LAMPIRAN

Lampiran 1
Lembar Identifikasi Tumbuhan

INSTITUT TEKNOLOGI BANDUNG
SEKOLAH ILMU DAN TEKNOLOGI HAYATI
Jalan Ganesha 10 Bandung 40132, Telp: (022) 251 1575, 250 0258, Fax (022) 253 4107
e-mail : sith@itb.ac.id http://www.sith.itb.ac.id

Nomor : 1624/I1.C02.2/PL/2019. 27 Maret 2019
Hal : Determinasi tumbuhan

Kepada Yth.
Ketua
Sekolah Tinggi Farmasi Bandung
Jalan Soekarno Hatta No. 754
Bandung

Memperhatikan surat permintaan Saudara dalam surat No. 1227/WKI-S1/X/2018 tanggal 17 Oktober 2018 mengenai determinasi tumbuhan, dengan ini kami sampaikan bahwa setelah dilakukan determinasi oleh staf kami, sampel tumbuhan yang dibawa oleh Sdr. Qonita Alya K. (NIM: 11151118), adalah :

Divisi	:	Magnoliophyta
Kelas	:	Magnoliopsida (Dicots)
Anak kelas	:	Asteridae
Bangsa	:	Solanales
Nama suku / familia	:	Solanaceae
Nama jenis / species	:	*Capsicum frutescens* L.
Sinonim	:	*Capsicum annuum* var. *frutescens* (L.) Kuntze
Nama umum	:	Cabai rawit (Indonesia)
Buku acuan	:	1. Djarwaningsih, T. 1983. Pemanfaatan jenis-jenis cabai (*Capsicum* spp.) sebagai tanaman hias. Buletin Kebun Raya 6 (2): 45 – 52.
		2. Djarwaningsih, T. 1986. Jenis-jenis *Capsicum* L. (Solanaceae) di Indonesia. Berita Biologi 3 (5): 225 – 228.
		3. Djarwaningsih, T. 2005. *Capsicum* spp. (Cabai): Asal, Persebaran dan Nilai Ekonomi. Biodiversitas 6(4): 292 – 296.
		4. Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York. pp. Xiii – Xviii.

Demikian yang kami sampaikan. Atas perhatian dan kerjasama yang diberikan, kami ucapkan terima kasih.

Wakil Dekan Bidang Sumber Daya,

Dr. I[illegible]wati
NIP 196205071988032001

Tembusan:
Dekan SITH ITB, sebagai laporan.

Lampiran 2
Pembuatan Medium *Nutrient Agar (*NA)

Media *Nutrient Broth* (NB) ditimbang sebanyak 1,6 gram ditambah 4 gram agar bacteriological kemudian dilarutkan dalam 200mL Reverse osmosis-Water dan dipanaskan. Selanjutnya media yang sudah jadi disterilkan dalam autoklaf pada suhu 121°C tekanan 2 atm selama 15 menit.

Lampiran 3
Isolasi Bakteri Endofit

(a) (b) (c) (d)

(e) (f) (g) (h)

Sterilisasi permukaan cabai rawit dengan perendaman (a) etanol 70%, (b) Natrium Hipoklorit 2,5%, (c) etanol, (d-h) akuades steril.

(a) (b)

Pengamatan pertumbuhan cabai rawit yang telah disterilkan (a) adanya pertumbuhan koloni dan (b) tidak adanya pertumbuhan koloni

Lampiran 4
Isolasi Bakteri Endofit

(a)

(b)

Uji Sterilitas Jaringan Cabai Rawit
(a) media NA berisikan akuades sterilis bilasan ke-5 serta (b) cabai rawit yang sudah steril

(a)

(b)

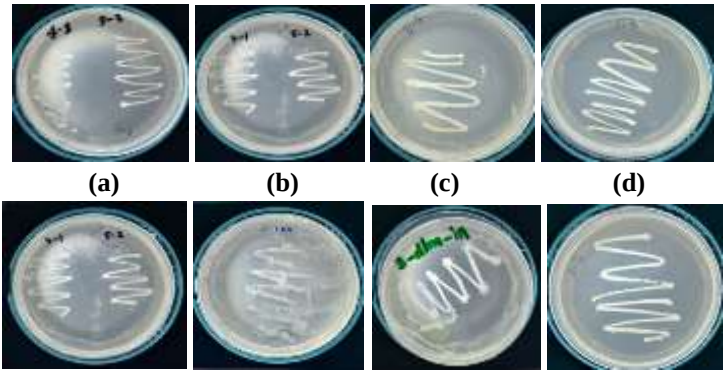
(c)

(d)

(e)

Isolasi bakteri endofit dengan pengenceran berseri (a) kultur stock, (b) 10^{-1} , (c) 10^{-2} , (d) 10^{-3} dan (c) 10^{-4}

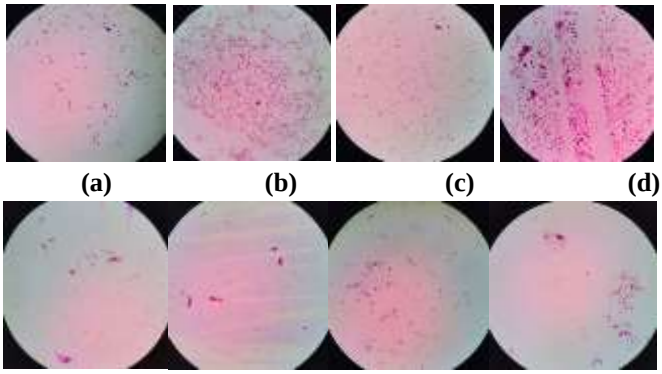
Lampiran 5
Identifikasi Bakteri Endofit



(a) (b) (c) (d)

(e) (f) (g) (h)

Isolat bakteri endofit cabai rawit
(a) BE1, (b) BE2, (c) BE3,
(d) BE4, (e) BE5, (f) BE6, (g) BE7, dan (h) BE8



(a) (b) (c) (d)

(e) (f) (g) (h)

Hasil pewarnaan Gram isolat bakteri endofit cabai rawit
(a) BE1, (b) BE2, (c) BE3,
(d) BE4, (e) BE5, (f) BE6, (g) BE7, dan (h) BE8

Lampiran 6
Isolasi DNA Bakteri Endofit

(a) Kit TriSure

Lampiran 7
Desain Primer dan Analisis *in silico*

(a)

(b)

Evaluasi primer

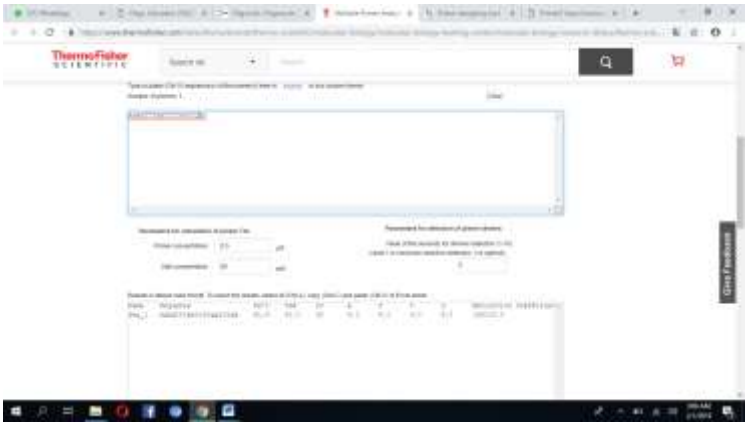
(a) forward 8F (b) Reverse 1429R menggunakan situs http://www.sciencelauncher.com/oligocalc.html

(a)

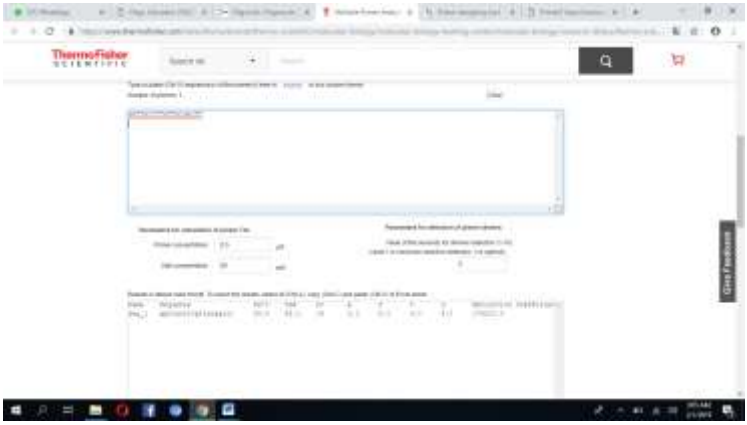
(b)

Evaluasi primer

(a) forward 8F (b) Reverse 1429R menggunakan situs http://biotools.nubic.northwestern.edu/OligoCalc.html



(a)

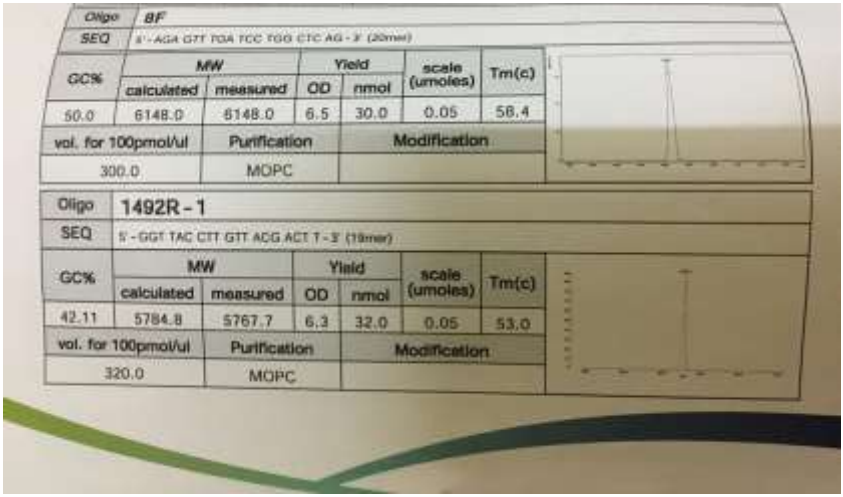


(b)

Evaluasi primer

(a) forward 8F (b) Reverse 1429R menggunakan situs https://www.thermofisher.com/id/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html

Lampiran 8
Identitas Primer



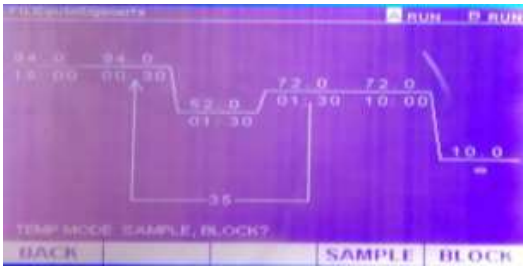
Oligo	BF					
SEQ	5' - AGA GTT TGA TCC TGG CTC AG - 3' (20mer)					
GC%	MW		Yield		scale (umoles)	Tm(c)
	calculated	measured	OD	nmol		
50.0	6148.0	6148.0	6.5	30.0	0.05	58.4
vol. for 100pmol/ul	Purification	Modification				
300.0	MOPC					

Oligo	1492R-1					
SEQ	5' - GGT TAC CTT GTT ACG ACT T - 3' (19mer)					
GC%	MW		Yield		scale (umoles)	Tm(c)
	calculated	measured	OD	nmol		
42.11	5784.8	5767.7	6.3	32.0	0.05	53.0
vol. for 100pmol/ul	Purification	Modification				
320.0	MOPC					

Lampiran 9
Amplifikasi DNA

(a)

(b)

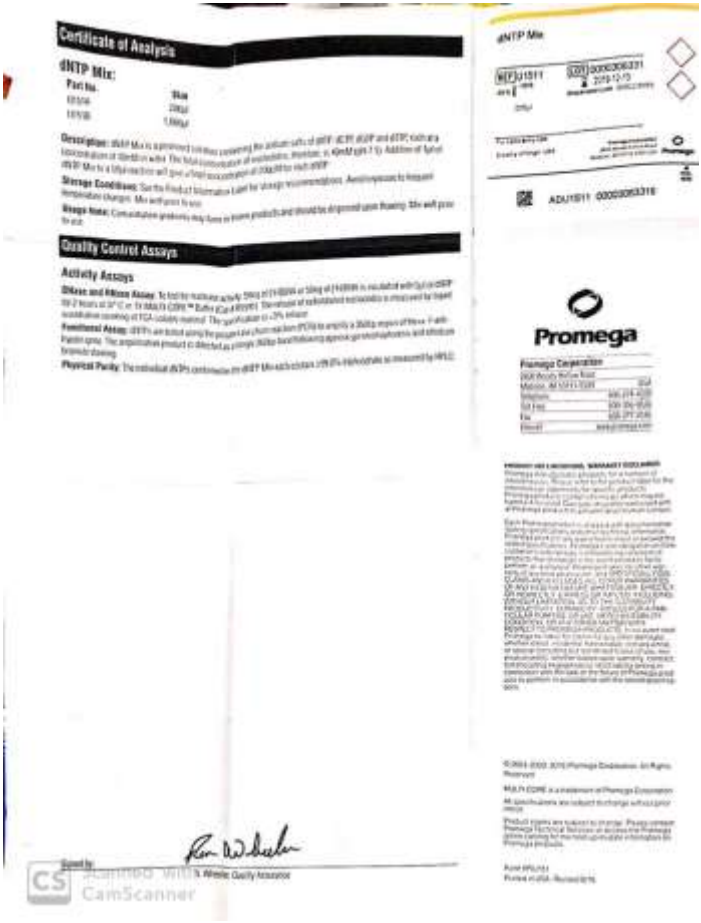


(c)

(a) ***Thermocycler*** **(b) Menempatkan tube pada** ***Thermocycler*** **(c) Suhu pada proses amplifikasi PCR**

Lampiran 10
Certificate Of Analysis Loading dye

Lampiran 11
***Certificate Of Analysis* dNTP's**



Lampiran 12
Certificate Of Analysis Taq Polymerase

Certificate of Analysis

GoTaq® Flexi DNA Polymerase:
Supplied With:

Cat.#	GoTaq® DNA Polymerase	5X Green GoTaq® Flexi Buffer	5X Colorless GoTaq® Flexi Buffer	Magnesium Chloride Solution, 25mM
M8291	100 units (M829A)	1ml (M891A)	1ml (M890A)	0.75ml (A351B)
M8295	500 units (M829B)	4 × 1ml (M891A)	4 × 1ml (M890A)	5 × 1.2ml (A351H)
M8295-C	500 units (M829B)			

Description: [illegible]

Biological Source: The enzyme is derived from bacteria.

Enzyme Concentration: 5u/µl.

5X Green GoTaq® Flexi Buffer (Part# M891A): [illegible]

5X Colorless GoTaq® Flexi Buffer (Part# M890A): Proprietary formulation supplied at pH 8.5. This buffer does not contain magnesium.

Magnesium Chloride Solution, 25mM (Part# A351B, A351H): Provided to allow users to optimize $MgCl_2$ concentration according to their individual requirements. **Vortex the $MgCl_2$ thoroughly after thawing and prior to use.**

Storage Conditions: See the Product Information Label for storage recommendations. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are specified below under Standard DNA Polymerase Assay Conditions.

Quality Control Assays

5X Green GoTaq® Flexi Buffer Migration Pattern: [illegible]

Enzyme Physical Purity: [illegible]

Functional Assay: [illegible]

Nuclease Assays: [illegible]

Standard DNA Polymerase Assay Conditions (Not PCR Conditions): [illegible]

PCR

PCR Satisfaction Guarantee

[illegible]

That's Our PCR Guarantee!

[illegible]

Signed by: R. Wheeler, Quality Assurance

GoTaq® Flexi DNA Polymerase

REF M8291 LOT 0000318700

ADM8291 0000318700

Promega

Promega Corporation

[illegible]

PRODUCT USE LIMITATIONS, WARRANTY DISCLAIMER

[illegible]

Promega

Usage Information

1. Standard Application

Reagents to Be Supplied by the User

- PCR Nucleotide Mix (Cat.# C1141, C1145)
- Nuclease-Free Water (Cat.# P1193)
- upstream primer
- downstream primer
- template DNA

1. In a sterile, nuclease-free microcentrifuge tube, combine the following on ice:

Component	Final Volume	Final Concentration
5X Green or Colorless GoTaq® Flexi Buffer¹	10µl	1X
$MgCl_2$ Solution, 25mM	2–8µl	1.0–4.0mM
PCR Nucleotide Mix, 10mM each	1µl	0.2mM each dNTP
upstream primer	Xµl	0.1–1.0µM
downstream primer	Yµl	0.1–1.0µM
GoTaq® DNA Polymerase (5u/µl)	0.25µl	1.25u
template DNA	Zµl	<0.5µg/50µl
Nuclease-Free Water to	50µl	

¹Thaw completely and vortex thoroughly prior to use.

2. If using a thermal cycler **without a heated lid**, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.
3. Perform PCR using your standard parameters. An example profile is given in Table 1. For the cycling protocol, we recommend the following:
   a. Reactions are placed in a thermal cycler that has been preheated to 95°C.
   b. The thermal cycling protocol has an initial denaturation step where samples are heated at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Initial denaturation of longer than 2 minutes at 95°C is unnecessary [illegible]
   c. The extension time should be at least 1min/kb target length.

Table 1. Recommended Thermal Cycling Conditions for GoTaq® DNA Polymerase-Mediated PCR Amplification. These guidelines are optimal for the Perkin-Elmer thermal cycler model 480 or comparable thermal cyclers.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	
Annealing	42–65°C*	0.5–1 minute	25–35 cycles
Extension	72°C	1min/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*Annealing temperature should be optimized for each primer set based on the primer T_m.

4. Separate the PCR products by agarose gel electrophoresis and visualize with ethidium bromide or any other means. For reactions containing the 5X Green GoTaq® Flexi Buffer, load the amplification reaction onto the gel directly after amplification. Reactions containing the 5X Colorless GoTaq® Flexi Buffer also can be loaded directly onto the wells of an agarose gel, but a loading dye will need to be added to monitor the progress of electrophoresis.

2. General Considerations

A. Enzyme Concentration

We have found that 1.25 units of GoTaq® DNA Polymerase per 50µl amplification reaction is adequate for most amplifications. [illegible] *Taq* DNA polymerase.

B. Buffer Choice

We recommend using the 5X Green GoTaq® Flexi Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green GoTaq® Flexi Buffer is not recommended for any downstream applications [illegible]

For reactions going directly from thermal cycler to an application using absorbance or fluorescence, the 5X Colorless GoTaq® Flexi Buffer is recommended. [illegible] Wizard® SV Gel and PCR Clean-Up System [illegible]

Both reaction buffers are compatible with common PCR additives such as DMSO and betaine. These additives do not change the color of the Green GoTaq® Flexi Buffer or affect dye migration.

C. Primer Design

PCR primers generally range in length from 15 to 30 bases and are designed to flank the region of interest. [illegible] Calculator is provided on the BioMath page of the Promega web site at: **www.promega.com/biomath/**

D. Amplification Troubleshooting

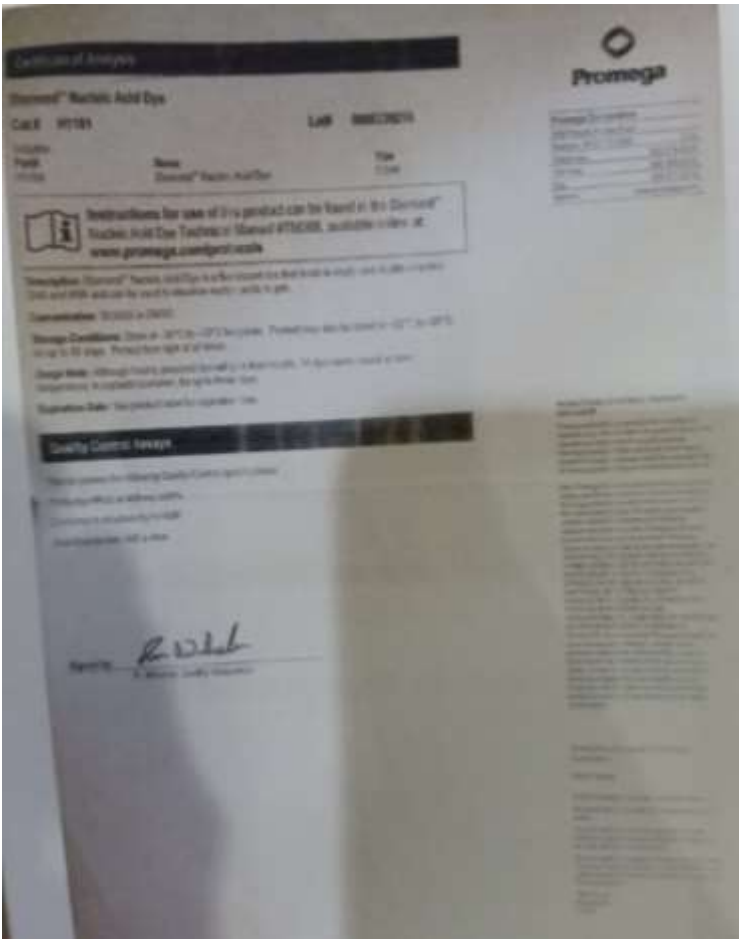
To overcome low yield or no yield in amplifications (e.g., mouse tail genotyping applications), we recommend the following suggestions:

- Adjust annealing temperature. [illegible] (**www.promega.com/biomath/**).
- Minimize the effect of amplification inhibitors. [illegible]
- Increase template DNA purity. [illegible]
- Add PCR additives. [illegible]

Lampiran 13
Certificate Of Analysis DNA Ladder

Lampiran 14

Certificate Of Analysis Analysis Diamond



Lampiran 15
Elektroforesis

(a)

(b)

(b)

(d)

(a) Agarose (b) agarose yang telah membeku (c) alat elektroforesis yang dijalankan pada 100V selama 30 menit (d)Fragmen DNA mulai bergerak

Lampiran 16
Form DNA Sekuensing Hasil PCR

1st BASE

DNA Sequencing Order Form

Print Date: 05/07/2019 3:16 pm

Collection Time	:		
Order ID Online	: 146739	Customer Name	: Ms. ZUHRIANI PUTRI
Order Date	: 05/07/2019 3:16PM	Principal Investigator	:
Type of Service	: Single Pass DNA Sequencing	Contact No.	: -082265534016
Type of DNA	: Purified PCR	Mail Address	: Jalan Soekarno Hatta No 754 Sekolah Tinggi Farmasi Bandung, Cijerah Bandung, 40514, Indonesia, Attn Ms. ZUHRIANI PUTRI
Total Template	: 5		
Total Primer	: 2	Payment Method	: Prepaid
Total Reaction	: 10	Payment Ref No	:
Order Remark	:	Payment Comment	:

Template Info

Online Template ID	Template		Total Rxn
	Name	Total Size (bp)	
1138652	3	1500	2
1138653	4	1500	2
1138654	5	1500	2
1138655	6	1500	2
1138656	52	1500	2

Primer Info

Online Primer ID	Primer		
	Name	Conc (uM)	Ta (°C)
356092	8F	10	52
356093	1492R	10	52

Reaction Info

Online Rxn ID	Sample Name	Primer Name	Unit Price (IDR)	Remarks
1673644	3	8F	0.00	
1673645	3	1492R	0.00	
1673646	4	8F	0.00	
1673647	4	1492R	0.00	
1673648	5	8F	0.00	
1673649	5	1492R	0.00	
1673650	6	8F	0.00	
1673651	6	1492R	0.00	
1673652	52	8F	0.00	
1673653	52	1492R	0.00	
		Subtotal Unit Price	0.00	

Page 1 of 2

Subtotal Price	0.00 (IDR)
Tax Rewards	0.00 (IDR)
Total Price	0.00 (IDR)
Prices above are indicative only and before prevailing taxes	

Note : All samples and sequencing data will be kept confidential. If not specified by the customer, all samples will be discarded 2 weeks after results are released.