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Technical challenges

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SSI
Public Health WGS workshop

With focus on Illumina

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- DNA preparation from cells
- Nextera XT Library prep
  - Short insert size
- Using different platforms, Illumina-Iontorrent-454-whatnot
  - HiSeq vs MiSeq
    - SNP pipeline
    - Gene by gene

- http://seqanswers.com/

How important is the DNA preparation?

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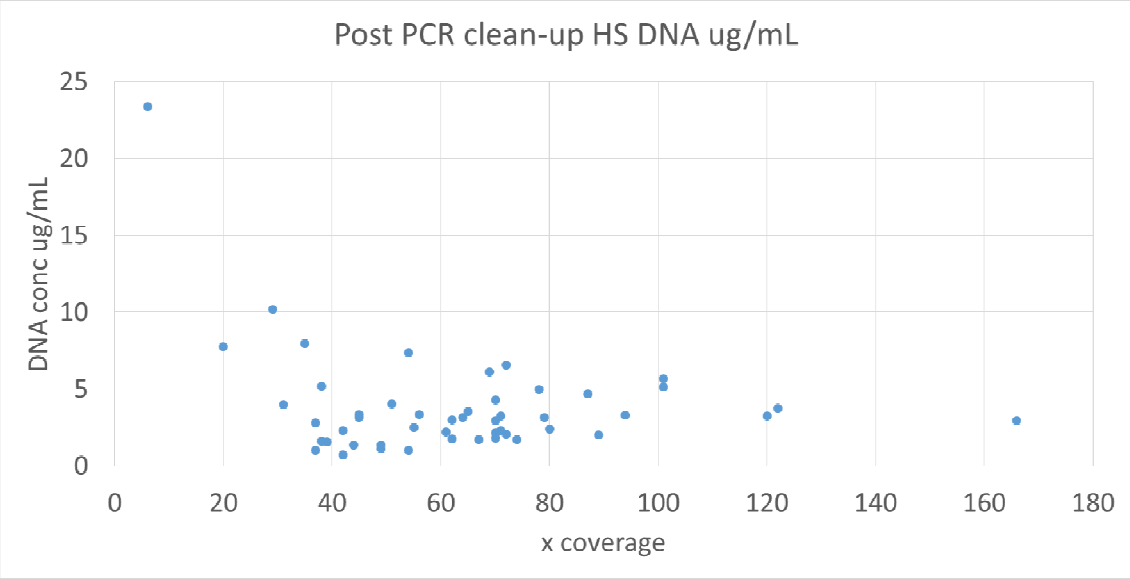
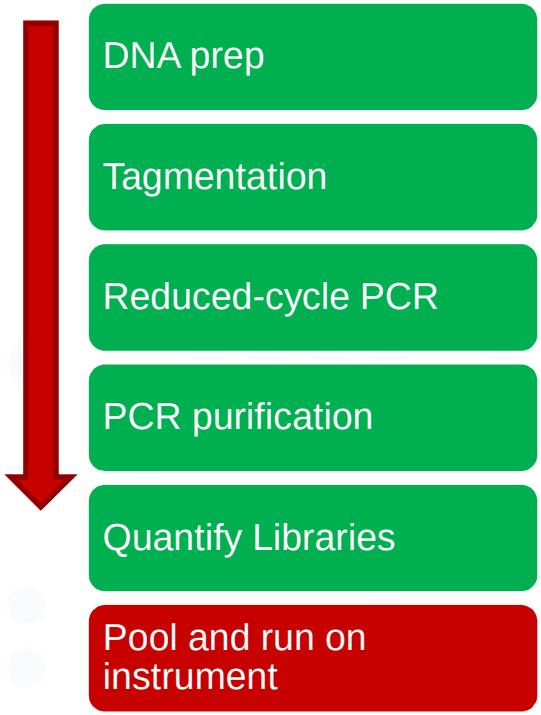
- We have tried
  - Promega Wizard SV
  - Qiagen Gentra Puregene Yeast/Bact. Kit
- Our limited observation is that Nextera XT preps are identical from the same DNA prep
- Need to look closer into why some DNA preps seemingly have ‘bad library properties’





Short insert size

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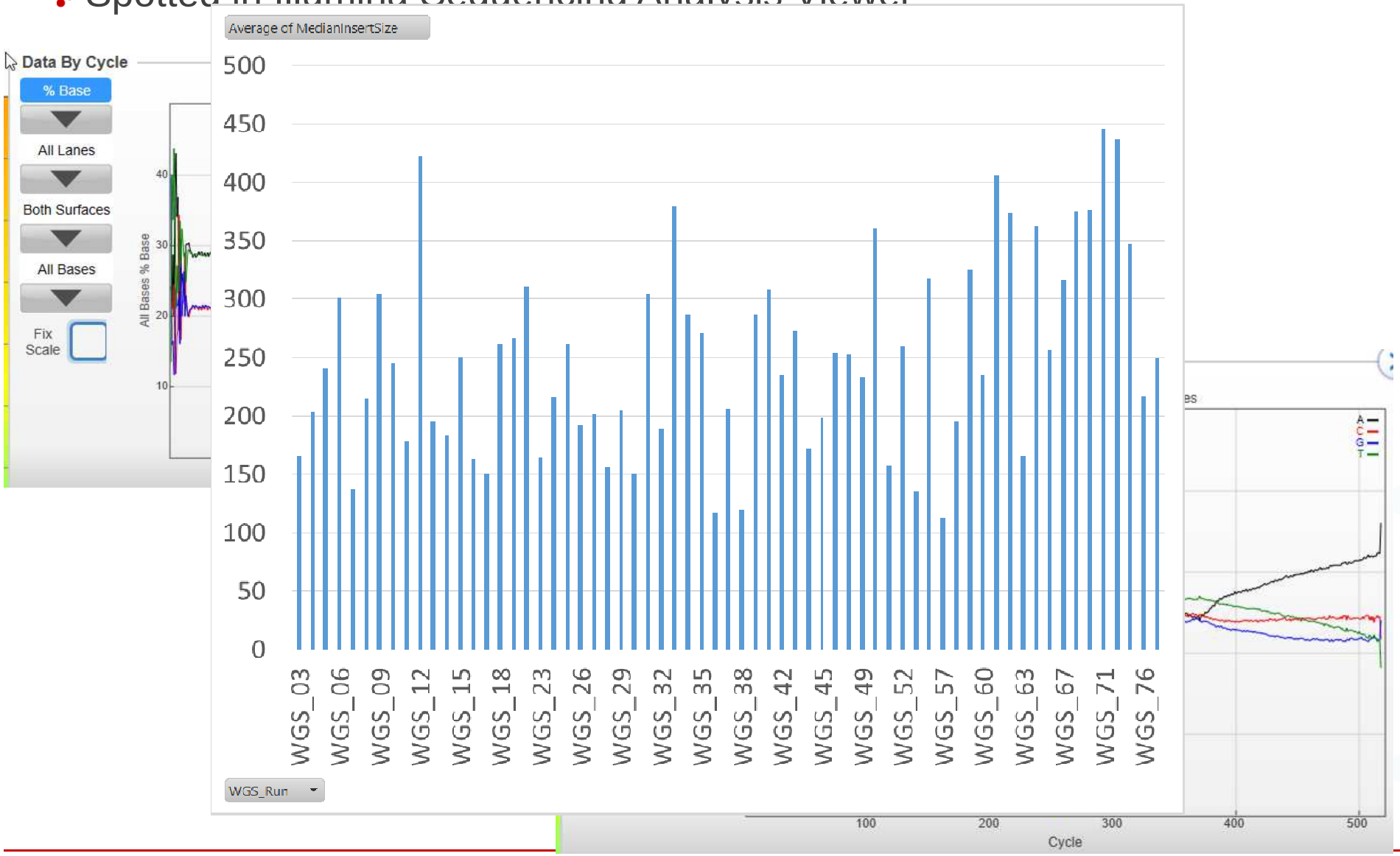
- Spotted in Illumina Sequencing Analysis Viewer



Short insert size

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- Insert sizes with the default Nextera XT protocol are much too small
- Changing from 25 to 20 µl AMPure XP beads in the PCR purification

DNA prep

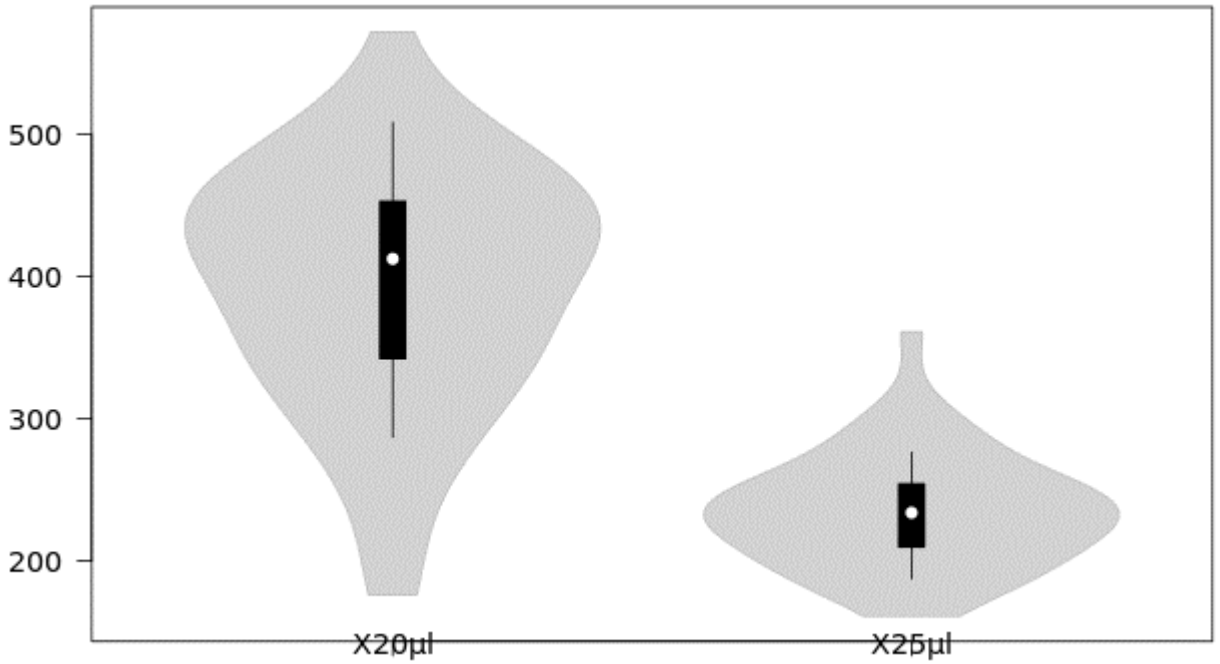
Tagmentation

Reduced-cycle PCR

PCR purification

Quantify Libraries

Pool and run on instrument



Normalization

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- We are now using a protocol from CDC without the bead-based normalization
- Single quantification with Qubit
- Simple dilution scheme
  - Compensate for genome sizes
- Skips microplate shaking/magnetic bead steps
- More even normalization