

Fire Monkey is a spin-column HMW extraction kit developed by RevoluGen



- **Fire Monkey** is a rapid and user-friendly **spin column HMW-DNA** extraction kit. It extracts DNA in **~1hr** from bacteria and mammalian cells
- The extract is 'not too short/not too long' so it can be used in library prep **without fragmentation or size selection**
- Fire Monkey DNA produces **N50s of over 40-50KB, and throughputs of up to 21Gb and 26Gb on LSK109/MinION** for mammalian and bacterial cells



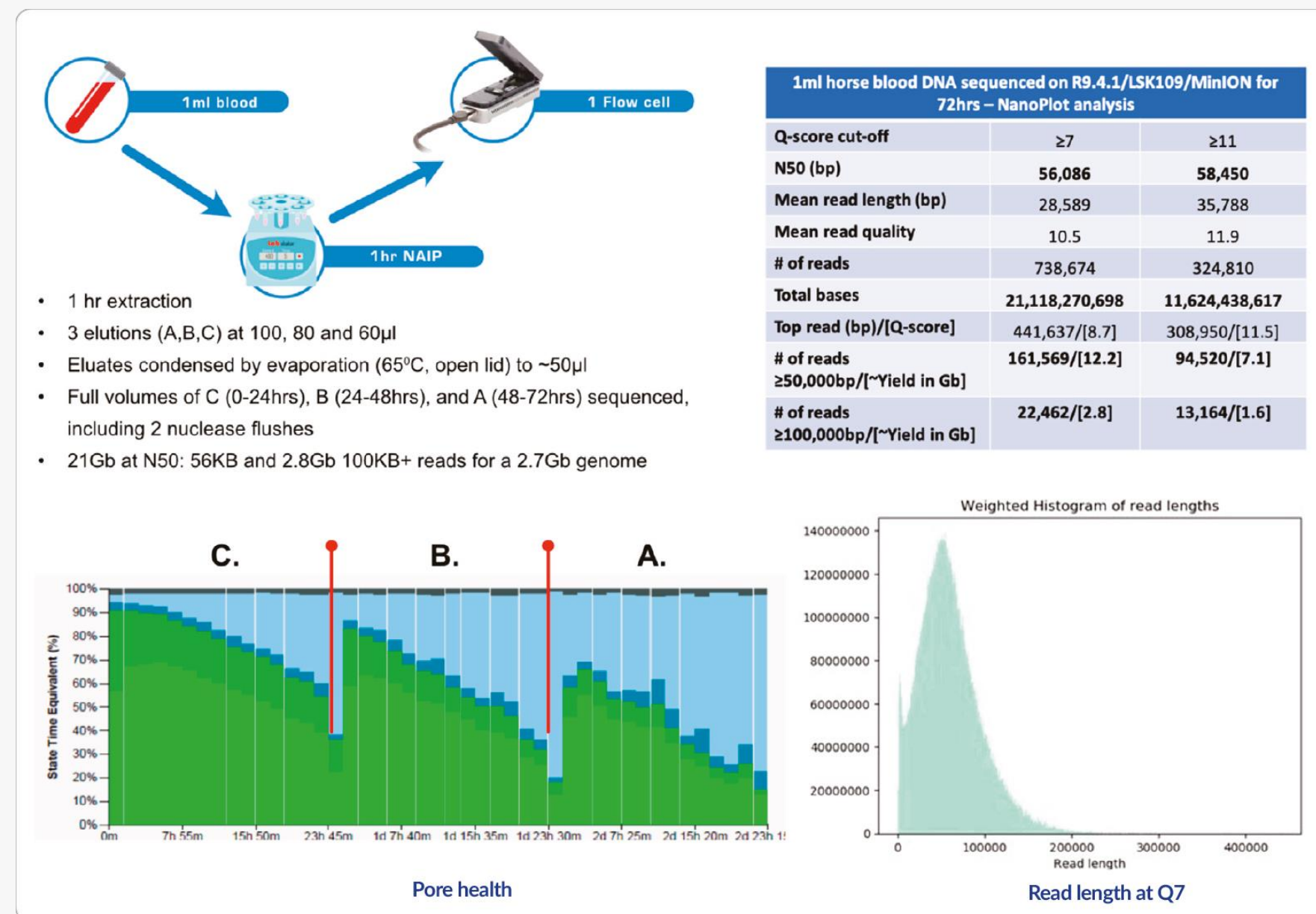
Standard equipment

From sample to DNA in 1h	Bacteria cells throughput up to ~26 Gb
Spin column Standard format	10-20kb Size selection Cut-off
> 100kb Average extract size	N50 mammalian cells up to 62 kb

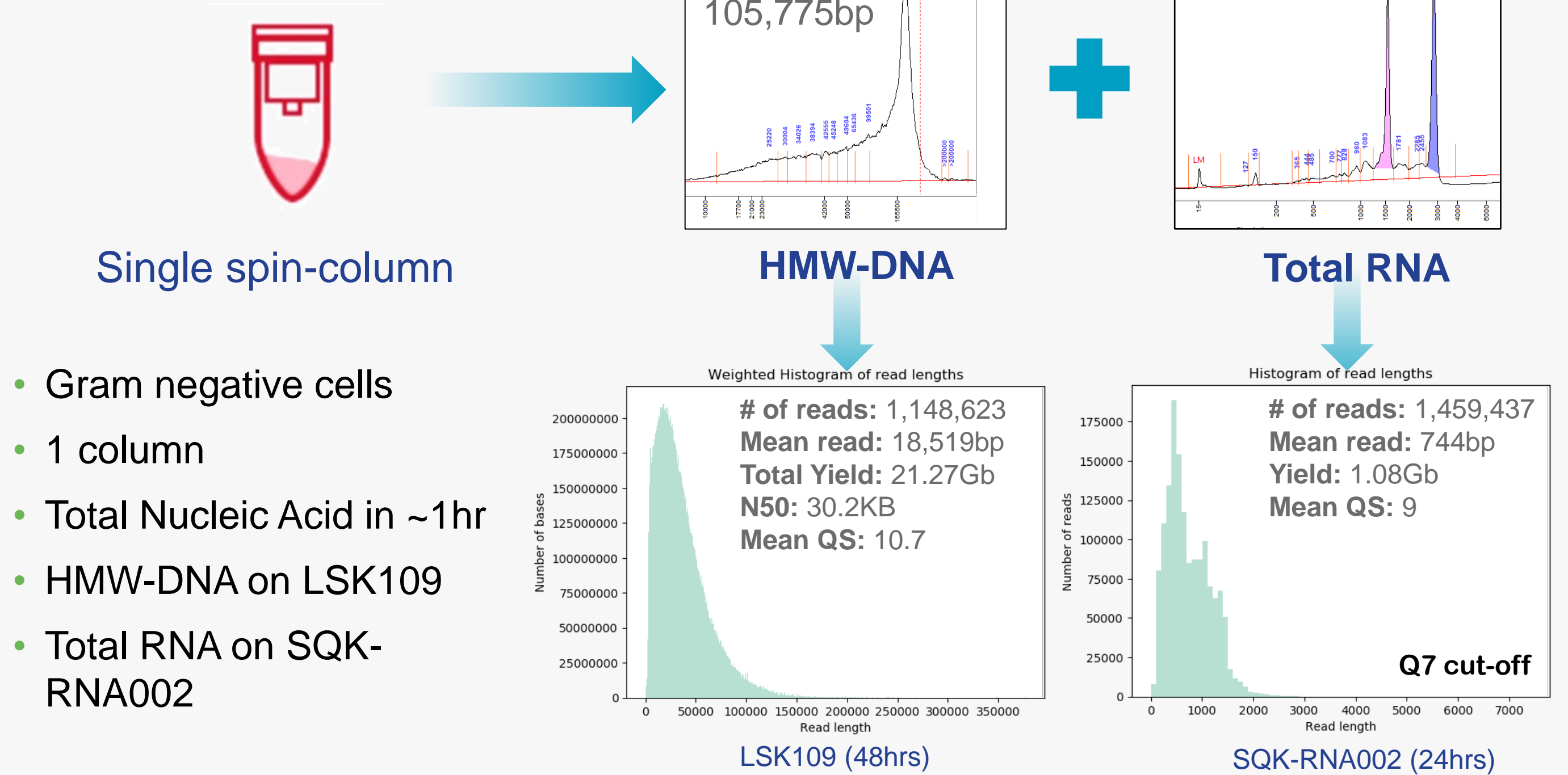
FIRE MONKEY HMW-DNA extraction kit

'Not too short/not too long DNA' generates 21.1Gb at an N50: 56KB on a single MinION flow cell!

- White blood cells
- 1 column
- 1hr extraction
- No fragmentation
- No size selection
- Single MinION flow cell
- 3x fractions
- 3x LSK109 libraries
- N50: 56KB
- Throughput: 21.1Gb
- 2.8Gb in >100KB reads

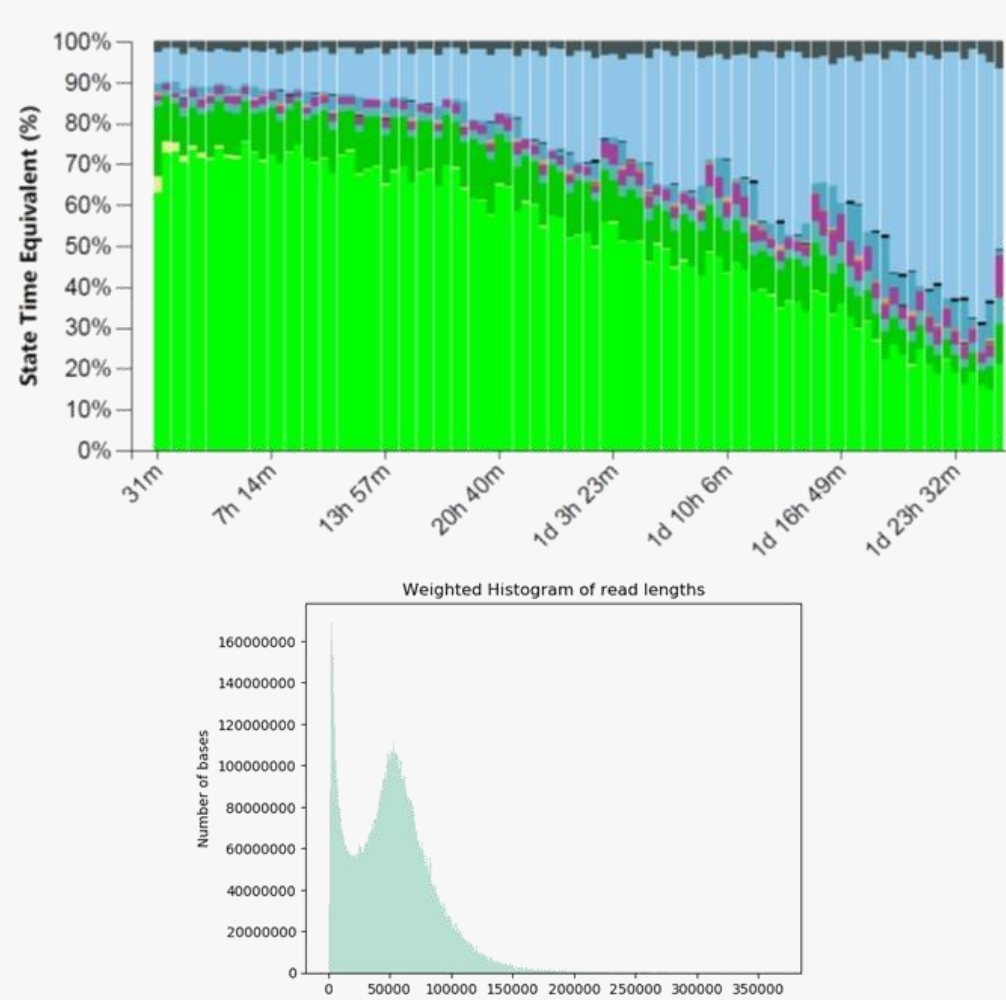


Single biological sample > single Fire Monkey prep > Total Nucleic Acid!



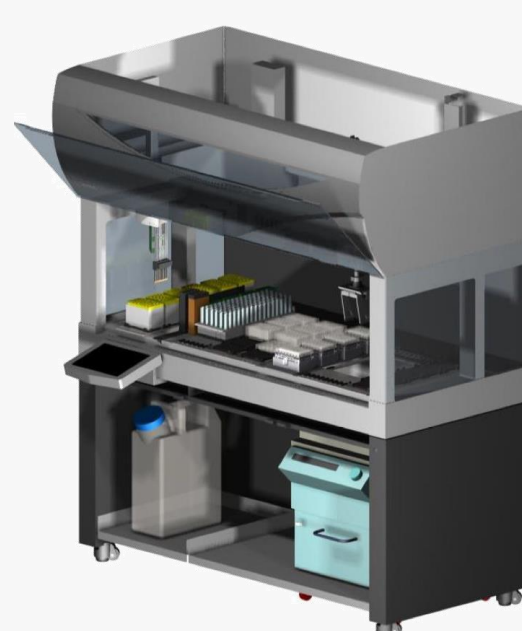
Gram positive bacteria performance; 15.7Gb at N50: 50.3KB!

- 2ml of an O/N *Bacillus subtilis* culture
- On step 2.a of Fire Monkey IFU (lysozyme treatment) substitute 20mg/ml of lysozyme in 1.2% Triton X-100 in (H2O) to 20mg/ml of lysozyme in 1.2% Triton X-100, 20mM Tris-Cl pH8, 2mM EDTA
- Use 48µl of Elution B in LSK109 (no post-extraction size-selection)
- 15.7Gb at N50: 50KB after 50hrs
- ~1.3Gb in reads >100kb for a ~4.3Mb genome



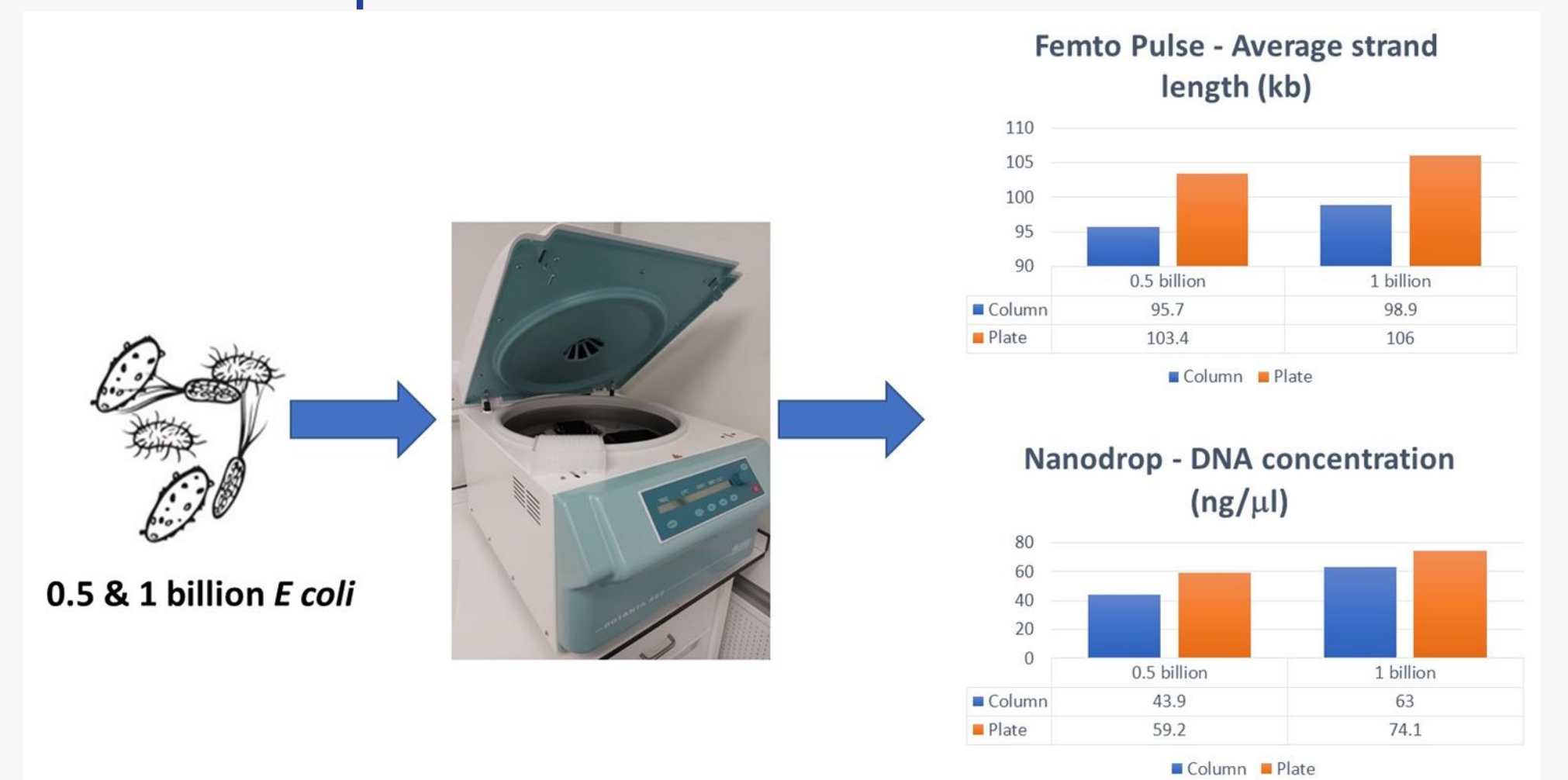
- Gram positive cells
- 15.7Gb at N50: 50.3KB
- 1.3Gb in >100KB reads

96-well filter plate adaptation & automation; Filter plate extractions proof of concept!



Fluent 780 with integrated centrifuge and heated shakers

- Tecan developed an automated Fire Monkey extraction platform
- 96-well filter plates to support multiplexed extraction and sequencing runs
- Installation pending



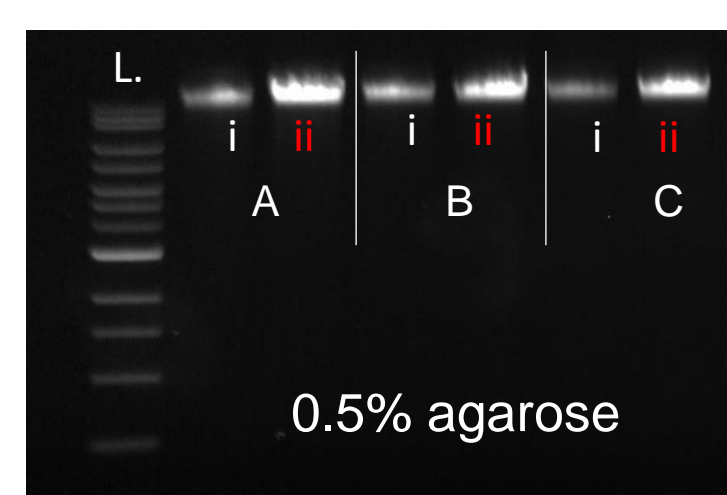
- 96-well filter plate & plate centrifuge proof of concept
- Same biological sample (bacteria) through Fire Monkey columns vs Fire Monkey filter plates
- Same average strand length & DNA concentration from column and filter plate

NEW! Fire Monkey protocol doubles HMW-DNA extraction yield and generates ~26Gb at N50: 45.8KB on LSK109/MinION!

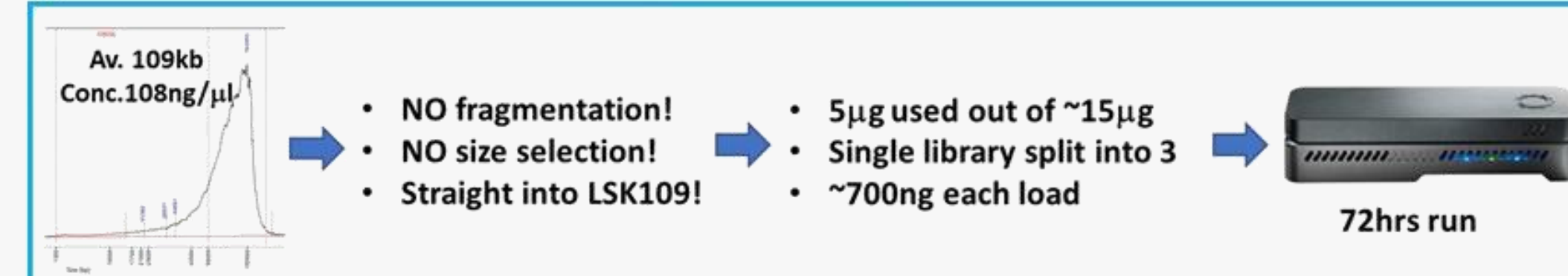
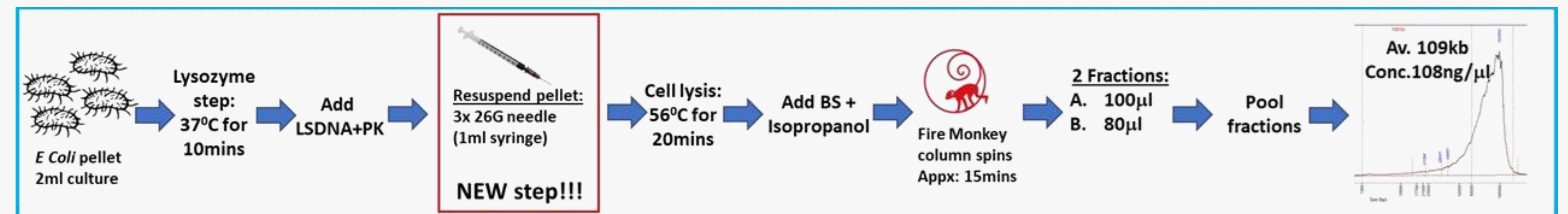
- Gram negative cells
- Needle resuspension guarantees efficient lysis
- Doubles extraction yield vs Standard protocol
- More 'not too short/not too long' HMW-DNA
- No fragmentation or size selection needed
- LSK109/MinION
- Generates 25.63Gb (27.17Gb for all reads) at N50: 45.8KB

Protocol Fraction	Average length (bp, Femto Pulse)	Average ng/ml (Nanodrop)
Standard_A	122,812	58.6
New_A	106,354	122.2
Standard_B	109,028	38.5
New_B	118,880	74.7
Standard_C	135,485	39
New_C	132,229	59.7

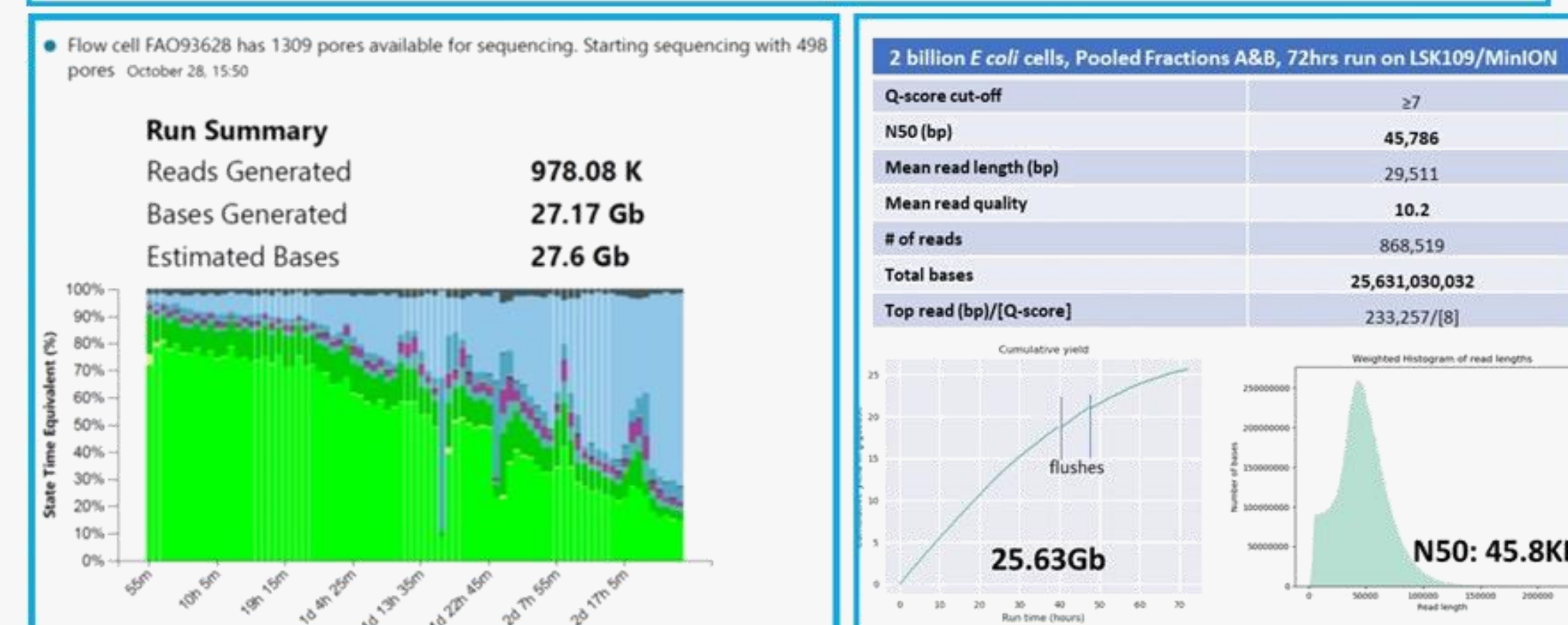
Standard vs New protocol average strand length and DNA concentration for E coli sample fractions A, B and C.



1µl of Standard (i) and New (ii) protocol Fractions A, B and C.



DNA repair time	10mins 20°C / 10mins 65°C
Ligation time	30mins RT
Last AMPure XP step temperature / elution volume	37°C / 30µl



Equipment needed

Fire Monkey is an extremely rapid HMW DNA extraction kit. It extracts average DNA fragment lengths of between 100-130kb (Femto Pulse), with significantly reduced numbers of small DNA fragments shorter than 10kb. This results in high throughput and high N50 values. **Fire Monkey** latest protocol doubles extraction yield and pushes LSK109/MinION throughput to 26Gb. Tecan supported automation is in development.



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