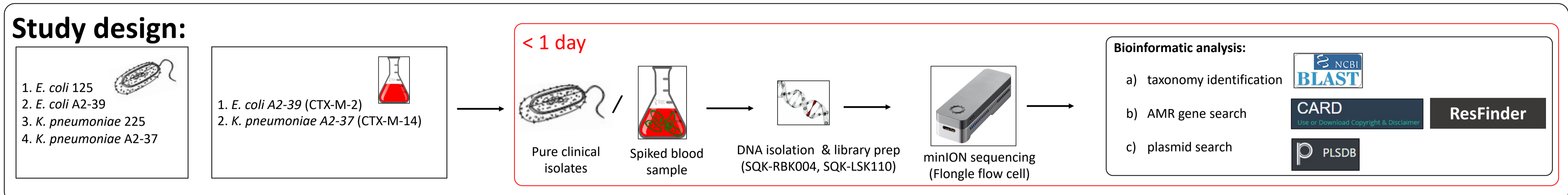
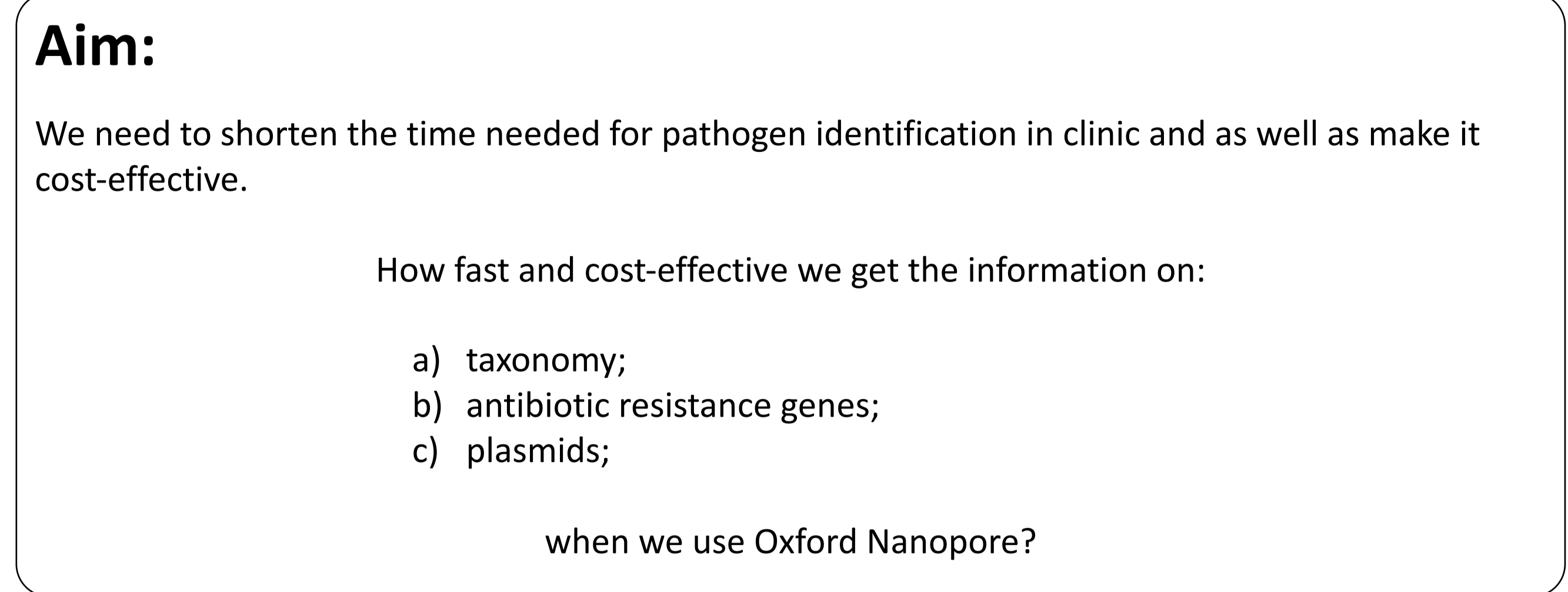
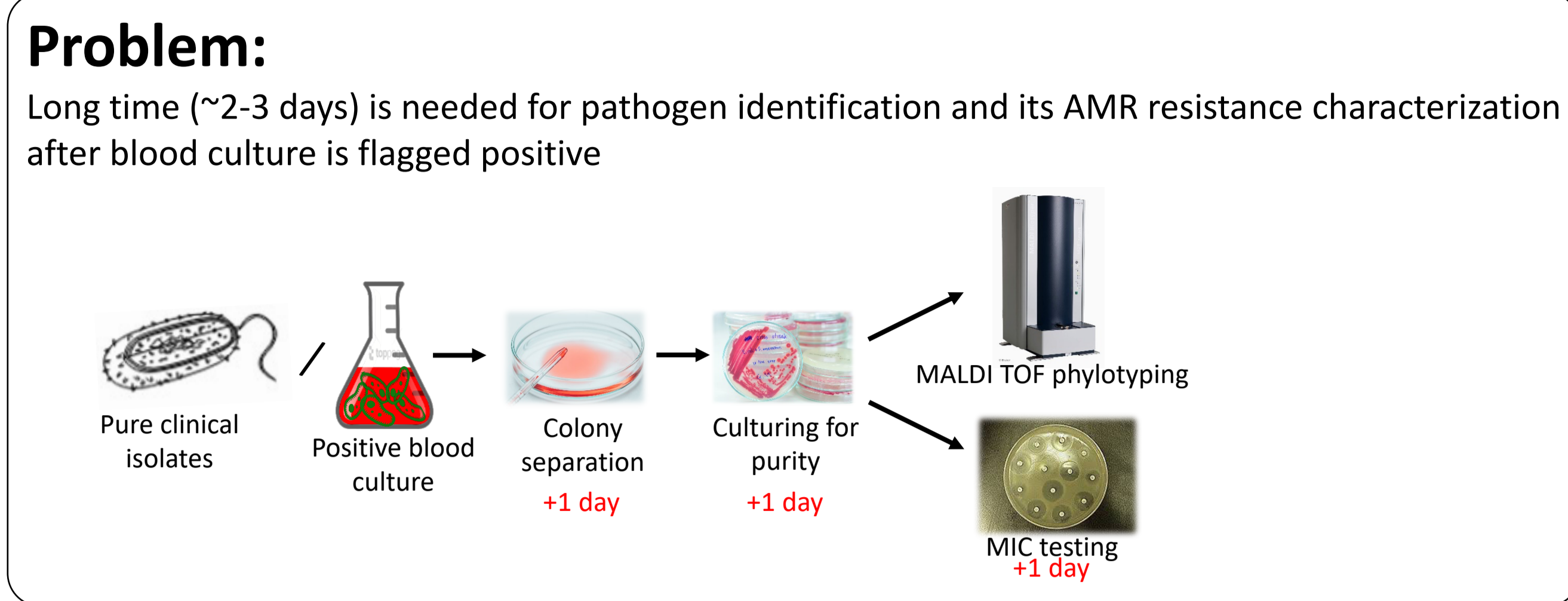


# Ultrafast and Cost-Effective Pathogen Identification and Resistance Gene Detection in a Clinical Research Setting Using Nanopore Flongle Sequencing

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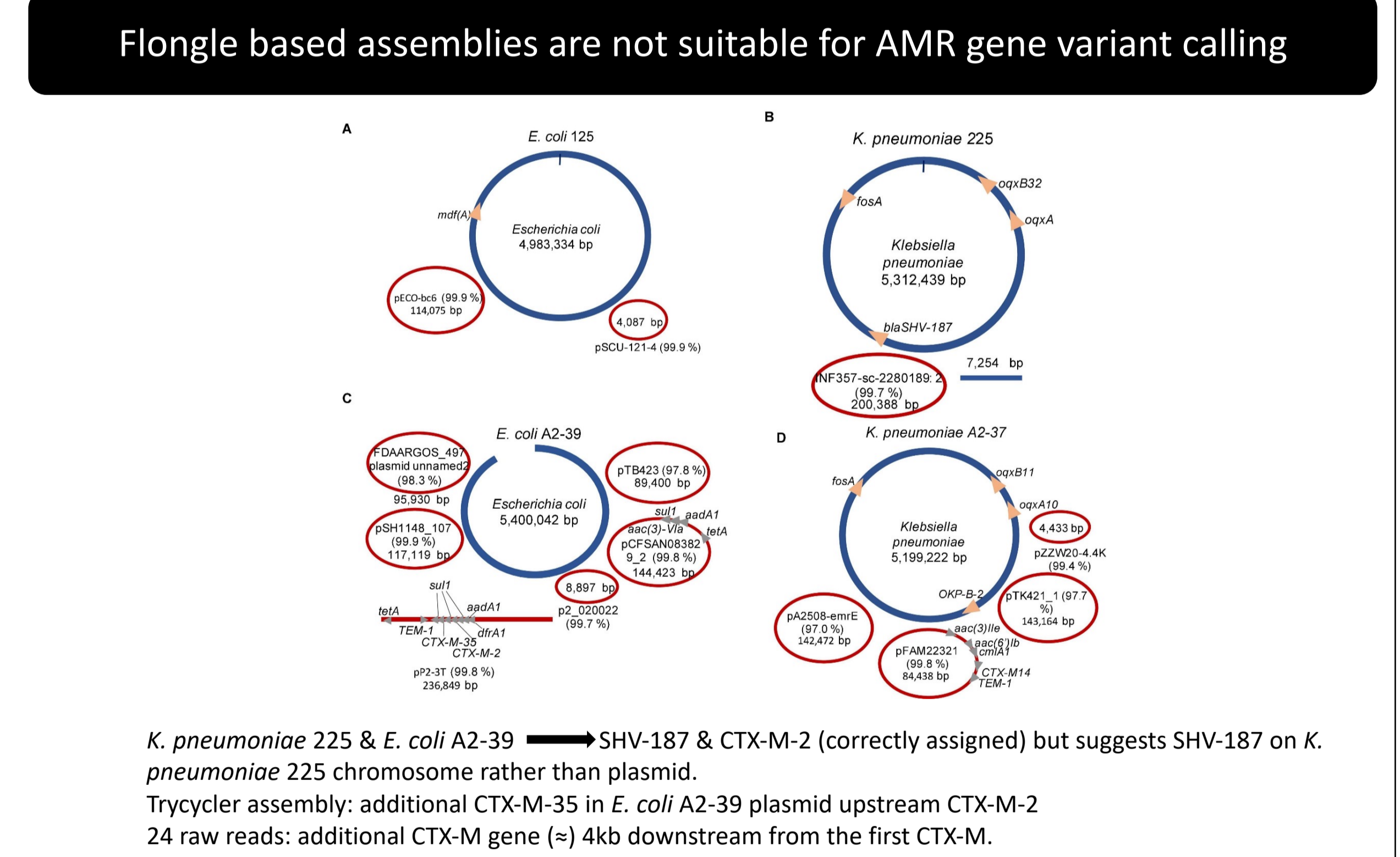
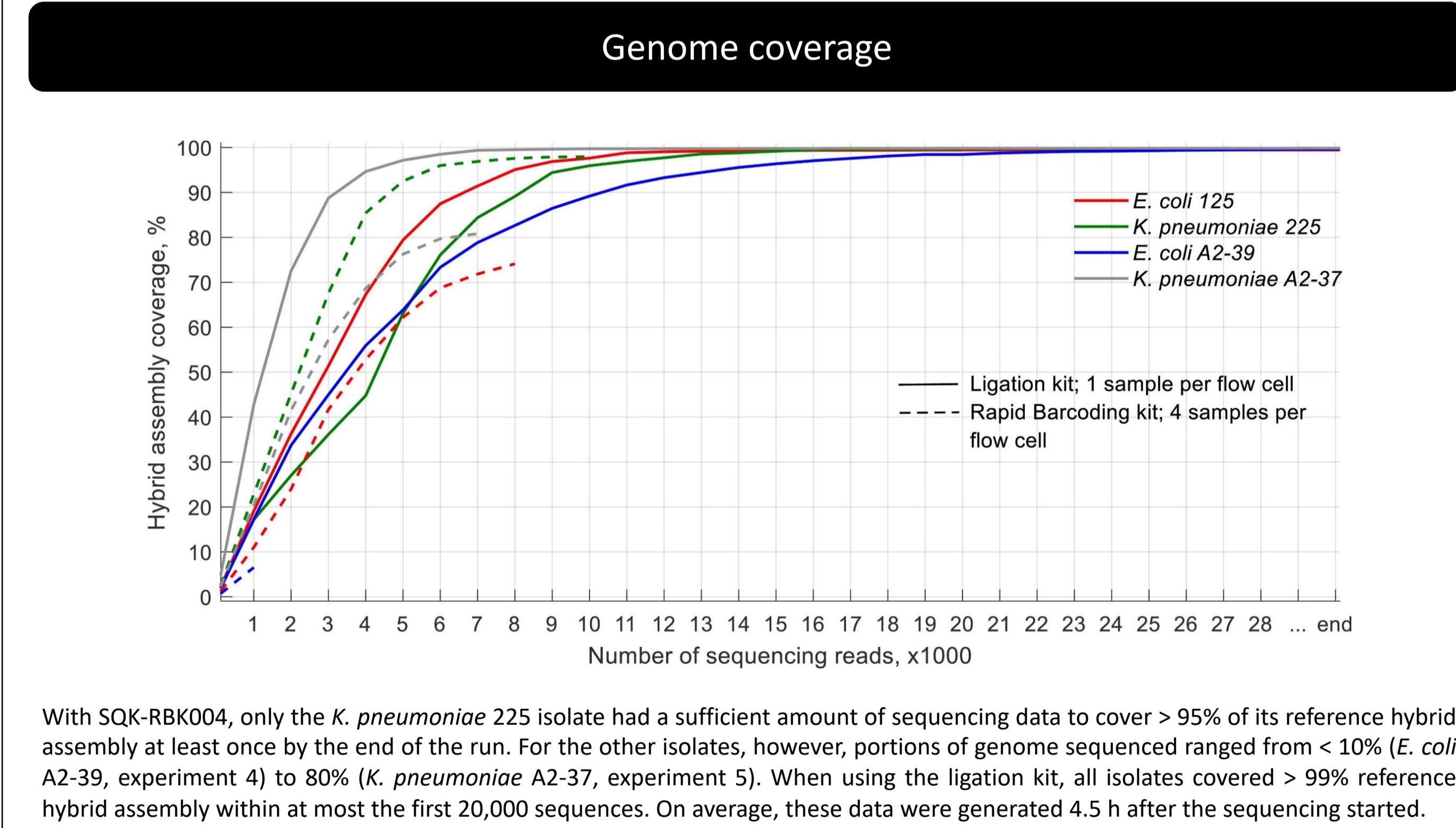
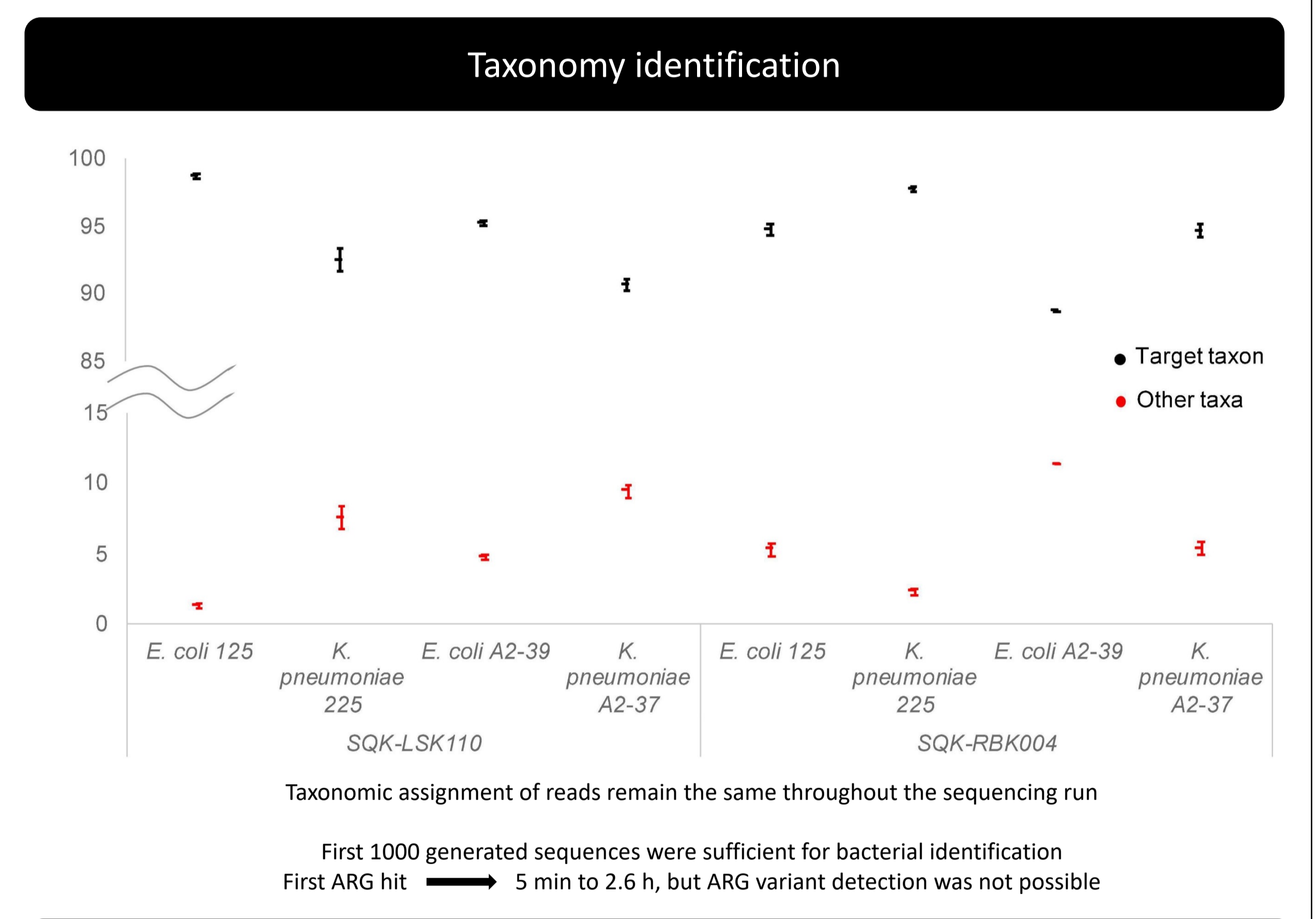
**Results:**

**General statistics of the ONT Flongle runs**

The number of active pores remained stable during cold storage four of which lasted up to 8 months

Exp Nr	ID	Sample type	ONT kit	Number of active pores	Number of reads	Total length [Mbp]	Longest read [bp]	N50 [bp]
1	E. coli 125	clinical isolates	SQK-RBK004	69	7840	25.6	89955	7956
	K. pneumoniae 225		9493	76.6	124329	21552		
2	E. coli A2-39	plasmid preparation	SQK-RBK004	67	879	1.9	58672	11868
	K. pneumoniae A2-37		6744	35.1	136325	14239		
3	E. coli 125	spiked blood culture	SQK-LSK110	70	105368	683.5	122281	13222
	K. pneumoniae 225		60	102714	904.0	127460	21738	
4	E. coli A2-39	spiked blood culture	SQK-LSK110	67	313395	861.6	124559	9871
	K. pneumoniae A2-37		59	193000	1082.9	146931	14684	
5	E. coli 125	spiked blood culture	SQK-LSK110	59	1144	1.7	24745	1626
	K. pneumoniae 225		59	189	0.8	44111	10015	
6	E. coli A2-39	spiked blood culture	SQK-LSK110	62	3575	6.1	51440	2359
	K. pneumoniae A2-37		59	5579	12.3	54951	3889	
7	E. coli A2-39	spiked blood culture	SQK-LSK110	62	293814	1273.8	40760	7044
	K. pneumoniae A2-37		59	127537	756.8	41029	7961	
9	E. coli A2-39	spiked blood culture	SQK-LSK110	39	95689	528.6	44214	7260
	K. pneumoniae A2-37		24	93892	583.5	39975	8339	

The longest generated reads ranged from 24,745 to 124,329 bp for SQK-RBK004 and from 40,760 to 146,931 bp for SQK-LSK110. The total length generated for clinical isolates using the SQK-LSK110 kit was on average 126x higher compared to SQK-RBK004 ( $p < 0.05$ ). When using SQK-RBK004, only one output file was generated after the whole sequencing was run for one of the isolates, i.e., it required >24 h to get data for that isolate. Additionally, 1.2% of the barcodes were misidentified during the demultiplexing step.



**Sequencing of plasmids from clinical isolates recovered < 9 kbp long plasmids only**

Plasmid Name	Total number of reads identified	Longest read length [bp]	Assembly	Assembly length [bp]	Assembly N50 [bp]	Plasmid ID	Plasmid length [bp]	DT%	AMR	Assembled in	Raw reads	Assembly
E. coli 125	1,166	26,745	Unicycler	5,644	31	E. coli pEAD125 (CP004252.1)	4,381	99.9	No	No	No	No
K. pneumoniae 225	188	44,511	Unicycler	5,295	39	K. pneumoniae pZ2W20-4.4K (CP004252.1)	5,302	99.8	No	No	No	No
E. coli A2-39	5,375	95,483	Unicycler	8,893	102	E. coli pEAD125 (CP004252.1)	4,381	99.9	No	No	No	No
K. pneumoniae A2-37	5,375	95,483	Unicycler	4,447	102	K. pneumoniae pZ2W20-4.4K (CP004252.1)	5,302	99.8	No	No	No	No
K. pneumoniae A2-37	5,375	95,483	Unicycler	4,447	102	K. pneumoniae pZ2W20-4.4K (CP004252.1)	5,302	99.8	No	No	No	No

In *K. pneumoniae* A2-37 Unicycler produced one circular plasmid of 4,447 bp closely related to *K. pneumoniae* plasmid pZ2W20-4.4K, 4,436 bp. Two of the three contigs produced by Flye gave the closest PLSDB match to plasmids 4–10 times shorter, including a match to *K. pneumoniae* plasmid pZ2W20-4.4K.

***K. pneumoniae* A2-37 plasmid data:** CTX-M detected in single read but was lost after assembly.

