An inexpensive method for collection of high quality RNA from blood of surgical patients

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Blood collection for gene expression studies

- Time delay in preserving blood leads to degradation of RNA
- Commercial kits are costly, don't scale well with small-scale projects
- Whole blood

RNA*later* ™ analog preserves whole blood, can be separated by centrifugation after freezing

- "RNAse Inhibition Buffer" or RI Buffer
  4M ammonium sulfate
- 100mM MES buffer
- 20mM EDTA
- PH 4.6
- Worley, Sullivan, Luo et al. "Genome-Wide Analysis of the TORC1...", G3: Genes|Genomes|Genetics, Vol 6, Feb 2026, pg 463

Blood cell pellet homogenized in TRIzol ™ and extracted via TRIzol Plus method

- TRIzol phase separated w/ chloroform
- Aqueous phase separated, combined w/ 1 volume 70% ethanol
- Run through PureLink ™ silica column per protocol, using on-filter DNAse digest step
- Post-extraction quality control test on Agilent Bioanalyzer ™

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Blood collection in hospital space

- 0.3mL anticoagulated blood added to 1.3mL RI Buffer
- Mix by inversion, keep at RT for one hour
- Refrigerate overnight
- Slowly freeze to –80C

Patient samples collected individually, then returned to core in batches



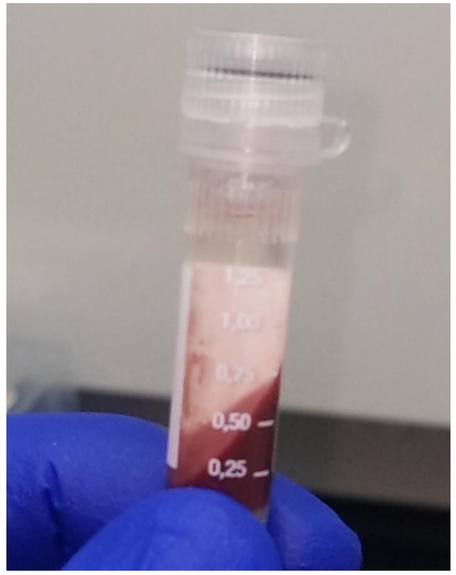


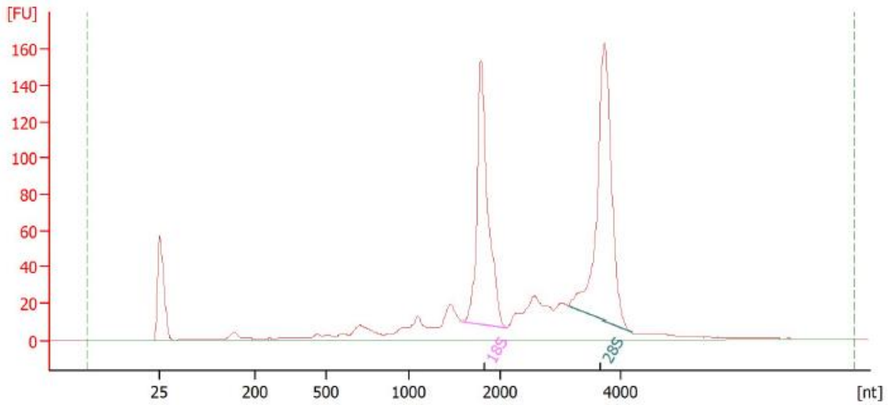


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Extraction of preserved blood cells

- 30 minute centrifugation at 800RCF, 4C works well to isolate blood cells
- TRIzol LS was tried, did not handle isolated cells as well as TRIzol reagent
- Some issues found with coagulation in tubes, seems to vary by patient
- RI Buffer preserved cells can be used for DNA extraction as well





Overall Results for sample 3 : A3-C8

RNA Area:	1,063.2	RNA Integrity Number (RIN):	9 (B.02.11)
RNA Concentration:	3,736 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.2	Result Flagging Label:	RIN:9

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