genes. The CT values showed the cycle that gene amplified through the threshold line (Zhu, Korabeˇ and Neuˇ, 2020). It proved that all three primers were able to amplify the gene using qRT-PCR method. Those data have not confirmed whether the amplified gene was the desired gene or not. It could be evaluated by analyzing the melting temperature graphs and values. Highly specific primer has single and sharp peak of melt curve. The sharper single peak performed, the more specific primer observed (Rahardianti and Nur, 2017). On the other hand, the Tm value of the probe played an important role in primer specificity. Concretely, Tm probe 6-10°C higher than Tm of primers is recommended (Simon *et al.*, 2019). This study showed that primer no. 5 and no.8 have a single peak with probe Tm range of 80-83°C. On the contrary, the primer no.7 has double peaks with different Tm values.

Non-template control (NTC) was important to set up to reduce the false positive on qRT-PCR. The false positive might come from technical error, contaminated primer, or other reagent stocks (Cahyadi *et al.*, 2018). Ideally, NTC should not be detected or undetermined in qRT-PCR reaction. For example, NTC of primer no.8 showed CT value at 36,938 cycles. However, NTC of primer no.5 was undetermined. It means that primer no.5 was relatively free from contamination compared to primer no.8. Therefore, it was assumed that primer no.5 is the best primer compared with the two other primers.

In silico study was conducted to obtain more convincingly results for Cx36 primer no.5. Fortunately, primer no.5 showed better results compared to the previously published Cx36 pre-existing primer in silico. Based on Beheshti's research, 2017 (Beheshti, Zeinali and Esmaeili, 2017); Calabrese, 2003(Calabrese *et al.*, 2003) and Nicholson, 2001 Cx36 primary sequences have relatively different Tm and %GC between forward and reverse. Therefore, it potentially affected the low specificity and effectivity of primer. In addition, primer no.5 has lower probability of conforming secondary structure compared with pre-existing primers. The secondary structure probability of primer is closely related to the occurrence of misspiming (non-specific sticking the primer) and primer dimer (primer sticked to its fellow). Once the primer is attached, it could be stronger and difficult to separate (Handoyo and Rudiretna, 2001). Therefore, primer no.5 designed in this study is expected to have higher specificity and effectiveness compared with pre-existing primer in silico.

CONCLUSION

As the conclusion, the primer designed in this study, especially primary no.5 (F: 5'-ATTTCCCGCTTCTACATCATCATCCAAG-3' and R: 5'-CACAGCAAACATGAACACCAGAAAG-3'), have good specificity and sensitivity to amplify the Cx36 gene of Rattus norvegicus' brain. Therefore, this primer is recommended for Cx36 gene amplification by using qRT-PCRR method.

SUGGESTION

Further research to detect the expression of the Cx36 gene in other organs or rat derived cell line is suggested to obtain maximum Cx36 expression results.

Septiasari, A., Ningsih, Sri S., Stujanna, Endin N., Avissa R., Ujianti I. 2024. **Specific Primer Design to Detect Connexin-36 Gene in *Rattus Norvegicus* Brain**. Surabaya : *The Journal of Muhamadiyah Medical Laboratory Technologist*. Vol: 7, No.1 (14-24). DOI : 10.30651/jmlt.v7i1.17985

REFERENCES

Beheshti, S., Zeinali, R. and Esmaeili, A. (2017) 'Rapid upregulation of the hippocampal connexins 36 and 45 mRNA levels during memory consolidation', *Behavioural Brain Research*, 320, pp. 85–90. doi: 10.1016/j.bbr.2016.11.048.

Cahyadi, M. *et al.* (2018) 'Specific primer design of mitochondrial 12S rRNA for species identification in raw meats', *IOP Conference Series: Earth and Environmental Science*, 102(1). doi: 10.1088/1755-1315/102/1/012038.

Calabrese, A. *et al.* (2003) 'Oscillations and Insulin Secretion in MIN6 Cells', *Diabetes*, 52(August 2002), pp. 2–9.

Deepthi Thomas and Joanne Mm Senecal (2020) 'Connexin36 localization along axon initial segments in the mammalian CNS', *International journal of physiology*, 12(6), pp. 153–165.

Dong, A., Liu, S. and Li, Y. (2018) 'Gap Junctions in the Nervous System: Probing Functional Connections Using New Imaging Approaches', *Frontiers in Cellular Neuroscience*, 12(September), pp. 1–9. doi: 10.3389/fncel.2018.00320.

Handoyo, D. and Rudiretna, A. (2001) 'Prinsip umum dan pelaksanaan Polymerase Chain Reaction (PCR)', *Unitas*, 9(1), pp. 17–29.

Lusian, R. (2021) 'Analisis Kualitas Dan Kuantitas Rna Total', pp. 102–108.

Nicholson, S. M. *et al.* (2001) 'Altered gene expression in Schwann cells of connexin32 knockout animals', *Journal of Neuroscience Research*, 66(1), pp. 23–36. doi: 10.1002/jnr.1194.

No, C. and Co, T. (2004) 'qPCR Mix'.

Praja, R. K. and Rosalina, R. (2021) 'Perancangan primer gen lktB pada Fusobacterium necrophorum untuk analisis PCR', *Jurnal Sains dan Teknologi Peternakan*, 2(2), pp. 47–55. doi: 10.31605/jstp.v2i2.960.

Promega (2004) 'SV Total RNA Isolation System. Catalog no. Z3100, TM048', p. 28.

Rahardianti, R. and Nur, E. M. (2017) 'Akurasi Metode Real Pcr untuk Analisa Ekspresi Gen PmVRP15', *Prosiding Pertemuan Teknis Teknisi Litkayasa Lingkup BBPBAP Jepara*, pp. 1–166.

Rimkute, L. *et al.* (2018) 'Modulation of connexin-36 gap junction channels by intracellular pH and magnesium ions', *Frontiers in Physiology*, 9(APR), pp. 1–12. doi: 10.3389/fphys.2018.00362.

Simon, P. W. *et al.* (2019) 'Carrot Carotenoid Genetics and Genomics', 1275, pp. 247–260. doi: 10.1007/978-3-030-03389-7_14.

Stujanna, E. N. *et al.* (2022) 'Collagen-VI Specific Primer Design Identification in Rats (Rattus norvegicus) Pancreas', *Indonesian Journal of Medical Laboratory Science and Technology*, 4(1), pp. 60–70. doi: 10.33086/ijmlst.v4i1.2515.

Teubner, B. *et al.* (2000) 'Functional expression of the murine connexin 36 gene coding for a neuron-specific gap junctional protein', *Journal of Membrane Biology*, 176(3), pp. 249–262. doi: 10.1007/s002320001094.

Toyobo (2004a) 'Instruction manual ReverTra Ace TM qPCR RT Master Mix with gDNA'. Available at: www.toyobo.co.jp/e/biotech_osaka @toyobo.jp.

Toyobo (2004b) 'ReverTra Ace TM qPCR RT Master Mix with gDNA remover2004 ReverTra Ace TM qPCR RT Master Mix with gDNA Remover'.

Zhu, H., Korabeˇ, M. and Neuˇ, P. (2020) 'Review PCR past , present and future', 69(4), pp. 1–9.

Septiasari, A., Ningsih, Sri S., Stujanna, Endin N., Avissa R., Ujianti I. 2024. **Specific Primer Design to Detect Connexin-36 Gene in *Rattus Norvegicus* Brain**. Surabaya : *The Journal of Muhamadiyah Medical Laboratory Technologist*. Vol: 7, No.1 (14-24). DOI : 10.30651/jmlt.v7i1.17985