Fire Monkey, a spin column HMW-DNA extraction kit

Dr. Georgios Patsos, CSO & kit inventor

RevoluGen

Spin columns for long reads

REVOLUGEN

Rapid & user-friendly extraction of HMW-DNA or RNA from mammalian cells or bacteria

~1hr

- Spin columns NAIPs are fast & user-friendly
- High g-force applied guarantees extraction speed/efficiency
- However, high g-force breaks DNA
- Fire Monkey patented chemistry & matrix overcomes that
- Protocols remain fast and user-friendly
- Filtration removes small fragments-no need for size selection
- Spinning homogenizes long fragment distribution
- 100kb+ average strand length-no need for fragmentation
- Extract is library-ready
- Parallel RNA extraction possible

Sample type	Throughput (Gb)	N50 (kb)
Gram negative	25.63	45.8
Gram positive	15.7	50.3
White blood cells	21.1	56

LSK109/MinION at ≥Q7

Kit

Equipment

From sample to DNA in 1h

Spin column Standard format

> 100kb Average extract size

FIRE MONKEY HMW-DNA extraction kit

Bacteria cells throughput up to ~26 Gb

10-20kb Size selection Cut-off

N50 mammalian cells up to 62

Oxford NANOPORE Technologies

Automation

Library ready DNA for multiplexed long reads

REVOLUGEN

Automation platform

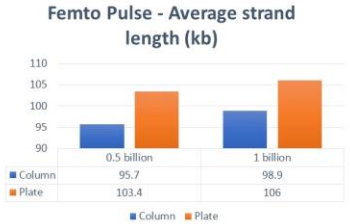
TECAN

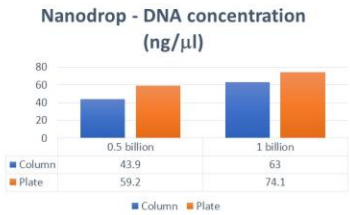
Fluent 780 with integrated centrifuge and heated shakers

- Tecan developed an automated platform for HMW-DNA extraction utilising Fire Monkey chemistry & matrix
- Plate centrifuge proof-of-concept
- Bacteria were processed using Fire Monkey 96-well filter plates vs Fire Monkey columns
- Generated extracts with very similar extraction yield and average strand length
- Fluent 780 installation and full workflow optimization is next

Filter plate proof of concept

0.5 & 1 billion *E coli*





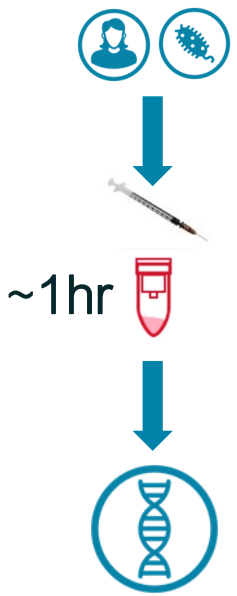
Automation/multiplexing applications

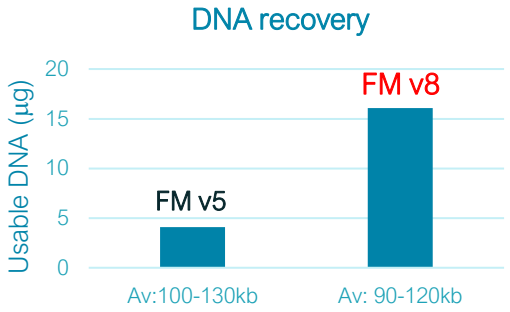
Oxford NANOPORE Technologies

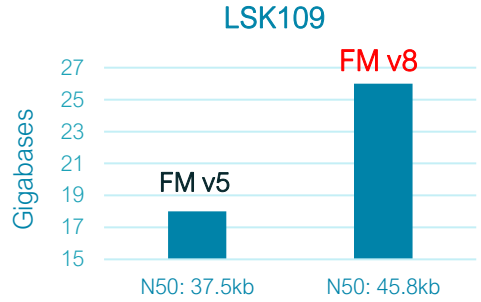
Updated extraction protocols

Efficient resuspension > major DNA recovery boost > more throughput

Needle cell resuspension-FM version 8



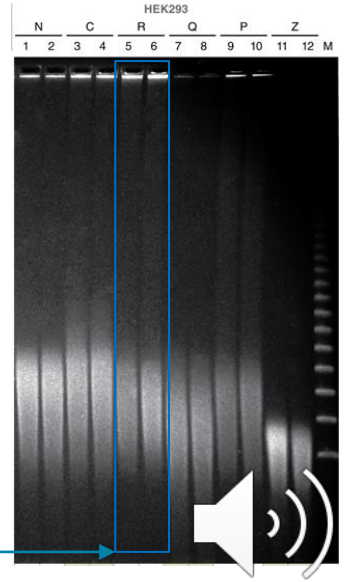




New protocol benefits

- Needle cell resuspension prior to Proteinase K lysis boosts DNA recovery
- New protocol version (v8) extracts ~4x more usable DNA than previous version (v5)
- DNA not 'too short/not too long' generates maximum throughput at a high N50
- Critical starting concentration (100ng/µl) achieved
- More DNA=more runs, or more loads per library
- More homogeneous HMW-DNA lowers $/Gb price

N: NEB
C: Circulomics
R: RevoluGen
Q: Qiagen
P: Promega
Z: Zymo Research



Old FM v5 version published by NEB

Thank you