Materials for CRC tissues RNA-seq and TIP-seq

0.3-0.5g will be held back from cryo-milling for TIP-seq
→ we need to assemble a protocol for this from Kathy

Cases to examine initially #
174 Metastatic breast carcinoma
185 MMMT ovary
197 Adenocarcinoma sigmoid colon
198 Adenocarcinoma right colon
268 Ovarian carcinoma
284 Clear cell carcinoma ovary

Numbers above 200 should be in rack B3 of the newest -80C, numbers below 200 should be in the chest freezer from Bronk 409. These are in numerical order by case#, 268 is the one Marty scored highest for Orf1 status.

I am emailing Marty separately about the other case #s he recommended that I don't think we have at RU. So there might be additional tumors to add to the list later, but please start with these for now.
We will also definitely be continuing with the three 162 Liver Mets that are already milled, and possibly 159, but again I will have to update you on the latter after checking with Marty.

Update on tumor tissues (Hua June 8, 2020)

162 Liver Mets (A, B, C), 162N
More than 2g of powder left for each one and more tissues can be ground

174 Metastatic breast carcinoma (still could not find this one)

185 MMMT ovary
VDE1858 Ovary mmmt, 4 full Falcon tubes of Tumor, No Normal

197 Adenocarcinoma sigmoid colon
1 Falcon tube of Tumor (~7g) and 1 Falcon tube of Normal (~7g)

198 Adenocarcinoma right colon
1 Falcon tube of Tumor (~7g) and 1 Falcon tube of Normal (~5g)

268 Ovarian carcinoma:
2 Falcon tubes: 1 black labeled "P1858 268 Normal spleen", 1 purple labeled "P1858 268 #268" (tumor?), ~30g each

284 Clear cell carcinoma ovary
2 Falcon: both labeled "P1858 284", ~40g each (tumor, no normal?)

Notes from Leila:

174: This should be in the chest freezer in Bronk 409. There are 5 falcon tubes...should not be hard to find unless it is mislabeled. The case # is S16-67317 so you could also look for that label.

The other potential cases I discussed with Marty are #267 (S17-80823), #251 (S17-63652), #273 (S18-16835), #298 (S18-49843), #? (S18-52430). The last one I don't think we have at RU, based on the date of the case (8/3/2018) and the date of the last shipment we received (7/30/2018).

Previous experiments of tissue RNA preps

This is the best protocol we have, I did not see anything with better results by Lars or Angelica:
23/01/2019_162T RNA Preparation - Zymo and Phaselock

When I initially did the RNA IP optimization experiments in tumor, we saw that sonication did not make a difference in RNA quality, but I never went back and checked protein quality/yield, although I definitely saved some protein samples for that purpose. I don't know if it is worth taking a look at them now, after over a year at -20. See here, lane 3 is no sonication.
08/01/2018_Extract RNA from Human Tissues (optimize tissue RNA prep conditions). (We ultimately concluded that superasin was not doing anything in our samples)

Leila's old experiment
23/01/2019_162T RNA Preparation - Zymo and Phaselock

Extraction/Wash buffer:
20mM HEPES, pH7.4, 500mM NaCl, 1% Triton X-100 Extraction/Wash buffer:

1. made with Nuclease-free water (the stock solutions are nuclease free) with
2. protease inhibitors used at 1x during extraction and washes
3. RNasin used at 1:40 each in extraction buffer and 1:250 during washes

- All tumor lines: sonicate 4x1 sec at 2 Amp (energy output ~10J)
- MT302: Sonicate for 5x2 sec at 2 Amp; (energy output ~20J) (had to repeat 1x)
- IP@ 4°C for 30'

Hua's old experiment
09/09/19 - RNVC Testing – RNA Prep from MT302 and 162T

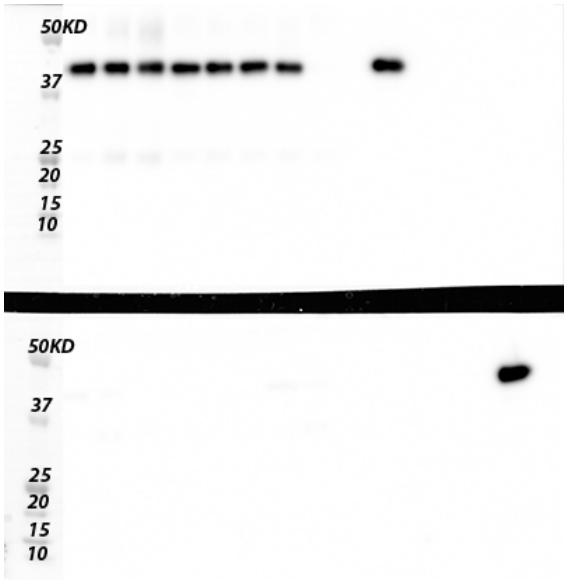
Best condition seems to be:
5) 162TA 1:40 RNasin EB; 1:250 RNasin wash
- Resuspend the powder by vortexing, no sonication
- IP @ 4°C for 5'

Optimization of tumor IP conditions

Hua's old experiment

05/28/19 - 162T and 162N anti-ORF1 IP_Sonication_Beads titration_Time course
Conclusion: no sonication (-sonication), IP for 5' is fine at protein level

Anti-ORF1 Western (top: elution; bottom: lysate)



Loading order
Top:

1) Prestained marker_5ul
2) 162T - sonication_10ul beads_5' IP
3) 162T - sonication_20ul beads_5' IP
4) 162T - sonication_25ul beads_5' IP
5) 162T - sonication_10ul beads_30' IP
6) 162T + sonication_10ul beads_5' IP
7) 162T + sonication_10ul beads_15' IP
8) 162T + sonication_10ul beads_30' IP (Positive control)
9) 162N + sonication_10ul beads_30' IP
10) Space
11) Positive control_10% of MT302 elution from 50mg IP_040319_(2ul out of 20ul total)

Bottom

1) Prestained marker_5ul
2) Sup_162T – sonication
3) Pellet_162T – sonication
4) FT_162T - sonication_10ul beads_5' IP
5) FT_162T - sonication_20ul beads_5' IP
6) FT_162T - sonication_25ul beads_5' IP

7) FT_162T - sonication_10ul beads_30’ IP
8) Sup_162T + sonication
9) Pellet_162T + sonication
10) FT_162T + sonication_10ul beads_5’ IP
11) FT_162T + sonication_10ul beads_15’ IP
12) FT_162T + sonication_10ul beads_30’ IP
13) Sup_162N + sonication_2ul
14) Pellet_162N + sonication_2ul
15) Positive control_10% of MT302 elution from 50mg IP_040319_(2ul out of 20ul total)