Notebook Week 8 (July 17-21)

Project: iGEM 2017
Authors: Locke Bonomo
Dates: 2017-07-17 to 2017-07-21

MONDAY, 7/17

- Review Meeting with Tim from Friday
- Skype Call with Aachen @ 9am
    - They sent us an email with all of the information they collected. They noted that Eastern Germany had a bigger problem, and said to let them know if we had any problems translating.
- Contact IDEXX
- MRS/Water Lead assay standard curves?

MRS Calculations:

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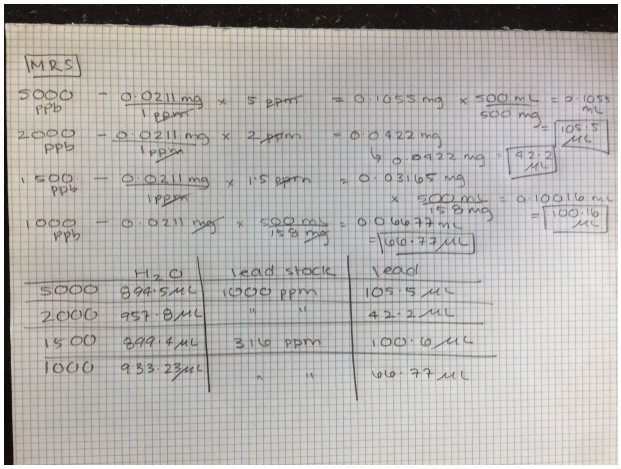

MRS Trial 1: Needs to be repeated due to variability for control.

MRS Trial 1.xlsx

MRS Trial 1, Take 2:
This trial was also very variable. The dilutions were found to be off by a 10 fold increase.

MRS Trial 1a.xlsx

MRS Trial 1, Take 3:

MRS Trial 1b.xlsx

The math for the dilutions, and the dilutions themselves were redone. This trial showed much more consistent values, but the 1,500 ppb value still seemed to be off. The 570nm run appeared to have the lowest R value when the 1,500 ppb time point was excluded. The math for this dilution will be checked tomorrow.

- Make 100,000 ppm Stock of Lead
    - filtered with a steriflip
    - Put 4.24 g (last of the lead) into 42.4 mL of DI water to obtain 100,000 ppm

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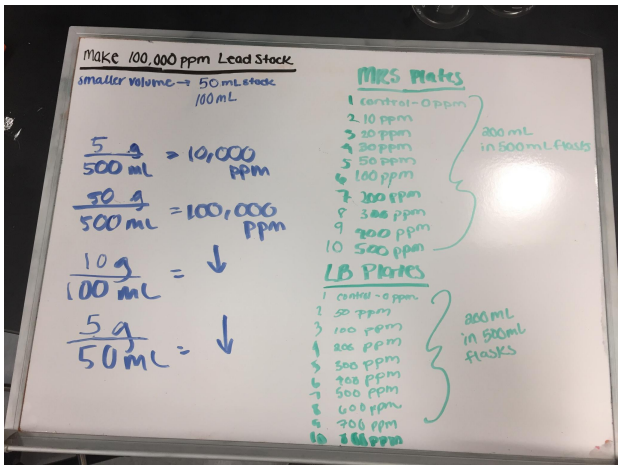

- Make LB and MRS plates
  - Autoclaving 200mL of media into 500mL flask (made 2 liters of both media)
    - 10 of each Lb and MRS
  - Added calculated amount of lead to each 500mL flask.. calculations seen below

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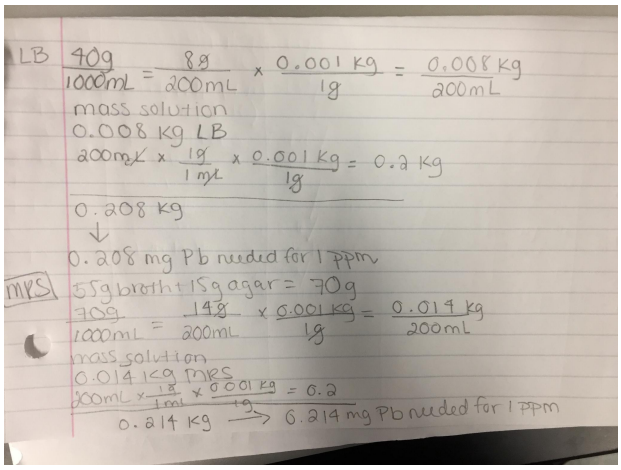

Calculating mg per 1 ppm with addition of the agar to the mass of the solution

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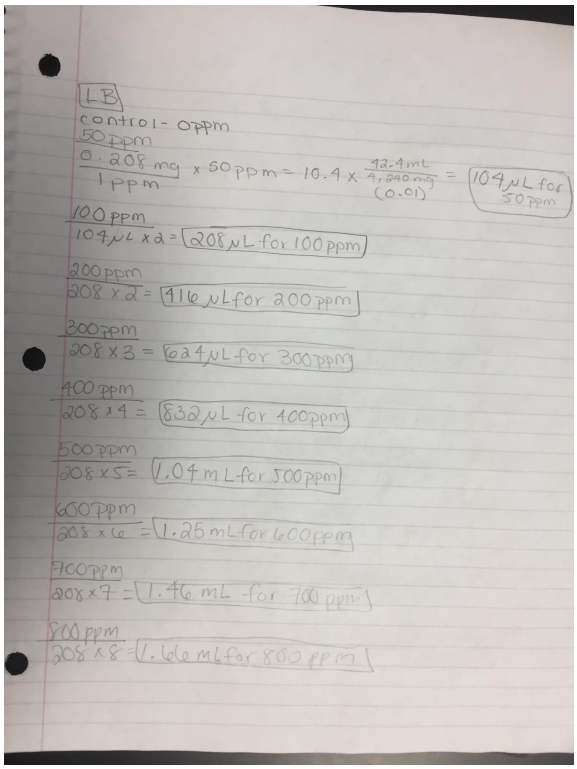

LB calculations for 50, 100, 200, 300, 400, 500, 600, 700, 800 ppm

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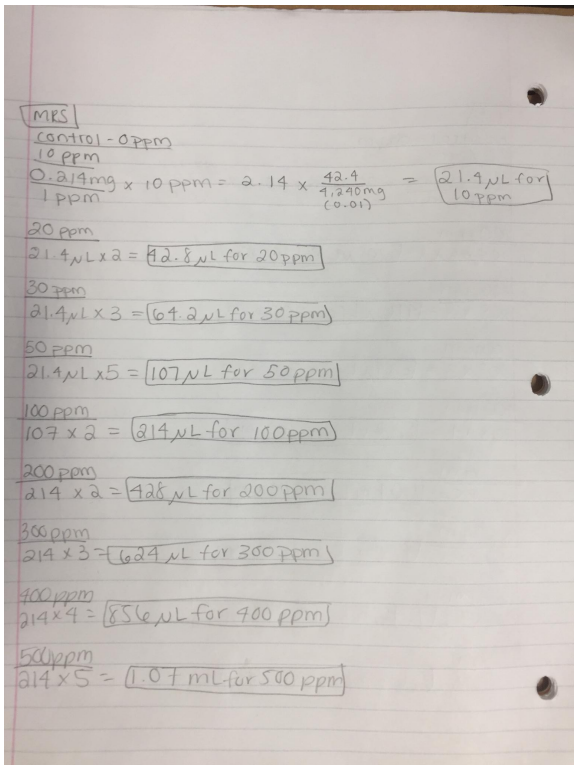

MRS calculations of 10, 20, 30, 50, 100, 200, 300, 400 and 500 ppm

- Restreak B.sub and Lactobacillus for fresh colonies to use tomorrow

- B. subtilis - why isn't transformation working? (from Fri). Next steps:
    - double check primers, ordered DNA, etc. for correct overlaps (20-25 bp from ds restriction site to vector on each end)
      Go to IDT order history and align that sequence with the designed plasmid in benchling

- Vector not linearizing? -> New minipreps, fresh material

  4C fridge has plate with colonies (pick 4 each from pET21a and pET42a) - prep those for tomorrow (setup cultures)

  Do fresh minipreps tomorrow and find yield tomorrow

  - Prepared 2 10-mL cultures of DH5a E. coli with pET21a and pET42a, respectively, as well as 10 uL of the respective antibiotic (amp and kan). Picked 4-5 colonies from each plate kept in the 4C fridge to start the cultures. Put in shaking incubator to grow overnight, will use for plasmid minipreps tomorrow.

TUESDAY, 7/18

- Women in Science: 1-4pm, 2 sessions, each 75min
  - Cut paper for DNA activity
  - Get ready for fruit DNA extraction activity
- Overnight culture of pET 21a didn't grow -> redo overnight culture
  - Setup pET21a liquid culture using several colonies from the same plate as was used last time, 10-mL culture + 10 uL AMP. Put them in shaking incubator to grow overnight.
- pET42a mini prepped, 33ng/µL
- Streak out B. sub and lactobacillus onto lead plates
  - 2 plates of each concentration
    - 20 plates total per bacteria
  - Lactobacillus didn't grow individual colonies -> need to make an overnight culture and do serial dilutions and streak the liquid culture onto the lead plates

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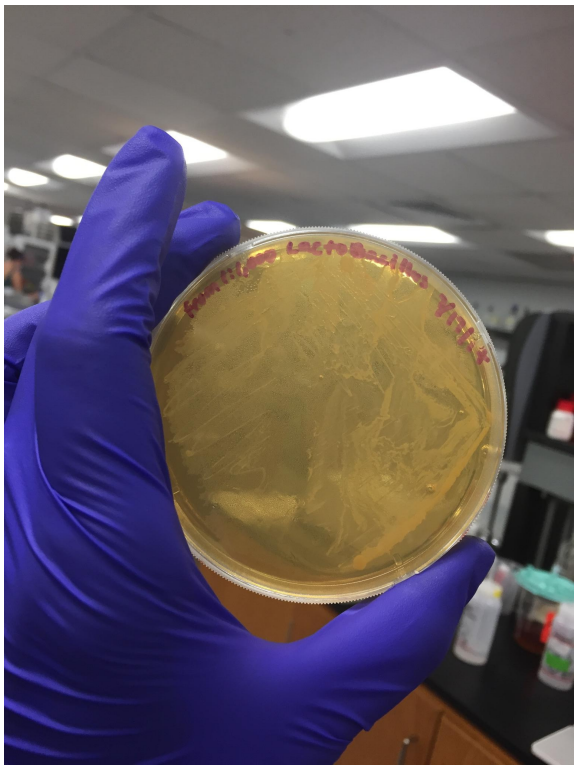

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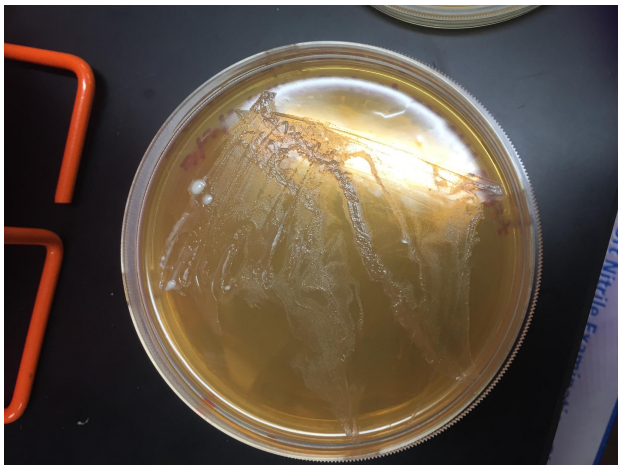

    - Fresh streaked B.subtilis wasn't put in incubator, placed in fridge by accident-> restreaked and placed into incubator
    - Will streak lead plates tomorrow with fresh bacteria
- Cat- Call Alisa about Water Testing
- Talk about survey/fix
    - Completed

Lead Contamination in Your Drinking Water Survey.pdf

- Go over IDT stuff for Gibson Assembly Problems
    - Completed
    - New primers under Inventory --> Chromoprotein primers in benchling
    - NdeI (same as before) and EcoRI (new) will be used as restriction enzymes

WEDNESDAY, 7/19

- Contact:
    - IDEXX
    - Pediatricians
    - Steve Pratt
    - Gary Ginsberg
- Streak lead plates and incubate them overnight and count colonies Thursday and Friday
- pPBR DNA should be in, only 3 out of the 4 pieces were completed
- mini prep pET21a
    - Did not grow, new plate from glycerol stock was done
- Vector digest
    - only pET42a
- FDA Bottled water regulations: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=165.110
- WHO Information on Lead (includes exposure, average daily intake, and health concerns)

WHO Lead Requirements.pdf

A World Health Organization Document was found that is very specific to lead exposure and poisoning. This document contains specific information on how one can be exposed to lead in the home, including through drinking water. It goes over average daily intake levels in different areas and why there can be differences. It also goes over the health effects of increased lead intake levels, and how lead exposure can affect animal and human health.

- WHO Drinking Water Requirements (Page 392 is for lead, suggestion is 10ppb, but that usually it is/should be lower):

WHO Drinking water Requirements.pdf

This was an informative document about the World Health Organization's recommendations for drinking water. It goes over what drinking water should and should not contain, and what levels the various chemicals and microorganisms can cause concern. It also goes over how water should be tested, what to do if the levels exceed the recommended values, and the health effects of those high values. It has several sections that are very specific to lead, and important to note for the project. The WHO's recommended lead levels are also different than the EPA standard. The WHO recommends 10ppb as the cause for conceern whereas the EPA's standard is 15ppb.

- Lead Assay:
    - check pH of phosphate buffer
        - phosphate buffer pH: 7.25
    - check pH of LB & MRS
        - LB pH: 6.77
        - MRS pH: 6.39
    - Test in water for molarities from original paper
    - Done with 37.5µL media, 25µL AuNPs, 12.5µL GSH

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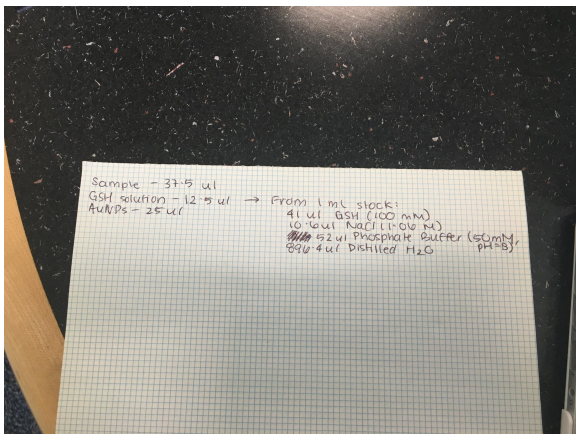

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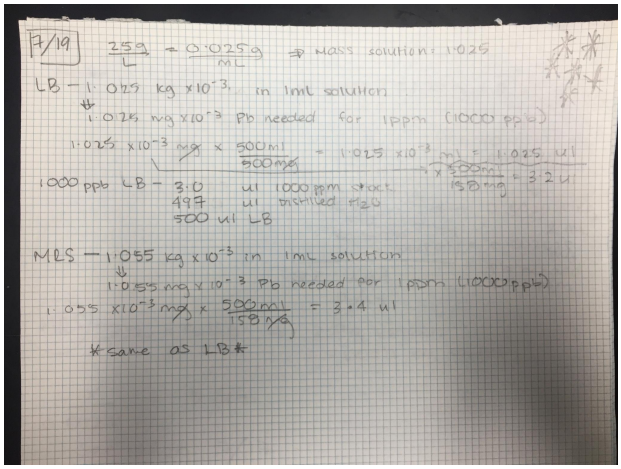


Results:

DIY Lead Assay 719.xlsx

Results from repeated experiment using 1000 ppb LB & MRS instead of water:

DIY Lead Assay LB and MRS 719.xlsx

- Plated Lactobacillus and B.subtilis on plates containing lead of various concentrations of lead
    - Lactobacillus:
        - Concentrations of 0, 10, 20, 30, 50, 100, 200, 300, 400, and 500 ppm
        - 100 microliters of a 1:1000 dilution made from an overnight culture of Lactobacillus were streaked onto the lead plates
    - B.subtilis
        - Concentrations of 0, 50, 100, 200, 300, 400, 500, 600, 700, and 800 ppm
        - Colonies were picked from a plate of B. Sub and streaked directly onto the lead plates

- pET21a liquid culture for miniprep
    - Liquid culture doesn't look very cloudy - checked OD to make sure it didn't grow, OD = 0, so no growth. Plate is probably too old and ran out of amp, so need to start from a fresh stock.
    - Streaked a glycerol stock of pET21a E. coli onto an LB AMP plate to grow at 37C all day/night, will check for growth tomorrow.
- Restriction digest - used pET42a miniprep from yesterday.
    - 4 tests - 10 uL reactions
        - Both enzymes
            - 5 ul plasmid
            - 1 ul EcoRI
            - 1 uL NdeI
            - 1 uL cutsmart
            - 2 ul water
        - NdeI
            - 5 ul plasmid
            - 1 uL NdeI
            - 1 uL cutsmart
            - 3 ul water
        - EcoRI
            - 5 ul plasmid
            - 1 ul EcoRI
            - 1 uL cutsmart
            - 3 ul water
        - Negative control
            - 5 ul plasmid
            - 1 uL cutsmart
            - 4 ul water
    - ~30 uL full reaction
        - 20-25 ul plasmid
        - 4 ul Cutsmart
        - 1 ul NdeI
        - 1 ul EcoRI
        - 4 ul DI water

      Left 30 uL reaction to sit overnight in 37C water bath, will finish tomorrow morning.
- Make more amp plates. Make more MRS plates.
    - Completed

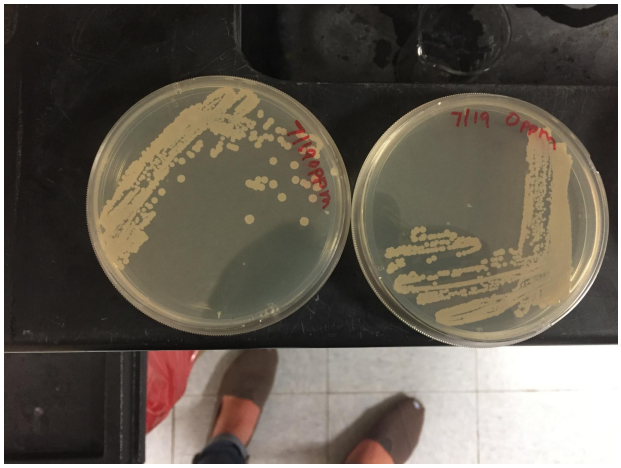
THURSDAY, 7/20

- Check lead plates for growth
    - 2 plates per concentrations

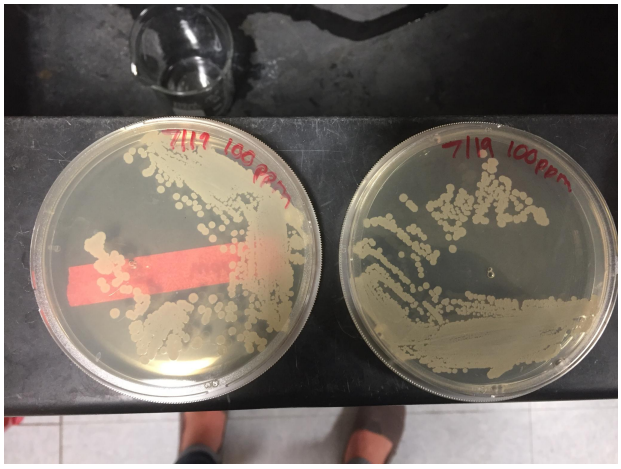
B.subtilis

	A	B
1	0 ppm	growth on both
2	50 ppm	growth on both- equal to 0 ppm
3	100 ppm	growth on both-> spike of growth (more than 0 and 50)
4	200ppm	growth on both -> consitent with 100ppm
5	300ppm	growth on both -> consitent
6	400 ppm	contamination, when streaking fragile agar
7	500 ppm	growth on both-> plates different see picture below
8	600 ppm	growth on both -> consitent
9	700 ppm	growth on both -> high growth (another spike)
10	800ppm	growth on both -> high growth, plates differ, see picture below

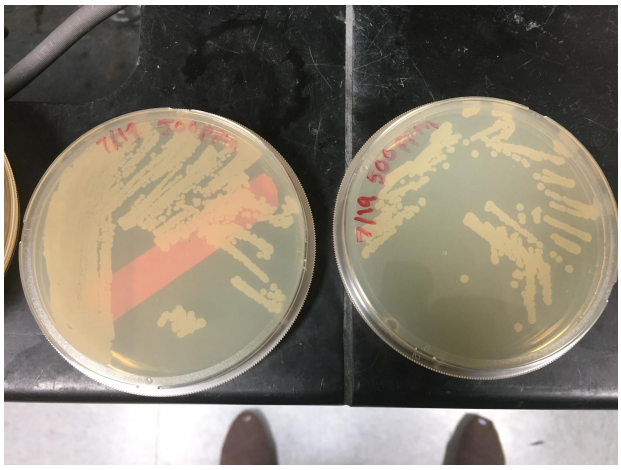
0ppm



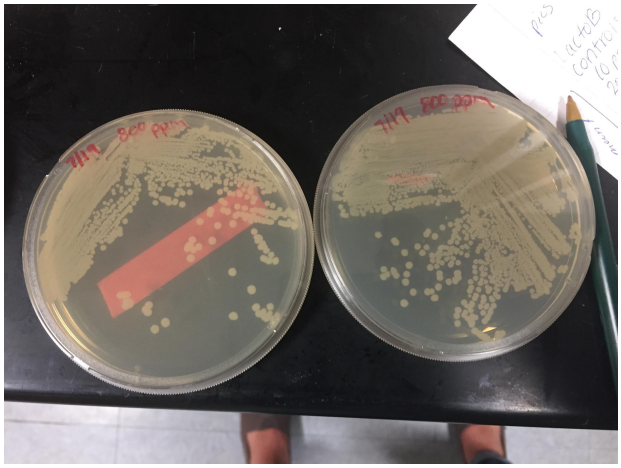
100ppm(spike growth)



500 ppm



800 ppm

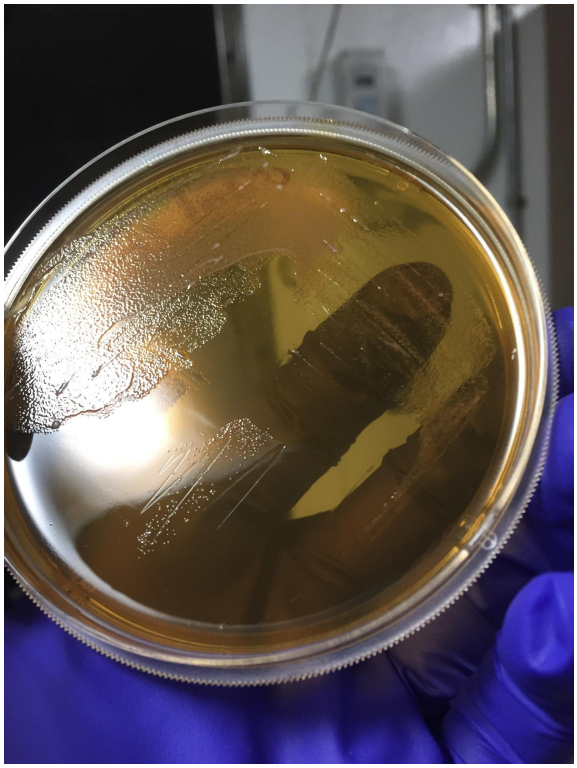


Lactobacillus

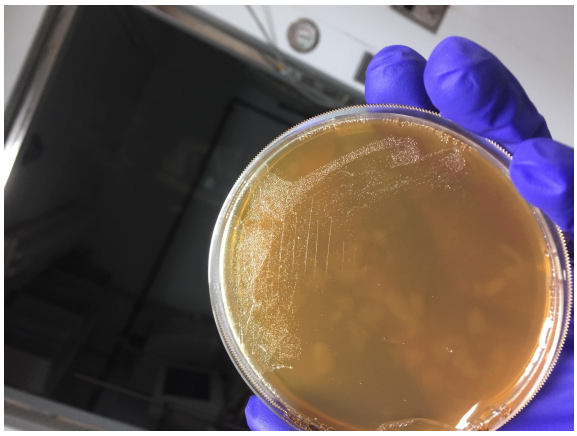
	A	B
1	Controls (4)	growth on all
2	10 ppm	Growth on both
3	20 ppm	Growth on both
4	30 ppm	Only one plate grew -> plate grew more than 20 ppm ~ equal to 10 ppm
5	50 ppm	growth on both, very small amount from where liquid cultures were placed
6	100 ppm	growth on both
7	200ppm	growth on both
8	300ppm	growth on both (one plate has a lot of growth) doesnt follow the pattern
9	400 ppm	growth on both
10	500 ppm	growth on btoh

A pattern was seen in the growth, at higher concentrations there were less or none individual small colonies, and just growth from where the liquid culture was places. Lactobacillus had really small colonies, that when seen in the light you can see the individual colonies.. see pictures. Keeping in incubator and checking the end of today and tomorrow am to see if growth improves

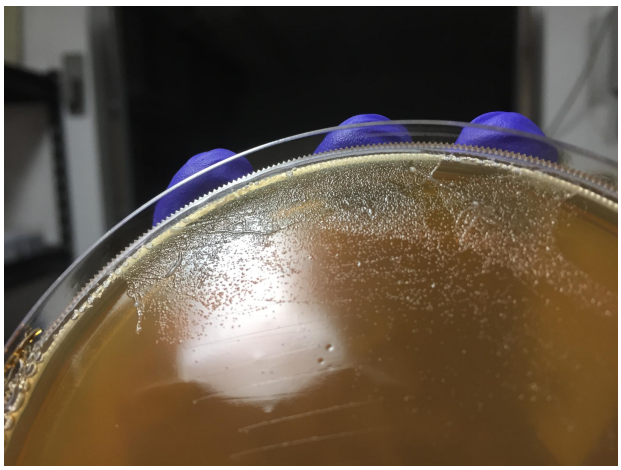
Control



500 ppm (highest concentration)

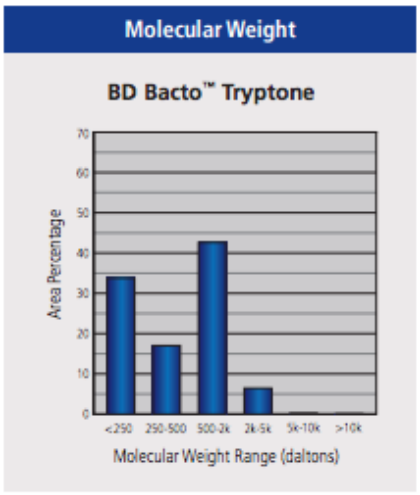


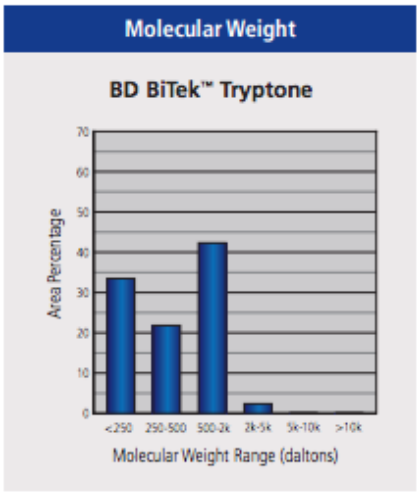
Close up to see individual colonies on 500 ppm



- Lead Assay
    - Looked at differences in media and water
    - Tried new GSH pH's

Screen Shot 2017-07-20 at 11.33.22 AM.png





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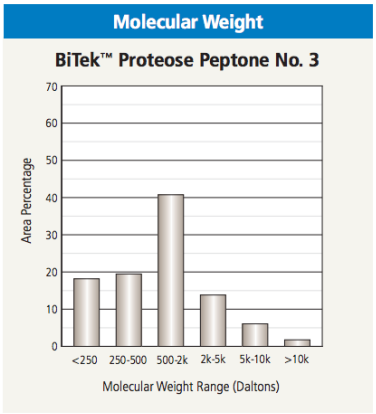

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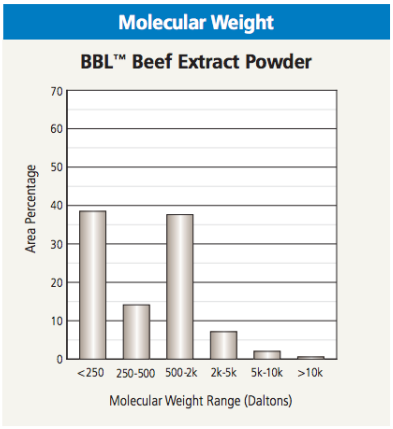

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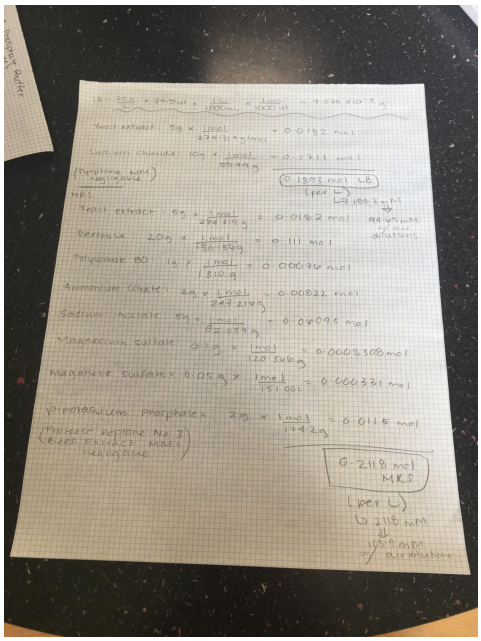

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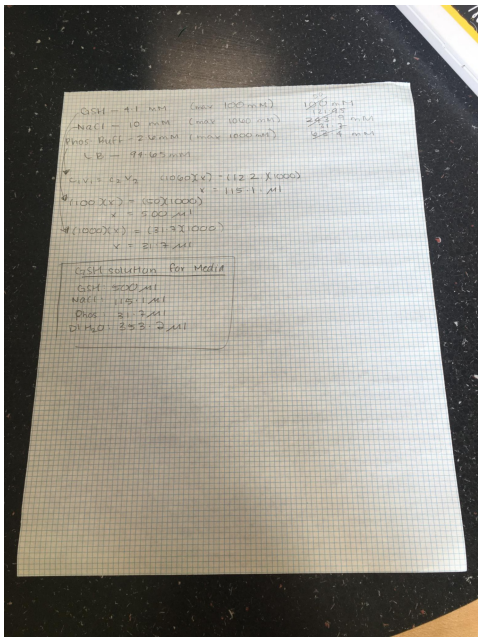


Results: LB works well with the adjusted GSH solution at 450 nm. When conducting standard curve trials, the absorbance at 405 nm and 450 nm will be measured. MRS trial was unsuccessful. Next step is to adjust the pH of the phosphate buffer so that it is more basic to compensate for the acidic MRS media.

LB and MRS new.xlsx

Results (phosphate buffer pH 8): Keep with neutral pH for LB. Try phosphate buffer pH 8.5 and 9 for MRS.

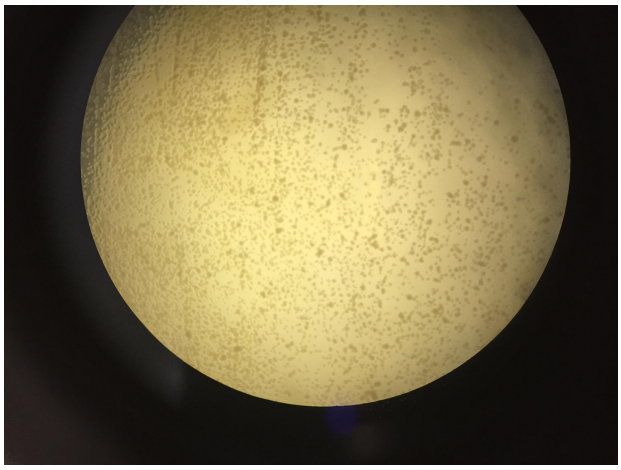
LB and MRS BASIC.xlsx

Results (phosphate buffer pH 8.6 and 9.05): Keep pH 8.6 570nm for MRS.

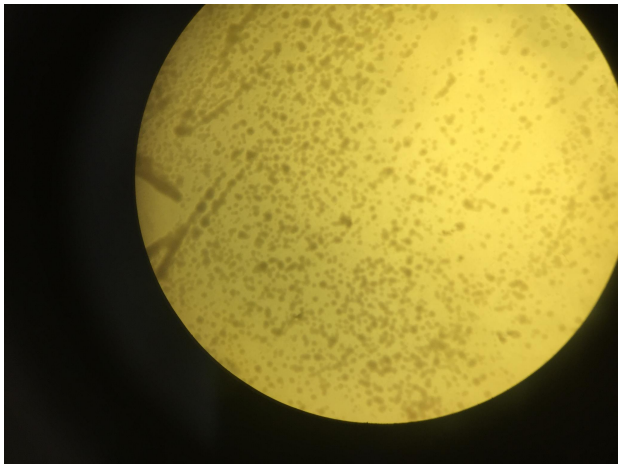
lb mrs 8.5 9.xlsx

- Contact
    - Steve Pratt Contacts
    - Pediatricians
    - Gary Ginsberg
- Look under microscope at both lactobacillus smaller colonies on both control and 500 ppm plates and b.subtilis to see if lead affected it any way such as deformities
    - Shape stays the same throughout but size of the colonies differ, 500 ppm colonies are larger than the control .. maybe because of the uptake of lead?

Control

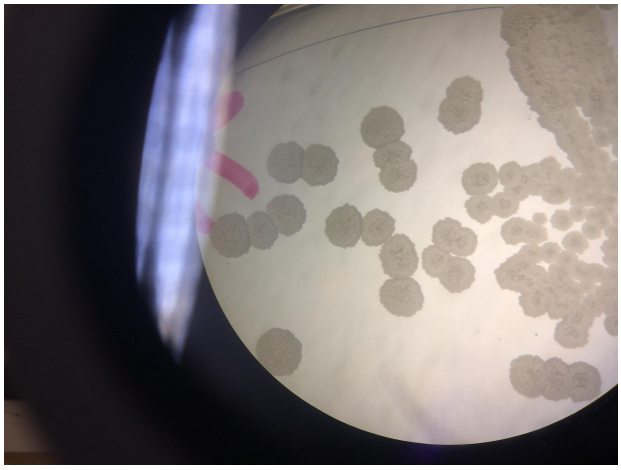


500 ppm

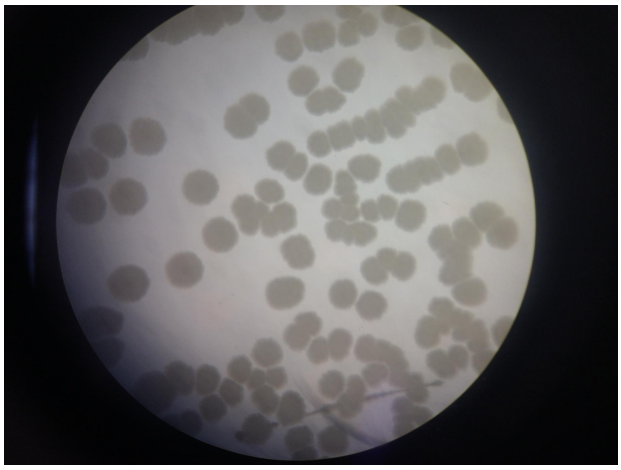


- Look under microscope at B. subtilis plates of 0, 200, and 800ppm

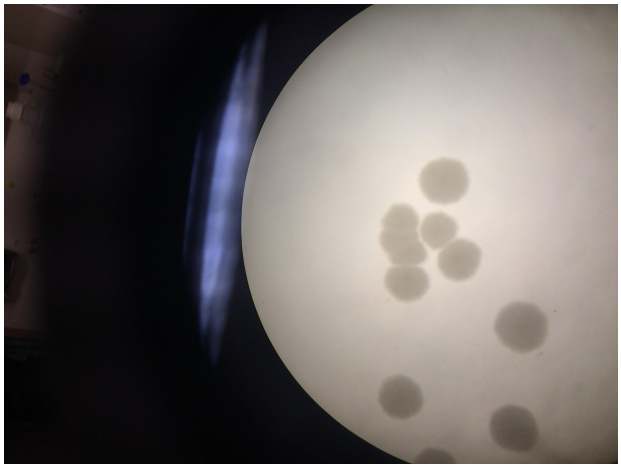
Control (0ppm)



200 ppm



800 ppm



Colony Counts

	A	B	C
1	**ppm**	**Plate 1**	**Plate 2**
2	Control	76	37
3	50	89	97
4	100	123	124
5	200	118	100
6	300	128	90
7	400	*	*
8	500	44	49
9	600	72	73
10	700	36	67
11	800	127	137

*Both 400 ppm plates were thrown into the biohazard because they were contaminated

- Proof of concept of lead plates
    - Lactobacillus
        - On control plates and 500 ppm (highest lead concentration)
            - going to create serial dilutions again, but use the 1:10 and 1:100 serial dilutions to see if we can get bigger colonies
    - B.subtilis
        - going to break up the same colonies and use half on a control and half on 800 ppm of lead
            - see if growth will differ, because although all colonies were picked from the same plate, they may not be the same colony

- Finish pET42a prep/purification
    - Product that sat overnight was ran on the gel
    - Size on gel looks good, so proceeded with the gel purification of our ~30 ul reaction from yesterday
    - Loaded ~35 uL (30 ul reaction + 5 ul dye) on gel with 10 uL 2-log ladder. Let run for 1 hour, then gel purified

pet42adigest.jpg

- Digest yielded 11.3 ng/uL
- Check pET21a plate for growth - it grew several colonies!
    - Picked ~6 colonies and made 5-mL liquid culture with amp to grow in shaking incubator overnight
    - Saved pET21a plate in fridge

FRIDAY, 7/21

- Miniprep of pET21a
- Vector Digest of pET21a
    - Had to make 2 log ladder with sybr
    - Yield was 16.1 ng/uL
- Contact
    - Figure out questions
    - Steve Pratt Contacts
    - Pediatricians
    - Gary Ginsberg
- Lead Assay
    - Standard Curves for MRS
- Aliquots of L-Glutamine
- Lead Plates Update
    - Lactobacillus need 2 days to fully grow -> got white colonies today when observed the plates from wednesday, which yesterday they just weren't fully grown
        - can still see pattern of more growth -> See excel file where the linear average on the graph is a negative slope from the control down to 500 ppm
    - Counted colonies

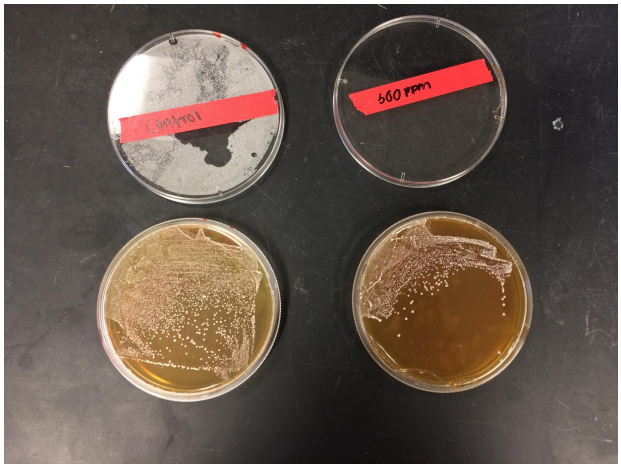
Table2

	A	B	C
1	ppm	Plate 1	Plate 2
2	control	251	138
3	10ppm	51	122
4	20ppm	53	69
5	30ppm*	178	0
6	50ppm	78	79
7	100ppm	105	126
8	200ppm	61	26
9	300ppm	92	31
10	400ppm	83	32
11	500ppm	70	78

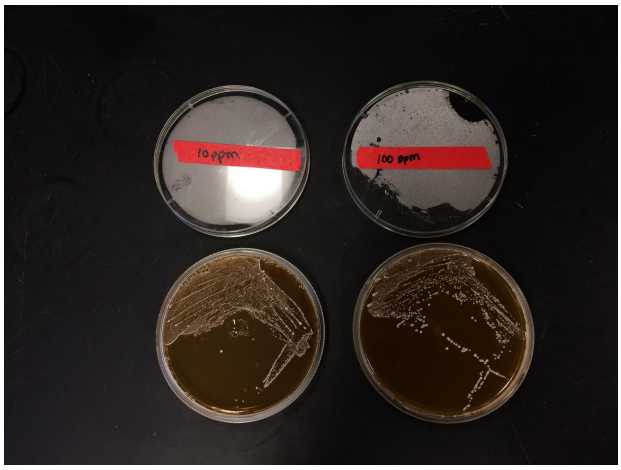
* think for the 30ppm, streaked twice on the same plate (200l of the liquid culture) and did not streak the second plate by accident

- Pictures:

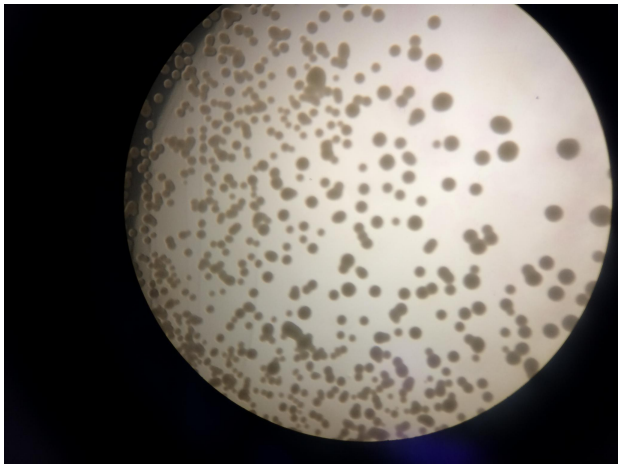
Control vs 500ppm lactob



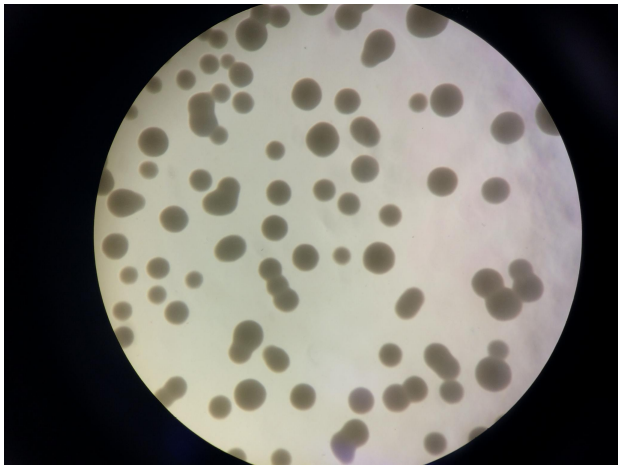
10ppm vs 100ppm lactob



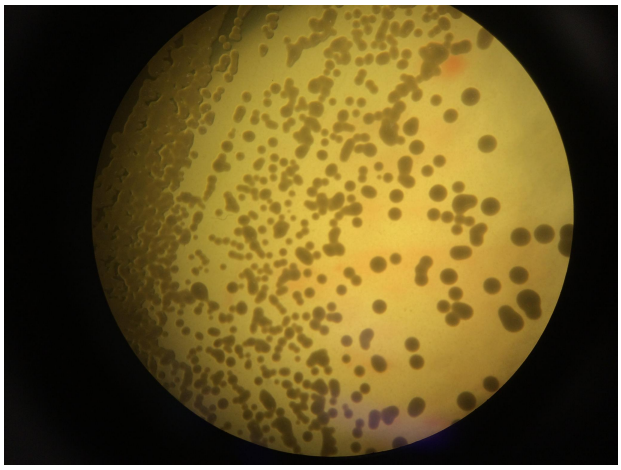
Control lactob



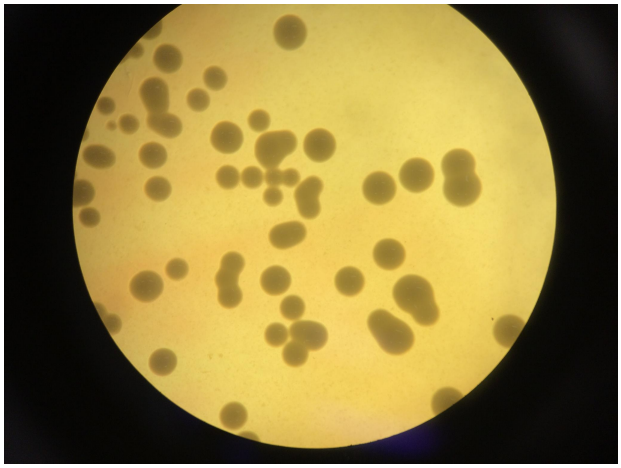
control lactob zoom



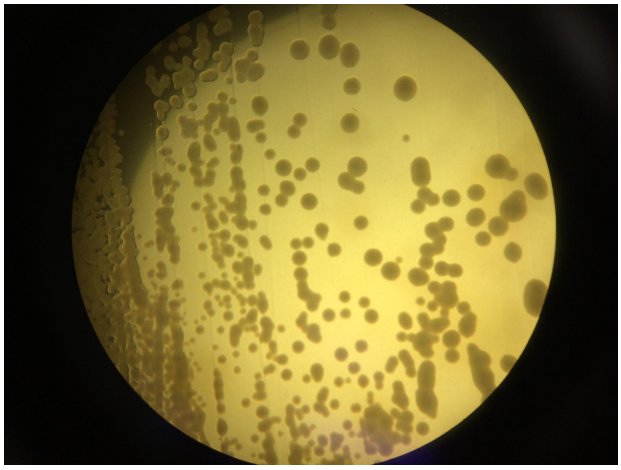
100ppm lactob



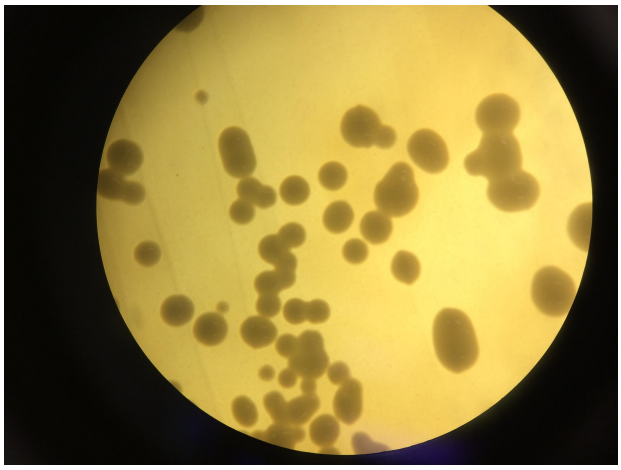
100ppm lactob zoom



500ppm lactob



500ppm lactob zoom



    - Cant see any differences in from the microscopic level of shape or size, but the counting shows that the higher the lead, the less growth of lactobacillus individual colonies

- Excel file with all the cell counting of both Lactobacillus and B.subtilis with graphs and averages

LeadPlatesColonyCounting.xlsx

- Miniprep, digest, and cleanup of pet21a
    - Culture grew so miniprepped entire volume.
    - Miniprep nanodrop = 61.2 ng/ul
    - Restriction digest: 60 uL reaction
        - Rxn Setup:
            - 50 ul plasmid
            - 6 ul Cutsmart
            - 2 ul NdeI
            - 2 ul EcoRI
        - Left rxn in 37C water bath for 1 hr
        - Gel ran for ~50 mins, then cut out band. Band was ~5kb, which was the expected size.
            - Weight of band = 306mg, so added 618 ul of NTI buffer
        - Followed gel cleanup "at-a-glance" protocol on lab bench
            - eluted in 25 ul NE buffer
    - Gel cleanup (final) nanodrop = 16.1 ng/ul
    - Saved in -20C freezer