Whole Genome Sequencing for TB diagnostics

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WGS applications in TB diagnostics

- **Resistance genotyping**
  - **Inform choice of treatment**

- **Surveillance**

WGS applications in TB diagnostics

- **Resistance genotyping**
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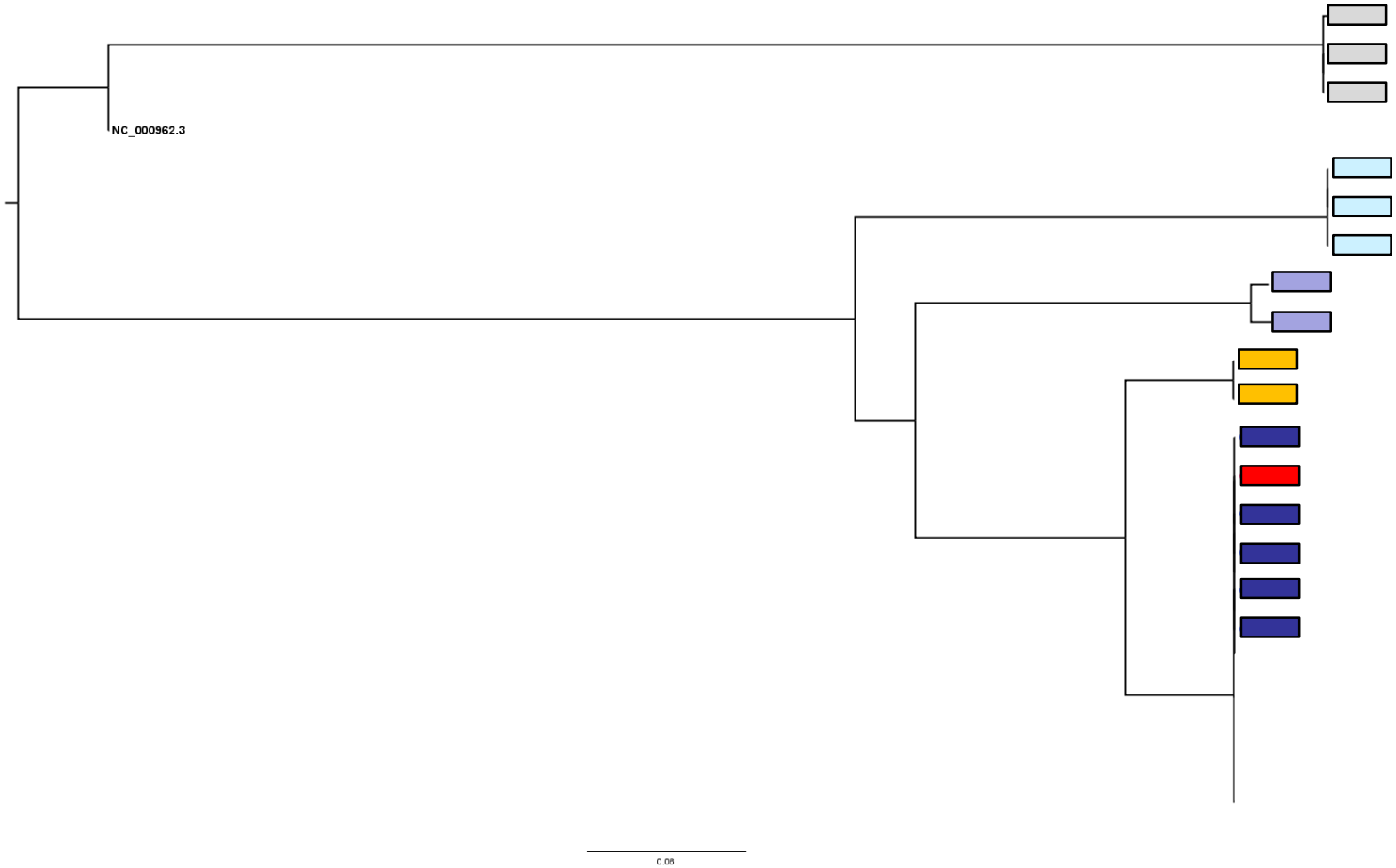
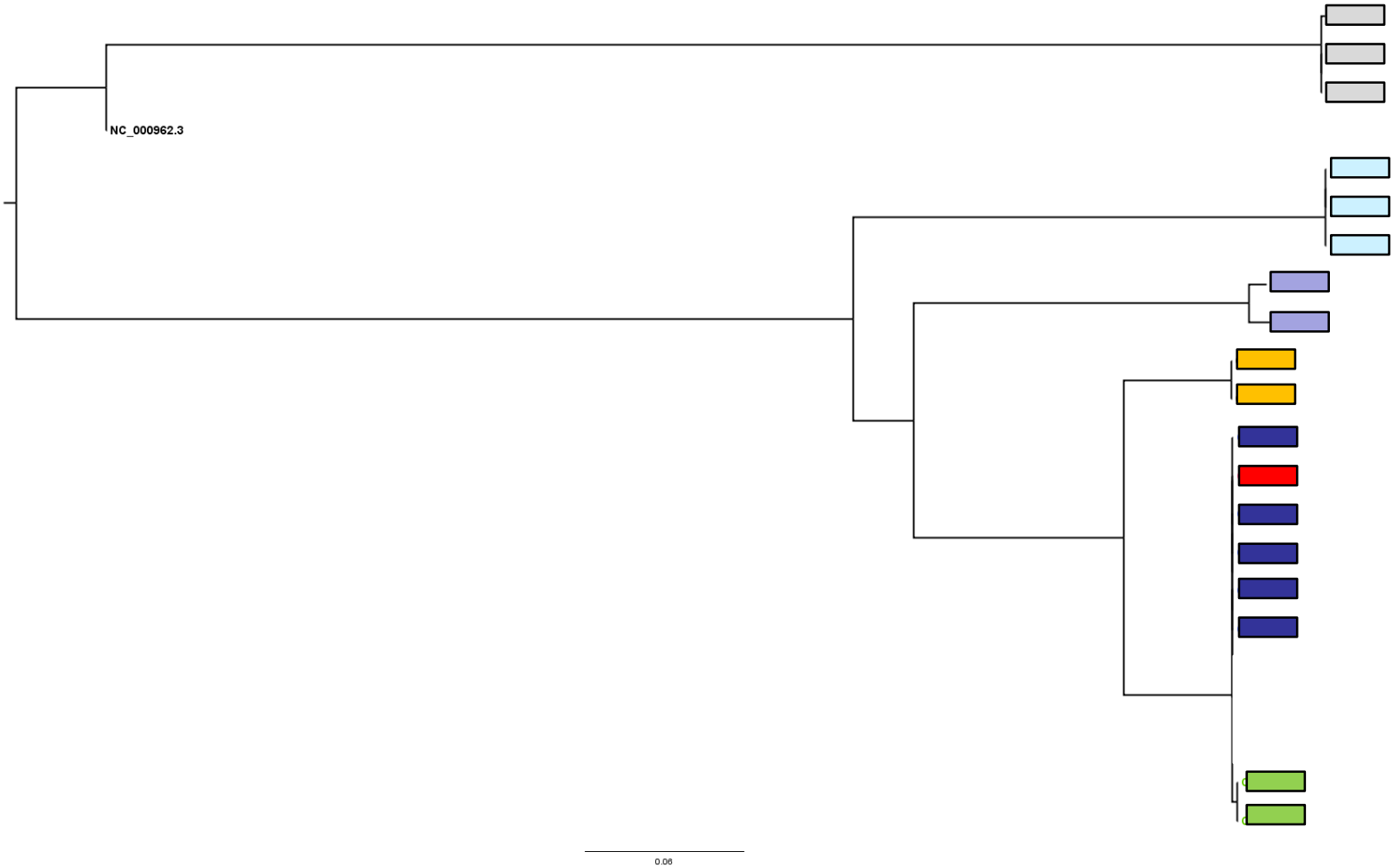
  - **Associate with outcomes**

- **Surveillance**
  - **Identify transmissions / outbreaks**
  - **Direct public health interventions**
  - **Inform choice of treatment**



Witney et al. *J Clin Microbiol* (2015)



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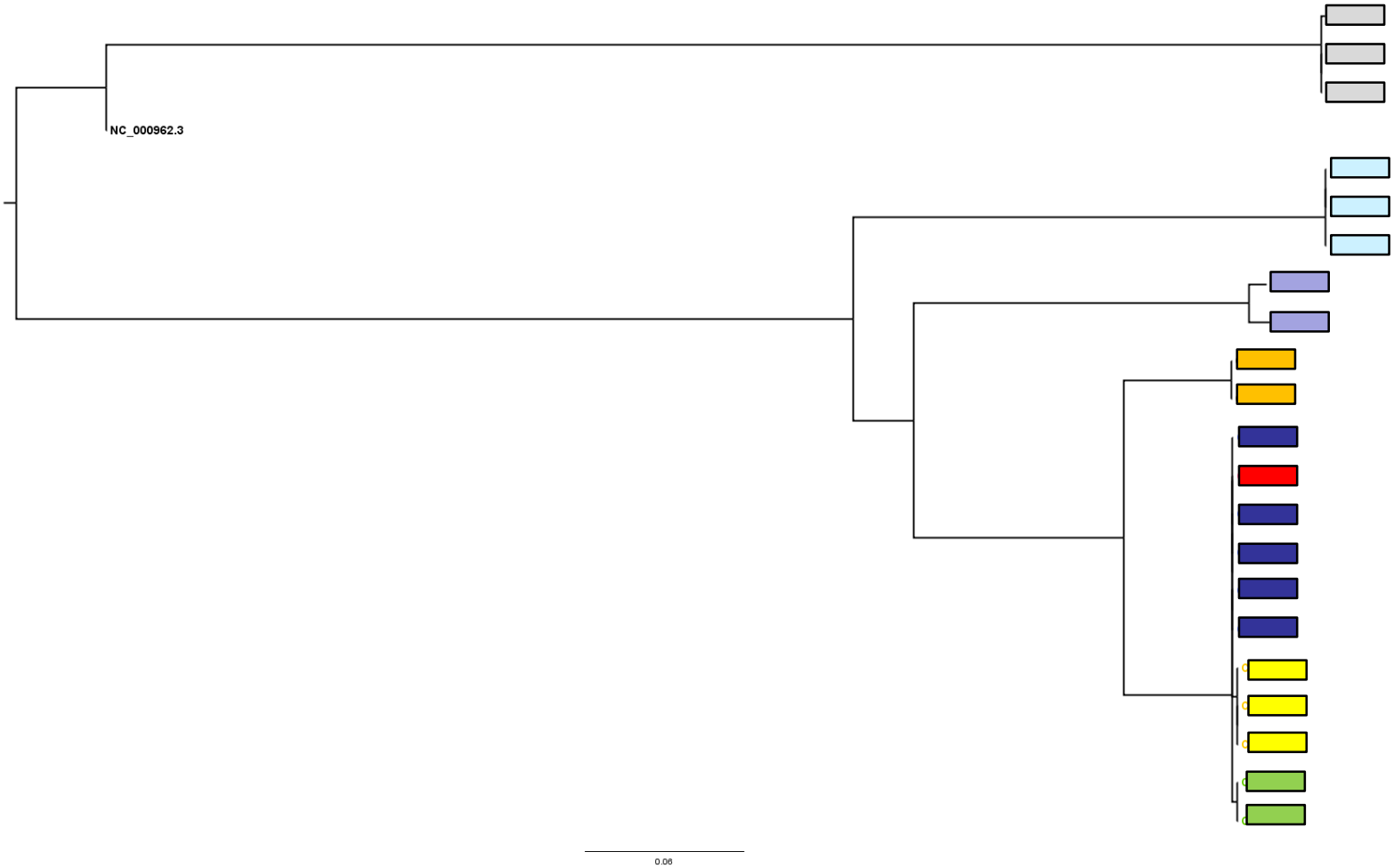

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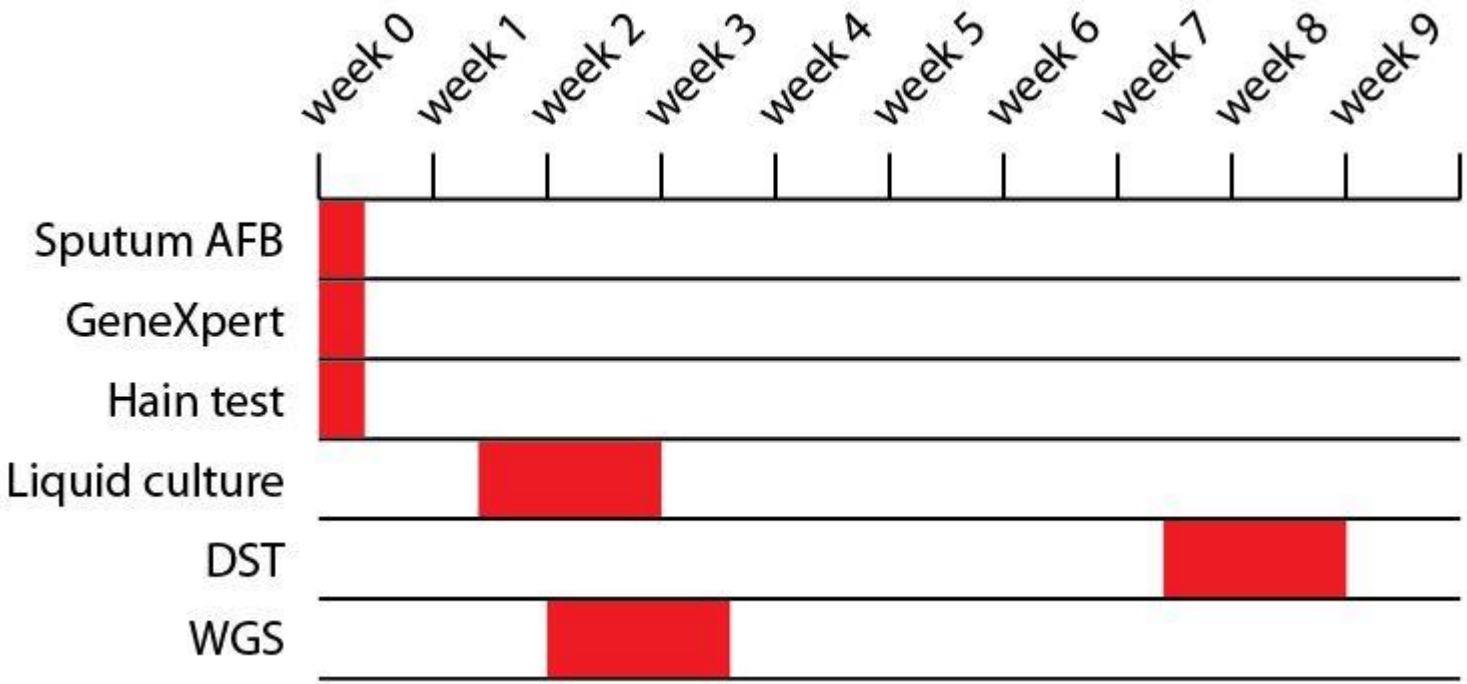
TB Diagnostic WGS

- **Where next?**
  - **Faster?**
  - **More accurate**
  - **More agile?**
  - **Cheaper**

TB Diagnostic timelines



TB Diagnostic workflow

Sample → Culture → DNA extraction → WGS → Bioinformatics

TB Diagnostic workflow

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- **Culture**
  - **Ensures sufficient TB DNA for WGS**
  - **Purifies out host and other organism contamination**
  - **But, adds extra time**

TB Diagnostic workflow

Sample → DNA extraction → WGS → Bioinformatics

- **Culture free**
  - **Metagenomic approach (Doughty et al, 2014)**
    - **Only low coverage achieved (< 0.7x)**
  - **SureSelect target enrichment (Brown et al, 2015)**
    - **Slow (2-3 days), expensive**
  - **MolYsis enrichment (Votintseva et al, 2017)**
    - **21/39 samples: >12x depth, >90% genome recovery**
    - **14/39 samples: <3x depth, <12% genome recovery**

TB Diagnostic WGS

- **Where next?**
  - **Faster?**
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TB Diagnostic workflow

Sample → Culture → DNA extraction → WGS → Bioinformatics

- **WGS platform**
  - **Speed**
  - **Accuracy**
  - **Cost**
  - **Agility**

Illumina

- **Current platforms**

	iSeq 100	MiniSeq	MiSeq	NextSeq	HiSeq 4000	HiSeq X	NovaSeq 6000
Run Time	9-17.5 hrs	4-24 hrs	4-55 hrs	12-30 hrs	1-3.5 days	<3 days	16-44 hrs
Output	1.2Gb	7.5Gb	15Gb	120Gb	1500Gb	1800Gb	6000Gb
Reads	4 million	25 million	24 million	400 million	5 billion	6 billion	20 billion
Read Length	2x150bp	2x150bp	2x300bp	2x150bp	2x150bp	2x150bp	2x150bp

- **50x coverage of 4.5Mb genome = 225Mb**

https://emea.illumina.com/systems/sequencing-platforms.html

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TB genomes	5	33	67	533*	6667*	8000*	26667*

- **50x coverage of 4.5Mb genome = 225Mb**

* can only multiplex up to 96 or 384 samples

https://emea.illumina.com/systems/sequencing-platforms.html

Ion (ThermoFisher)

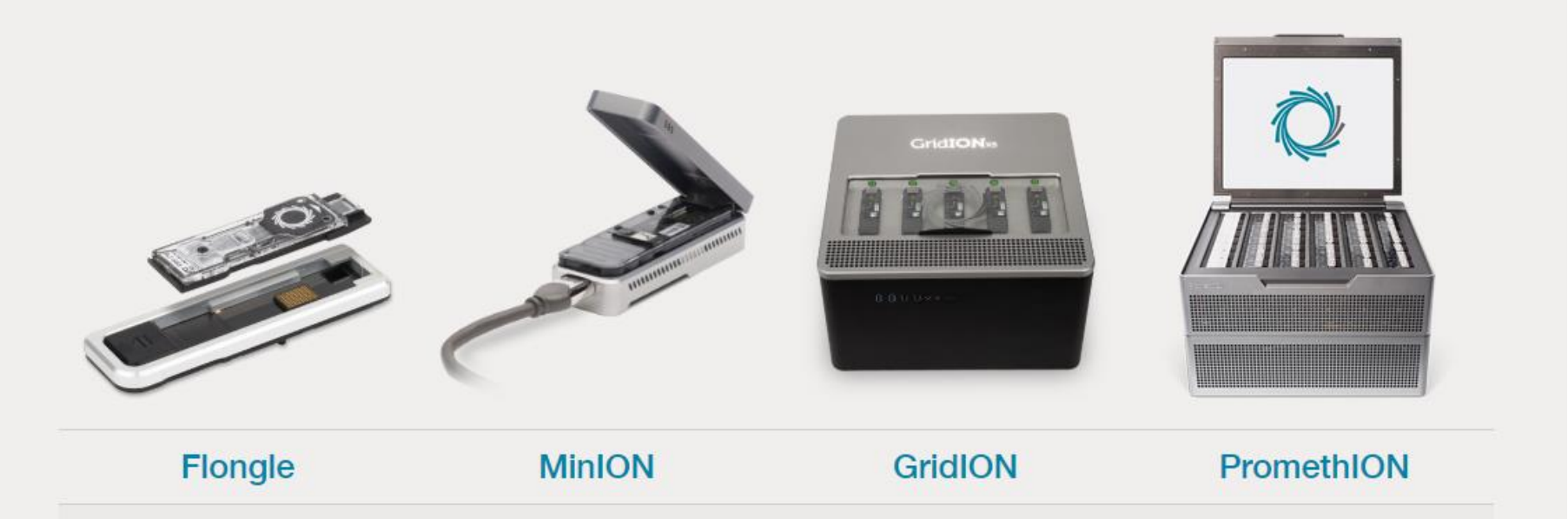
- **Current platforms**

	Ion 314	Ion 316	Ion 318	Ion PI
Run Time	2-4 hrs	3-5 hrs	4-8 hrs	2-4 hrs
Output	30-100Mb	300-1000Mb	600-2000Mb	15Gb
Reads	500,000	3 million	5 million	60-80 million
Read Length	200/400bp	200/400bp	200/400bp	200bp
TB genomes	1 (22x)	4	8	67

- **50x coverage of 4.5Mb genome = 225Mb**

https://www.thermofisher.com

Oxford Nanopore Technologies



SmidgION

https://nanoporetech.com/products

Oxford Nanopore Technologies

- **Current platforms**

	Flongle	MinION	GridIONx5	PromethION (1 flow cell)	PromethION (48 flow cells)
Run Time	1min-16hrs	1min-48hrs	1min-48hrs	1min-64hrs	1min-64hrs
Output	3.3Gb (1Gb)	40Gb (22Gb)	200Gb (110Gb)	315Gb (150Gb)	15Tb
Available		yes	yes		
TB genomes		98 (96*)	489 (96*)		

- **Read length variable (2Mb reads reported)**
- **50x coverage of 4.5Mb genome = 225Mb**

* can only multiplex up to 96 samples

https://nanoporetech.com/products

Oxford Nanopore Technologies

- **Highly portable options**
- **Real time, data starts in minutes**
- **No fixed run time, can be run until enough data generated**
- **Pay as you go model available, no capital expenditure required**

- **Long reads (up to 2Mb reads reported)**
- **Can detect base modifications e.g. methylation**

TB Diagnostic workflow

Sample → Culture → DNA extraction → WGS → Bioinformatics

- **WGS platform**
  - **Speed**
  - **Accuracy: Error rate = 0.8% / 5-30% (MiSeq/MinION)**
  - **Cost: ~£400-500 per sample (Pankhurst et al (2016))**
  - **Agility**

Agile - Where should WGS be performed?

- Central Public Health labs
  - Economies of scale
  - National surveillance
  - Response time
- Local/regional hospitals
  - Closer to the physician/patient
  - Response time
- Answer may be different for different countries?

TB Diagnostic workflow

Sample → Culture → DNA extraction → WGS → Bioinformatics

- **WGS platform**
  - **Speed**
  - **Accuracy**
  - **Cost**
  - **Agility**

Choice of platform

- **Local requirements**
- **Priorities**

TB Diagnostic workflow

Sample → Culture → DNA extraction → WGS → Bioinformatics

- **Bioinformatics**
  - **Knowledgebase**
  - **Data Management**
  - **Software Tools**

Bioinformatics - Knowledgebase

- **TBDreamDB (https://tbdreamdb.ki.se/Info/)**
  - **Sandgren et al. *PLoS Med* (2009)**
- **Coll et al. *Genome Medicine* (2015)**
  - **TB-Profiler (http://tbdr.lshtm.ac.uk/)**
- **Walker et al. *Lancet Infect Dis* (2015)**
- **Bradley et al. *Nature Comm* (2015)**
  - **Mykrobe Predictor**
- **ReSeqTB (https://platform.reseqtb.org/)**
- **CRyPTIC (http://www.crypticproject.org/)**

WGS resistance prediction accuracy

Drug	Sensitivity (%)	Specificity (%)
Isoniazid	92.8 (89.9-95.7)	100 (100-100)
Rifampicin	96.2 (93.9-98.5)	98.1 (96.8-99.4)
Ethambutal	88.7 (83.6-93.8)	81.7 (77.6-85.8)
Pyrazinamide	70.9 (62.4-79.4)	93.9 (90.6-97.2)
Streptomycin	87.1 (82.7-91.5)	89.7 (86.0-93.4)
Ethionamide	73.6 (66.7-80.5)	93.3 (89.6-97.0)
Moxifloxacin	60.0 (29.6-90.4)	68.7 (52.6-84.8)
Ofloxacin	85.5 (79.1-91.9)	94.9 (91.8-98.0)
Amikacin	82.9 (74.4-91.4)	98.3 (96.0-100)
Capreomycin	60.7 (50.6-70.8)	90.7 (87.2-94.2)
Kanamycin	87.3 (81.3-93.3)	93.4 (89.9-96.9)

Coll et al. *Genome Medicine* (2015)
TB-Profiler (http://tbdr.lshtm.ac.uk/)

Bioinformatics – Data Management

- **Public release?**
  - **European Nucleotide Archive (EBI)**
  - **Short Read Archive (NCBI)**
  - **ReSeqTB (https://platform.reseqtb.org/) ?**
- **Which meta-data?**

Bioinformatics – Software Tools

- **TBDreamDB (https://tbdreamdb.ki.se/Info/)**
- **TB-Profiler (http://tbdr.lshtm.ac.uk/)**
- **PhrResSE (https://bioinf.fz-borstel.de/mchipscgi/phy/phyresse)**
- **TGS-TB (https://gph.niid.go.jp/tgs-tb/)**
- **ReSeqTB (https://platform.reseqtb.org/)**

- **Mykrobe Predictor (http://www.mykrobe.com/)**
- **KvarQ (https://github.com/kvarq/kvarq)**

TB and beyond?

- **Will WGS become a standard test in diagnostic labs?**
  - **TB**
  - **Salmonella**
  - **HIV**
  - **Gram negatives?**
- **When?**
  - **To replace culture, WGS should be < 30hrs and < £15 per sample**

Resistance determination in the future…?

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