

Polysaccharide Precipitation

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Purpose: Remove excess polysaccharides with a differential precipitation protocol (high salt, low ethanol.) Polysaccharides will precipitate leaving the DNA in the supernatant, which can be recovered by adding additional ethanol.

Based on the protocol at:

<https://www.pacb.com/wp-content/uploads/2015/09/Shared-Protocol-Guidelines-for-Using-a-Salt-Chloroform-Wash-to-Clean-Up-gDNA.pdf>

Input:

Four aliquots of extracted DNA:

Aliquot	Qubit	Conc (ng/uL)	Volum e (uL)	Mass (ng)	
DD_02C	2.53	12.65	58	734	
DD_03C	2.15	10.75	57	613	
DD_09C	1.96	9.8	55	539	
DD_10C	3.04	15.2	58	882	
				2767	ng total mass

- 1) Into a new 1.5mL LoBind DNA tube (Eppendorf), bring the DNA to a final concentration of 1M NaCl, 10 mM EDTA and 10mM Tris at pH 8.0:

gDNA	228	uL
5M NaCl	100	uL
500mM EDTA	2	uL
PacBio EB	170	uL
Total	500	uL

- 2) Add 0.3X volumes of pure ethanol: 150 uL
- 3) Gently invert 10X until fully mixed
- 4) Spin 15 minutes at max speed (21.3k RCF) at 20C in an Eppendorf 5425R benchtop centrifuge. (*Polysaccharides will pellet to the bottom of the tube*)
- 5) Remove supernatant to a new 2mL LoBind tube, check volume, and add 1.7X volumes pure ethanol:

- | | |
|------|-----------|
| 646 | uL Sample |
| 1098 | uL EtOH |
- 6) Gently invert 10X until fully mixed

- 7) Spin 15 minutes at max speed (21.3k RCF) at 20C in an Eppendorf 5425R benchtop centrifuge. (*DNA will pellet to the bottom of the tube*)
- 8) Remove supernatant and gently add 500 uL 80% EtOH (*washes off excess salt*)
- 9) Spin 15 minutes at max speed (21.3k RCF) at 20C in an Eppendorf 5425R benchtop centrifuge.
- 10) Remove supernatant, quick spin tube and remove any residual wash.
- 11) Let pellet dry with open cap ~1 min at room temperature.
- 12) Resuspend in 100 uL LowTE buffer and let rehydrate ~1hr at room temperature with slow rotation.

Qubit:	16.0	ng/uL
		ng
	1600	total
	58%	yield

- 13) Shear DNA in a Megaruptor 3 (Diagenode) using settings:

Concentration: 16 ng/uL

Volume: 100 uL

Setting: 28

- 14) Add 2uL 10% Triton (*sequesters residual SDS*) and 1X volume SMRTbell Cleanup Beads, incubate on rotator @ RT 10 min, wash 2X with 1mL 80% EtOH, elute in 47 uL Low TE buffer.

Qubit:	33.2	ng/uL
		ng
	1527	total
	95%	yield

- 15) Prepare SMRTbell library as detailed in the manufacturer's protocol

<https://www.pacb.com/wp-content/uploads/Procedure-checklist-Preparing-whole-genome-and-metagenome-libraries-using-SMRTbell-prep-kit-3.0.pdf>

- 16) Size select library on the PippinHT instrument with a high-pass cutoff of 14000 bp.

- 17) Final mass after size selection: 241 ng (15 uL @ 16.1 ng/uL, 15% library yield), mean library length 18,631 bp by FEMTO Pulse

- 18) Sequence on PacBio Revio using all available mass for a final OPLC 197 pM. (30 hour movie time, adaptive loading, Revio chemistry v1, SMRTLink v13.1)