

Q & A

Why is the NFR peak at ~200 bp?

Protocol steps correspond to the Chromium Next GEM Single Cell ATAC Reagent Kits v2 User Guide (CG000496)

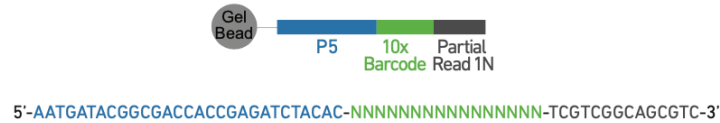
Protocol Step 1 – Transposition

Transposed DNA
Product

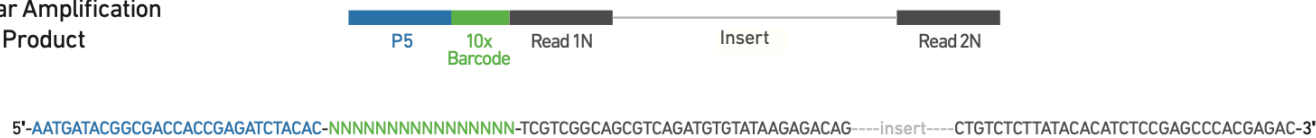


Protocol Step 2.5 – GEM Incubation

Gel Bead Oligo
Primer
PN-2000210



Linear Amplification
DNA Product



-> around 150 bp EXCLUDING the insert

Protocol Step 4.1 – Sample Index PCR

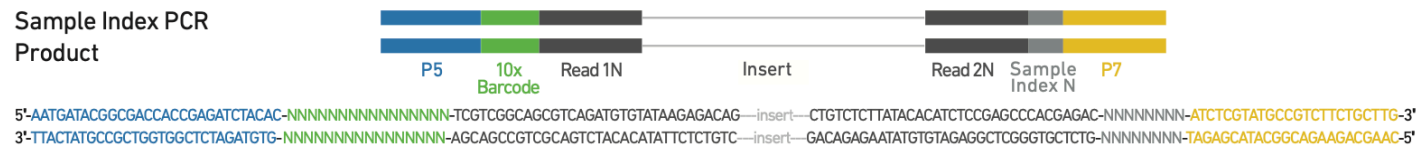
SI-PCR Primer B
PN-2000128

Forward Primer:
5'-AATGATACGGCGACCACCGAGA-3'

Reverse Primer:
5'-CAAGCAGAGACGCGCATACGAGAT-NNNNNNNN-GTCTCGTGGGCTCGG-3'

Single Index Plate N
Set A
PN-3000427

Sample Index PCR
Product

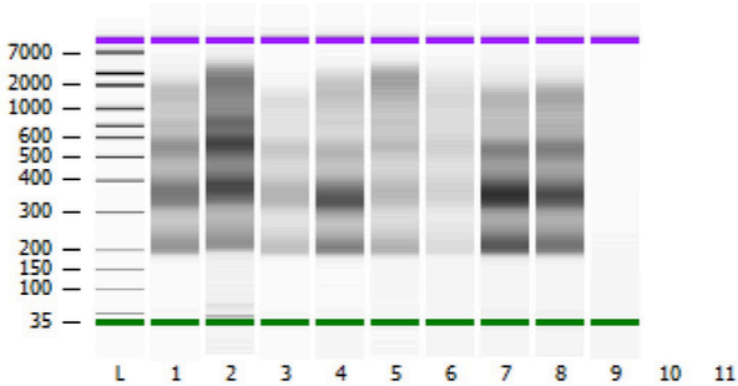


~75 bp

~70 bp

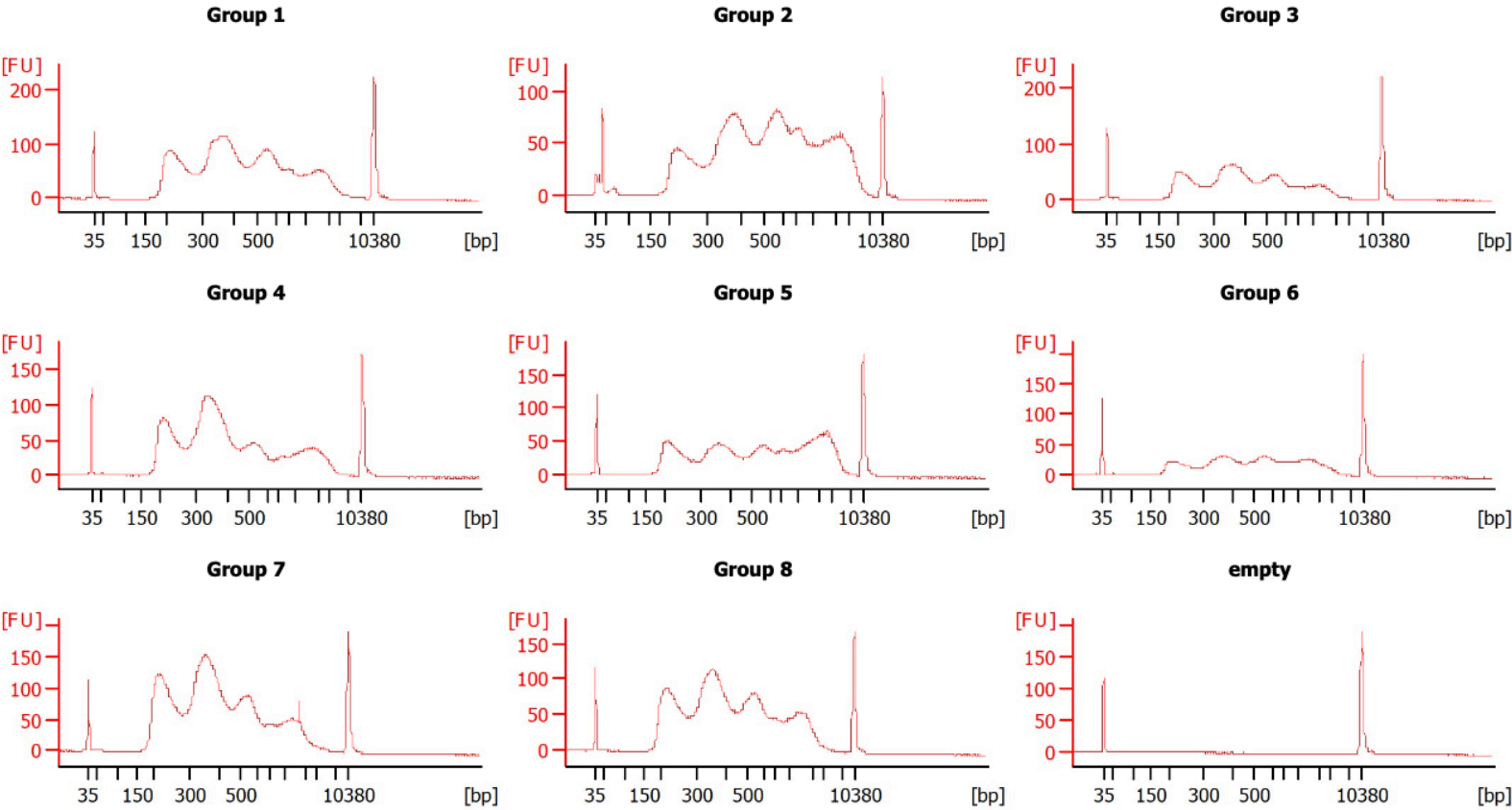
TN5 covers at least 9bp plus steric interference

BioA results



version: 1.0.3
Assay Comments: Copyright © 2003-2010 Agilent Technologies

Chip Information:
Chip Lot #:
Reagent Kit Lot #:
Chip Comments:

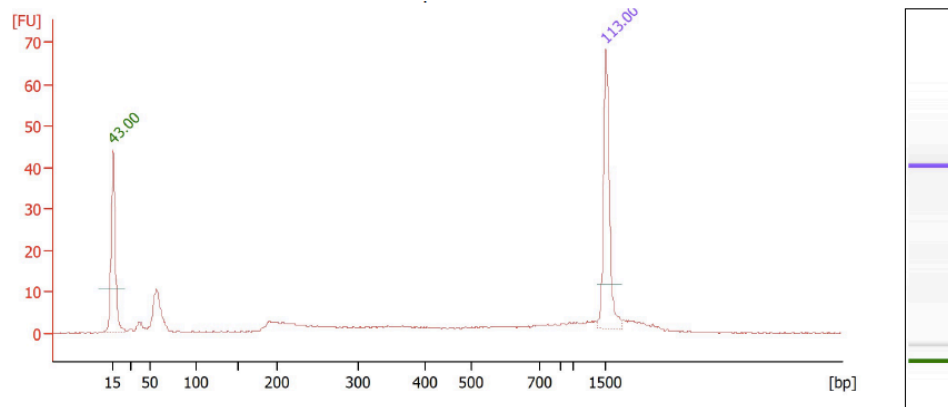


Pre-Sequencing QC

transposition of dead cells

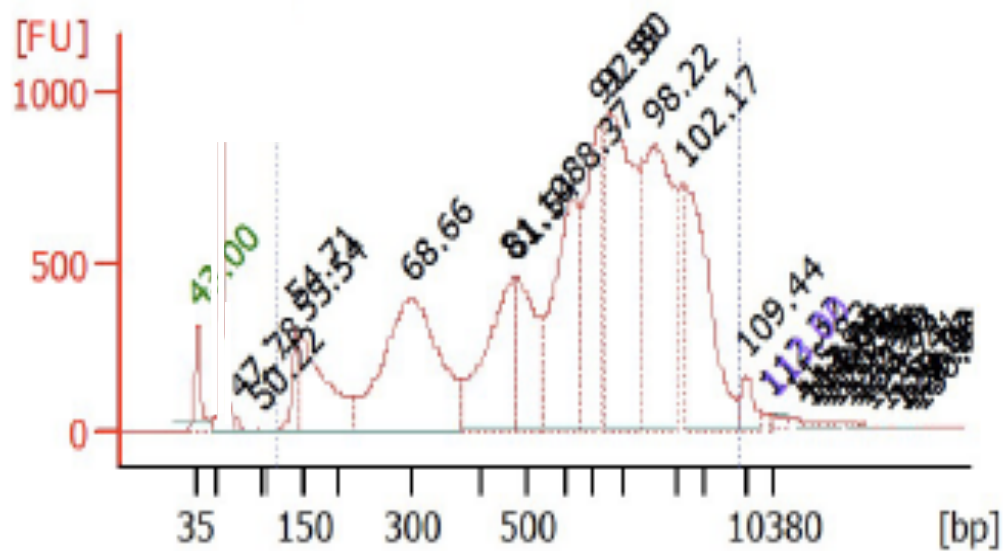
A part of dead cells in a sample is sometimes already sufficient to spoil the whole sample

Bioanalyzer showing no nucleosome 'bumps', usually high primer dimer amount

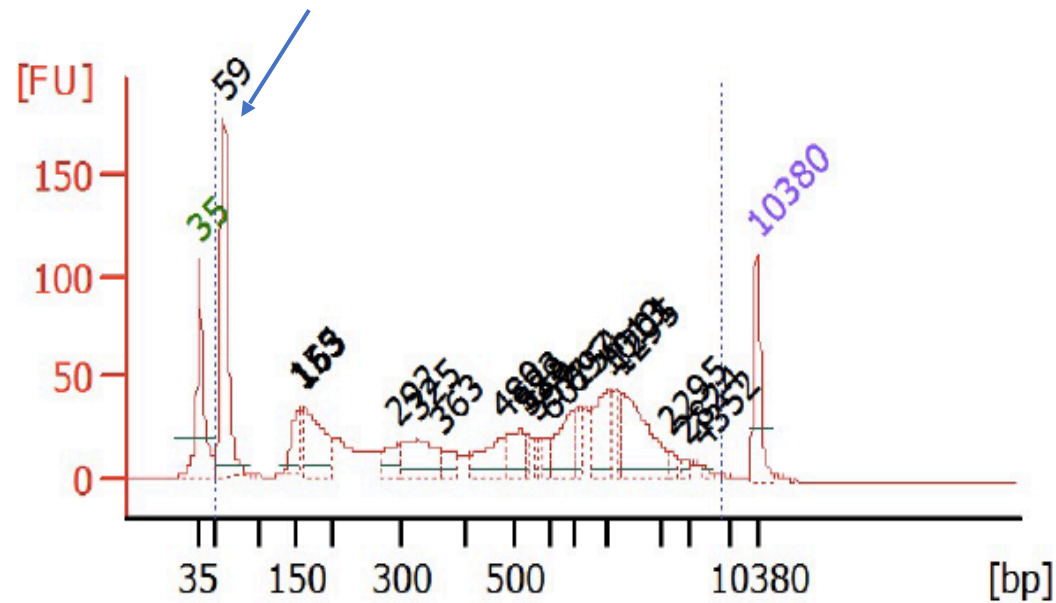


To remove dead cells:
Ficoll gradient, sorting, DNase treatment

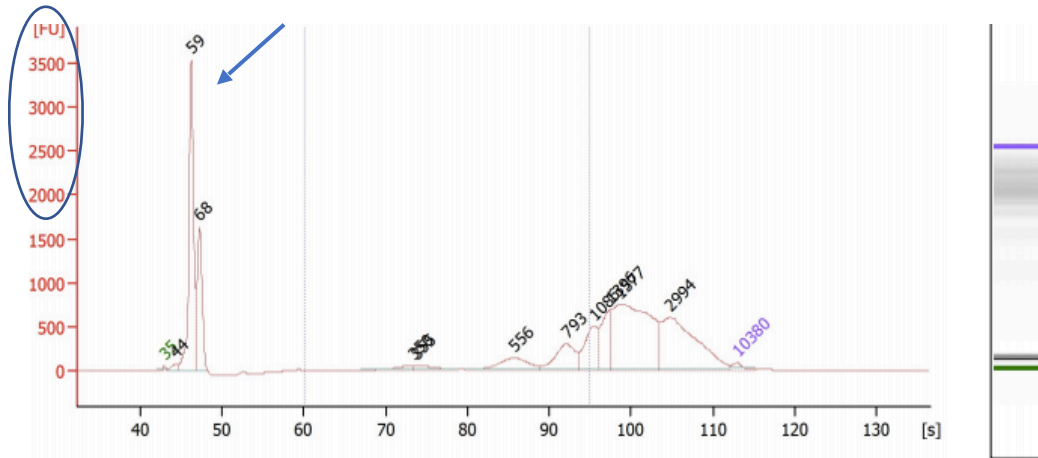
High molecular weight BioA



High primer amount BioA



Overloaded BioA Chip



Looked totally good after primer cleanup!

Reasons:
overloaded?
Overloaded before your sample?
Ethanol leftover after cleanup?

Uneven baseline

