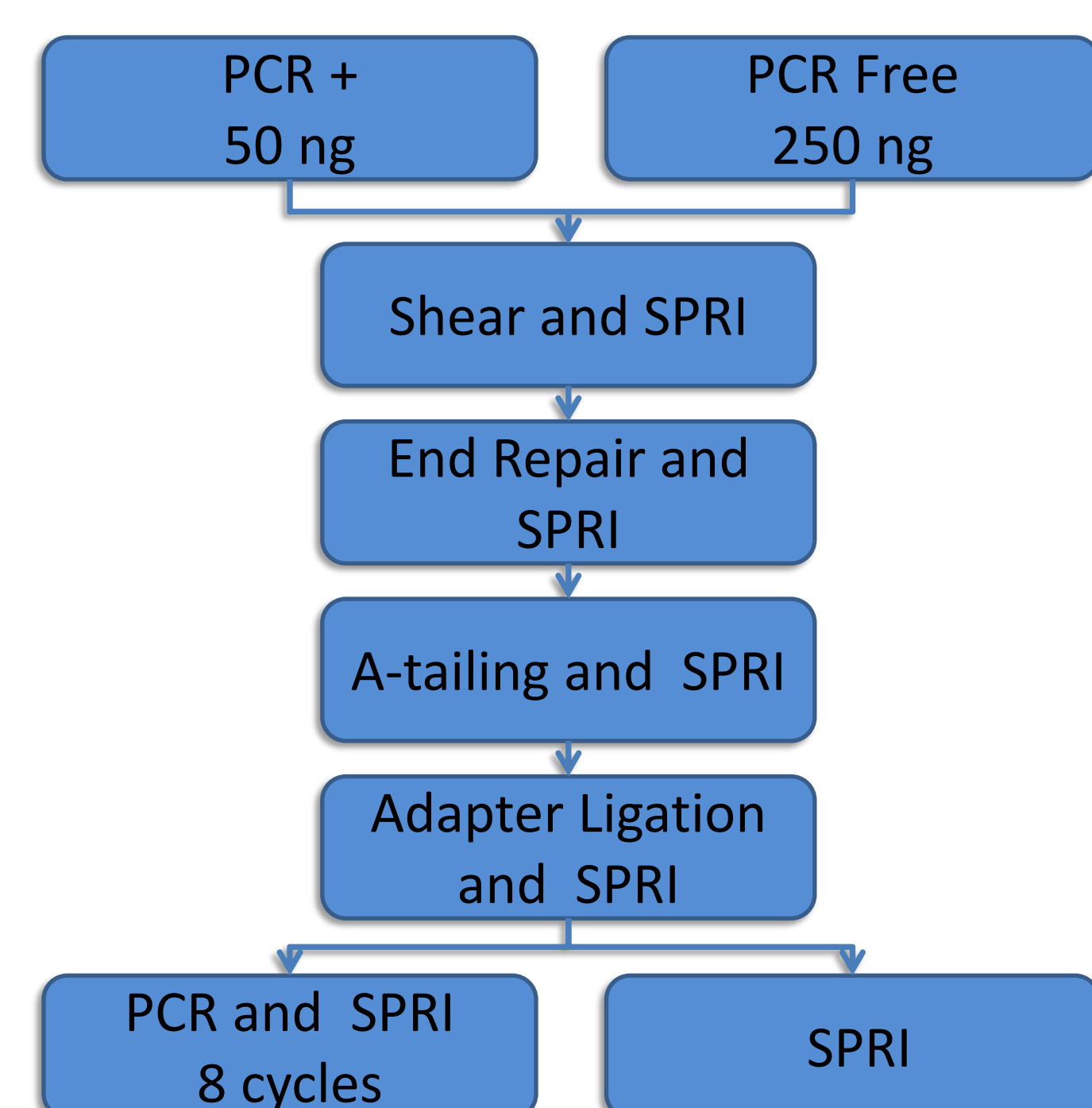


PCR-free human whole genome libraries sequenced on the Illumina HiSeq X are the current NGS gold standard for population-scale genomics, Mendelian disease gene discovery, and understanding the genetic components of common diseases. Within the last year we have implemented a high throughput whole genome pipeline which:

- Uses a streamlined and unified workflow
- Allows for a reduction in gDNA input
- Allows for the creation of more complex sequencing pools and more even library representation
- Increases per lane yield (Gb) on the HiSeqX

## Increasing Scale with Unified Automated Workflow

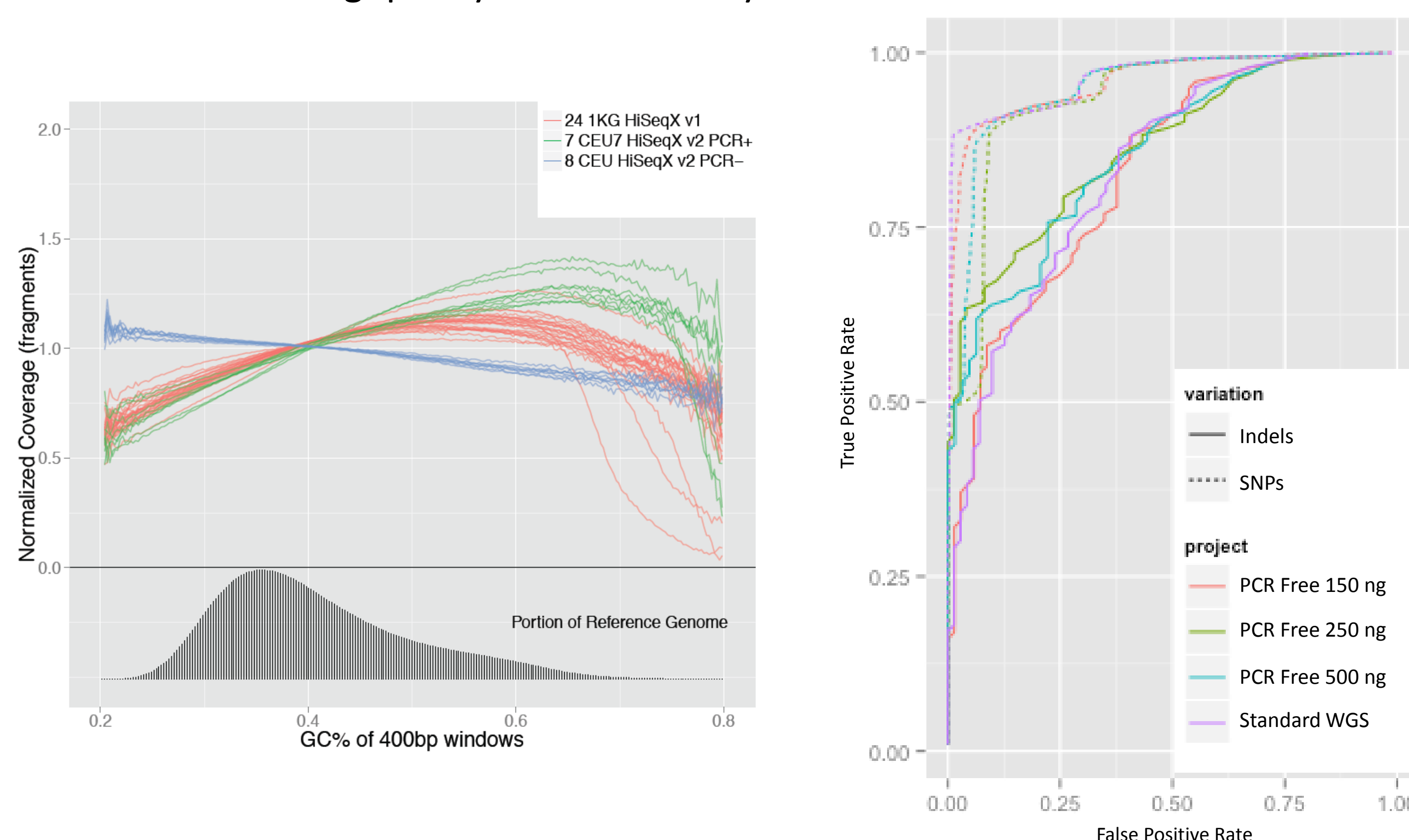


- Single, simplified workflow for both PCR free and PCR plus protocols
- Allows a single full time employee to create up to 192 whole genome libraries per day
- Protocol is dictated by amount of available input material
  - PCR Free input: 250 ng
  - PCR+ input: 50 ng

**Figure 1: Process map.** Single unified library preparation for all WGS samples

## Reducing Input into WGS Library Preparation

- PCR Free library prep results in improved coverage across the genome and reduction in base specific biases that are attributed to DNA polymerases
- Optimized PCR Free library preparation allows for a reduction of gDNA input while maintaining quality and sufficient yield



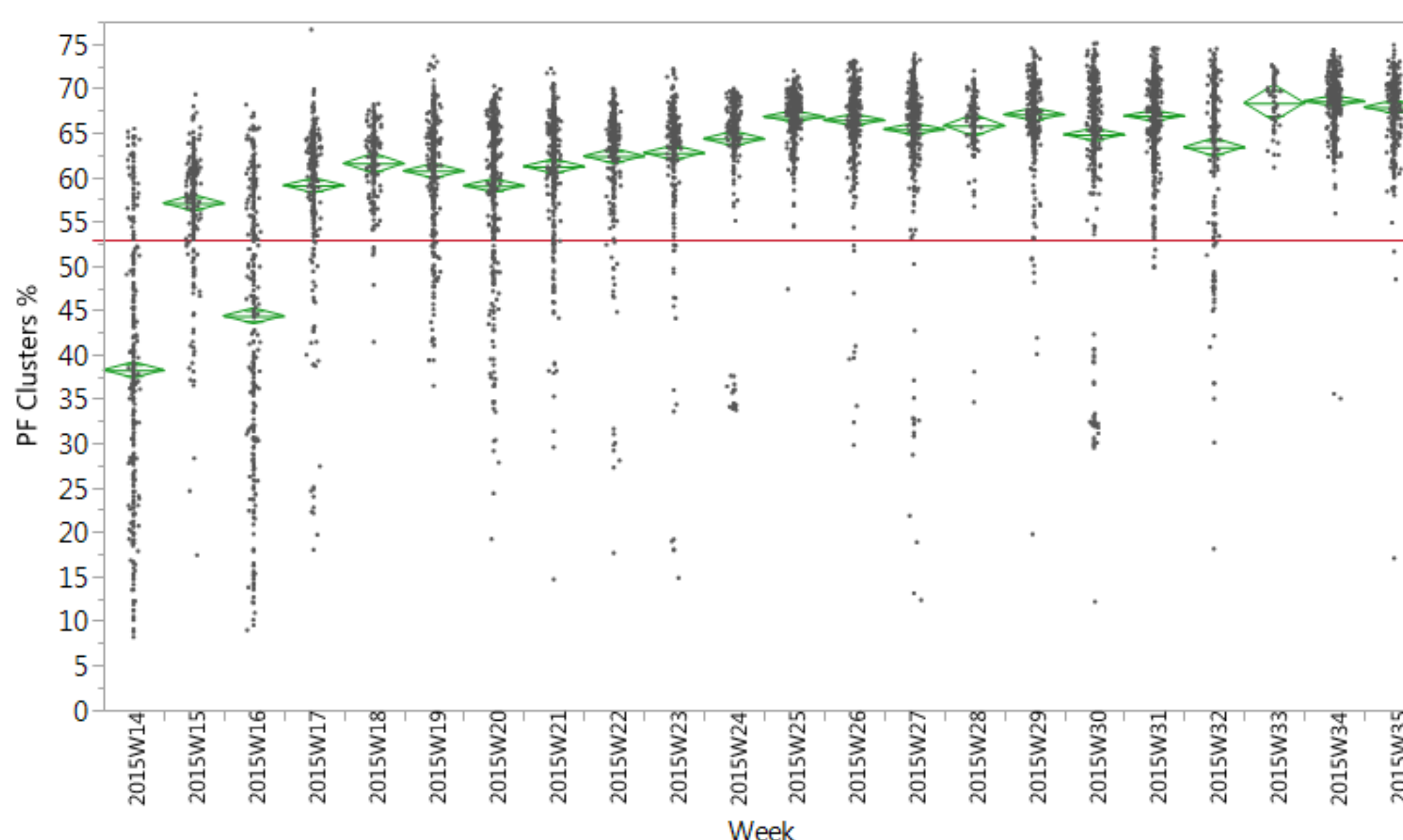
**Figure 2: GC bias comparison.** PCR-free WGS libraries (blue) show significantly more even coverage across the GC spectrum when compared to traditional WGS libraries (red and green).

**Figure 3: SNP and Indel Calling.** Analysis has shown no significant difference in SNP and Indel calling between inputs of 500 ng and 250 ng gDNA into PCR Free library prep

## Increasing Sequencing Yield

Increased sequencing yield achieved through:

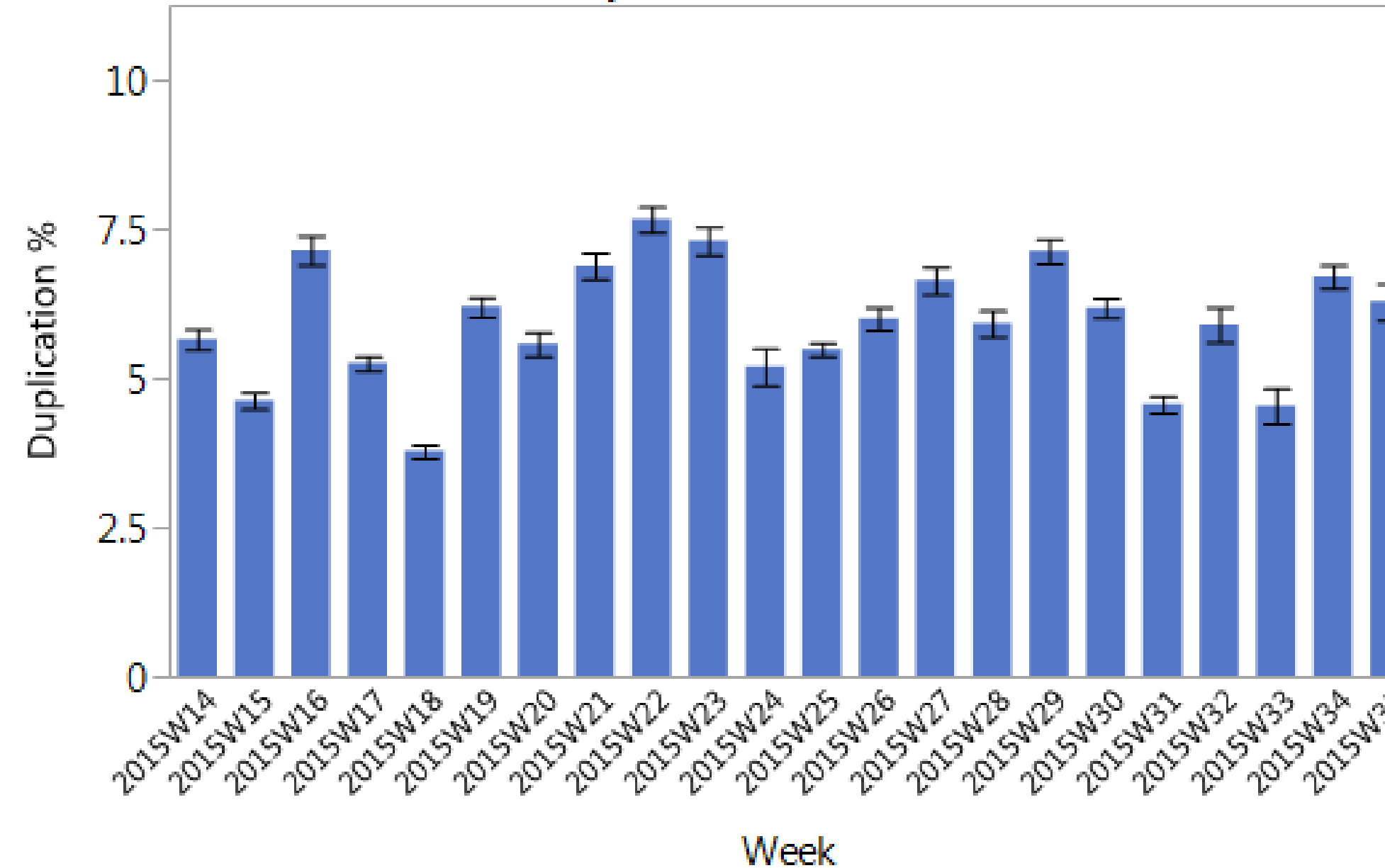
- Improvements to automated steps and liquid handling
- Reduced downtime between strip tube creation and cluster amplification
- Identification of optimal loading concentrations by sample type to increase %PF while keeping optical duplication levels low



**Figure 4: %PF Clusters.** Process improvements increase %PF over time while decreasing variability

- Optical duplication by pad hopping is specific to the arrayed flowcell technologies. With insufficient DNA loading, a strand of DNA will “hop” from an already seeded well to a neighboring one causing identical clusters.

**Mean(Duplication %) vs. Week**

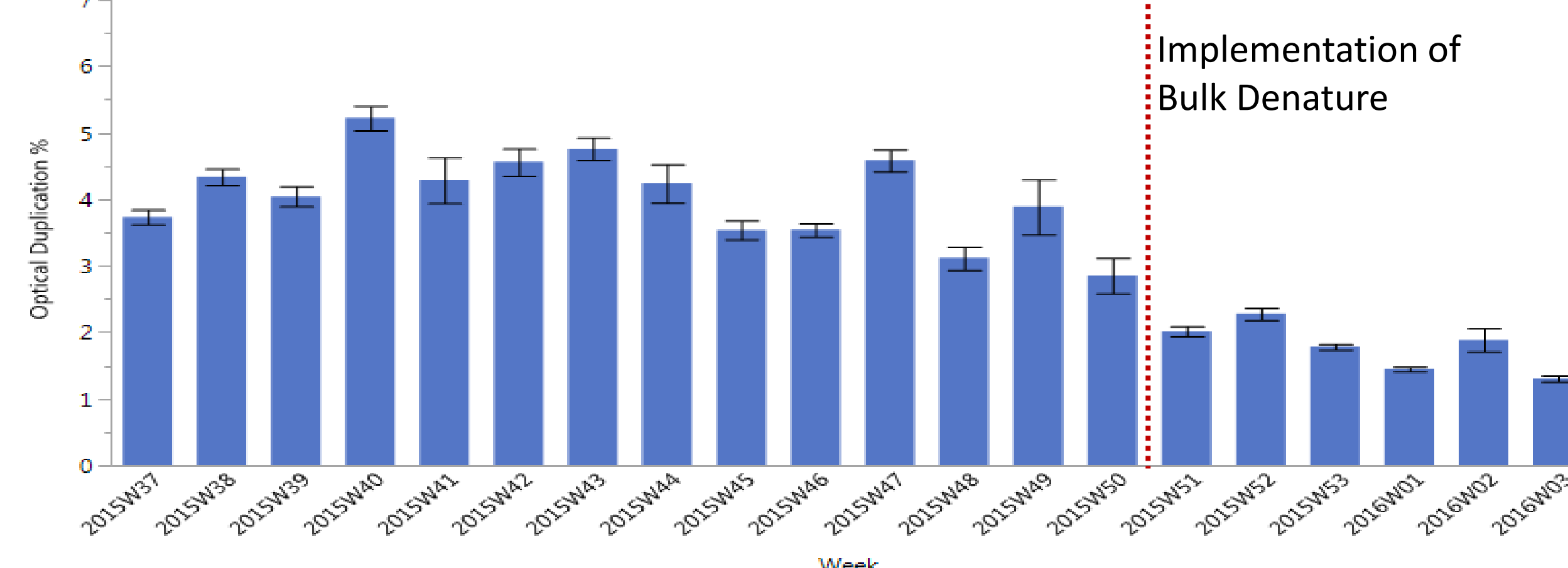


**Figure 5: Duplication %.** As %PF increased there was no change in overall duplication and more importantly optical duplication

Bulk denature of all lanes of a single pool allows for:

- Reduction in variability lane to lane
- Increased loading concentration resulting in a reduction of optical duplication %

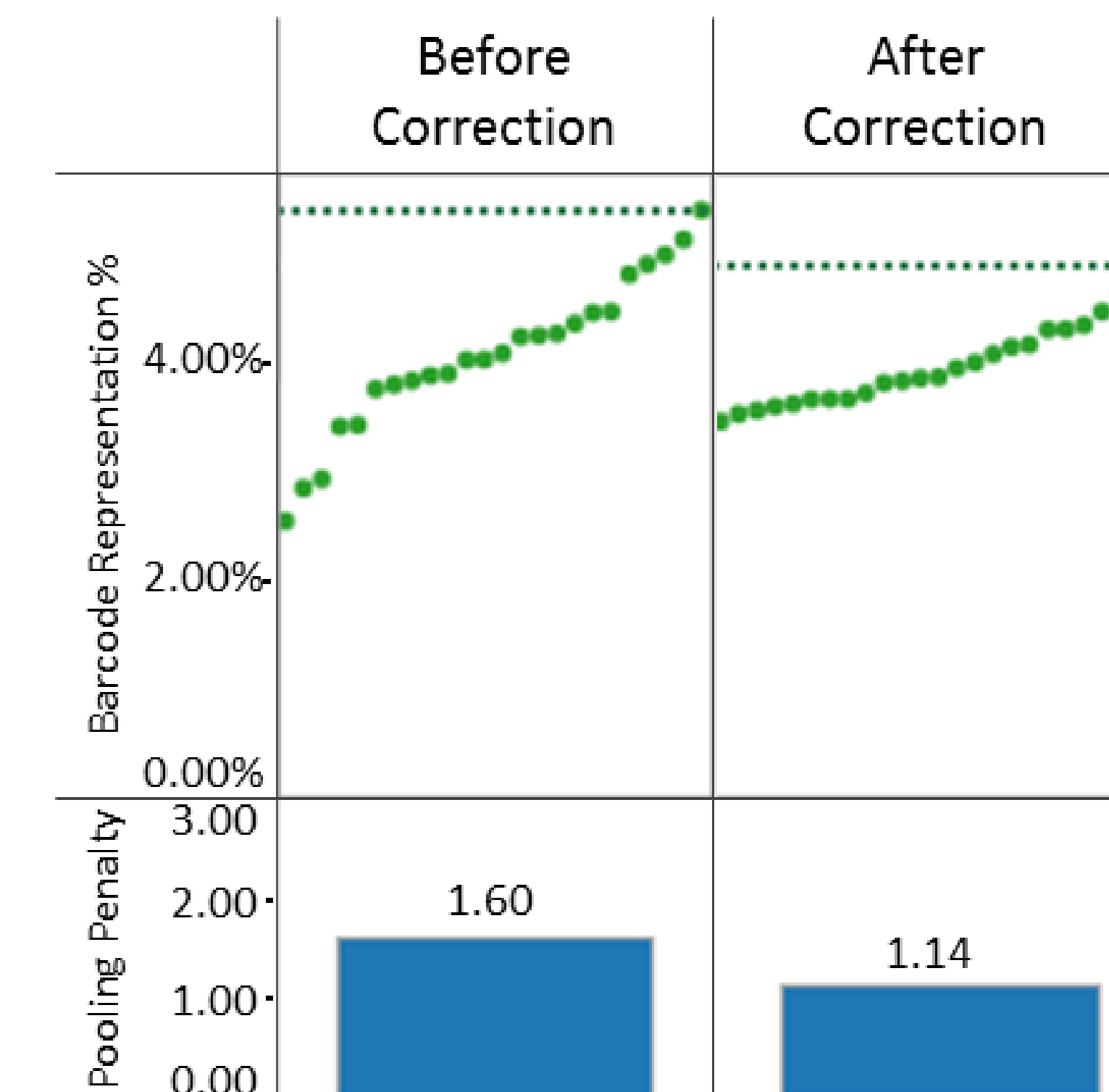
**Mean(Optical Duplication %) vs. Week**



**Figure 6: Optical Duplication %.** Bulk denature allows for sequencing lower concentration pools at a higher loading concentration leading to an overall drop in optical duplication %.

## Increasing Pooling Plexity

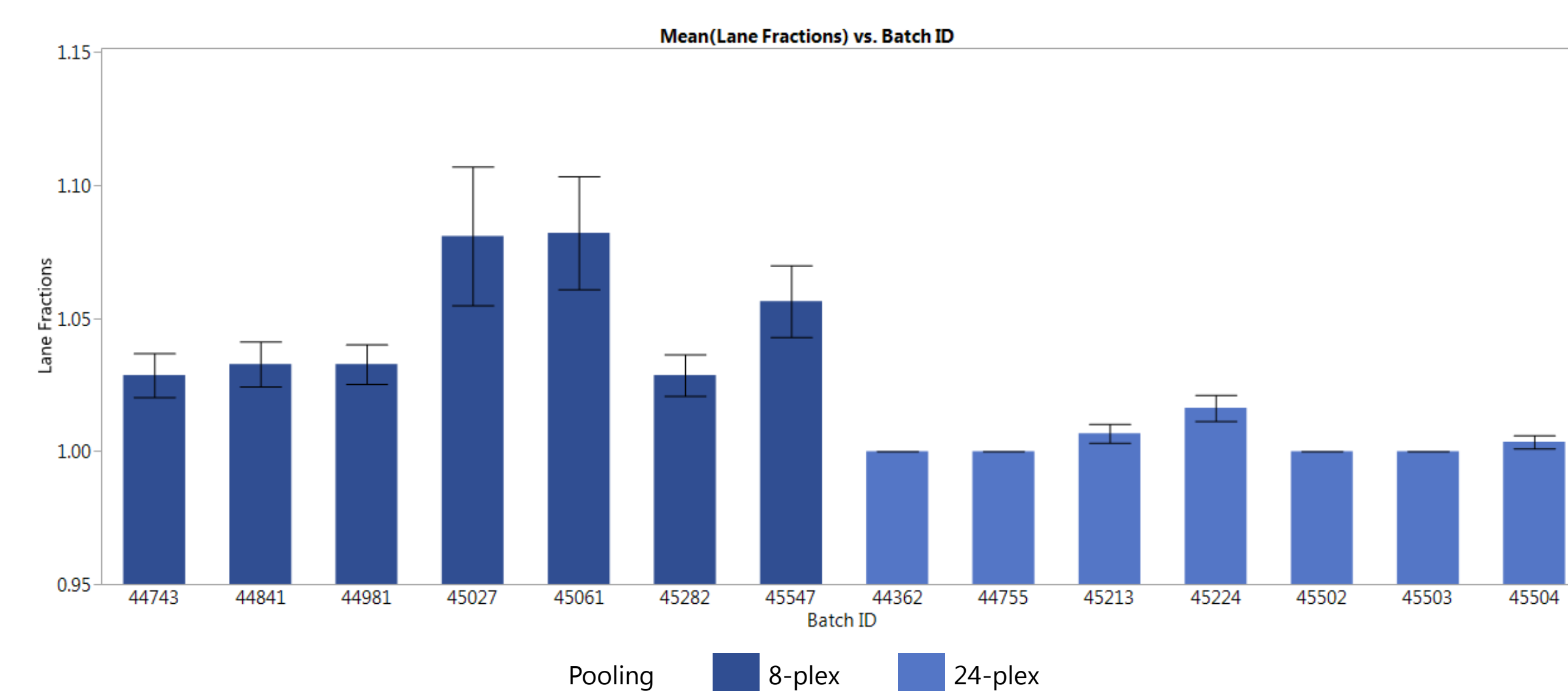
- Ability to pool up to 24 unique whole genome libraries prior to sequencing
- Leverage single lane of HiSeqX sequencing to assess the evenness of library representation within pools
- Ability to correct for underrepresented libraries within pools to ensure desired coverage is met while minimizing the amount of sequencing necessary



**Figure 7: Pool Correction.** Pool Correction strategy improves library representation from original pool (left) to adjusted pool (right).

Pool Correction results in more even pools which:

- Increases number of samples that hit coverage goal on first pass
- Leads to shorter turn around time for projects
- Reduces overall number of lanes of sequencing required per batch



Batch ID	44743	44841	44981	45027	45061	45282	45547	44362	44755	45213	45224	45502	45503	45504	
Pooling	8-plex							24-plex							
1 <sup>st</sup> Pass success	86.4	84.2	80.6	81.1	80.0	85.1	79.8	100	100	95.6	89.5	100	100	97.9	

**Figure 8: Pool Correction of 24 Plex Pools.** Corrected pools result in more samples hitting coverage on first pass and fewer overall lanes being sequenced. 24 plex pools are corrected, 8 plex are not.

## Acknowledgements

