Week 1

Friday, 30 July 2021 13:45

GENOMIC SEQUENCING

- BACTERIAL TYPING
- APPLICATIONS OF **WGS** → WHOLE GENOME SEQUENCING
- NEXT-GEN SEQUENCING
- DE-NOVO ASSEMBLY

BIOINFORMATICS TOOLS → PROCESSING DATA FROM SEQUENCING

SPECIES IDENTIFICATION
MLST TYPING
RESISTANCE GENE DETECTING
SEROTYPING
BACTERIAL ANALYSIS PIPELINE
SNP TREE CONSTRUCTION → SINGLE NUCLEOTIDE POLYMORPHISM
WGS RESULTS

1) WHAT DOES "TYPING MEAN"?
HOW DO WE PERFORM GENOTYPING?
WHAT METHOD SHOULD WE USE?

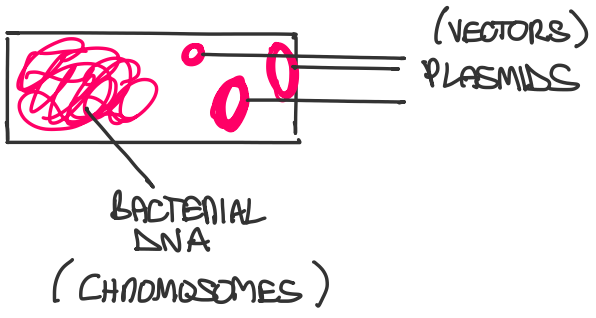
BACTERIAL TYPING

CHARACTERIZING BACTERIAS

- DNA **BANDING-PATTERN**
  DNA FRAGMENTED, FRAGMENTS SEPARATED
  PATTERN ARE ANALYSED

TYPING DIFFERENTIATIONS BEYOND SPECIES & SUB-SPECIES

EX: ALL HUMANS ARE DIFFERENT, YET THEY ARE FROM THE SAME SPECIE HOMO SAPIENS SAPIENS

PHENOTYPIC METHODS	GENOTYPING METHODS
EXPRESSED TRAITS (OLD WAY)	GENETIC CONTENT (DNA)
– FERMENTATION – CHANGE IN COLOR IN MEDIUM – MICROBIAL RESISTANCE PATTERNS	
BASED ON WHAT WE SEE	

FROM ISOLATE TO STRAIN

ISOLATE PURE CULTURE DERIVED FROM SINGLE COLONY THA ARISES FROM A SINGLE BACTERIUM

STRAIN SET OF ISOLATE THAT WHEN TYPED ARE INDISTINGUISHABLE F EACH OTHER

GENOTYPING

IN BOTH CASES WE START W/ BACTERIAL CELLS

BAND BASED

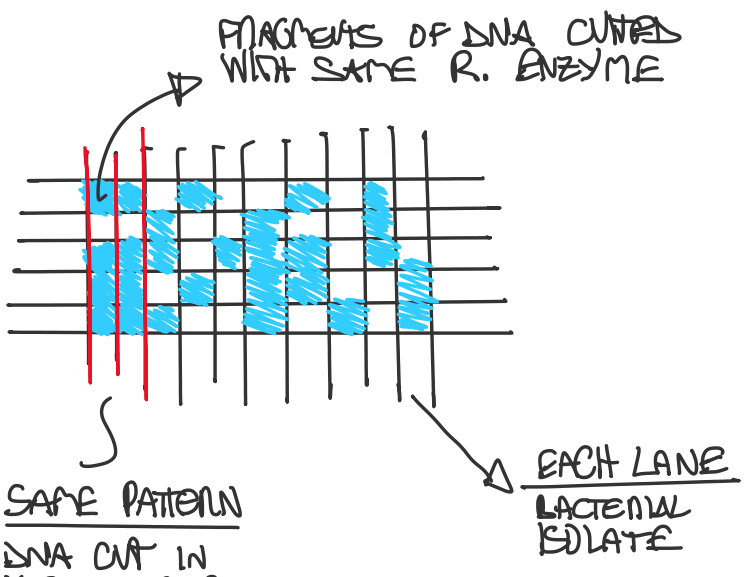
ELECTROPHORESIS

EACH LANE = BACTERIAL ISOLATE W/ SAME RESTRICTION ENZYMES

SOLVE DNA IN BANDS
- AMPLIFICATION BY PCR
- CUTTING W/ RESTRICTION ENZYMES

OUTPUT
DNA FRAGMENTED IN PIECES OF DIFFERENT SIZE

ELECTROPHORESIS



SAME PATTERN
DNA CUT IN THE SAME PLACES
=
STRAINS ARE RELATED TO EACH OTHER

SEQUENCE BASED

PCR — ORGANISM SEQUENCE

NEEDS TO BE ANALIZED

DNA EXTRACTED FROM CELL AND THEN SEQUENCE

STANDARD WGS: WHOLE GENOME SEQUEN

WHAT TO ANALYZE?
- SNP
- REPEAT TRAITS

AAAATCG AAATT
AAG AG AG AG AG AG AG A

YOU CAN CARACTHERIZE ON MANY DIFFERENT LEVELS

HOW TO CHOOSE A GENOTYPING METHOD ?

- TYPEABILITY — ABILITY TO TYPE MANY STRAINS
- DISCRIMINATORY POWER — P(DIFFERENT TYPES FOR 2 UNRELATED STRAINS IN POPULATION)
- REPRODUCIBILITY — SAME RESULTS OVERTIME
- RAPIDITY
- COST
- LAB CAPACITY

} PRACTICAL

FINER RESOLUTION BETWEEN BACTERIAL INDIVIDUALS

PURPOSE ?

- TRACING AN OUTBREAK (PANDEMIC)
  - HIGH DISCRIMINATORY-POWER
    WE WANT TO KNOW IF STRAINS IN DIFFERENT INDIVIDUALS ARE RELATED OR NOT
- LONG-TERM SURVEILLANCE

WE DON'T WANT FINE DETAILS
ONLY GENERAL PICTURE

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE USING WGS

- SURVEILLANCE
- BIOINFORMATICS TOOL
- GENOTYPING MONITORING

WHY → OBTAINING DATA FOR ACTION

MOLECULAR DIAGNOSTIC

PHENOTYPIC DATA — RELIABLE BUT TAKE LONGER TO DO & ARE PRONE TO MISTAKES

OLD CONVENTIONAL MICROBIOLOGICAL DIAGNOSTICS
NEW PLUG & PLAY BIOINFORMATICS TOOLS

K-MER FINDER
SEQ-SERO
NESS-FINDER
PLASMID-FINDER
VIRULANCE-FINDER
SNP-TREE

GENOTYPIC METHODS ARE BETTER

WEAKNESS OF WGS (DATABASES)

CANNOT DETECT NEW GENES
DB HAS TO BE MAINTAINED