

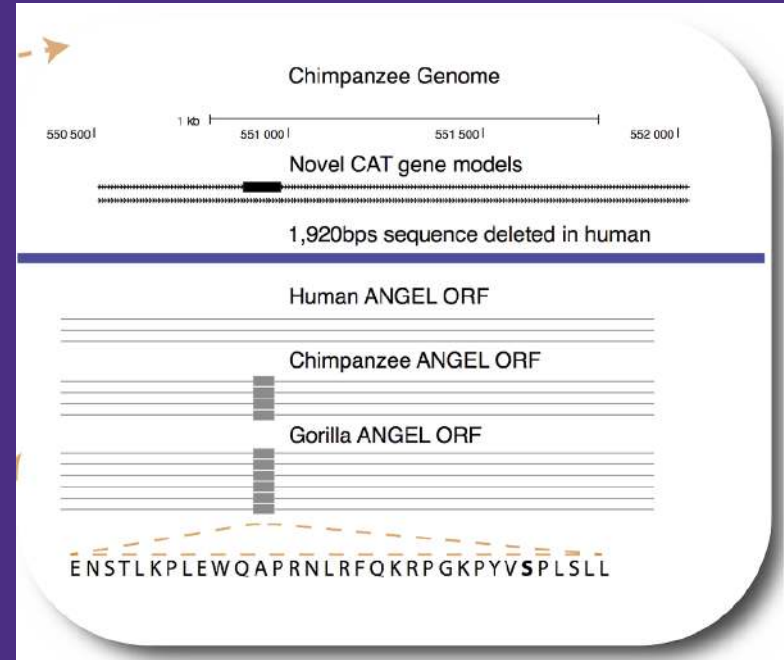
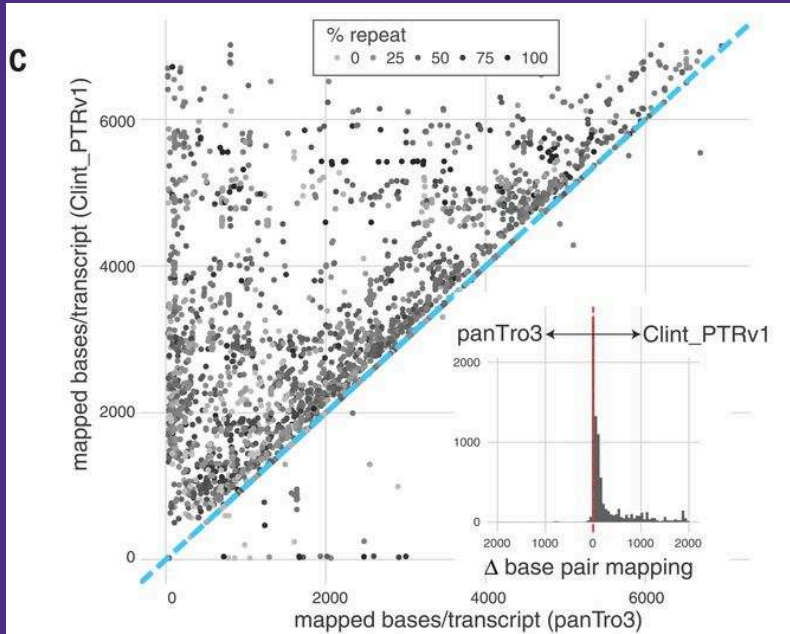
# Iso-Seq as a Service: Pro Tips for Starting from RNA

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# Better annotations make better genomes



# Challenges to a successful Iso-Seq experiment

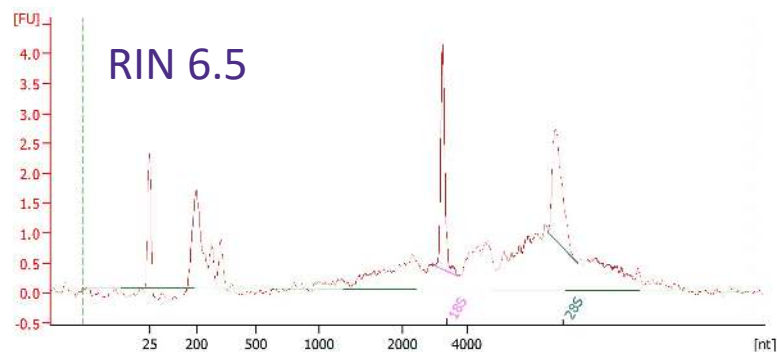
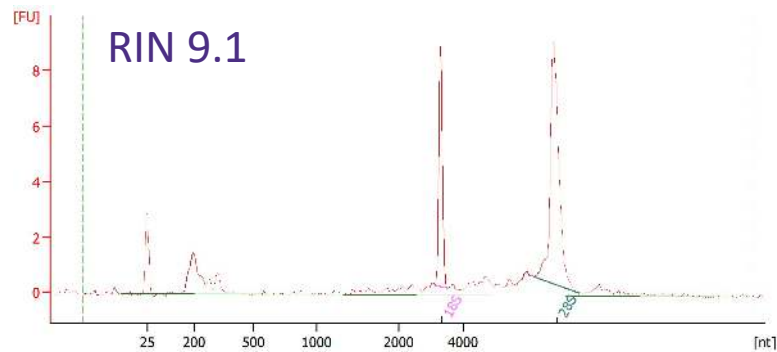
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- > RNA quantity and quality
- > PCR overamplification artifacts
- > Sequencer loading parameters
- > Insufficient read length

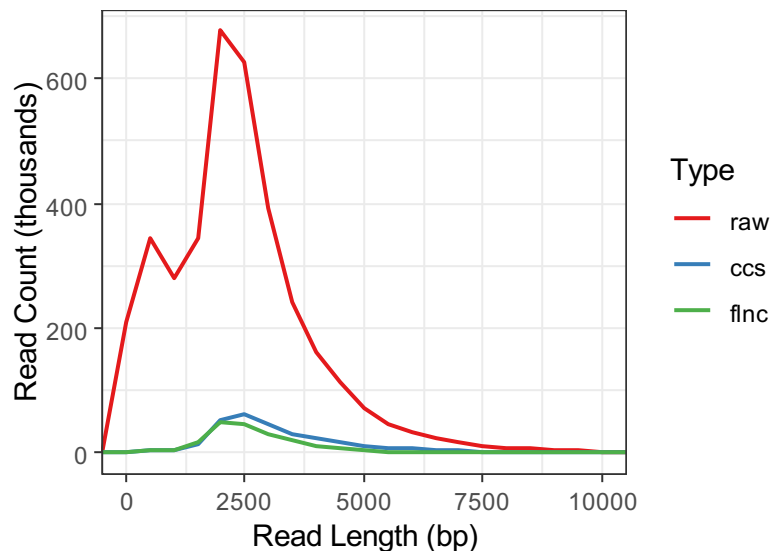
# Testing the effect of input amount and RIN score on Iso-Seq data

- Intentionally degrade high-quality RNA
- Barcode samples and process in parallel
- Pool for SMRTbell prep and sequencing

	1	2	3	4	5	6
A	RIN 9.1	RIN 8.5	RIN 8	RIN 7.4	RIN 7	RIN 6
	1000 ng	1000 ng	1000 ng	1000 ng	1000 ng	1000 ng
B	RIN 9.1	RIN 8.5	RIN 8	RIN 7.4	RIN 7	RIN 6
	750 ng	750 ng	750 ng	750 ng	750 ng	750 ng
C	RIN 9.1	RIN 8.5	RIN 8	RIN 7.4	RIN 7	RIN 6
	500 ng	500 ng	500 ng	500 ng	500 ng	500 ng
D	RIN 9.1	RIN 8.5	RIN 8	RIN 7.4	RIN 7	RIN 6
	250 ng	250 ng	250 ng	250 ng	250 ng	250 ng



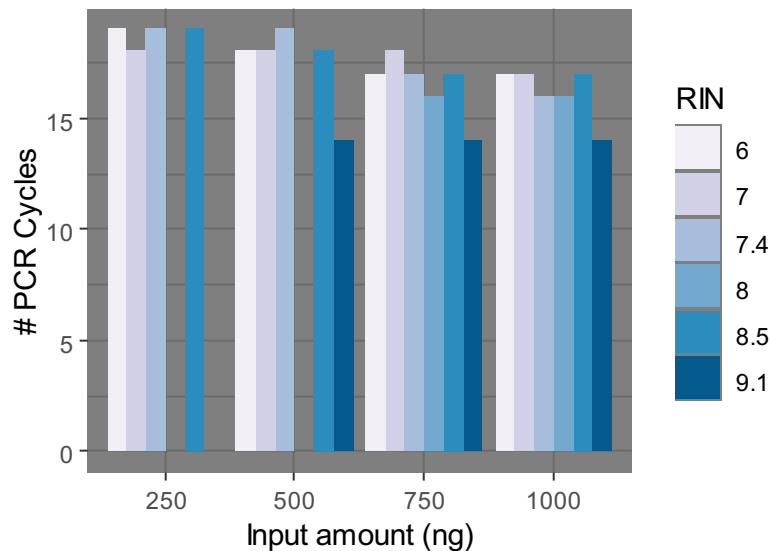
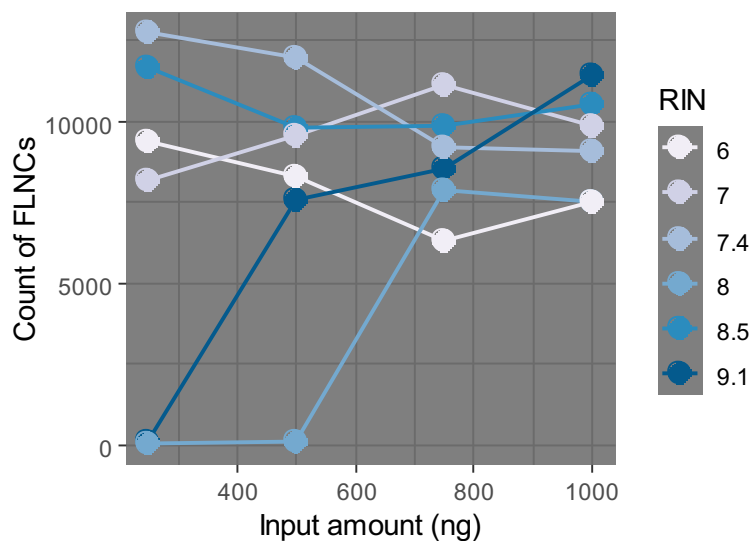
# Overall read yield for Iso-Seq run



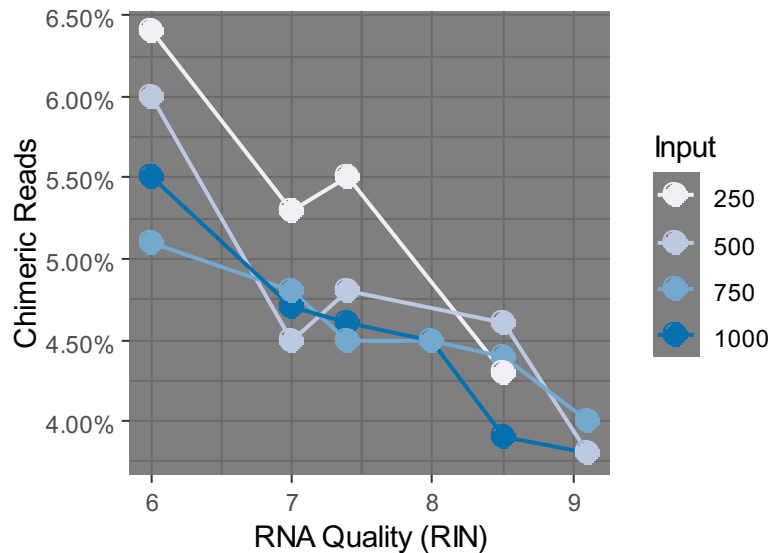
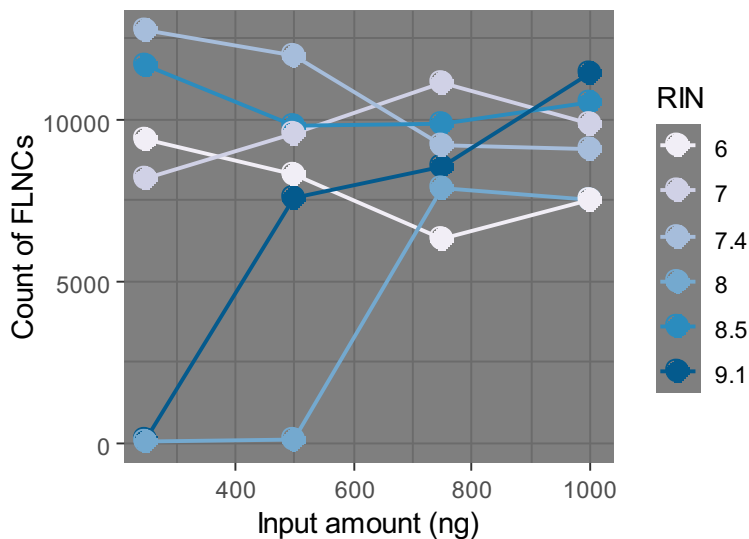
	# Reads	% of previous step	% of raw reads
ZMWs	1,019,235		
<b>Pol Reads</b>	<b>521,388</b>	<b>51%</b>	<b>100%</b>
<b>CCS Reads</b>	<b>302,861</b>	<b>58%</b>	<b>58%</b>
<b>Demultiplexed, full length reads</b>	<b>208,078</b>	<b>69%</b>	<b>40%</b>
<b>FLNCs</b>	<b>199,748</b>	<b>96%</b>	<b>38%</b>
<b>FLNC w/ polyA</b>	<b>198,187</b>	<b>99%</b>	<b>38%</b>

*Sequel chemistry 2.0/2.1, loading clean-up beads, MagBead loading at 75 pM, 2 hr pre-extension, 10 hr movie*

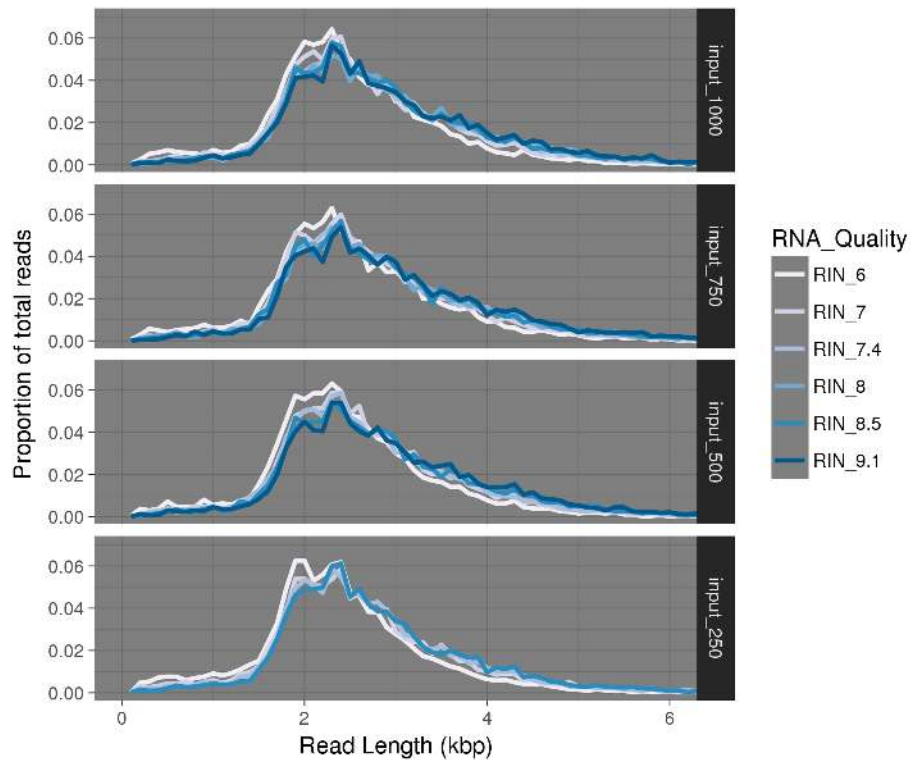
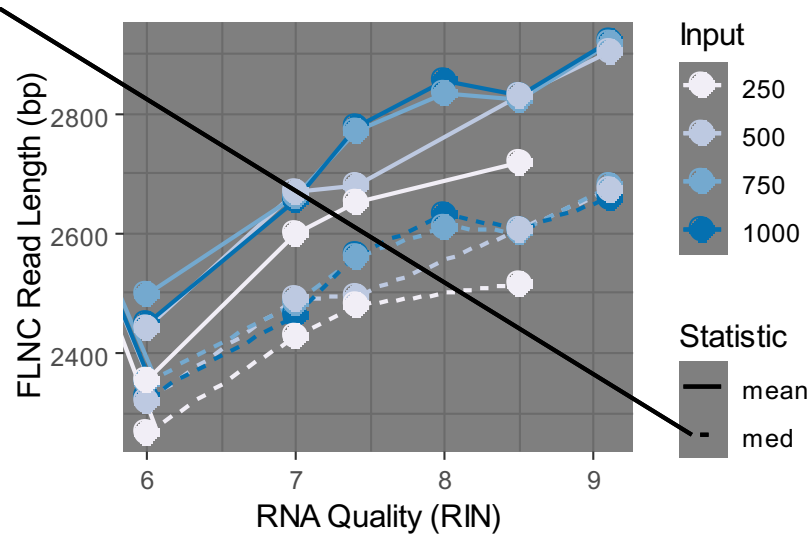
# Input amount has a minor effect on Iso-Seq data quality



# Input amount has a minor effect on Iso-Seq data quality



# RNA quality affects transcript length



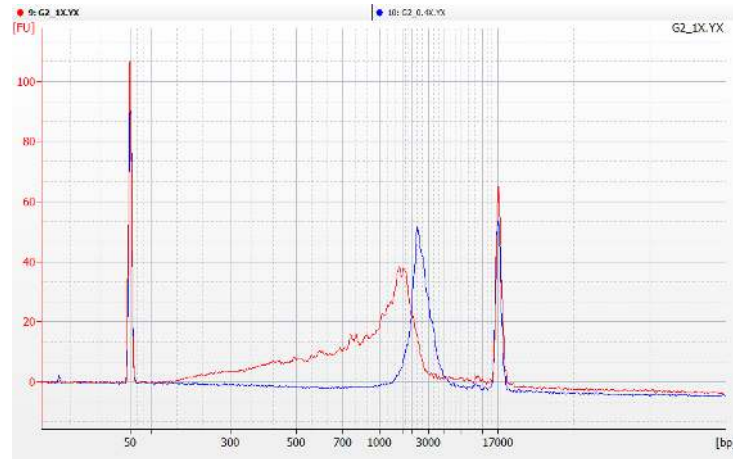


# Minimum requirements for RNA submission for Iso-Seq

- > RIN score of 7.5 or better
- > 5 µg submitted material for total RNA prep
  - (10 µg requested if performing polyA+ selection)
- > Sample barcoding available

## Protocol changes from standard

- > Prepare 3-4 first-strand synthesis reactions @ 1 µg each, pool for SMRTbell prep
- > If polyA+ RNA, 200 ng input into one reaction
- > After adding SMRTbell adapters, split and perform size selection (1X/0.4X AMPure)
- > Repool size fractions equimolar and/or sequence separately

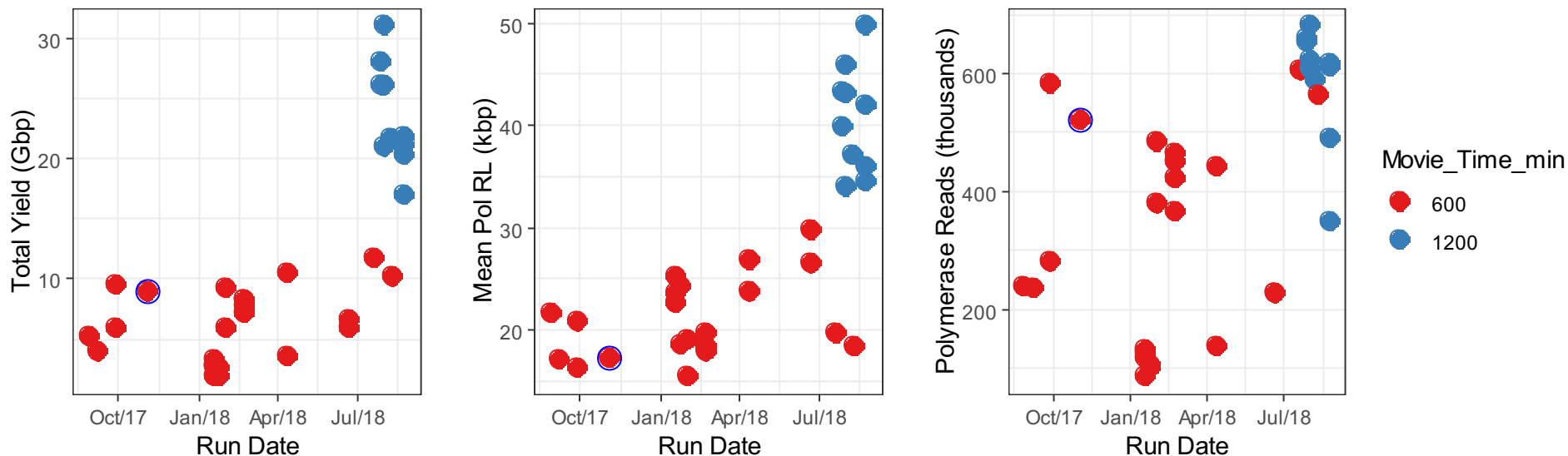


# Recent improvements increase yield and transcript length

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- Sequel chemistry 2.1 → overall read length
- Post-bind cleanup with AMPure beads → improved yield and much quicker
- Loading by diffusion → less library needed, improved P1, and quicker to set up
- 20-hour movies on LR chips → read length!!

# 20-hour movies offer improved base yield over same number of reads



We expect to see improved base quality and better yields of longer transcripts.

# Acknowledgments

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- > Alexandra Lewis
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- > Kristin Mars
- > Matthew Seetin

<https://pacbio.gs.washington.edu/>