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Publications for Accel-NGS® 2S DNA Library Kits

List of Publications

1. A comparative study of ChIP-Seq library preparation methods (*Sundaram, A.Y.M. et al., BMC Genomics, 2016)*
2. Cell cycle progression in *Caulobacter* requires a nucleoid-associated with high AT sequence recognition (*Ricci, D.P. et al., PNAS, 2016)*
3. Distinct sub-clonal tumor responses to therapy revealed by circulating, cell-free DNA (*Gremel, G. et al., Annals of Oncology, 2016)*
4. Digital sorting of pure cell populations enables unambiguous genetic analysis of heterogenous formalin-fixed, paraffin-embedded tumors by next generation sequencing (*Bolognesi, C. et al., Scientific Reports, 2016)*
5. Application of sequencing, liquid biopsies, and patient-derived xeonografts for personalized medicine in melanoma (*Girotti, M.R. et al., Cancer Discov, 2016)*
6. Vasculogenic mimicry in small cell lung cancer (*Williamson, S.C. et al., Nature Communications, 2016)*
7. Genome sequence of *Klebsiella pneunoniae* urinary tract isolate Top52 (*Johnson, J.G. et al., Genome Announc, 2014)*
8. Genome sequence of *Klebsiella pneunoniae* respiratory isolate IA565 (*Johnson, J.G. et al., Genome Announc, 2014)*

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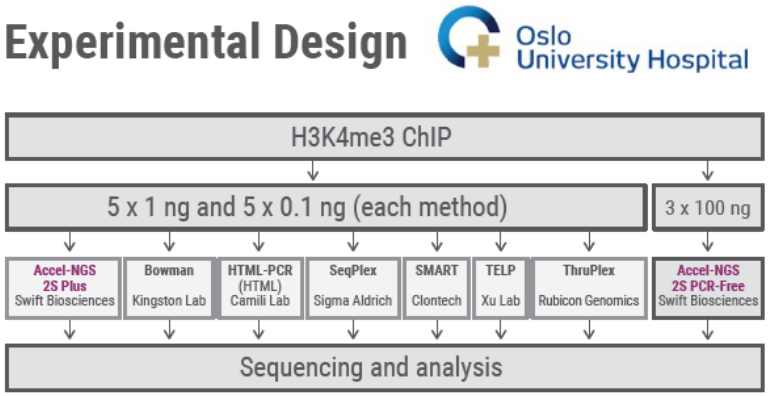
A Comparative Study of ChIP-Seq Library Preparation Methods *(Sundaram, A.Y.M. et al., BMC Genomics, 2016)*

- Seven methods designed for low input DNA/ChIP-seq sample preparation (Accel-NGS® 2S, Bowman-method, HTML-PCR, SeqPlex, DNA SMART, TELP, and ThruPLEX) were performed on five replicates of 1 ng and 0.1 ng input H3K4me3 ChIP material and compared to a gold standard reference PCR free dataset.



- The Accel-NGS 2S PCR-Free Kit was chosen for the PCR-free dataset. The only PCR-free kit tested that was able to construct libraries from 100 ng inputs.
- All samples resembled closely existing H3K4me3 datasets. The Accel-NGS 2S Kit shows the most consistent high scores at both 1 ng and 0.1 ng input levels.
- The highest peak calling, sensitivity, and specificity values were recorded for the Accel-NGS 2S Kit.

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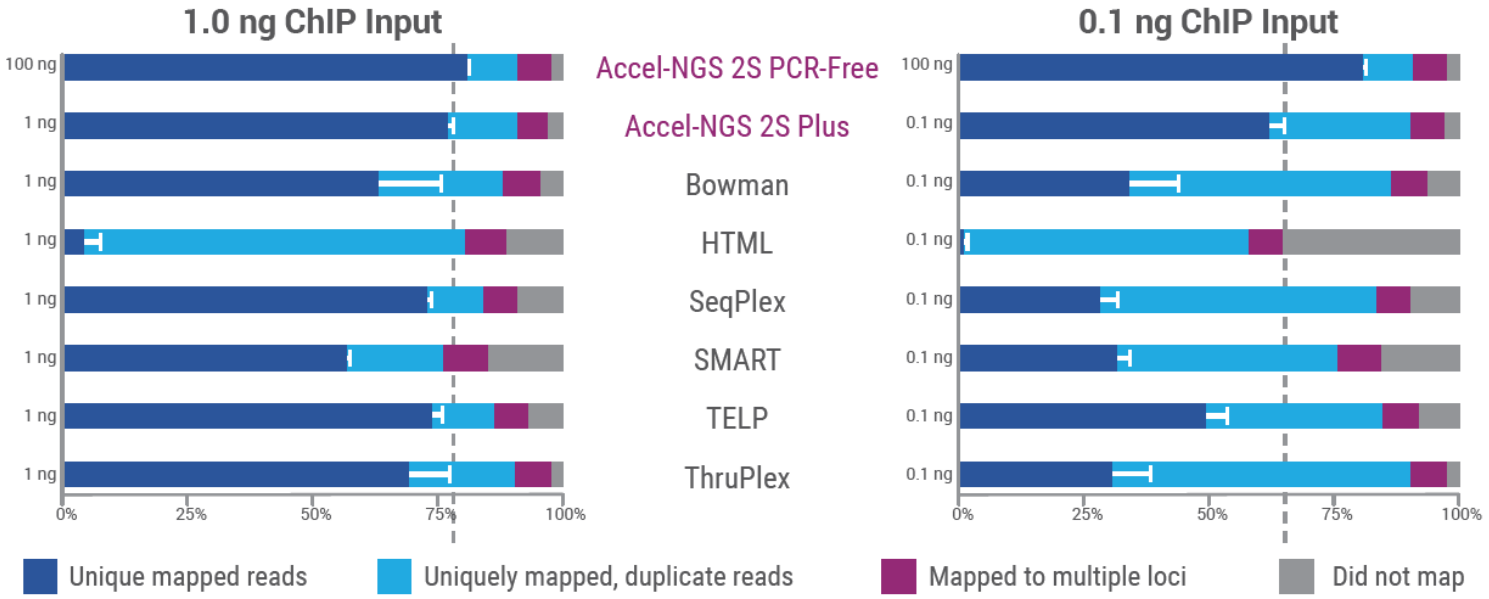
A Comparative Study of ChIP-Seq Library Preparation Methods *(Sundaram, A.Y.M. et al., BMC Genomics, 2016)*

- Accel-NGS® 2S technology enables the use of ultralow input samples for ChIP-Seq applications by utilizing a unique chemistry for the most efficient preparation of NGS libraries.
- At 0.1 ng DNA input, the greatest complexity was retained by the Accel-NGS 2S Plus Kit with the highest % unique mapped reads.



Results shown are the mean of 5 replicates for each method, using 25 million reads per replicate.

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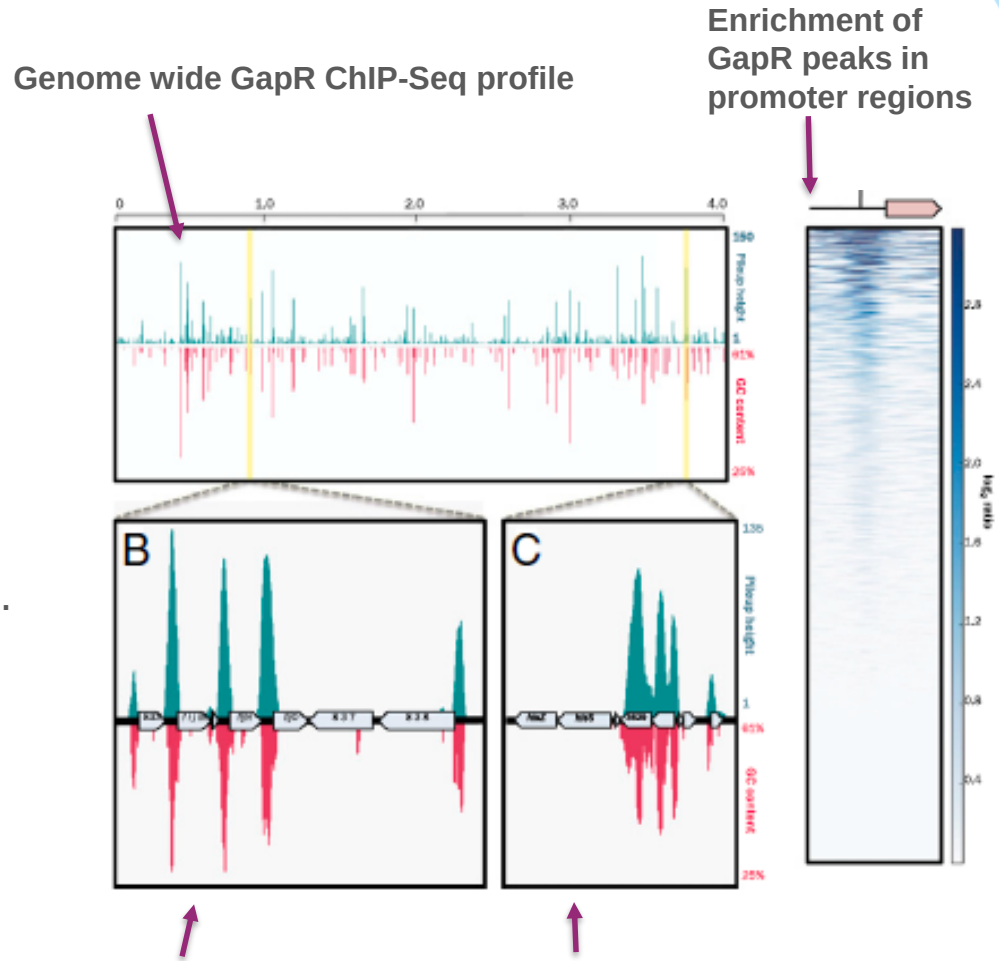
Cell Cycle Progression in *Caulobacter* Requires a Nucleoid-Associated with High AT Sequence Recognition (*Ricci, D.P. et al., PNAS, 2016)*

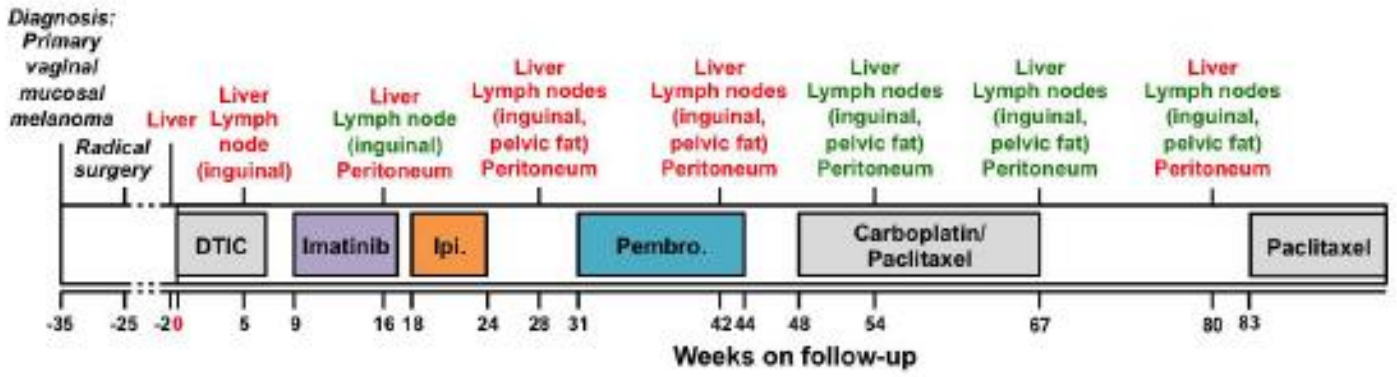
- Deep sequencing of ChIP DNA demonstrated that GapR, an essential DNA-associated protein, selectively associates with AT-rich DNA, yet it does not localize preferentially to the swamer compartment when expressed exogenously in *Caulobacter,* suggesting that recognition of AT-rich DNA is not sufficient for the asymmetric accumulation of GapR.
- ChIP libraries were prepared and multiplexed using the Accel-NGS® 2S Plus DNA Library Kit, taking advantage of its remarkable evenness of coverage for AT-/GC-rich regions.



Distinct Sub-Clonal Tumor Responses to Therapy Revealed by Circulating, Cell-Free DNA

(Gremel, G. et al., Annals of Oncology, 2016)

- NGS of circulating, cell-free DNA (cfDNA) was used to monitor the response of a *KIT* p.L576P-mutant metastatic vaginal mucosal melanoma to sequential targeted, immunotherapy and chemotherapy.
- Whole exome sequencing (WES) and targeted longitudinal analysis of cfDNA was performed.
- Sequencing libraries were generated from 10 ng or 25 ng cfDNA, or 200 ng sheared DNA using the Accel-NGS® 2S Hyb DNA Library Kit.
- Below is the overview of patient's treatment history, including information on initial diagnosis (35 weeks prior to follow-up) and surgery.



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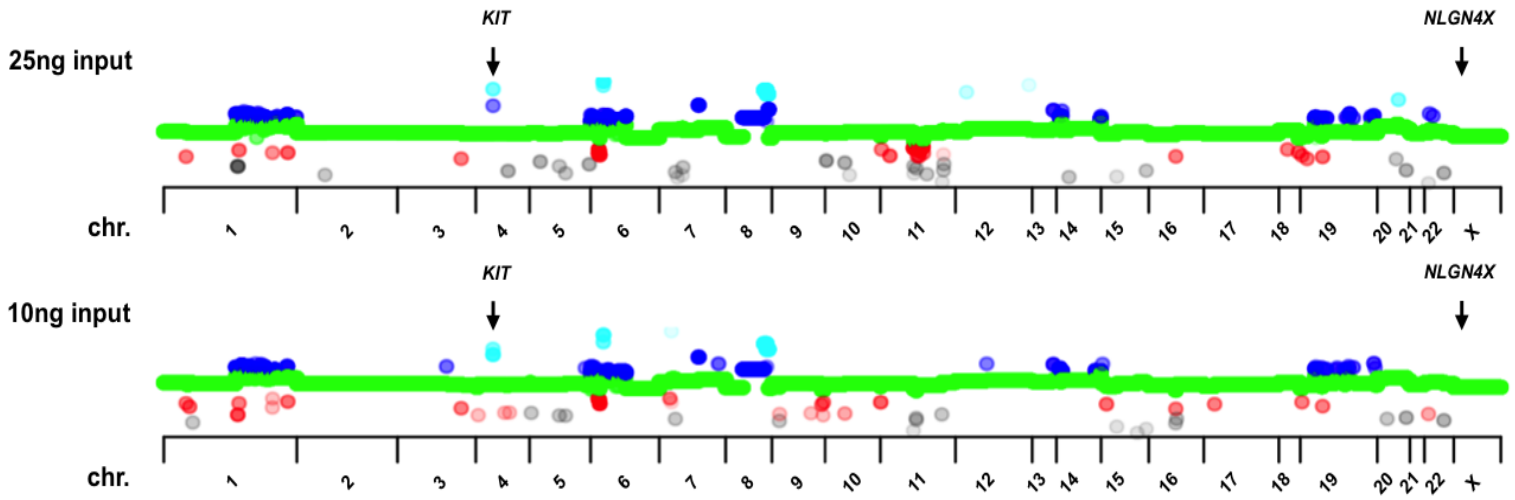
Distinct Sub-Clonal Tumor Responses to Therapy Revealed by Circulating, Cell-Free DNA

(Gremel, G. et al., Annals of Oncology, 2016)

- cfDNA reveals two tumor sub-clones with distinct responses to therapy.
- Copy number variation by chromosome (chr.) based on WES from the two different input cfDNA amounts isolated at week 37 of follow-up. Grey and red dots indicate allele loss (both and one, respectively), green dots indicate normal copy number state, blue and light blue dots indicate copy number gains (one and two, respectively)



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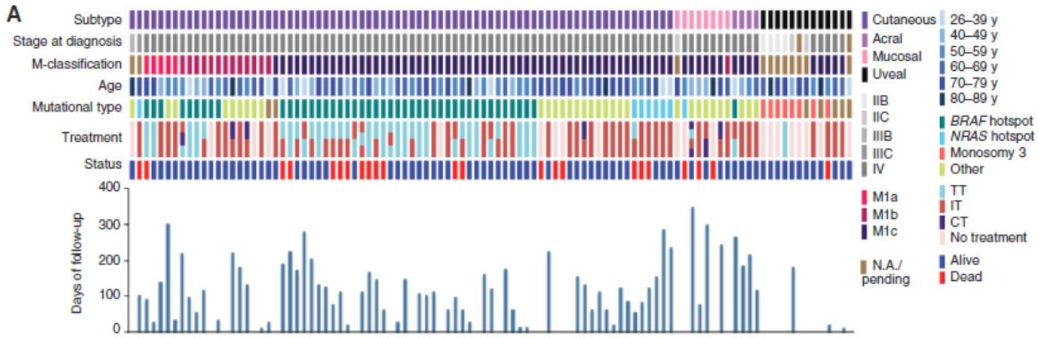
Application of Sequencing, Liquid Biopsies, and Patient-Derived Xeonografts for Personalized Medicine in Melanoma *(Girotti, M.R. et al., Cancer Discov, 2016)*

- WES of circulating tumor DNA (ctDNA) provided insight into mechanisms of resistance in many melanoma patients. ctDNA was used to monitor patient response.
- Overview of 101 patients with cutaneous, acral, mucosal, and uveal melanoma at different disease stages.



- ctDNA was extracted with QIAamp® Circulating Nucleic Acid Kits (Qiagen). Libraries were prepared using the Accel-NGS® 2S Hyb DNA Library Kit.

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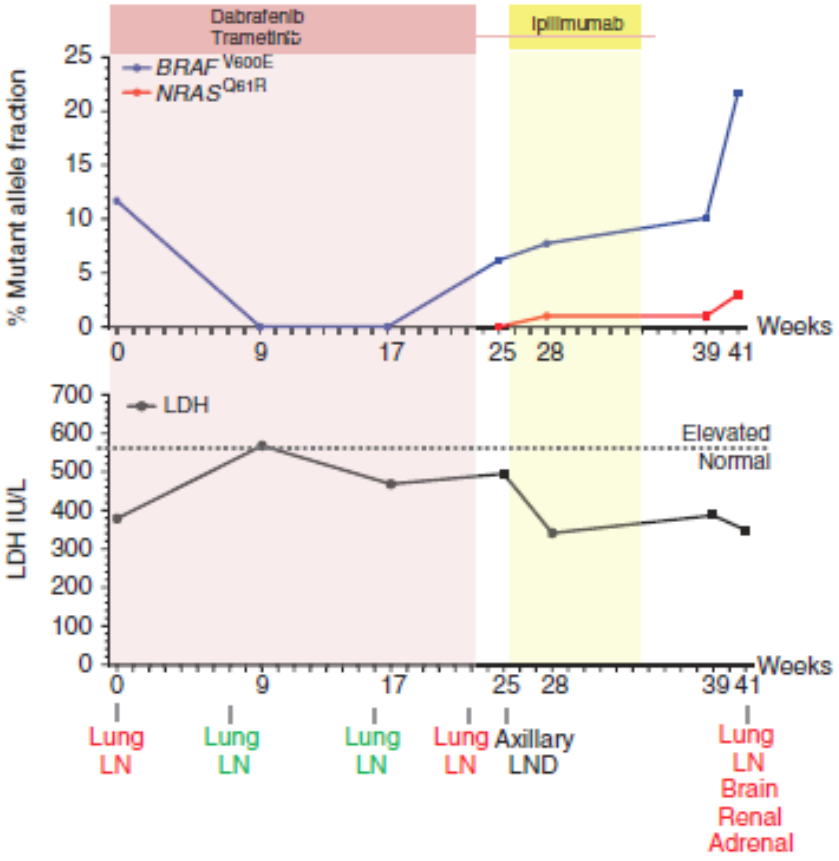
Application of Sequencing, Liquid Biopsies, and Patient-Derived Xeonografts for Personalized Medicine in Melanoma *(Girotti, M.R. et al., Cancer Discov, 2016)*

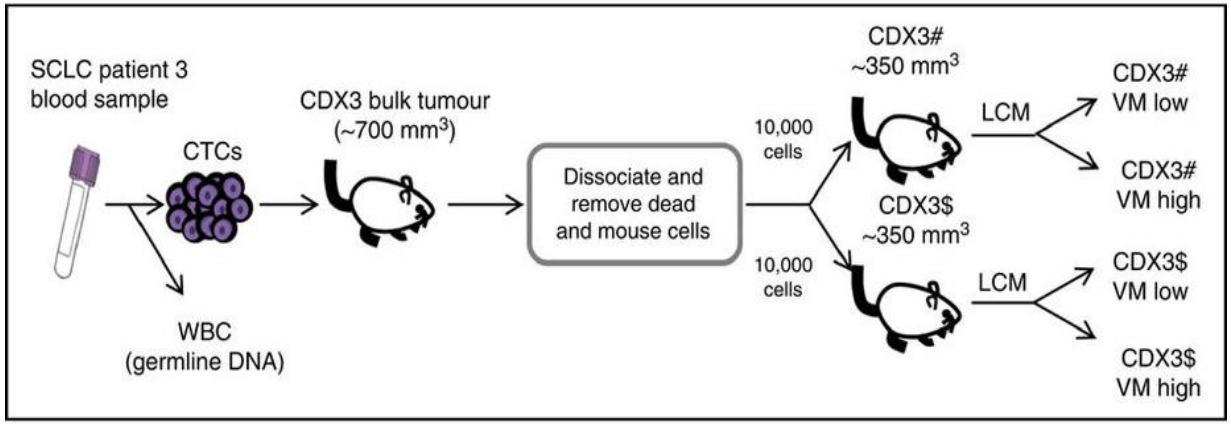
- WES of ctDNA was used as a tool to monitor 5 patients' responses to treatment over a course of monitoring
- Representative time courses for *BRAF*^{V600E} variant allele fractions (VAF; blue), *NRAS*^{Q61R} VAF (red), and serum LDH (black) in patient 1
- Providing mechanisms of resistance even in the absence of prior knowledge of the underlying molecular processes.



Vasculogenic Mimicry in Small Cell Lung Cancer

(Williamson, S.C. et al., Nature Communications, 2016)

- This paper has shown that patients with lung cancer have rare circulating tumor cells (CTCs) that are expressing growth factors that are consistent with endothelial cell-like (cells that make up the capillaries) vessels.
- The Accel-NGS® 2S Plus DNA Library Kit was used to construct libraries from 25 ng DNA extracted from CTC patient-derived explant (mouse models) laser captured microdissection and subject to low pass NGS with all sequencing reads that aligned to the mouse genome eliminated from the analysis to identify the presence of human-specific sequences.



Vasculogenic Mimicry in Small Cell Lung Cancer

(Williamson, S.C. et al., Nature Communications, 2016)

- This data strongly supports that in SCLC, VE-cadherin positive vessels are tumor cell-derived and not the manifestation of co-option of murine host endothelial cells via anastomosis. Sequencing data from the 8 different samples used for genomic analysis demonstrating minimal mouse cell contamination and predominantly human genomes in dissected tissues.
- The data all together suggest that even though rare, SCLC cells may be able to promote outgrowth of chemo-resistant clones preceding treatment failure.

Index	Description	Percentage reads aligned to human	Percentage reads aligned to mouse	Percentage reads aligned to both species	Percentage mouse DNA contribution (using germline control as baseline)
1	CDX3$ VM low	87.87	17.94	15.92	5.53
2	CDX3$ VM high	81.33	15.13	13.06	2.66
3	CDX3# VM low	84.08	15.25	12.69	2.3
4	CDX3# VM high	80.80	18.04	12.44	2.04
5	Patient 3 germline	79.95	11.25	10.40	0
6	CDX3 bulk tumor	63.18	12.67	9.92	-0.48
7	Mouse stroma	14.59	85.26	12.70	
8	Mouse muscle	13.51	85.02	11.77	

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Genome Sequence of *Klebsiella pneunoniae* Urinary Tract Isolate Top52 (*Johnson, J.G. et al., Genome Announc, 2014)*

- gDNA of *K. pneumoniae* IA565 was isolated using the Qiagen DNeasy Blood and Tissue Kit. gDNA was fragmented to an average size of 550 bp using a Covaris M220.
- Fragments were subsequently size selected by Pippin Prep, and whole genome libraries were made using the Accel-NGS® 2S DNA Library Kit.
- These libraries were sequenced on the MiSeq® and approximately 4 million FASTQ reads were used for *de novo* genome assembly using MIRA 4.

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Genome Sequence of *Klebsiella pneunoniae* Respiratory Isolate IA565 (*Johnson, J.G. et al., Genome Announc, 2014)*

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