

Инновации в диагностики ТБ. Секвенирование полного генома

Др. Гиртс Шкендерс

Врач лаборатории Рижской Восточной клинической университетской
больницы

ВОЗ Центр сотрудничества по обучению и исследованию МЛУ-ТБ,

WHO & Foundation for Innovative New Diagnostics (FIND)

Скрининг и сортировка на туберкулез

Test/tool (Manufacturer)	Type	Sensitivity*/ Specificity*/ AUC**	Lowest level of use	Time to results	Price per image/ test/tool	Stage of development/ WHO review
Delft Light (Delft Imaging)	Portable X-ray	—	Community	10 seconds ¹²	~US\$83,000 (including portable solar panel) ¹³	Commercially available
FDR Xair (Fujifilm)	Portable X-ray	—	Community	2–3 seconds	US\$70,000 to US\$100,000 ¹⁴	Commercially available
Impact (MinXray)	Portable X-ray	—	Community	~4 seconds ¹⁵	US\$47,000 to US\$50,000 ¹⁶	Commercially available
HandMed (JLK Inspection)	Portable X-ray	—	Community	< 3 seconds ¹⁷	Varies ¹⁸	Commercially available
CAD4TB v6 (Delft Imaging)	CAD	SE: 95% SP: 80% AUC: 0.92 ¹⁹	Community	< 20 seconds ²⁰	Image: US\$0.82 to US\$1.11; Offline unit: US\$7,000 ²¹	WHO review: 2020
qXR v3 (qure.ai)	CAD	SE: 95%*** SP: 82%*** AUC: 0.94 ²²	Community	< 1 min	Image: Volume-based pricing; ²³ Offline unit: price not available	WHO review: 2020
INSIGHT CXR (Lunit)	CAD	SE: 95% SP: 76% AUC: 0.94 ²⁴	Community	20 seconds	Image: Volume-based pricing; ²⁵ Offline unit: price not available	WHO review: 2020
JLD-02K JVIEWER-X (JLK Inspection)	CAD	Not yet available	Community	< 10 seconds	Varies ²⁶	Projected year of WHO review: 2021
TB Breathalyser (Rapid Biosensor Systems)	Breath test	SE: > 95% SP: > 95% (early data from field trials) ²⁷	Community	2 min	< US\$5 per test; < US\$2,000 for the reader ²⁸	Early stage development
Aeonose (The eNose Company)	Breath test	SE: 81% SP: 60% ²⁹	Community	Rapid	< US\$10 ³⁰	Early stage development

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POC - Тест на биомаркер ТБ, не требующий сбора мокроты

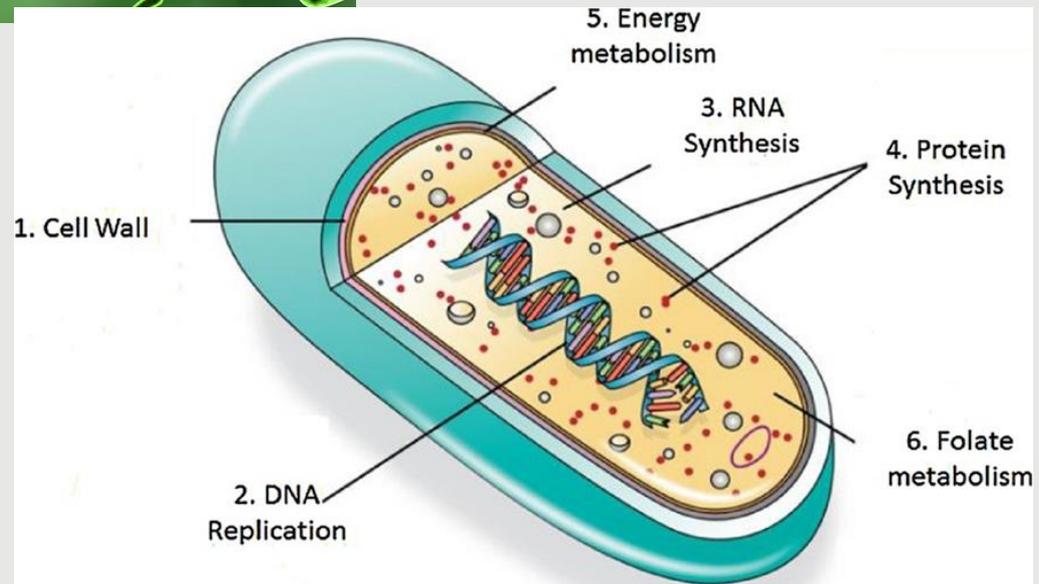
Test/tool (Manufacturer)	Type	Sensitivity*	Specificity*	Lowest level of use	Time to results	Price per test	Stage of development/ WHO review
SILVAMP TB LAM (Fujifilm)	Urine LAM test	87.1% 0-100 CD4 cells/mm ³ ; 62.7% 101-200 CD4 cells/mm ³	80.5% 0-100 CD4 cells/mm ³ ; 95% 101-200 CD4 cells/mm ³ ³⁷	Community	< 60 min	Not yet available	WHO review: 2021
Next-generation sensitive LAM (Abbott)	Urine LAM test	Not yet Available	Not yet available	Community	Not yet available	Not yet available	Early development ³⁸
Next-generation sensitive LAM (Gates Foundation/ Salus Discovery)	Urine LAM test	90-95% target sensitivity ³⁹	Not yet available	Community	Not yet available	Not yet available	Early development
Next-generation sensitive LAM (Global Good/ Biopromic)	Urine LAM test	Not yet Available	Not yet available	Community	Not yet available	Not yet available	Early development ⁴⁰

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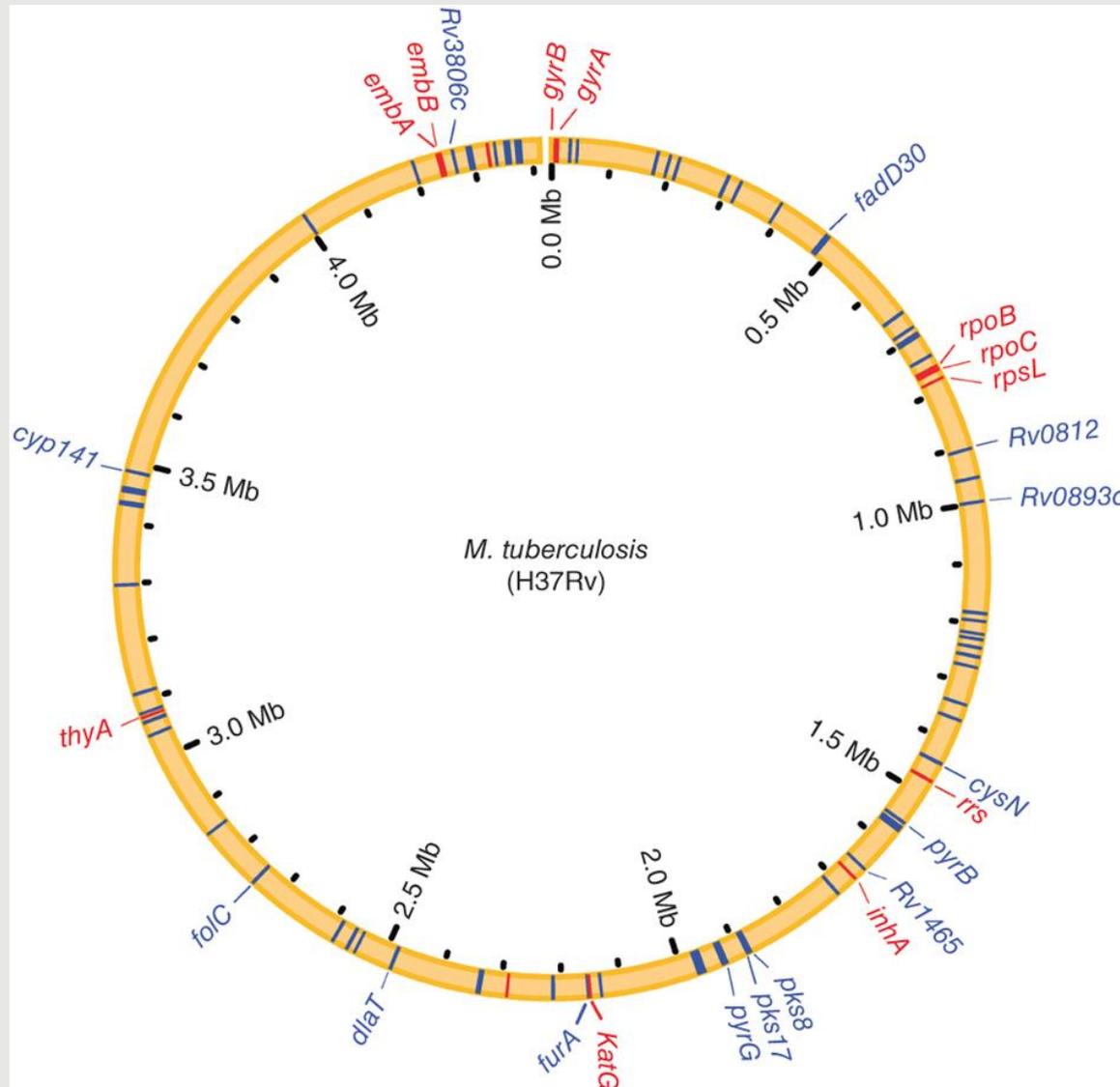
Тесты для раннего выявления туберкулеза и оценки эффективности лечения

Test/tool (Manufacturer)	Type	Potential use case	Sensitivity* / Specificity* / AUC**	Time to results	Price per test	Stage of development
Xpert-MTB-HR (Cepheid)	Host blood RNA response/PCR	Incipient TB/ Treatment monitoring	Incipient TB triage (PLWHA): SE: 90% SP: 55.8% AUC: 0.89 ¹¹³	Not yet available	Comparable to pricing of other Xpert tests ¹¹⁴	Early development; Expected launch in 2023 ¹¹⁵
RISK6 signature assay (QuantuMDx)	Host blood RNA response/PCR	TB infection/ Incipient TB	Not yet available	< 30 min ¹¹⁶	PCR-based test pricing	Early development/ Projected year of WHO review: 2022 ¹¹⁷
FilmArray Assay (bioMérieux)	Host blood RNA response/PCR	Treatment monitoring	Not yet available	< 1 hour ¹¹⁸	PCR-based test pricing	Early development ¹¹⁹

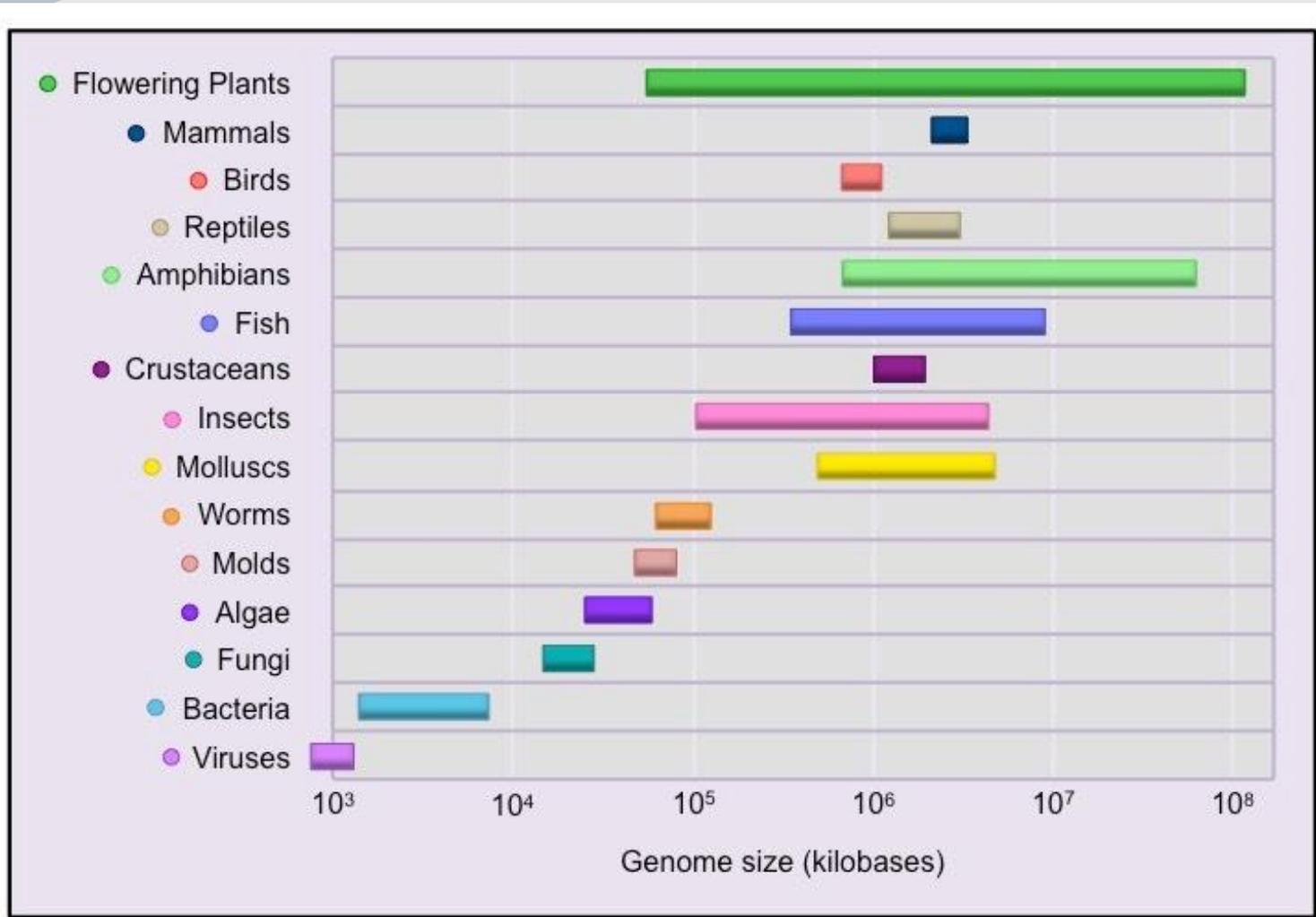
Mycobacterium tuberculosis



M.tuberculosis



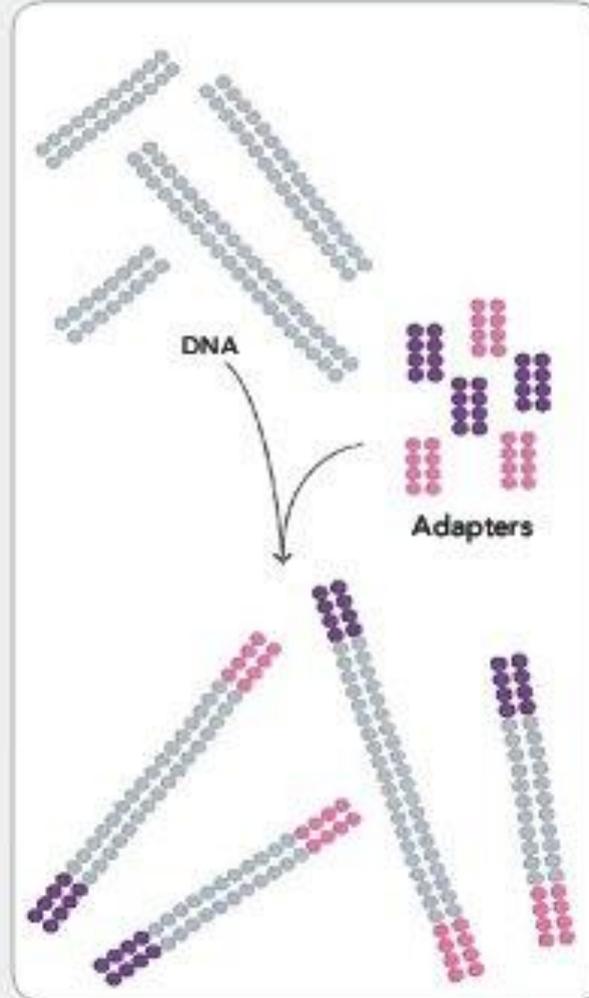
Сравнительные значения генетического материала, в килобазах



Крупномасштабное параллельное секвенирование (NGS – Next Generation Sequencing)

- крупномасштабное параллельное секвенирование – основной принцип пробоподготовки:
 - Полученные большие количества с клонально амплифицированными фрагментами ДНК
 - Полученные клонально амплифицированные фрагменты ДНК секвенируют параллельно путем секвенирования каждого клона отдельно.

1. PREPARE GENOMIC DNA SAMPLE



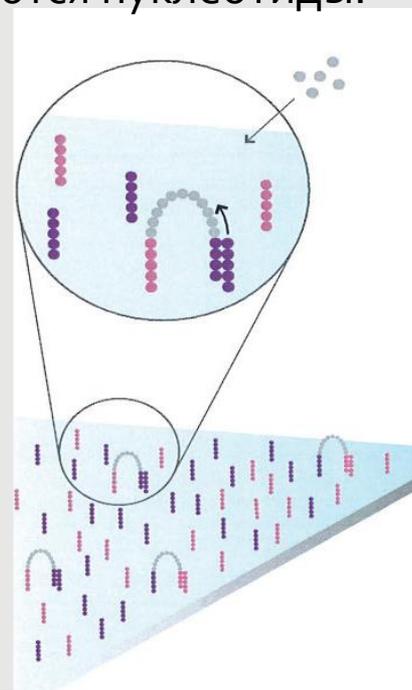
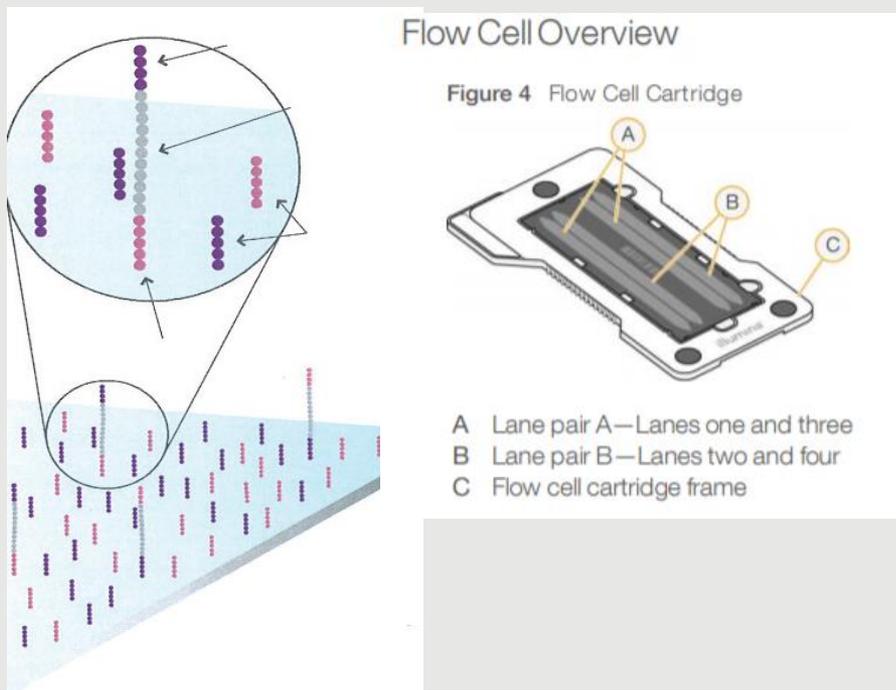
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

технология секвенирования

- Образцы оцДНК наносят на внутреннюю поверхность проточной кюветы (flow cell). Адаптеры крепятся к слайду.

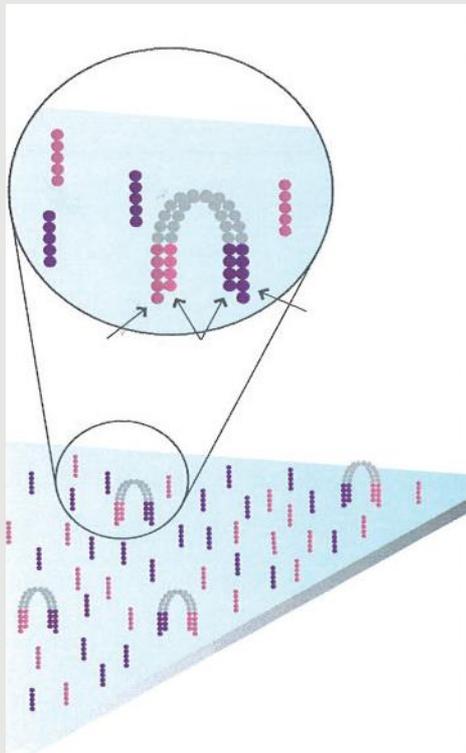
Свободный адаптер гибридизуется с праймером, прикрепленным к поверхности проточной кюветы, образуя структуру моста.

Добавляются нуклеотиды.

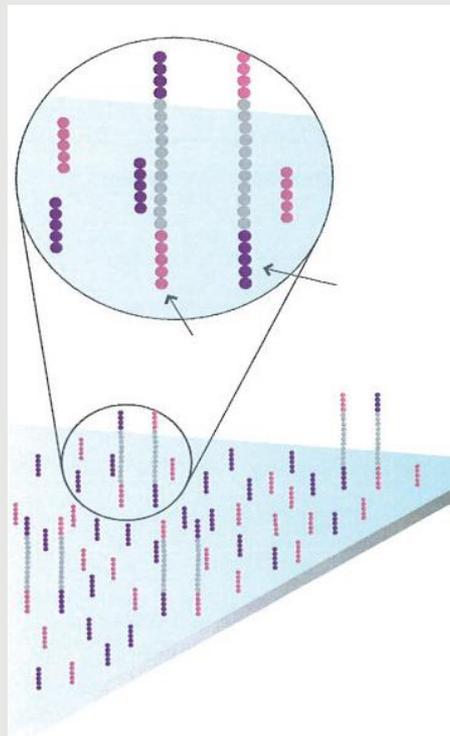


технология секвенирования

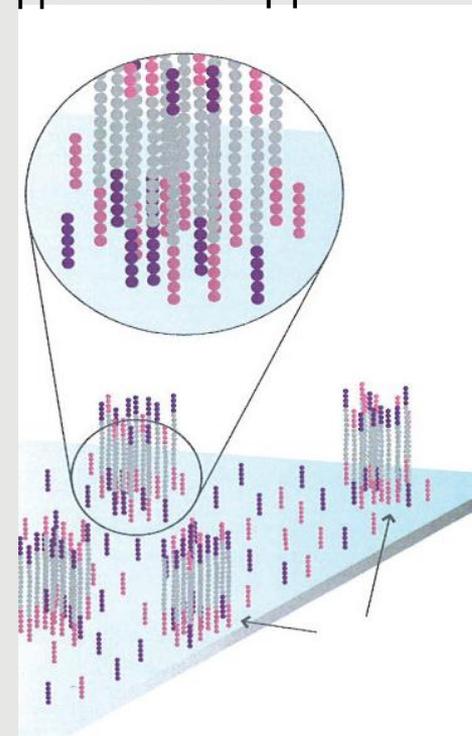
Полимераза синтезирует комплементарную цепь - образуется мостик дцДНК.



дцДНК денатурирует, образуется оцДНК.



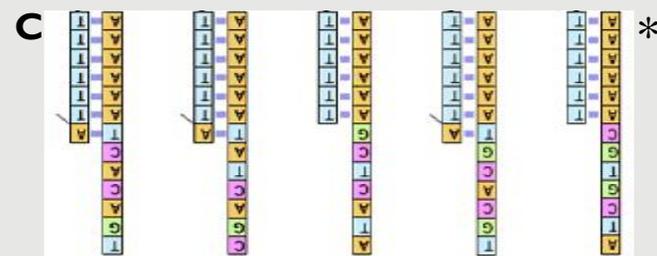
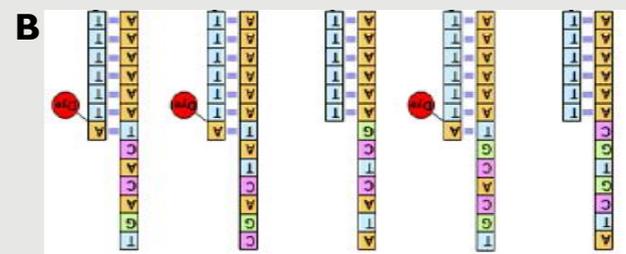
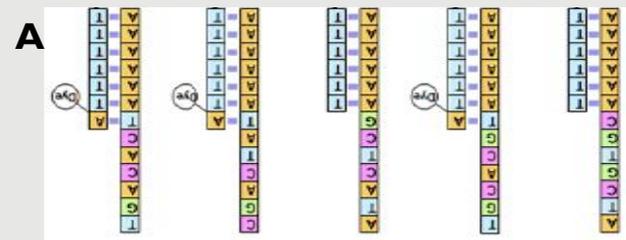
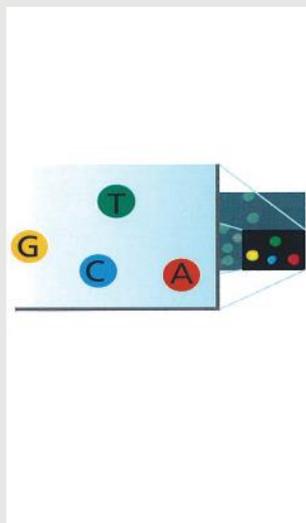
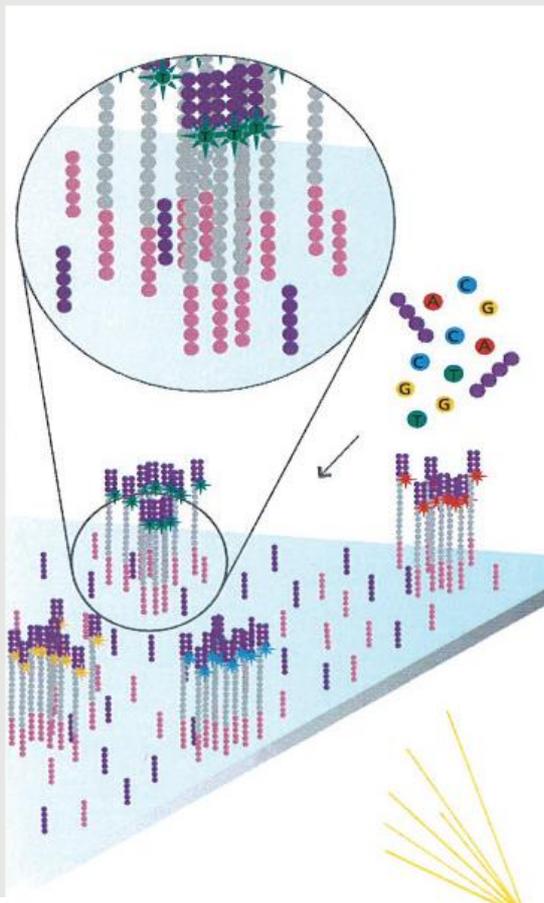
Аmplification завершено. На поверхности имеются плотные скопления фрагментов ДНК.



ТЕХНОЛОГИЯ СЕКВЕНИРОВАНИЯ

Добавляется все четыре меченых нуклеотида, праймеры, ДНК-полимеразу. Полимераза связывает нуклеотиды.

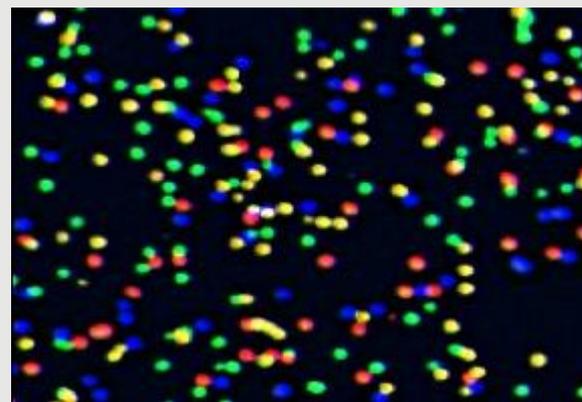
Излишки реагентов удаляются. Образец облучают лазером. Регистрируют флуоресцентный сигнал и определяют первое основание.



ТЕХНОЛОГИЯ СЕКВЕНИРОВАНИЯ

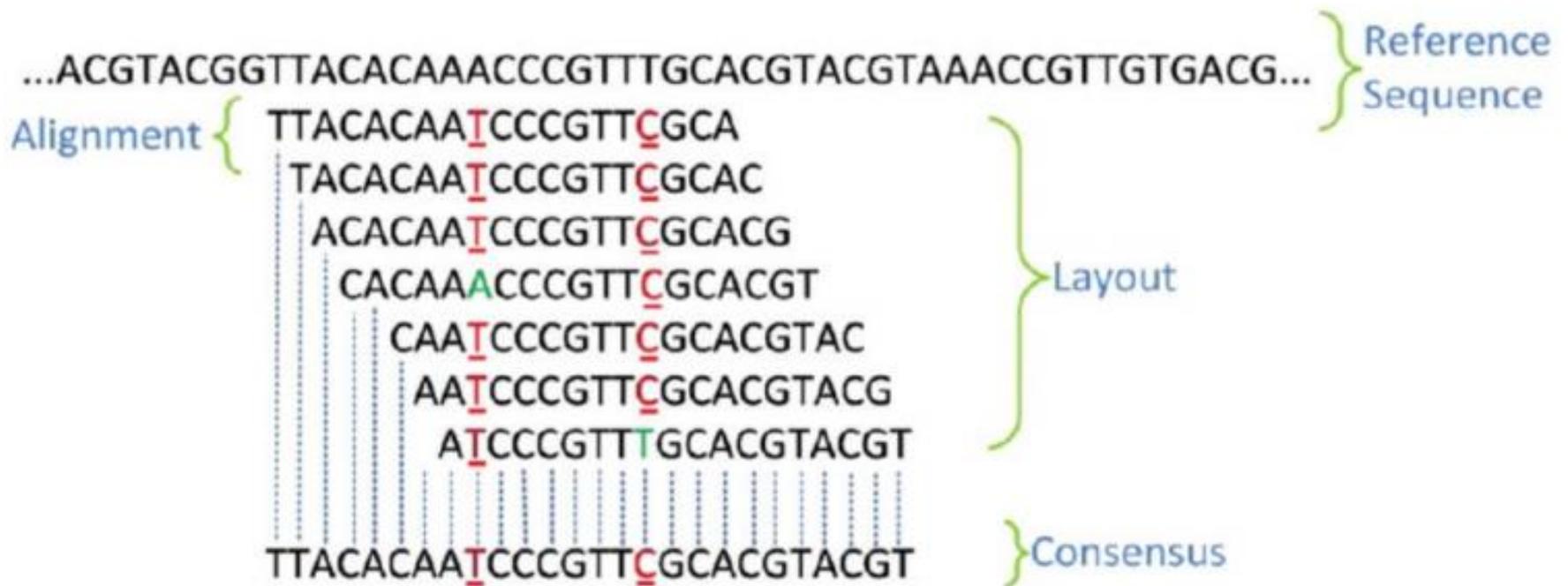


Illumina NextSeq
550Dx

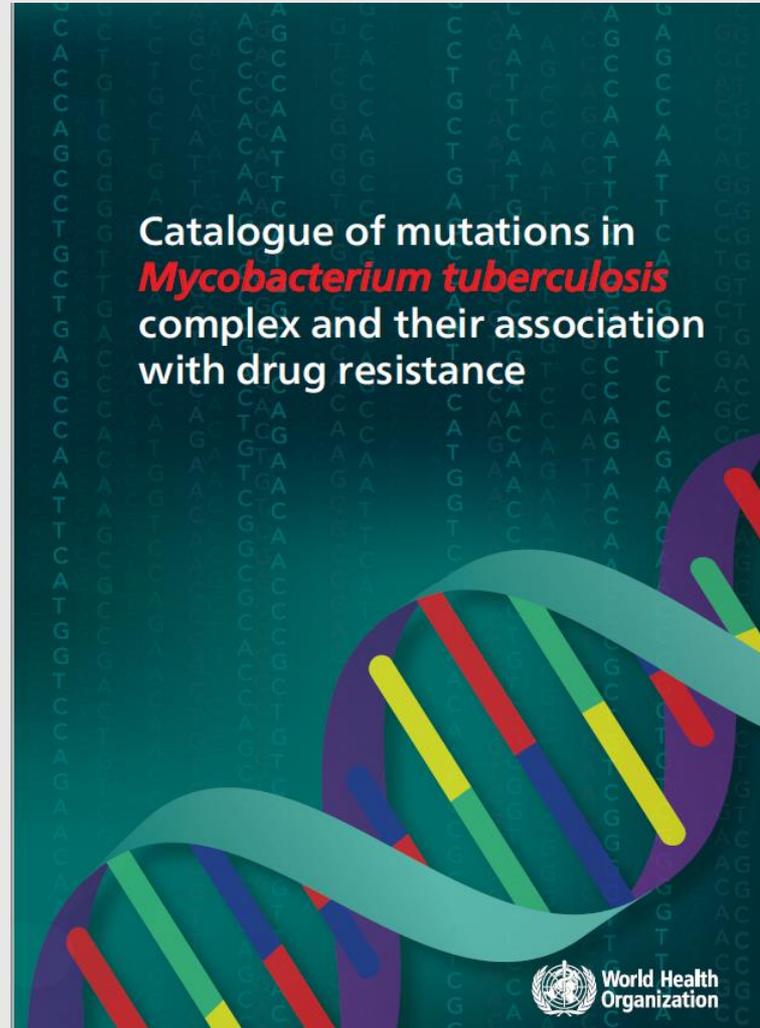


Флуоресценция
регистрируется цифровой
камерой.

Биоинформатика - анализ полученных данных



База данных мутаций, связанных с устойчивостью к *M.tuberculosis*



База данных мутаций, связанных с устойчивостью к M.tuberculosis



Mutation named as described in the chapter: Detailed methods

Final confidence grading of a mutation

Drug	Variant (common name)	Present_S	Absent_S	Present_R	Absent_R	SENSITIVITY	SPECIFICITY	PPV	PPV SOLO	PPV SOLO_lb	PPV SOLO_ub	OR SOLO	INITIAL CONFIDENCE GRADING	DATASET(S)	ADDITIONAL GRADING CRITERIA	FINAL CONFIDENCE GRADING
RIF	rpoB_S450L	74	24473	6536	3333	66.2%	99.7%	98.9%	98.6%	98.3%	98.9%	584.342	Assoc w R	ALL+WHO		1) Assoc w R
RIF	rpoB_L430P	103	24330	106	9743	1.1%	99.6%	50.7%	23.1%	16.3%	31.2%	0.806	Uncert. Sig.	ALL+WHO	Borderline	1) Assoc w R
RIF	rpoB_V695L	55	20207	52	6678	0.8%	99.7%	48.6%	1.8%	0.0%	9.6%	0.058	Not assoc w R	WHO		5) Not assoc w R

Drug in focus

Additional grading criteria applied when relevant to reach the Final confidence grading



База данных мутаций, связанных с устойчивостью к M.tuberculosis

Drug	Dataset*	Total no. of isolates	Resistant isolates No.	Percentage (95% CI)
Rifampicin	WHO	27 063	6 736	24.9 (24.4–25.4)
	ALL	34 375	9 868	28.7 (28.2–29.2)
Isoniazid	WHO	26 727	8 440	31.6 (31.0–32.1)
	ALL	34 437	12 199	35.4 (34.9–35.9)
Ethambutol	WHO	23 706	3 615	15.2 (14.8–15.7)
	ALL	30 708	4 900	16.0 (15.5–16.4)
Pyrazinamide	WHO	15 903	2 329	14.6 (14.1–15.2)
	ALL	15 902	2 329	14.6 (14.1–15.2)
Levofloxacin	WHO	10 305	2 019	19.6 (18.8–20.4)
	ALL	18 277	3 108	17.0 (16.5–17.6)
Moxifloxacin	WHO	6 904	1 094	15.8 (15.0–16.7)
	ALL	13 351	1 869	14.0 (13.4–14.6)
Bedaquiline	WHO	88	3	3.4 (0.7–9.6)
	ALL	8 321	73	0.9 (0.7–1.1)
Linezolid	WHO	1 131	9	0.8 (0.4–1.5)
	ALL	11 018	123	1.1 (0.9–1.3)
Clofazimine	WHO	3 635	23	0.6 (0.4–0.9)
	ALL	10 179	125	1.2 (1.0–1.5)
Delamanid	WHO	89	2	2.2 (0.3–7.9)
	ALL	7 778	82	1.1 (0.8–1.3)
Amikacin	WHO	8 040	664	8.3 (7.7–8.9)
	ALL	16 978	1 288	7.6 (7.2–8.0)
Streptomycin	WHO	9 043	2 562	28.3 (27.4–29.3)
	ALL	13 984	4 635	33.1 (32.4–33.9)
Ethionamide	WHO	2 184	884	40.5 (38.4–42.6)
	ALL	13 918	2 965	21.3 (20.6–22.0)
Kanamycin ^a	WHO	7 381	688	9.3 (8.7–10.0)
	ALL	16 154	1 481	9.2 (8.7–9.6)
Capreomycin ^a	WHO	9 103	702	7.7 (7.2–8.3)
	ALL	11 526	970	8.4 (7.9–8.9)

Пример резултата секвенирования

Mutations associated with antibiotic resistance detected for SM, RMP, PZA, INH, FQ, EMB.

Sequencing quality overview:

Total number of reads – 2,915,508

Mapped read fraction – 99%

Q30 fraction – 92%

Average coverage depth – 94x

GC content – 65.31%

Resistance conferring mutation list:

Position	Ref	Type	Variant	Cov	Qual20	Freq	Subst	Gene	GeneName	Product	ResistanceSNP	InterestingRegion
1472359	A	SNP	C	122	119	98,36		Rvnr01	-	rRNA	streptomycin (SM)	streptomycin (SM)
761155	C	SNP	T	177	176	100	Ser450Leu (tcg/tTg)	Rv0667	rpoB	DNA-directed RNA polymerase subunit beta	rifampicin (RMP)	rifampicin (RMP)
2289054	T	SNP	C	220	219	100	Asp63Gly (gac/gGc)	Rv2043c	pncA	pyrazinamidase/nicotinamidase PncA	pyrazinamide (PZA)	pyrazinamide (PZA)
2155168	C	SNP	G	211	211	100	Ser315Thr (agc/aCc)	Rv1908c	katG	catalase-peroxidase	isoniazid (INH)	isoniazid (INH)
1673425	C	SNP	T	178	177	100					isoniazid (INH)	isoniazid (INH)
7563	G	SNP	T	198	197	99,49	Gly88Cys (ggc/Tgc)	Rv0006	gyrA	DNA gyrase subunit A	fluoroquinolones (FQ)	fluoroquinolones (FQ)
4248003	A	SNP	G	185	183	100	Gln497Arg (cag/cGg)	Rv3795	embB	arabinosyltransferase B	ethambutol (EMB)	ethambutol (EMB)
4408156	A	SNP	C	210	209	100	Leu16Arg (ctt/cGt)	Rv3919c	gid	rRNA small subunit methyltransferase G		streptomycin (SM)
781395	T	SNP	C	191	191	100						streptomycin (SM)
1834836	T	SNP	C	151	149	100	Met432Thr (atg/aCg)	Rv1630	rpsA	30S ribosomal protein S1		pyrazinamide (PZA)
7585	G	SNP	C	203	203	100	Ser95Thr (agc/aCc)	Rv0006	gyrA	DNA gyrase subunit A		fluoroquinolones (FQ)
8040	G	SNP	A	201	197	100	Gly247Ser (ggc/Agc)	Rv0006	gyrA	DNA gyrase subunit A		fluoroquinolones (FQ)
7362	G	SNP	C	228	228	100	Glu21Gln (gag/Cag)	Rv0006	gyrA	DNA gyrase subunit A		fluoroquinolones (FQ)
9304	G	SNP	A	214	213	100	Gly668Asp (ggc/gAc)	Rv0006	gyrA	DNA gyrase subunit A		fluoroquinolones (FQ)
4242643	C	SNP	T	188	188	100	Arg927Arg (cgc/cgT)	Rv3793	embC	arabinosyltransferase C		ethambutol (EMB)

Method:

DNA was extracted from MGIT cultures with 30 minute inactivation at 95°C, followed by incubation with lysozyme at 37°C for 16 hours and extraction with Qiagen DNeasy kit.

Libraries were prepared using Illumina DNA Prep library preparation kit and sequenced on Illumina iSeq 100.

СПАСИБО ЗА ВНИМАНИЕ!