Webinar Sesi Perkongsian Latihan Luar Negara Siri 4 / 2025

REGIONAL TRAINING ON DETECTION OF ESBLS AND WHOLE GENOME SEQUENCING FOR AMR SURVEILLANCE IN TERRESTRIAL ANIMALS

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VET AMR
CHULALONGKORN UNIVERSITY

Food and Agriculture Organization of the United Nations

Latarbelakang latihan

Lokasi / tempoh:

Fakulti Sains Veterinar, Universiti Chulalongkorn Bangkok Thailand

17 – 21 Mac 2025

Dibiayai oleh:

European Union

Projek Regional Tripartite AMR

Dengan Kerjasama:

FAO dan CUVET AMR

Peserta Kursus

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Matlamat latihan

Melatih peserta dalam mengukuhkan keupayaan makmal dalam penghasilan data kerintangan antimikrob (AMR).

Meningkatkan penggunaan teknik molekular dalam sistem pemantauan dan survelan AMR

Membiasakan peserta dengan penggunaan WGS (Whole Genome Sequencing) untuk sistem survelan AMR

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Isi kandungan kursus

- Lecture / classroom (45%)
- Labwork (45%)
- Computer lab (10%)

Regional training on detection of ESBLs and whole genome sequencing for AMR surveillance in terrestrial animals
17-21 March 2025
Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Monday 17 March 2025		
1st floor, Demonstration room		
08:15-08:30	Registration	CU VET AMR
08:30-08:45	- Opening remarks from FAO - Welcome speech from CU - Group photo	Dr. David Sutherland, FAO RAP Prof. Rungtip Chuanchuen, CUVETAMR
08:45-09:00	- Introduction to the workshop - Participant introduction	CU VET AMR
09:00-09:30	FAO's work to address AMR in food and agriculture sector for sustainable agrifood systems transformation	Dr. Usman Zaheer, FAO RAP
09:30-10:00	The prevalence and surveillance of AMR in food animal and companion animal in China	Prof. Shaolin Wang, College of Veterinary Medicine China Agricultural University
10:00-10:30	**Coffee break**	
10:30-11:00	Pretest	CU VET AMR
11.00-12:00	Clinical and epidemiological importance of ESBL, AmpC and car-bapenemase-producing Gram-negative	Pattrarat Chanchaithong
12:00-13:30	**Lunch**	
7th floor, Laboratory room 701		
13.30-14.30	Detection of ESBLs	Rungtip Chuanchuen
14.30-16:30	**Lab safe** **Lab 1:** Phenotypic detection of ESBLs **Demonstration:** AmpC and carbapenamese detection	CU VET AMR
16:30-17:00	Wrap up of the day	
Tuesday 18 March 2025		
8th floor, Smart classroom-2		
08:30-09:00	Recap	CU VET AMR
09:00-10:00	Basic molecular biology for AMR testing and WGS	Rungtip Chuanchuen
10:00-10:30	**Coffee break**	
10.30-12:00	Principle of PCR	Rungtip Chuanchuen
12:00-13:30	Lunch	
12th floor, Laboratory room 1217		
13.30-14.30	**Lab 2:** Phenotypic detection of ESBLs (Cont.)	CU VET AMR
14.30-16:30	**Lab 3:** PCR detection of ESBL genes	
16:30-17.00	Wrap up of the day	
Wednesday 19 March 2025		
8th floor, Smart classroom-1		
08:30-09:00	Recap	CU VET AMR
09:00-09:30	Towards genomic AMR surveillance	Rungtip Chuanchuen
09:30-09:50	Principle and application of WGS	Rungtip Chuanchuen
09:50-10:10	Overview of the Illumina platform	Pattrarat Chanchaithong
10:10:10:30	Overview of the nanopore sequencing platform	Rungtip Chuanchuen
10.30-11:00	**Coffee break**	
11:00-11:30	Genomic DNA preparation for WGS	Rusmin Indra
11:40-13:00	**Lunch**	
12th floor, Laboratory room 1217		
13.00-16:30	**Lab 4:** PCR detection of ESBL genes (Cont.) **Lab 5:** Genomic DNA preparation for WGS **Demonstration:** Genomic DNA preparation	CU VET AMR
16:30-17.00	Wrap up of the day	
Thursday 20 March 2025		
12th floor, Laboratory room 1217		
08:30-09:00	Recap	CU VET AMR
09:00-10:30	DNA library preparation for nanopore sequencing	Chavin Leelapsawas
	• **Lab 6:** Hands-on DNA library prep by rapid barcoding kit • VDO for native barcoding ligation/adapter ligation	CU VET AMR
10:30-11:30	• VDO for software set up and sequencing run • **Hands on:** Priming and loading on the flow cells • VDO for nanopore sequencing/base calling/demultiplex-ing/adapter trimming/ sequence quality check /assembling	
11:30-12:00	Sequence quality check	Chavin Leelapsawas/ Jiratchya Pu-angseree
12:00-13:30	**Lunch**	
8th floor, Computer lab		
13.30-14:00	Tools for genetic analysis of AMR	Jiratchya Puangseree
14:00-14.20	**Bioinformatic analysis 1**- Genetic analysis of AMR	Jiratchya Puangseree
14:20-15:00	**Lab 7:** Hands-on exercise on genetic analysis of AMR	CUVETAMR
15:00-15:30	**Coffee break**	
15:30-17:00	**Lab 7: (cont.)**	
Friday 21 November 2024		
8th floor, Computer lab		
08:30-09:00	MLST for AMR tracking	Pattrarat Chanchaithong
	Bioinformatic analysis 2 - Bacterial strain typing	Pattrarat Chanchaithong
09:00-10:00	**Lab 8:** Hands-on exercise for Bioinformatic analysis -2	CUVETAMR
10:00-10:30	Posttest	
10:30-11:00	**Coffee break**	
11:00-11:30	How to correlate AMR gene profile to bacterial sequence type	Pattrarat Chanchaithong
11:30-12:00	Wrap up and closing	CUVETAMR
12:00-13:30	**Lunch**	

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Pengalaman pembelajaran

- ESBLs detection at phenotypic and genotypic level
- Basic molecular biology for AMR testing and WGS
- WGS for AMR surveillance

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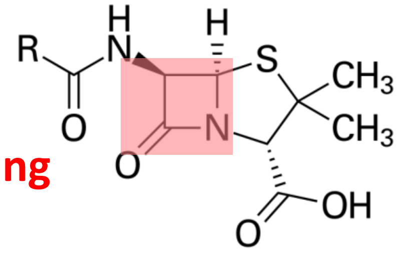
Apakah dia β-lactams and β-lactamases?

β-lactams ialah satu kumpulan besar antibiotik yang mempunyai struktur cincin β-lactam (eg Penicillins, Cephalosporins, Carbapenems, Monobactams).

Fungsi utama:

Menghalang pembentukan dinding sel bakteria → bakteria pecah dan musnah.



β-lactam ring

Penicillin

β-lactamases pula ialah enzim yang dihasilkan oleh bakteria untuk memecahkan cincin β-laktam pada antibiotik tersebut.

Bila cincin ini rosak → antibiotik tidak berfungsi → bakteria menjadi resistant.

Jenis-jenis β-lactamases:

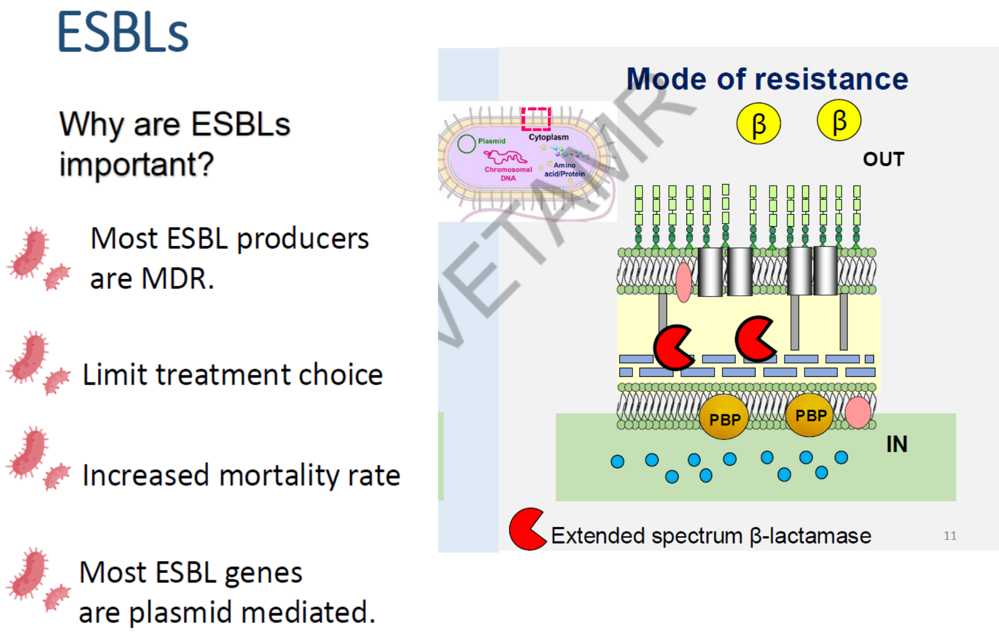
- Penicillinases
- Cephalosporinases (AmpC)
- Extended-Spectrum β-Lactamases (ESBL)
- Carbapenemases

ESBL (Extended-Spectrum Beta-Lactamase)

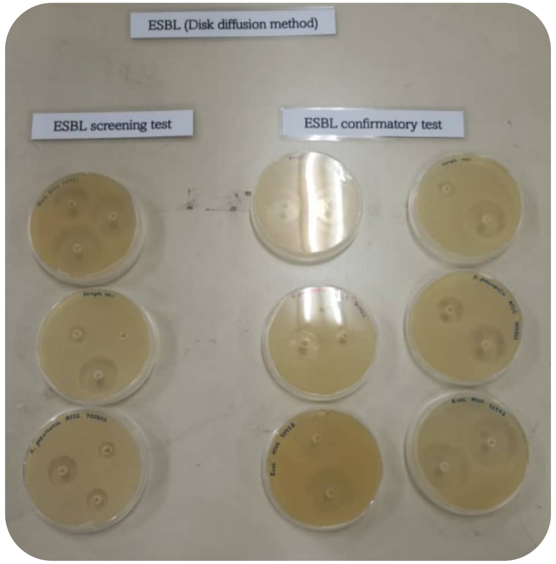
Jenis beta-lactamase yang lebih kuat dan lebih luas spektrum — mampu memecahkan banyak antibiotik beta-lactam generasi lebih tinggi (cephalosporin 3rd / 4^{th} gen), menjadikan bakteria lebih kebal.

Most common ESBL-producing bacteria:

Enterobacterales (*E. coli*, *Klebsiella pneumoniae*, *Salmonella*, etc.)



Phenotypic detection



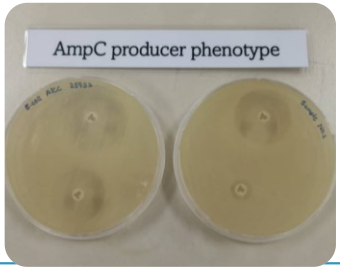
- Screening tests: detection of resistance to 3rd generation cephalosporins (3GC) - CTX, CAZ, CPD
- Confirmatory tests: Combination disk test (CDT) – CTX, CTX/CA, CAZ, CAZ/CA

ESBLs

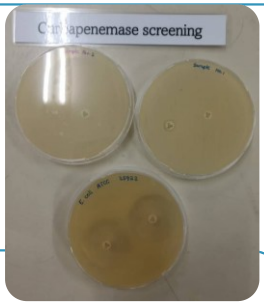
Positif ESBL: Report positif ESBLs
Negatif ESBL: Suspect AmpC/carbapenem.
Teruskan dengan pengesanan AmpC.

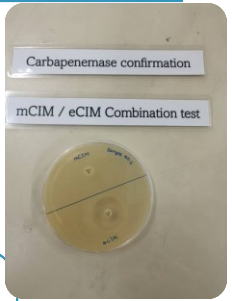
AmpC

- AmpC tests – FOX



Positif AmpC: Report positif AmpC.
Negatif AmpC: Suspect carbapeneme.
Teruskan dengan pengesanan carbapenem.

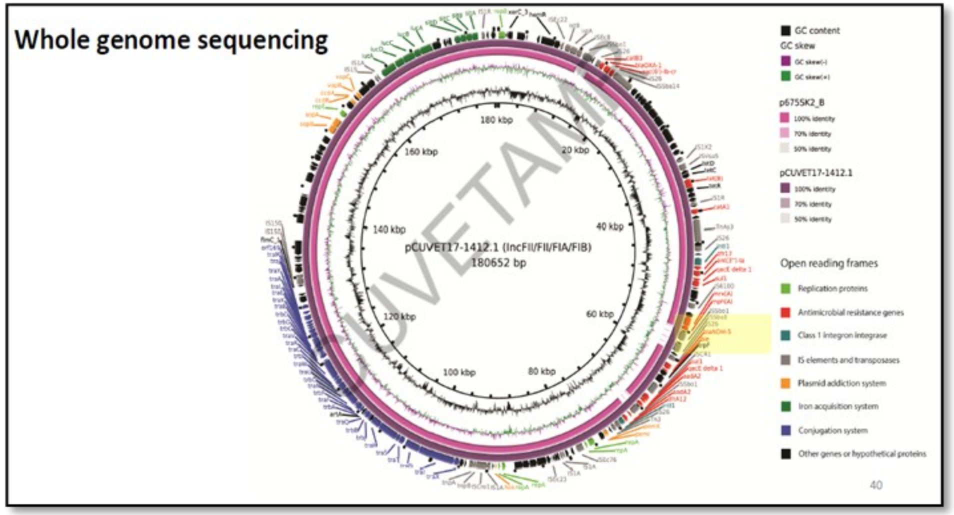




- Screening tests
- Confirmatory: Detection of carbapenemase production and activity (Carba NP test and mCIM test)

Carbapenemases

Tarikh labwork:
17-18/03/2025
(Day 1-2)



Whole Genome sequencing

Identifikasi menyeluruh bagi gen AMR, mutasi, dan elemen mobil, serta analisis filogenetik ber resolusi tinggi.

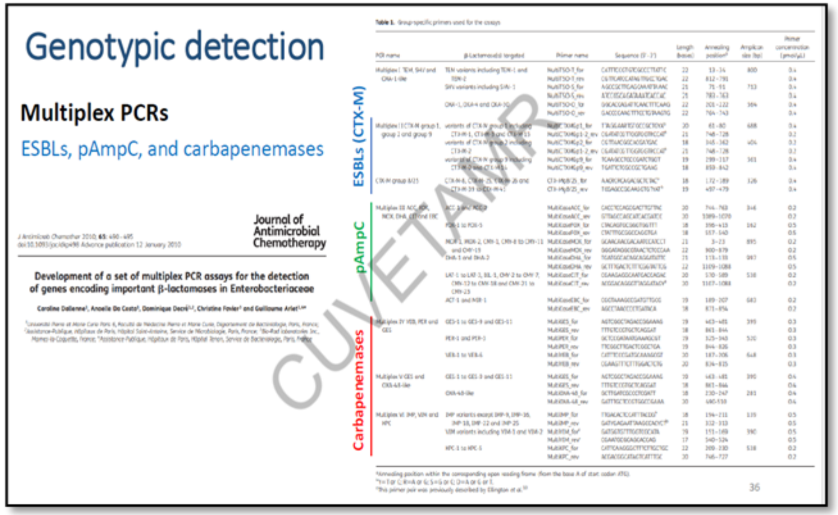
- Sesuai untuk surveillance, outbreak investigation, tracing sumber pencemaran, dan characterization lengkap strain.

Multiplex PCR

Genotypic detection

WGS

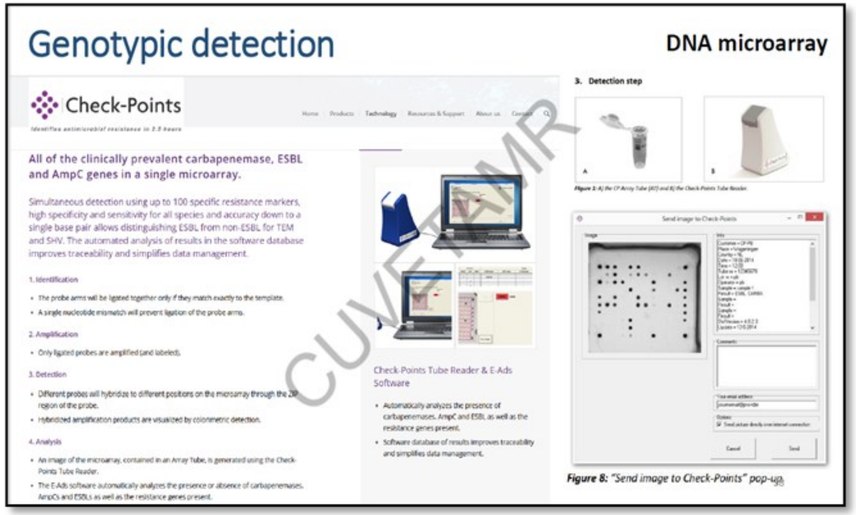
DNA microarray



Multiplex PCR

Pengesanan gen AMR spesifik.

- Sesuai untuk screening targeted apabila senarai gen sudah diketahui atau dijangka ada dalam populasi bakteria.

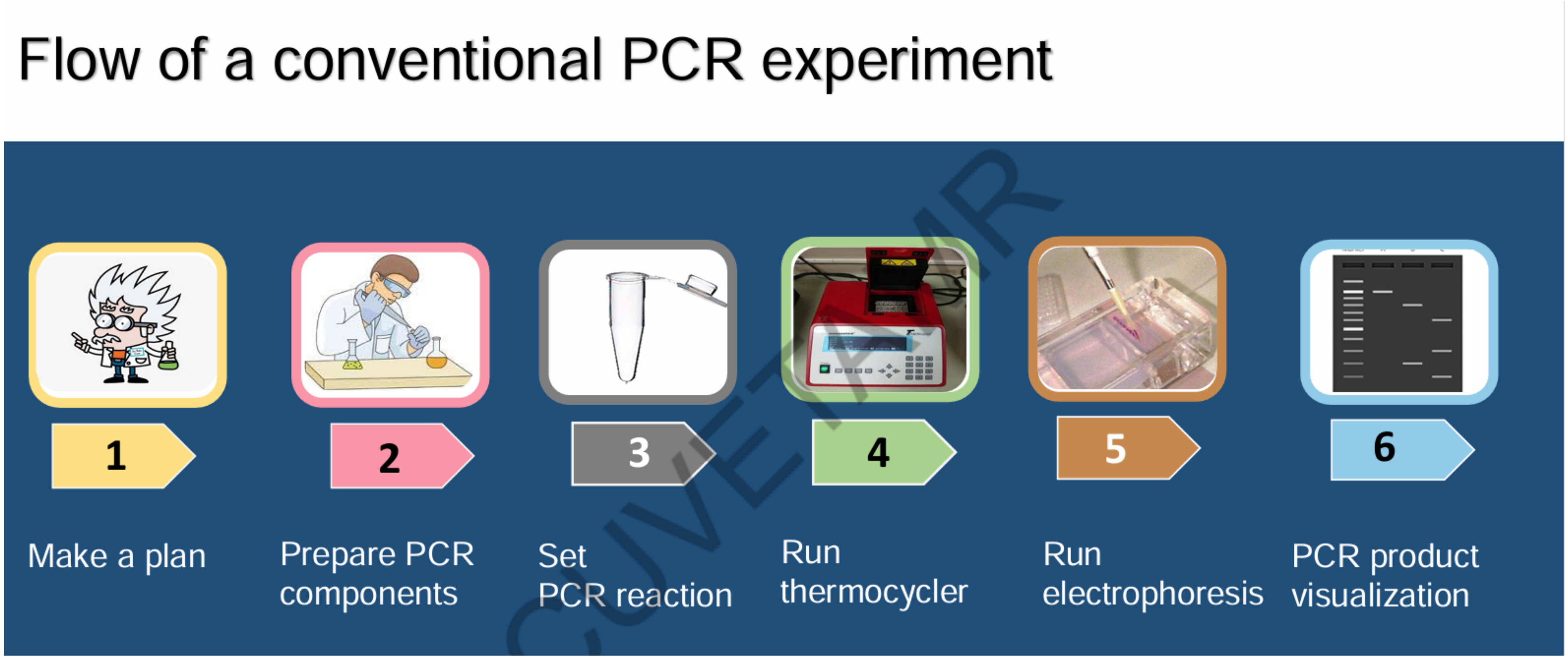


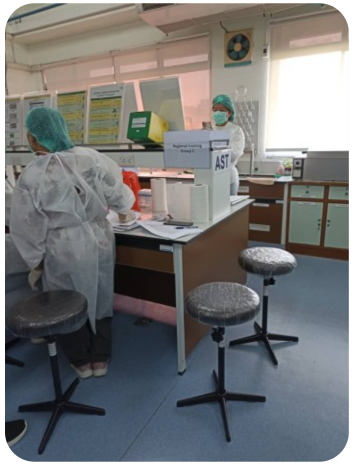
DNA microarray

Penentuan profil AMR/virulens .

- Sesuai untuk profil rintangan yang lebih luas, meliputi ratusan gen dalam satu analisis.

PCR – Detection of ESBL genes (bla$_{CTX\text{-}M}$ universal)

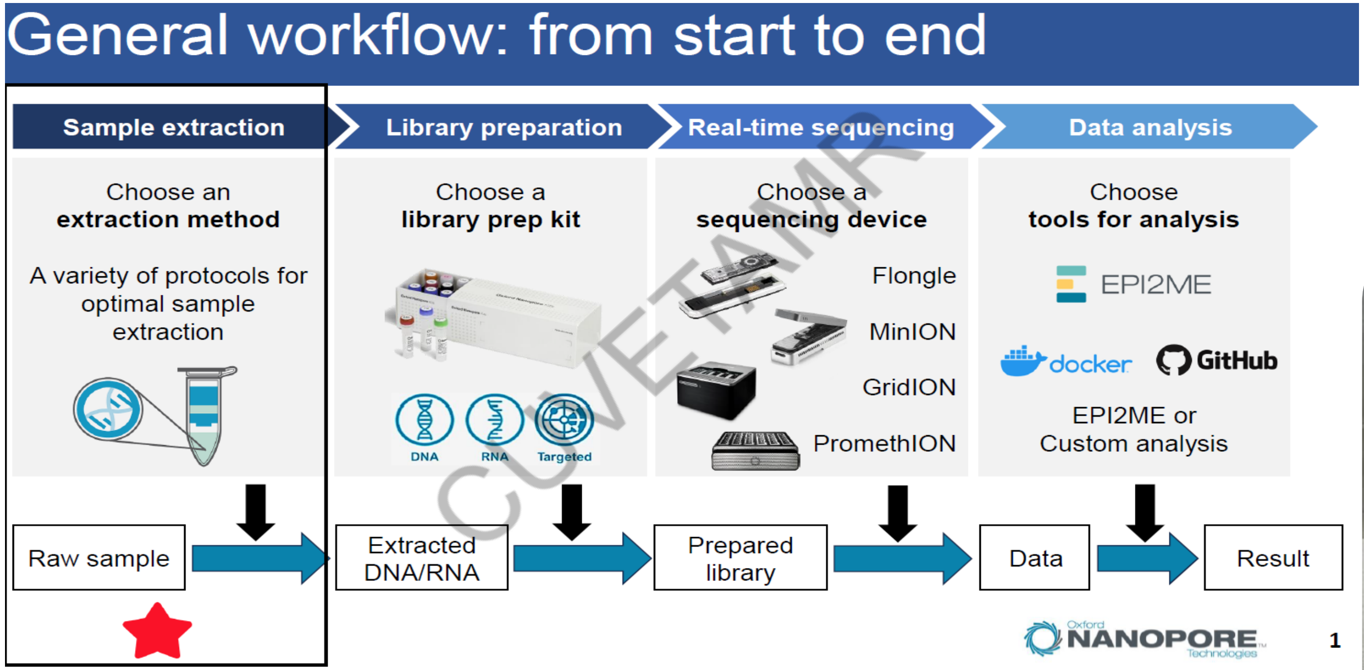




Detection bla$_{CTX\text{-}M}$ universal : PCR product 585 bp

Tarikh labwork:
18-19/03/2025
(Day 2-3)

Whole Genome Sequencing

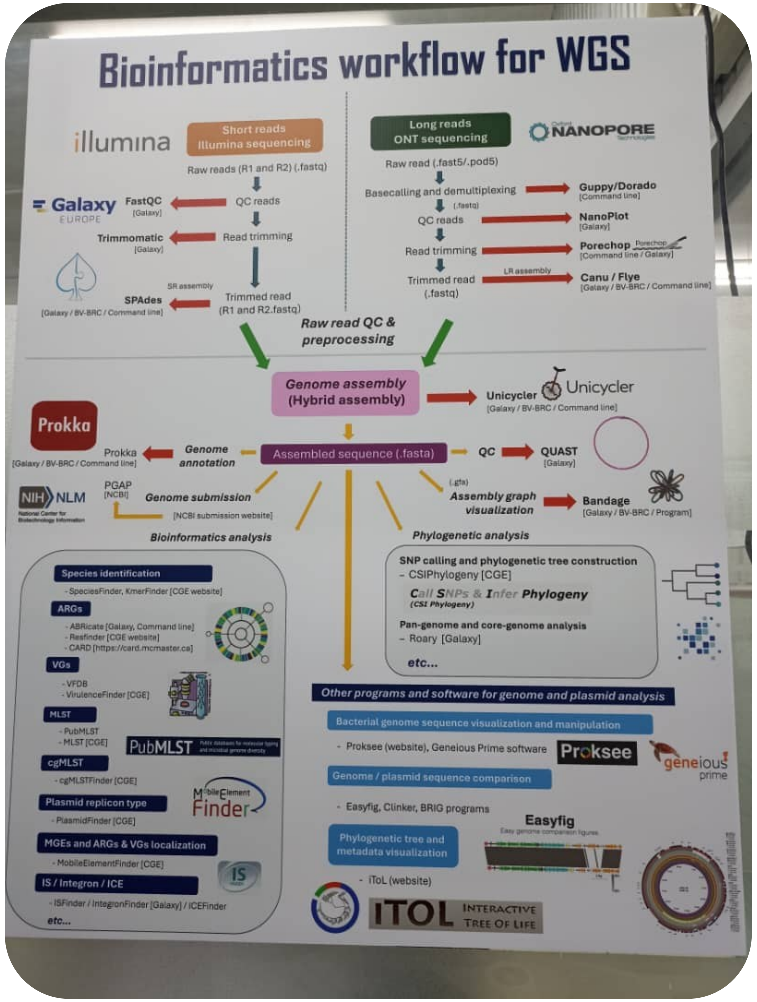


Magnetic beads
ZymoBIOMICS™ DNA Miniprep Kit

Rapid barcoding kit (RB01-24)

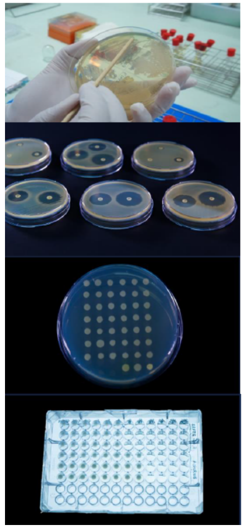
Oxford Nanopore (MinION)

Bioinformatics workflow for WGS



Tarikh labwork:
21-20/03/2025
(Day 3 -4)

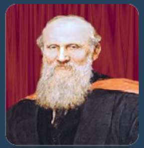
Phenotypic AST have limitations.



- Ensuring accurate results requires maintaining appropriate temperature, pH, atmospheric conditions, and ion concentrations in the culture medium.
- Some antibiotics, such as colistin, are not suitable for disk diffusion testing.
- Challenges for slow-growing or fastidious bacteria
- Standard approaches may not be applicable to anaerobic or rare bacterial species.
- Clinical breakpoints may be unavailable

These limitations are not addressed by WGS.

Potential benefits of WGS in AMR



"If you cannot measure it, you cannot improve it"

Lord Kelvin, 1824-1907

- Predict phenotypic traits based on the presence of specific genetic markers or mutations in the genome.
- Detection of novel molecular markers of drug resistance
- High-resolution tracing of microbial transmission events and rapid, accurate identification of outbreaks
- Linked to epidemiological and clinical metadata
- Rapid identification of AMR strains for better decision making
- Ready standardization and sharing of raw data for "big data" approaches;
- Potential facilitation of development of targeted diagnostics and novel antimicrobial treatments and vaccines.

AMR characterization according to surveillance objectives

The first step in selecting tests for AMR surveillance is defining the objectives.

AST Phenotypic methods

- Analysis of trends in AMR rates,
- Assessment of the frequency of AMR infections and their impact on human health,
- Data to inform the national list of essential antimicrobial medicines
- Data to inform treatment guidelines can be fully met by using phenotypic methods.

These can be fully met by using phenotypic methods.

WGS WGS as complement of phenotypic methods:

- To understand the genetic basis of AMR mechanisms
- To differentiate phenotypically identical isolates with the same AST profile.
- To allow the localization of AMR determinants on the bacterial chromosome or in plasmids
- To facilitate linkages during the early investigation phase of outbreaks.

WGS is not the method of choice in all circumstances.

How WGS, AST and clinical and epidemiological data together can result in action for public health!!

Epidemiology — Population and individual data

Clinical data — Patient and prescribing data

WGS data — Reference database

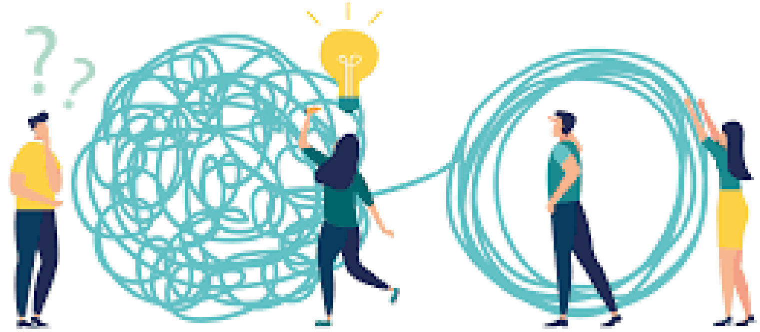
AST data — Reference database

→ **Combined analysis** → **Public health action**: Prevention and control of infections; Community health interventions

- Linkage of comprehensive genome databases to epidemiological and clinical metadata would be invaluable for public health, medical research and clinical care.

Cabaran yang dihadapi sepanjang latihan

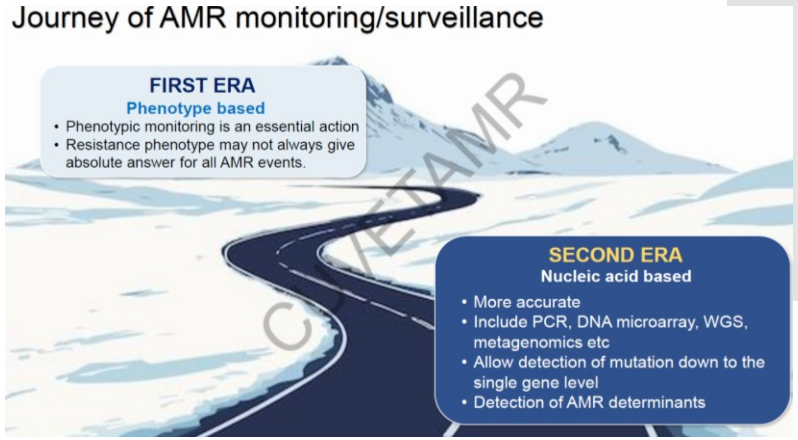
- Kepadatan modul latihan
- Masa yang singkat untuk pelajari WGS dan penggunaan perisian bioinformatik yang kompleks
- “Language barrier”



Cadangan penambahbaikan

Penambahbaikan keupayaan makmal

- ✓ Teknik pengesanan phenotypic (Disk diffusion - ESBLs, AmpC, carbapenemase detection).
- ✓ Pengesanan genotypic menggunakan PCR untuk ESBLs dan gen AMR lain.
- ✓ Peralatan untuk analisis gen AMR/WGS.
- ✓ Komputer berkapasiti tinggi untuk analisis bioinformatic WGS.



Kesimpulan

- Melalui latihan ini, negara dapat memperkukuh aktiviti survelan AMR dengan meningkatkan keupayaan makmal dalam menghasilkan data yang berkualiti dan standard.
- Penerapan teknik molekular dan *Whole-Genome Sequencing* (WGS) membolehkan pengesanan awal serta pemantauan AMR yang lebih tepat, menyokong pembuat dasar membuat keputusan berasaskan bukti dalam kesihatan awam veterinar.
- Selain itu, pembangunan kepakaran tempatan mengurangkan kebergantungan kepada sumber luar, sekaligus memperkasakan keupayaan negara dalam menangani ancaman AMR di peringkat nasional dan antarabangsa

TERIMA KASIH