Princess Margaret Genomics Centre
101 College Street
PMCRT, Rm 9-601A
Toronto, ON, M5G 1L7

PMGC GENOMIC DNA EXTRACTION FOR SHRNA AND SGRNA SCREENS

Genomic DNA (gDNA) Extraction

gDNA extraction is performed using the QIAamp Blood Maxi kit (Cat#51194) essentially as described in the kit manual. Follow the instructions below, using the indicated volumes and refer to the kit manual for extra details if required.

This protocol is optimized for cell pellets containing 20-50x10^6 cells.

It is very important to use a swinging-bucket rotor in a centrifuge that is capable of attaining the required g-force. Failure to do so will result in low yield and dirty genomic DNA

Prepare 70°C water bath with rack for your tubes.

Ensure that buffers are prepared and ready to use.

Thaw cell pellets in 37°C water bath for 5-10 min.

Label samples and pre-label tubes and columns to be used in extraction.

Use plugged pipette tips for all steps – contamination of the gDNA is to be avoided!

- Add 4.5ml of sterile PBS to each cell pellet. Seal tubes tightly and vortex thoroughly to disperse cells. Pipette to disrupt cell clumps if needed – no clumps of cells can remain or the prep will not work well.
- Add 500µl of Qiagen protease solution to each sample. Mix briefly by swirling.
- Add 6ml Buffer AL. Cap tubes and mix by inversion for 2 min.
- Incubate tubes in 70°C water bath for 15 min.
- Let tubes cool to ~40°C, add 5ml 100% ethanol. Mix by shaking and inverting for 2 min.
- Carefully pour all of the solution into a pre-labeled QIAamp maxi column placed in the provided 50ml centrifuge tubes. Take care not to spill onto the rim of the column.
- Centrifuge tubes at 1850g for 3 min.
- Remove the QIAamp maxi column, vacuum aspirate the filtrate (**do not** pour out), and place the column back into the tube.
- Add 5ml Buffer AW1 to the column, being careful to not spill onto the rim.
- Cap the tubes and centrifuge at 4500g for 2 min.
- Add 5ml Buffer AW2 to the column, being careful to not spill onto the rim.
- Cap the tubes and centrifuge at 4500g for 20 min.

Tanja Durbic, for sample drop off
(647) 764-4144
Tanja.Durbic@uhn.ca

Dr. Troy Ketela, Head of Operations
(416) 634-8816
Geneservice@pmgenomics.ca

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- Check the tubes – if any buffer remains on the inside edge of the column, or if the filter appears to be wet, uncap the columns and dry in a warm incubator for ~15 minutes (or remove by pipette/aspiration – for wet edge only, NOT wet filter.
- Place the QIAamp maxi columns into clean 50ml centrifuge tubes, and discard the filtrate and the previous tubes.
- Add 600µl of room temperature Buffer AE directly onto the membrane and cap the tubes.
- Incubate the tubes at room temperature for 10 min, then centrifuge at 4500g for 3 min.
- Pipette the flow-through (less than 600µl) back onto the membrane, and dispense an additional 150µl of Buffer AE onto the membrane.
- Incubate the tubes at room temperature for 10 min, then centrifuge at 4500g for 10 min.
- Collect flow through and transfer 500µl to eppendorf tubes (save extra eluate in case you need it).
- Quantitate DNA and measure purity by spectrophotometry (we suggest using a Nanodrop instrument. Use 4µl of 2mg/ml DNAse-free RNAse A for every ~30µg, mix well by pipetting. Incubate at 37°C for 1hr.

Genomic DNA Precipitation

- Estimate total quantity of gDNA being precipitated based on volume and measured concentration. Assume ~75% of the concentration that you got from the nanodrop reading prior to RNAse A treatment was from DNA.
- Add 5M NaCl to a final concentration of 0.2M and 2 volumes of -20°C 96-100% ethanol. Do **NOT** use sodium acetate as residual acetate interferes with our PCR reaction.
- Invert tubes 15 times, then spin at 13,000rpm for 15 minutes at 4°C in a table-top centrifuge.
- Aspirate supernatant, being careful to avoid DNA pellet.
- Add 500µl -20°C 70% ethanol.
- Wash pellet by inverting tube 10 times, then centrifuge at 13,000rpm for 10 minutes at 4°C.
- Aspirate supernatant. Pulse-spin down any remaining liquid and aspirate it, being careful to avoid the DNA pellet.
- Air dry pellet ~ 5-10 minutes (not longer unless it is still wet) and resuspend with repeated pipetting in buffer EB (10mM Tris-HCl pH 7.5) to a final estimated concentration of 450ng/µl.
- Heat samples at 50°C for 1 hour. Pipette and vortex repeatedly to fully resuspend/solubilize the DNA. **Make sure that DNA is fully solubilized with no lumps or clumps.**

IMPORTANT:

- Check concentration and quality of DNA samples via Nanodrop and dilute to 400ng/µl.
- OD 260/230 ratio **SHOULD** be >2.0 and OD 260/280 ratio **SHOULD** be 1.8 +/- 0.05.

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Sample Drop-off / Shipping

If dropping off samples: Please **schedule your drop off date and time in advance** with your PMGC contact person.

- Your PMGC contact will meet you at the **9th floor elevator lobby** of the Princess Margaret Cancer Research Tower (PMCRT) at your pre-arranged time. PMCRT is the East Tower of the MaRS building, near the corner of College and Elizabeth Street entrance.
- Email or call/text when you are at the designated meeting area and your PMGC contact will come to collect the samples.
- REMINDER: Transport samples using appropriate means of storage (e.g. on dry ice for frozen samples, wet ice for fresh samples). Please confirm with PMGC if any questions.

If shipping samples: Please ship out on **Monday/Tuesday** to prevent weekend delays. Place a generous supply of dry ice to ensure dry ice will remain for the duration of the delivery time. For international clients, we recommend shipping with World Courier. Within Canada, or if shipping DNA/RNA, we recommend FedEX Next Day Priority services.

Shipping address:

Attn: Tanja Durbic
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Canada

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