

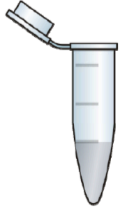
Single molecule, Real-Time (SMRT) DNA sequencing – Sequel PacBio®

Application Whole Genome sequencing

Input : gDNA

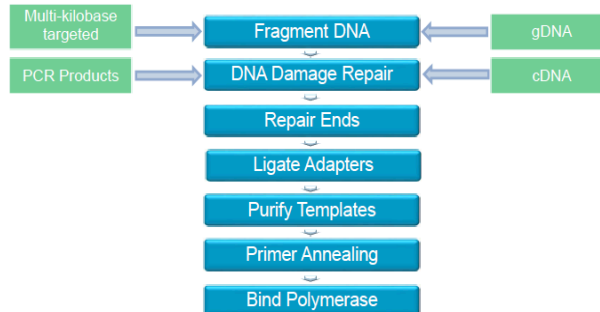
Library construction

SAMPLE CONDITIONS THAT LEAD TO HIGHER QUALITY LIBRARIES



- Double-stranded DNA Sample (dsDNA)
- Minimized freeze-thaw cycles
- No exposure to high temp (>65° C)
- No exposure to pH extremes (<6 or >9)
- OD260/280 between 1.8 and 2.0
- OD260/230 between 2.0 and 2.2
- No insoluble material
- No RNA contamination
- No exposure to UV or intercalating fluorescent dyes
- No chelating agents, divalent metal cations, denaturants, or detergents
- No carryover contamination (e.g. polysaccharides) from starting organism

- Kit HMW DNA Qiagen or Macherey
- DNA input 5-10µg (min 50 kb size)



1 Million wells called Zero-mode waveguides (ZMWs)



Performance Sequel

Throughput: 4-5 Gb
Average read length: 10-18 kb
Movie times: 30 mins to 10 hours

Quality control of gDNA

- Nanodrop and Qubit measurements
- Fragment Analyzer profil (to ~50kb)
- Pulse Field Gel Electrophoresis

First results obtained with the Sequel chemistry v2.0 (February 2017)

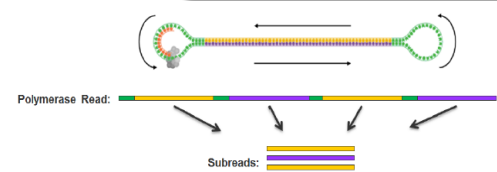
1 SMRT Cell per library – 10 hours movie

Metrics Sequel	Sample 1 Bacteria Gram ⁺ GC% 67.7	Sample 2 Bacteria Gram ⁺ GC%: 64.1	Sample 3 Bacteria Gram ⁺ GC%: 52.1	Sample 4 Fungi
Genome size	4.086 Mb	4.8 Mb	5.06 Mb	14.58 Mb
Size selection library	15 Kb	10 Kb	15 Kb	15 Kb
Nbr of bases	2.7 Gb	2.64 Gb	6.72 Gb	4.77 Gb
Nbr of Polymerase reads	186,254	317,385	514,545	390,011
Polymerase read length (Mean)	14,512	8,342	13,064	12,234
Polymerase read N50	23,750	13,250	21,250	20,250
Insert length (Mean)	11,832	6,512	10,982	10,262

Sample 1 Resequencing analysis – SMRT Link v4

Metrics	Value
Number of subread bases mapped	2,333 Gb
Number of subreads (mapped)	187,999
Subread length Mean (mapped)	12,410
Subread length N50 (mapped)	16,821
Subread length Max (mapped)	47,623
Mapped concordance (mean)	86.63

Consensus accuracy: 99.998
Mean base coverage: 545



Future

Microbial Multiplexing sequencing

1 SMRT Cell / 16 microbes (<2 Mb) - 1 SMRT Cell / 12 microbes (<5 Mb)