# КU инэпае иноветнае SATREPS Development of the Duckweed Holobiont Resource Values towards **Thailand BCG Economy**

Japanese side: Hokkaido U, Tohoku U, U Yamanashi, Kyoto U, Osaka U, National Institute for Environmental Studies (NIES), Saraya Co. Ltd.

Thai side: Kasetsart U, Mahidol U, Khon Kaen U, Chulalongkorn U, Nakhon Pathom Rajabhat U, Ramkhamhaeng U, NBT, BIOTEC, NANOTEC, NSTDA, Advanced Greenfarm

Period of Project: October 2021 - September 2026 (5-year)

## **Be-HoBiD Previous Output 2021-2023**

#### **Publications:**

- 1. Saimee et al. 2021. IJSEM, 71 (11): 005106.
- 2. Yoksan et al. 2022. Int J Biol Macromol 203: 369-378.
- 3. Duangjarus et al. 2022. Foods, 11, 2348.
- 4. Bunyoo C et al. 2022. Plants. 11, 2915.
- 5. Kajadpai et al. 2023. AIMS Microbiology. 9(3): 486-517.

#### Conference:

- 1. The 14th Botanical Conference of Thailand, Khon Kaen University, 11-12 June 2022
- 2. International Conference of Genetics Society of Thaland (iGST2022), 21-22 June 2022.
- 3. Proceeding of the 11th International Conference on Environmental Engineering, Science and Management, Environmental Engineering Association of Thailand, 12 May 2022
- 4. The Proceedings of the 59th KU Annual Conference, 2002. 655-662

## **Be-HoBiD Previous Output 2021-2023**

#### Research grant:

3. Δ

1.	Fundamental Fund, KURDI, KU	2022 = <b>4,574,100</b> THB
2.	SRU, KU	2022 = <b>300,000</b> THB
3.	Kurita Water and Environment Fund	2022 = <b>400,000</b> JPY
4.	Research Grant for New Scholar, KURDI, KU	2023 = <b>300,000</b> THB
Sch	olarship:	
1.	Canada-ASEAN scholarships and Educational Exc	change for Development (SEED), EduCanada (Ph.D)
2.	Post-Doc Scholarship, KU Reinventing U	2022 = <b>590,000</b> THB (1y)
3.	KU Graduate School Scholarship	2021-2024 = <b>800,000</b> THB (2y) (4x)
4.	KU Graduate School Scholarship	2022 = <b>300,000</b> THB (3y)
5.	Post-Doc Fellowship, KU	2022 = <b>480,000</b> THB (1y)
6.	Doctoral Scholarship from Fac. Engineering, KU	2022 = <b>786,000</b> THB (3y)

- 7. KKU scholarship for ASEAN & GMS Countries 2021 = 228,000 THB (2y)
- 8. Scholarship from Fac. Engineering, KKU 2023 = 120,000 THB (2y)
- 9. TA Scholarship, KKU 2023 = 30,000 THB (1y)

Awards: Good level academic oral presentation award, The 11th National Animal Science Conference of Thailand (2022)

## **Project Monitoring Sheet I** (2024) (Annex 1-1, 1-2 Form 3-2)





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(Annex 2)



## (Annex 2)

Be-HoBiC

			(Annex 1-1, 1-2 Form 3-2)
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Overall Goal			
Research activities at the Duckweed Holobiont Resource & Research Center (DHbRC) are continued and further developed, and the research activities will be applied to biologica resources other than duckweed, so that the project's contribution to the Bio-Circular-Green (BCG) 2 economy will be recognized.	1. Number of cases where DHbRC provided host organisms, associated microorganisms (groups), DNA Isequence data, and related information (at least 10 cases related to duckweed) (at least 5 cases other than duckweed)	>DHbRC and related organizations' materials >Interviews with DHbRC and related organizations	
	2. Number of technologies and cases applied socially by DHbRC and related organizations (at least 3)		
	3. Amount of R&D funding (budget) and project funding (budget) acquired by DHbRC and related organizations (3M THB)		2023 800,000 THB (KURDI), 2024-2025 = 600,000 THB (NRCT) 2024 = 1,000,000 THB (KURDI), 600,000 THB (NRCT), 400,000 THB (PMUB)

Narrative Summary	<b>Objectively Verifiable Indicators</b>	Means of Verification	Achievement
Project Purpose			
research development base is tablished for the development of ackweed industrial technology and s practical use which contributes to aailand BCG economy.	1. Number of human resources trained (at least 20)		Training in Japan: Long term training: G3:2pp, G4-1:1pp, G5/G6: 1pp (total 4pp) Short term training: G4-1:1pp G5/G3:1pp (Total 2pp)
	2. Number of valuables developed using duckweed holobiont resources (at least 3)	>Project report	
	3. Number of entities (universities, government agencies, companies) and a number of individual farmers for which DHBRC provided duckweed, associated microorganisms, DNA sequence data, and related information (at least XX entities, at least 3 individuals).	>Monitoring report >Interview, survey and materials from related entities	

#### (Annex 1-1, 1-2 Form 3-2)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Outputs			
1. Duckweed Holobiont Resource & Research Center (DHbRC) is established at Kasetsart University.	1. Establishment of DHbRC	>Project report >Established DHbRC >DHbRC's homepage >Constructed duckweed plant factory in DHbRC >Interviews with DHbRC and research groups	JICA 1st and 2nd batch equipment were delivered and operational. DHbRC opening ceremony was held on 18/3/2024.
	2. Construction of a duckweed plant factory in DHbRC		Completed installation of Plant Factory Phase 1 (JICA Equipment).
	3. Quantity of produced duckweed holobiont biomass to supply to the research groups (adequate amount for research requirement)		
	4. Establishment of functions and services of DHbRC as a common laboratory		



## (Annex 1-1, 1-2 Form 3-2)



2<sup>nd</sup> Floor, Princess Chulabhorn 60<sup>th</sup> Birthday Anniversary Science Research Center Building













คณะเกษตร









#### **Duckweed Culture Room**













**Duckweed Plant Factory** 





### (Annex 1-1, 1-2 Form 3-2)

Narrative Summary	<b>Objectively Verifiable Indicators</b>	Means of Verification	Achievement
2. Duckweed holobiont collection is created	1. Number of preserved individual duckweeds, associated microorganisms, associated microbial communities, and their DNA sequence data (at least 20 plant specimens, 400 microbial strains, 20 microbial communities, 400 DNA sequence data)	>Project report >Created catalog	
	<ol><li>Creation of a catalog of preserved organisms and relevant information</li></ol>	papers related to the project	
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		2 Publications (Arch Microbiol, Cur Microbiol) 1 MS (IJSEM, under review), 2 MS (in prep)

#### Biodiversity of Thai Duckweed Species G2-1

#### 2020-2024 Output

**Research Plan** 

• Funding KURDI FF(KU)4.64, 65

#### • Data

- · 126+ DNA sequences
- · Ecological data from 10 sites Temporal Dynamic from 1 site
- Optimal Media for 4 species
- · Morphology from 42 plants
- HPLC profiles for 4 species
- LC MS/MS QTOF data for 4 species

- <u>Research</u> · 3 prepared MS
- 4 Bachelor Theses
- 1 PhD Student, 2 MS Student

#### Awards/Talks

- 1 International Internship
- 3 International Conference
- 1 National Conference Award
- 1 National Scholarship 4 National Conference Talks





**Research Team** 

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Athita Siwaporn Surapong Kraichak Senayai Jansantia Kosum Group PI PhD Student MS Student MS Student

## 5999 - 0.000 - 0.00 Morphology & Genetics Phytohemistry Physiology



Publication: 2, Manuscript under review : 1, Master students: 5

Number of Species PGP potential identification (traits/abilities) isolates 880 249 15/50 573 200 552/14 252 252 252/0 1.705 701 819/64

Co-cultivation of associated microbes with duckweeds

Bacterial and actinobacterial strains isolated from duckweeds were co-cultivated with axenic duckweed to assess the effectiveness of these strains to increase duckweed frond number and biomass.

The promising PGPB can promote duckweed growth range from 1.50-1.77 folds in Wolffia globosa (15 isolates), and 2.00-2.43 folds in Spirodela polyrhiza (41 isolates). Five PGPB isolates are able to enhance frond proliferation in both duckweeds.

Saimee, Y., Butdee, W., Boonmak, C., Duangmai, K. 2024. Actinomycetosoora lemnae so. nov., a novel actinobacterium isolated from Lemna aeauinoctialis able to enhance duckweed growth. Current Microbiol 81:92. sommak, C., Joursey, M., Buranathong, B. Morikawa, M., Duangmal, K. 2024. Duckword-associated bacteria as plant growth-promotor to enhance growth of Spirodelp polyhiza in wastewater effluence from a poultry farm Arch Microbiol 206, 43.

Butdee, W., Saimee, Y., Suriyachadkun, C. Duangmal, K. 2024. Pseudonocardia spiradelae sp. nov., isolated from duckweed and formal proposal to reclassify Pseudonocardia antarctica as a later heterotypic syno Pseudonocardia anni and reclassify Pseudonocardia corboxydivarans as Pseudonocardia anni subsp. corboxydivarans. Int. J. Syst. Evol. Microbiol. (under review)

			(Annex 1-1, 1-2 Form 3-2)
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
3. Technology base is developed for function enhancement of the duckweed holobiont.	<ol> <li>Number of potential duckweed holobionts for function enhancement and wastewater treatment (at least 10)</li> <li>Number of omics 6 databases of duckweed and associated microorganisms at DHbRC (at least 5)</li> <li>Number of analyses of duckweed and associated microorganisms' interaction (at least 2)</li> </ol>	>Project report >Published academic papers related to the project	1 duckweed genome data with long-read and short-read data 2 Genome/Ct genomes of Wolffia globosa and Lemna aequinoctalis 1 potential duckweed holobionts for function enhancement and wastewater treatment
	4. Number of technologies developed for improvement of duckweed holobionts for growth/stress tolerance (at least 2) 5. Number of active substances discovered/identified (at least 5) 6. Number of academic papers (including peer-reviewed conference proceedings) published (at least 10)		4 MS (in prep), 1 (Sci. Rep., under review), 1 Proceedings





Narrative Summary	<b>Objectively Verifiable Indicators</b>	Means of Verification	Achievement
<ol> <li>Technology base is developed for manufacturing valuables using duckweed as a raw material.</li> </ol>	1. Number of technologies developed for manufacturing valuables using duckweed as a raw material (at least 3)		
	2. Number of products of verified selected valuables at the laboratory level or bench plant scale (at least 3)	>Project report >Published academic journals related to the project	
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		3 Publications (Ind Crops Prod, Thai J Anim Sci, Khon Kaen Agric J), 1 MS (Trends Sci, accepted), 3 MS (under review), 4 MS (in prep)

#### **G4-1 BioFUEL : methane ENKK**U Group Target : Bio-fuel (bio-methane) production from Thai duckweed cultivated by wastewater 5 years plan Output for 3 years, going to the 4<sup>th</sup> year November 6, 2024) 3) Moving toward 1) Established duckwood 2) Start up and find out operating condition Lab scale bio-methane ge Bench scale biosupply system at KKU with the laboratory-scale reactors methane (harvesting >200 g (Finished the experiments) Bench scale generator DW/week) n from Duckwood Co-digested A 100AG 0 011000 01-0 ail 1 MATERIALS & METHODS 4) 1 Master student Objectives **N B** 5) 3 International conferences Materials are 1.s., F 1 6) 2 Manuscripts RESULTS 7) 1 master student, 1 doctoral student, 4 undergraduate students had graduated 8) 5 research grants and 3 scholarships for students ۲ 9) 1 Poster presentation award "Differentiation" "Differentiation

# G4-2: Bioplastics from duckweed biomass

#### PACKAGING & MATERIALS TECHNOLOGY 1. To add the value and expand the applications 2. To reduce the cost of bioplastics by replacing with renewable GOALS: in bioplastics of duckweed (DW) biomass low-cost DW materials which has little impact on the food chain Assoc. Prof. Rangrong Year 1 Year 2 Year 5 Year 3 Year 4 Yoksan epartment of Packaging and Basic research Technology development erials Technology, Faculty of Agro-Industry Research to feasibility (proof of concept) Prototype development asetsart University 0 1 Fabrication of DW-based bioplastics with improved performances RA: 2 Investigation of the relationship between the composition and properties of the obtained DW-based bioplastics Dr. Dang Minh Khanh (Post-doc) + Other components DW-based bioplastic resins/pellets Khanh DM, Yoksan R\*, Matsumoto K, Morikawa M. Duckweed biomass as an alternative to Technologies: Duckweed biomass (DW) Direct plasticization cassava starch in manufacturing TPS/PHBV/PBAT ternary blend (in prep) Municipal and agricultural ossible plastic converting processe Blending with other bioplastics and/or commodity plastics wastewater stream-treated DW Injection molding Low cost cultivation DW Compression molding 1 Formular of duckweed biomass-based bioplastic for melt-Sheet extrusion & the noformina Ċ extruded cast sheel Preparation of product prototype 3 ble and sustaina of DW-based bioplastics C /4% DW-based bioplast produc Evaluation of preliminary cost 4 of DW-based bioplastics

#### (Annex 1-1, 1-2 Form 3-2)





			(Annex 1-1, 1-2 Form 3-2)
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
5. Low-carbon effect of the water purification system using duckweed holobiont is verified.	1. Energy saving rate of the entire system (Energy consumption lower than the Business-As-Usual (BAU))		
	2. Amount of green-house-gas (GHG) emission reduction (GHG emission lower than the BAU)	>Project report >Created database/handbook	
	3. Database/handbook for design and operation management of duckweed holobiont water purification system	>Published academic journals related to the project	
	4. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		2 MS (in press), 1 MS (under review), 1 MS (in prep)

(Annual 4 4 2 France 2 2)

KASETSART UNIVERSITY		G5: Dι	ickweed-ba	ased waste	water treat	ment
		PI: Chart Chiemon Environmental Eng Team: Wilai Chier doctoral researche students (1 Ph.D.,	aisri, <i>Dept.</i> g. Faculty of Eng. nchaisri, 3 Post- er, 3 graduate 2 master)			Ventor 2
	2021	2022	2023	2024	2025	2026
	Preparation	Evaluate duckweed performance at lab livestock farm & for	d base WWT oratory scale using od processing WW	Practical water purit based WWT at bend operation at targeter Dissemination of re	ication of duckweed ch or field scale d WW source esearch results	Database & guidelines of technology
N	lain findings	(up to Oct24)				
-	<ul> <li>Optimal operating condition (duckweed species, hydraulic retention time, wastewater strength (organic &amp; nutrient loading) of duckweed wastewater treatment applied to different wastewaters (campus, food processing factory &amp; pig farm WW) identified.</li> </ul>					
-	Duckweed g	rowth yield in real w	astewater operated	under actual field er	nvironment known	
-	<ul> <li>Improved duckweed biomass yield and pollutant removals through development of duckweed associated microorganisms observed. Long-term performance investigation under field operating conditions is on-going.</li> </ul>					
R	Research output (up to Oct 24) 1 M.S. student graduated					

- C. Chiemchaisri, W. Chiemchasri, L. Saksukol, C. Chandaravithoon, C. Witthayaphirom, V. Boonyaroj, T. Toyama, K. Mori, M. Morikawa (2025), Duckwed-based waste stabilization ponds for wastewater treatment, In: Low-cost Water and Wastewater Treatment Systems, Elsevier, ISBN: 978-0443-236624.
- Chiemchaisri W, Chiemchaisri C, Towprayoon S, Ogata Y, Ebie Y, Ishigaki T., 2024. Biological oxidation for mitigating methane emissions from urban wastes and wastewater. Natural Based Solutions for Water Sustainability, International Water Association (in press)

			(Annex 1-1, 1-2 Form 3-2)
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
6. Support for duckweed production in farms and practical application of technology using duckweed are promoted.	1. Number of publication/dissemination/public relations activities of results from each research group (at least 3)	>Project report	Panel discussion on "Pham and duckweeds, new economical plants: from laboratory to GAP" ThailandLab, BITEC, 11 Sep 2024.
	2. Number of support activities for duckweed production (at least 3)	>Dissemination and public relations activity reports >Created technical manuals and proposals	
	3. Number of created technical manuals and proposals of technology using duckweed (at least 3)		



## **Be-HoBiD Output 2024**

## (Annex 2)

**Be-HoBiD** 

#### **Publications:**

- Boonmak, C., Kettongruang, S., Buranathong, B. Morikawa, M., Duangmal, K. 2024. Duckweed-associated bacteria as plant growth-promotor to enhance growth of *Spirodela polyrhiza* in wastewater effluent from a poultry farm. Arch Microbiol 206, 43. https://doi.org/10.1007/s00203-023-03778-4
- Saimee, Y., Butdee, W., Boonmak, C., Duangmal, K. 2024. Actinomycetospora lemnae sp. nov., a novel actinobacterium isolated from Lemna aequinoctialis able to enhance duckweed growth. Cur Microbiol 81:92. https://doi.org/10.1007/s00284-023-03595-4.
- Onsri P, Leetanasaksakul K, Saengkrit N, Thamchaipenet A (2024) Amino acid composition and antioxidant activity of protein extract from *Lemna aequinoctialis*. Proceedings of the 8th Biotechnology International Congress (BIC 2024).
- Yoksan R, Boontanimitr A. 2023. Effect of calcium carbonate on the performance of poly (butylene adipate-coterephthalate) filled with duckweed biomass. Ind Crop Prod, 205, 117442.
- Thongthung H, Thepparak S, Petchpoung K, Chungopast S, Kaewtapee C. (2024). Amino acids digestibility of duckweed and black soldier fly larvae in cecectomized laying hens. Khon Kaen Agri J. 52(Suppl. 2): 68–77.
- Thongthung, H., Chungopast, S., Kaewtapee, C. 2023. Chemical composition and in vitro protein digestibility of duckweed. Thai J Anim Sci. 4(Suppl. 1): 312-319.

## Be-HoBiD Output 2023-2024 (Annex 3)

#### Conference:

- Kraichak, E. (2024, Panelist) Genetics in Global Boiling Era. The 23rd International Conference of the Genetics Society of Thailand (iGST 2024).
- 2. Onsri P, Leetanasaksakul K, Saengkrit N, Thamchaipenet A (2024) Amino acid composition and antioxidant activity of protein extract from Lemna aequinoctialis. The 8th Biotechnology International Congress (BIC 2024)
- Thamchaipenet A, Bunyoo C, Phonmakham J, (2024) Duckweed microbiome: from nature to stress environment. 15th International Fundamental Science Congress, Malaysia.
- Bunyoo C, Phonmakham J, Thamchaipenet A (2024) Metagenome-assembled genomes (MAGs) display a persistent core microbiome associated with Wolffia globosa under long-term cultivation. The 23rd International Conference of Genetics Society of Thailand (iGST 2024).
- Phonmakham J, Phetlueam A, Narayam P, Thamchaipenet A (2024) Isolation and Identification of duckweedassociated bacteria. The 23rd International Conference of Genetics Society of Thailand (IGST 2024)
- 6. Thananusak R, Bunyoo C, Phonmakham J, Thamchaipenet A (2024) Unraveling Thai duckweed plastome in thailand using long-read data. The 23rd International Conference of Genetics Society of Thailand (iGST 2024)
- Thamchaipenet, A. (2023) Plant microbiome: source of beneficial microbes enhance environmental resilience in plants. ThailandLab, 6-8 September 2023, BITEC, Thailand

#### (Annex 2)

#### (Annex 2)

## Be-HoBiD Output 2023-2024 (Annex 3)

#### Conference:

- Bunyoo C, Roongsattham P, Khumwan S, Phonmakham J, Thamchajpenet A (2023) High resolution profiling of bacterial consortia associated with duckweeds using V3-V4 and full-length 16S metagenomic sequencing. International Conference of the Genetics Society of Korea 2023, 18-20 October 2023, Busan, Korea
- Patumanon, W., Onishi, K. & Imaram, W. MS-based metabolomics study of Spirodela. Pure and Applied Chemistry International Conference 2024 (PACCON 2024)
- Lertchaiyongphanit, R. & Imaram, W. Chemical profile of hexane fraction of hydrosol from fresh Wolffia globosa. Pure and Applied Chemistry International Conference 2024 (PACCON 2024)
- Thongthung H, Thepparak S, Petchpoung K, Chungopast S, Kaewtapee C. (2024). Amino acids digestibility of duckweed and black soldier fly larvae in cecetomized laying hens. The 12th National Animal Sciences Conference of Thailand.
- Thongthung H, Chungopast S, Kaewtapee C. (2023). Chemical composition and in vitro protein digestibility of duckweed. The 11th National Animal Science Conference of Thailand
- 6. Chandaravithoon C, Witthayaphirom C, Chiemchaisri C, Chiemchaisri W, Toyama T, Mori K, Morikawa M, 2023. Piggery wastewater treatment with resource recovery and greenhouse gas mitigation effect using duckweed and its associated microorganisms. The 16th Annual Conference on the Challenges in Environmental Science and Engineering, 5-9 November 2023, Perth, Australia.

No. of postgrad students & RA:

12 MS, 5 PhD, 5 Post-doc, 5 RA



(Annex 2)

## Be-HoBiD Output 2023-2024 (Annex 3)

#### Awards:

- Excellent research presentation award to Bunyoo, C. International Conference of the Genetics Society of Korea 2023, 18-20 October 2023, Busan, Korea
- The 1st runner-up award in the field of Applied Engineering Project at the 26<sup>th</sup> Bachelor Degree Student's Project Competition, Fac. Engineering, KKU, 22 February 2024
- Good level academic oral presentation award at the 12th National Animal Science Conference of Thailand.
- Good level academic oral presentation award at the 11<sup>th</sup> National Animal Science Conference of Thailand.

#### **Research grant:**

- 1. KUSpecial Research Incubator Unit from KURDI 2024 = 1,000,000 THB
- 2. PMUB, 2024 = **400,000** THB
- 3. Research Grant for New Scholar, NRCT, 2024-2025 = 600,000 THB
- 4. Research Grant for New Scholar, KURDI, 2023 = **300,000** THB
- 5. Research support fund for MS student's thesis, Faculty of Engineering, KKU, 2024 = 15,000 THB
- 6. Research Center for Environmental and Hazardous Substance Management, KKU, 2024 = 100,000 THB
- 7. Research Project Fund, Faculty of Engineering, KKU, 2023 = 5,000 THB
- 8. Fundamental Fund, KURDI, 2023 = 500,000 THB



## Be-HoBiD Output 2023-2024

#### Scholarship:

- 1. PhD Research Scholarship from the Rama VII Memorial Foundation, 2024 = 10,000 THB
- 2. Canada-ASEAN scholarships and Educational Exchange for Development (SEED), EduCanada (Ph.D)
- 3. 2x KU graduate School Scholarship, 2022-2023 = 200,000 THB (2y)
- 4. KU Graduate School Scholarship, 2023-2024 = 200,000 THB (2y)
- 5. KU Graduate School Scholarship, 2022-2024 = 300,000 THB (3y)
- 6. 80th year Kasetsart U Anniversary PhD Scholarship, 2023-2025 = 613,000 THB
- 7. Postdoc Fellowship, SciKU 2023 = 90,000 THB (2m)
- 8. Postmaster Fellowship, PMUB 2024 = 400,000 THB (1y)
- 9. Graduated Scholarship from Fac. Engineering, KKU, 2023-2024 = 120,000 THB (2y)

10. TA Scholarship, KKU, 2024 = 30,000 THB (1y)

- 11. TA Scholarship, KKU 2023 = 30,000 THB (1y)
- 12. Doctoral scholarship from Faculty of Engineering, KU, 2022-2024 = 786,000 THB (3y)



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# Congratulations

## Faculty of SCIENCE

## Mr. Chakrit Bunyoo

PhD Student, Doctor of Philosophy Program in Bioscience (International Program), Faculty of Science, Kasetsart University

Under supervision of

Prof. Dr. Arinthip Thamchaipenet for

Excellent Research Presentation Award for The Outstanding Oral Presentation

International Conference of the Genetic Society of Korea 2023 CCSK 2023
DCtober 18 – 20, 2023
BEXCO. Busan, Korea







(Annex 2)

