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Published data/DBS gDNA extraction Insight paper

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DBS Preparations for Figure 1

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:26 PM EST

16-Mar-2021: Preparation of DBSs

Goal: Prepare multiple DBSs for future experiments.

Procedure:

1. Made 24 903 cards
    1. 75uL of blood
    2. 12 EDTA + 12 Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Naming convention: "#1-12, M(march), E or H"

Naming of the spots:

-For 903: A-E from left to right

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:26 PM EST

12-May-2021: Preparation of DBSs #13-25(EDTA) or 28 (Heparin)

Goal: Prepare multiple DBSs for future experiments.

Procedure:

1. Made more 903 cards
    1. 75uL of blood
    2.
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Figure 2: DBS elution pre-treatment variations DNA

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24-Mar-2021: DBS elution protocol variations

Goal: Test alternative methods for the PBS elution protocol.

- After viewing the eluates following the PBS elution protocol using a hemocytometer, nothing appears to be there. So we don't think there is anything coming off the DBSs.

We are testing 2 protocols for DBS elution:

(1) PBS/ 0.05% Tween-20/ 0.08% Sodium Azide, shake overnight

- Made 3mL of the 0.05% Tween 20, 0.08% sodium azide, and PBS solution...
- For 1mL PBS:
  - For Sodium Azide. p = m/V -> 1.85g/cm^3 = m/0.08mL -> 1.85*0.08 = m = **0.148g = 148mg Sodium Azide**
    - cm^3 = mL
  - Tween20: 0.05*1mL = **50uL Tween-20**

w/V; V/V meaning: https://www.chemicals.ie/blog/what-do-vv-ww-wv-mean/

Also found here: Mössner, B. K., Staugaard, B., Jensen, J., Lillevang, S. T., Christensen, P. B., & Holm, D. K. (2016). Dried blood spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life Prospective Study. World Journal of Gastroenterology, 22(33), 7604–7612. https://doi.org/10.3748/wjg.v22.i33.7604

N. Grüner, O. Stambouli, and R. S. Ross, “Dried blood spots - Preparing and processing for use in immunoassays and in molecular techniques,” *J. Vis. Exp.*, vol. 2015, no. 97, pp. 1–9, 2015.

---

(2) 150uL PBS/600uL Lysis Buffer, shake for 2hr at RT

- A. J. Buckton *et al.*, “Development and optimization of an internally controlled dried blood spot assay for surveillance of human immunodeficiency virus type-1 drug resistance,” *J. Antimicrob. Chemother.*, vol. 62, no. 6, pp. 1191–1198, Sep. 2008.

We used Lysis buffer #1 from the DNA kit & 1X PBS for this experiment today

---

Tested the above protocols using M3E 903 sample

- 903A: (2) protocol, 6mm punched circles x 2
- 903B: (2) protocol, a whole circle
- 903C: (1) protocol, 6mm punched circles x 2
- 903D: (1) protocol, a whole circle

1) observe 25mL of samples every 30 minutes under the microscope to check for the presence of the cells

2) for control under microscope, used PBS sample and the wall was clear. for comparison, used solution from protocol (2).

Microscope observations...:

- 903A/B:
  - after 0.5hr, blue spider-like shape (debris) similar to what is seen with only the solution from protocol (2)
  - after 1hr, no chnages
  - after 1.5hr, no changes
- 903C/D:
  - after 0.5hr, similar to what is seen from 903A/B
  - after 1hr, no changes

After 2 hours, took #1 and put into freezer (-20degC)

#2 shook overnight and then was put into freezer (-20degC)

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28-Mar-2021: Elution of DNA from pre-treated DBSs (Pre-treatments #1, #2, #3)

Goal: Elute DNA from pre-treated DBSs (see 24-Mar)

Procedure:

1. Extracted all samples using the NucliSENS protocols... **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf + Nuclisens_Lysis_Buffer_200292_89130-532.pdf**
2. Prepared pre-treatment #3, using just the Kantor lab PBS only protocol for pre-treatment and tested that as well
    1. A: 2 x 6mm spots (s)
    2. B: whole spot (Ws)
3. Measured on Nanodrop
4. Stored at -20degC

Sample Name	Concentration [ng/uL]	260/280	260/230
3ME, A,s, #1	0.8	0.64	0.01
3ME, B, Ws, #1	3.9	1.77	0.01
3ME, C, s, #2	5.4	1.86	0.01
3ME, D, Ws, #2	1.8	1.31	0.01
3MH, A, s, #3,1	1.5	1.21	0.00
3MH, B, Ws #3, 1	5.5	1.81	0.01

Will re-do #2 with NucliSENS Lysis Buffer. Will also re-do #1 with newer reagents

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1-Apr-2021: Pre-treatment protocols #2, #3 for EDTA, and #4; Elution of #3 & #4

Goal: Pre-treat samples using protocols E2, #3 for EDTA, and #4

Procedure:

Prep work... left the temp controlled shaker on overnight. Need to measure temp with a thermometer b/c the display is not correct.

1. Warmed 8 NucliSENS Lysis Buffer to 37degC for 30min.
2. Shook #3 in 750uL 1x PBS for 30 min. [Kantor protocol: **DBS_Elution_Protocols.docx]**
    1. Moved the 750uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    2. Then followed: **DBS_Elution_Protocols.docx**
    3. Used 80uL for Elution Buffer
        1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
        2. Samples sat for 10min at RT after elution b/c I was working on other samples
    4. Sample Names:
        1. 2ME, E, s #3
        2. 3ME, E, Ws #3
3. For #2: Mixed 150uL of PBS & 600uL of NucliSENS Lysis Buffer and shook for 2hrs.
    1. Stored sample in -80degC for extraction tomorrow.
    2. Sample Names:
        1. 4ME, C, s #2
        2. 4ME, D, Ws #2
        3. 4MH, C, s #2
        4. 4MH, D, Ws, #2
4. For #4: NucliSENS Lysis Buffer only, on shaker at room temp. for 1 hour
    1. Removed the spots from the lysis buffer then added the beads to the lysate
    2. Proceeded with the **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    3. Used 80uL for Elution Buffer
        1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
        2. Samples sat for 10min at RT after elution b/c I was working on other samples
    4. Sample Names:
        1. 4ME, A, s #4,1
        2. 4ME, B, Ws #4,1
        3. 4MH, A, s #4,1
        4. 4MH, B, Ws #4,1

Sample Name	Concentration (ng/uL)	260/280	260/230
2ME, E, s #3,1	9.8	1.31	0.02
3ME, E, Ws #3,1	20.8	1.28	0.11
4ME, A, s, #4,1	5.4	1.32	0.01
4ME, B, Ws, #4,1	0.6	0.44	0.01
4MH, A, s #4,1	4.1	1.27	0.01
4MH, B, Ws #4,1	12.4	1.58	0.03

SourceS for protocol #4:

2hrs at RT, directly into 9mL NucliSENS Lysis Buffer: Kantor, R., DeLong, A., Balamane, M., Schreier, L., Lloyd, R. M., Injera, W., Kamle, L., Mambo, F., Muyonga, S., Katzenstein, D., Hogan, J., Buziba, N., & Diero, L. (2014). HIV diversity and drug resistance from plasma and non-

plasma analytes in a large treatment programme in western Kenya. *Journal of the International AIDS Society*, *17*(1), 1–12. https://doi.org/10.7448/IAS.17.1.19262

1hr at RT, directly into 2mL of NucliSENS Lysis Buffer: Guichet, E., Serrano, L., Laurent, C., Eymard-Duvernay, S., Kuaban, C., Vidal, L., Delaporte, E., Ngole, E. M., Ayouba, A., & Peeters, M. (2018). Comparison of different nucleic acid preparation methods to improve specific HIV-1 RNA isolation for viral load testing on dried blood spots. *Journal of Virological Methods*, *251*, 75–79. https://doi.org/10.1016/j.jviromet.2017.10.014

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2-Apr-2021: Pre-treatment protocol #5; Elution for #5 & #2 (pre-treated 1-Apr-2021)

Goal: Do the pre-treatment & elution for #5 protocol; just do the elution for #2.

#5: 2mL of NucliSENS Lysis Buffer at 37degC, shaking for 30min

Source: Soetens, O., Vauloup-Fellous, C., Foulon, I., Dubreuil, P., De Saeger, B., Grangeot-Keros, L., & Naessens, A. (2008). Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infections. *Journal of Clinical Microbiology*, *46*(3), 943–946. https://doi.org/10.1128/JCM.01391-07

Procedure:

1. For #2, since it was frozen, I put the tubes at 37degC for 30min (see **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**) and then began the elution protocol by adding the magnetic beads...**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    1. I believe I spilled the D, Ws of both EDTA and Heparin, so will have to re-do
    2. Sample names:
        1. 4ME, C, s, #2, 1
        2. 4ME, D, Ws, #2, 1 ***X***
        3. 4MH, C, s, #2, 1
        4. 4ME, D, Ws, #2, 1 ***X***
2. For #5: Let the lysis buffer sit at 37degC for 15min at least (to dissolve crystals), then added DBSs and continued with the protocols: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    1. Sample Names:
        1. 6ME, C, s, #5, 1
        2. 6ME, D, Ws, #5, 1
        3. 6MH, C, s, #5, 1
        4. 6MH, D, Ws, #5, 1

Nanodrop:

Sample Name	Concentration (ng/uL)	260/280	260/230
4ME, C, s, #2, 1	9.6	1.63	0.02
4ME, D, Ws, #2, 1 ***X***	6.2	1.51	0.01
4MH, C, s, #2, 1	11.9	1.57	0.02
4ME, D, Ws, #2, 1 ***X***	14.1	1.51	0.04
6ME, C, s, #5, 1	1.9	1.32	0.01
6ME, D, Ws, #5, 1	2.0	1.53	0.01
6MH, C, s, #5, 1	26	1.43	0.23
6MH, D, Ws, #5, 1	10.2	1.61	0.02

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#2-Apr-2021: A - #3 pre-treatment and elution ; #2 and #4 pre-treatment

For protocol #3, 1000µL of 1x PBS on the shaker for 30 mins [Kantor protocol: **DBS_Elution_Protocols.docx]**

1. Moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
2. Then followed: **DBS_Elution_Protocols.docx**
3. Used 80uL for Elution Buffer
    1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
    2. Samples sat for ~5min at RT to remove solutions on the lid
4. Prepared samples for protocol #2, #4, #5
    1. for #2, put them on the shaker for 2 hours at RT
    2. for #4, put them on the shaker for 1 hour at RT
    3. for #5, put them on the thermoshaker for 30 mins at 37degC
    4. stored the samples at -80degC
5. Sample Names:
    1. 5ME, C, s, #3, 2
    2. 5MH, D, Ws, #3, 2
    3. 5MH, C, s, #3, 2

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, C, s, #3, 2	4.3	1.57	0.01
5MH, C, s, #3, 2	9.9	1.99	0.02
5MH, D, Ws, #3, 2	12.9	1.85	0.03

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REF 200293

IVD

EN

NucliSENS® Magnetic Extraction Reagents

For in vitro diagnostic use.

INTENDED USE

NucliSENS® Magnetic Extraction Reagents are intended to be used for the extraction of total nucleic acids from biological specimens.

SUMMARY AND EXPLANATION

Nucleic acids prepared using NucliSENS® Magnetic Extraction Reagents are suitable for a variety of nucleic acid amplification methods, provided that their use in conjunction with a particular method has been properly validated. The reagents are intended to be used with samples lysed using a 3 mol/l GuSCN lysis Buffer (e.g. NucliSENS® Lysis Buffer) and a magnetic extraction instrument (e.g. NucliSENS® miniMAG).

PRINCIPLE OF THE METHOD

The nucleic acid extraction method is based on Boom chemistry [1] using magnetic silica particles. Briefly, under high salt conditions, nucleic acid will bind to the silica particles. These silica particles act as a solid phase and non-nucleic acid components are removed by several washing steps performed in the NucliSENS® miniMAG. Finally, nucleic acids are eluted from the solid phase.

REAGENTS

For in vitro diagnostic use.

Contents

Catalogue number 200293

Contents	Component	Description
6 x 6.6 ml	R1	**Silica:** suspended in biocide solution *Color code: white*
6 x 9 ml	Wash Buf 1	**Wash Buffer 1:** contains guanidine thiocyanate* *Color code: transparent*
6 x 11 ml	Wash Buf 2	**Wash Buffer 2:** contains organic buffer and biocide solution. *Color code: red*
12 x 6 ml	Wash Buf 3	**Wash Buffer 3:** contains inorganic buffer. *Color code: yellow*
12 x 1 ml	Elution Buf	**Elution Buffer:** contains inorganic buffer. *Color code: yellow*

1 package insert downloadable from www.biomerieux.com/techlib

Warnings and Precautions

*Signal Word: **WARNING**

Hazards statement

EUH031: Contact with acids liberates toxic gas.
H302 + H312 +H332: Harmful if swallowed, in contact with skin or if inhaled.
H412: Harmful to aquatic life with long lasting effects.

Precautionary statement

P261: Avoid breathing dust/fumes/gas/mist/vapours/spray.
P273: Avoid release to the environment.
P280: Wear protective gloves/ protective clothing/eye protection/face protection.
P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P302 + P352: IF ON SKIN: Wash with plenty of water.

For further information, refer to the Material Safety Data Sheet

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Biomerieux_NucliSENS_Full_Kit_Protocol.pdf (276 kB)

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REF 200292

IVD

EN

NucliSENS® Lysis Buffer

For in vitro diagnostic use.

INTENDED USE

NucliSENS® Lysis Buffer is intended to be used for the release of total nucleic acid from biological specimens.

SUMMARY AND EXPLANATION

Biological samples prepared using NucliSENS® Lysis Buffer are suitable for use in nucleic acid isolation procedures based on Boom chemistry [1,2]. The lysis procedure described below has been designed as a generic laboratory protocol. bioMérieux has performed validation for a limited number of human specimen types using NucliSENS® Magnetic Extraction Reagents and the NucliSENS® miniMAG.

PRINCIPLE OF THE METHOD

Biological specimen is added to NucliSENS® Lysis Buffer, which contains guanidine thiocyanate. Any viral particles or cells in the specimen will be disrupted, releasing all nucleic acids that may be present. RNases and DNases in the specimen will be inactivated [3].

REAGENTS

For in vitro diagnostic use.

Contents

Catalogue number 200292
NucliSENS® Lysis Buffer (2 ml) contains sufficient reagents for up to 40 isolations.

Contents	Component	Description
40 x 2 ml	LYS	**Lysis Buffer** Contains 50 % guanidine thiocyanate*, <2 % Tris x 8-100, <1 % EDTA.

1 package insert downloadable from www.biomerieux.com/techlib

Warnings and Precautions

*Signal Word: **WARNING**

Hazards statement

EUH031: Contact with acids liberates toxic gas.
H302 + H312 +H332: Harmful if swallowed, in contact with skin or if inhaled.
H412: Harmful to aquatic life with long lasting effects.

Precautionary statement

P261: Avoid breathing dust/fumes/gas/mist/vapours/spray.
P273: Avoid release to the environment.
P280: Wear protective gloves/ protective clothing/eye protection/face protection.
P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P302 + P352: IF ON SKIN: Wash with plenty of water.

For further information, refer to the Material Safety Data Sheet

- **Certain reagents contain guanidine thiocyanate.** Refer to the hazard statements "H" and the precautionary statements "P" above.
- **Warning:** Buffers containing guanidine thiocyanate should not be mixed with cleaning solutions containing bleach. Liquid waste from extraction and isolation procedures containing guanidine thiocyanate must not be mixed with other laboratory waste. This will prevent potentially harmful chemical reactions from occurring.
- Reagent spillages should always be dealt with appropriately (see Procedural Precautions).

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Nuclisens_Lysis_Buffer_200292_89130-532.pdf (257 kB)

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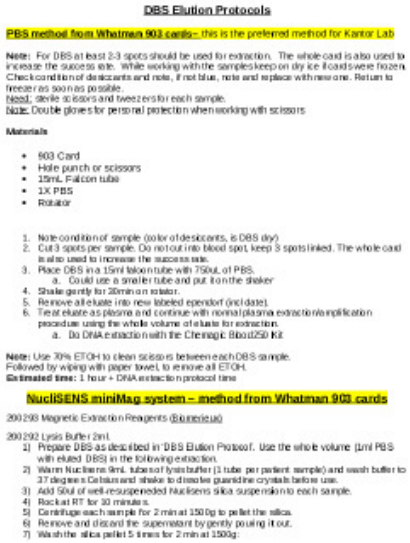

DBS Elution Protocols

PBS method from Whatman 903 cards– this is the preferred method for Kantor Lab

Note: For DBS at least 2-3 spots should be used for extraction. The whole card is also used to increase the success rate. While working with the samples keep on dry ice if cards were frozen. Check condition of desiccants and note, if not blue, note and replace with new one. Return to freezer as soon as possible.
Need: sterile scissors and tweezers for each sample.
Note: Double gloves for personal protection when working with scissors

Materials

- 903 Card
- Hole punch or scissors
- 15mL Falcon tube
- 1X PBS
- Rotator

1. Note condition of sample (color of desiccants, is DBS dry)
2. Cut 3 spots per sample. Do not cut into blood spot, keep 3 spots linked. The whole card is also used to increase the success rate.
3. Place DBS in a 15ml falcon tube with 750ul of PBS.
   a. Could use a smaller tube and put it on the shaker
4. Shake gently for 30min on rotator.
5. Remove all eluate into new labeled eppendorf (incl date).
6. Treat eluate as plasma and continue with normal plasma extraction/amplification procedure using the whole volume of eluate for extraction.
   a. Do DNA extraction with the Chemagic Blood250 kit

Note: Use 70% ETOH to clean scissors between each DBS sample.
Followed by wiping with paper towel, to remove all ETOH.
Estimated time: 1 hour + DNA extraction protocol time

NucliSENS miniMag system – method from Whatman 903 cards

200298 Magnetic Extraction Reagents (Biomerieux)

200292 Lysis Buffer 2ml

1) Prepare DBS as described in "DBS Elution Protocol". Use the whole volume (1ml PBS with eluted DBS) in the following extraction.
2) Warm Nuclisens 9ml. tubes of lysis buffer (1 tube per patient sample) and wash buffer to 37 degrees Celsius and shake to dissolve guanidine crystals before use.
3) Add 50ul of well-resuspended Nuclisens silica suspension to each sample.
4) Rock at RT for 30 minutes.
5) Centrifuge each sample for 2 min at 1500g to pellet the silica.
6) Remove and discard the supernatant by gently pouring it out.
7) Wash the silica pellet 5 times for 2 min at 1500g:

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DBS_Elution_Protocols.docx (19.8 kB)

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ANOTHER PROTOCOL

- Lofgren, S. M., Morrissey, A. B., Chevallier, C. C., Malabeja, A. I., Edmonds, S., Amos, B., Sifuna, D. J., von Seidlein, L., Schimana, W., Stevens, W. S., Bartlett, J. A., & Crump, J. A. (2009). Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities. *AIDS*, *23*(18), 2459–2466. https://doi.org/10.1097/QAD.0b013e328331f702

Protocol: Two DBS were transferred to a 50 mL conical tube with 1.7 mL of lysis buffer (Abbott m Sample Preparation System buffer, Abbott Laboratories, Abbott Park, IL). These tubes were incubated at room temperature for 2 hours with intermittent mixing;

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4-Apr-2021: A - #3 pre-treatment and elution ; #2, #4, #5 elution

procedure:

1. For #2, #4, and #5, I put the tubes at 37degC for ~45 mins on the thermoshaker and then began the elution protocol by adding the beads directly to the tubes (**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**).
    1. Transferred the lysis buffer solution into a 1.5mL tube, added the beads directly to the lysate
    2. Then followed: **DBS_Elution_Protocols.docx**
    3. Used 80uL for Elution Buffer
        1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
        2. Samples sat for <10min at RT
2. Prepared samples for protocol #3 (WS only) - followed the same steps as **2-Apr-2021**
3. Sample names:
    1. 5ME, A, s, #2, 2
    2. 5ME, B, Ws, #2, 2
    3. 5MH, A, s, #2, 2
    4. 5MH, B, Ws, #2, 2
    5. 7ME, A, Ws, #3, 2
    6. 5ME, E, s, #4, 2
    7. 6ME, C, Ws, #4, 2
    8. 5MH, E, s, #4, 2
    9. 6MH, C, Ws, #4, 2
    10. 6ME, D, s, #5, 2
    11. 6ME, E, Ws, #5, 2
    12. 6MH, D, s, #5, 2
    13. 6MH, E, Ws, #5, 2

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, A, s, #2, 2	2.4	2.68	0.01
5ME, B, Ws, #2, 2	12.7	1.62	0.03
5MH, A, s, #2, 2	1.5	2.43	0.01
5MH, B, Ws, #2, 2	2.4	2.61	0.01
7ME, A, Ws, #3, 2	1.2	14.69	0.01
5ME, E, s, #4, 2	1.9	1.23	0.01
6ME, C, Ws, #4, 2	4.8	2.44	0.01
5MH, E, s, #4, 2	0.8	1.53	0.00
6MH, C, Ws, #4, 2	2.7	1.72	0.01
6ME, D, s, #5, 2	2.9	1.55	0.01
6ME, E, Ws, #5, 2	4.8	1.57	0.01
6MH, D, s, #5, 2	6.2	1.93	0.01
6MH, E, Ws, #5, 2	1.1	0.95	0.01

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7-Apr-2021: Elution of #2, 1 re-dos and pre-treatment and elution of #2, 3

Goal: Do elution of #2, 1 re-dos and pre-treatment and elution of #2, 3

Procedure:

1. For #2, 1s , since it was frozen, I put the tubes at 37degC for 30min (see **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**) and then began the elution protocol by adding the magnetic beads...**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    1. Samples:
        1. 4ME, E, Ws, #2, 1
        2. 4MH, E, Ws, #2, 1
2. For #2, 3s, were prepared by A today
    1. Samples:
        1. 7ME, D, s, #2, 3
        2. 7ME, E, Ws, #2, 3
        3. 7MH, C, s, #2, 3
        4. 7MH, D, s, #2, 3

Sample Name	Concentration [ng/uL]	260/280	260/230
4ME, E, Ws, #2, 1	2.25	2.44	0.02
4MH, E, Ws, #2, 1	2.6	2.66	0.01
7ME, D, s, #2, 3	4.9	1.66	0.01
7ME, E, Ws, #2, 3	2.1	2.70	0.01
7MH, C, s, #2, 3	8.9	1.94	0.02
7MH, D, Ws, #2, 3	2.0	1.96	0.01

For RNA:

Sample Name	Concentration [ng/uL]	260/280	260/230
7MH, C, s, #2, 3	7.3	1.75	0.02
7MH, D, Ws, #2, 3	1.5	2.20	0.01

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 5:25 PM EST

8-Apr-2021: A - #4, #5 extraction:

Goal: 1) check whether I used the correct 1.5mL tubes

2) Do extraction of #4, and #5 (#3- need more lysis buffer)

Procedure:

1. Re-measured the samples collected from **4-Apr-2021** to check whether I used the correct 1.5mL tubes

Sample Name	Concentration (ng/uL)	260/280	260/230
5ME, A, s, #2, 2	2.4 --> 3.1	2.68 --> 1.56	0.01 --> 0.01
5ME, B, Ws, #2, 2	12.7 --> 13.2	1.62 --> 1.65	0.03 --> 0.04
5MH, A, s, #2, 2	1.5 --> .8	2.43 --> 2.65	0.01 --> 0.01
5MH, B, Ws, #2, 2	2.4 --> 3.0	2.61 --> 1.20	0.01 --> 0.02
7ME, A, Ws, #3, 2	1.2	14.69	0.01
5ME, E, s, #4, 2	1.9 --> 1.7	1.23 --> 1.52	0.01 --> 0.00
6ME, C, Ws, #4, 2	4.8 --> 5.6	2.44 --> 1.66	0.01 --> 0.01
5MH, E, s, #4, 2	0.8 --> 1.1	1.53 --> 0.80	0.00 --> 0.00
6MH, C, Ws, #4, 2	2.7 --> 2.9	1.72 --> 1.35	0.01 -> 0.01
6ME, D, s, #5, 2	2.9 --> 3.3	1.55 --> 1.04	0.01 --> 0.01
6ME, E, Ws, #5, 2	4.8 --> 4.9	1.57 --> 1.88	0.01 --> 0.01
6MH, D, s, #5, 2	6.2 --> 6,5	1.93 --> 1.53	0.01 --> 0.01
6MH, E, Ws, #5, 2	1.1 --> 1.8	0.95 --> 1.02	0.01 --> 0.01

2. thaw the tubes at 37degC for ~30 mins on the thermoshaker

3. began the elution protocol by adding the beads directly to the tubes [**Biomerieux_NucliSENS_Full_Kit_Protocol.pdf].**

1. removed the spots from the lysis buffer, added the beads directly to the lysate
2. then followed: **DBS_Elution_Protocols.docx**
3. used 80µL of elusion buffer
    1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
    2. Samples sat for 7mins at RT

4. Sample Names:

1. 7MH, E, s, #4, 3
2. 8MH, A, Ws, #4, 3
3. 8ME, A, s, #4, 3
4. 8ME, B, Ws, #4, 3
5. 8MH, B, s, #5, 3
6. 8MH, C, Ws, #5, 3

7. 8ME, C, s, #5, 3
8. 8ME, D, Ws, #5, 3

Sample Name	Concentration (ng/uL)	260/280	260/230
7MH, E, s, #4, 3	5.0	1.99	0.01
8MH, A, Ws, #4, 3	1.4	2.20	0.07
8ME, A, s, #4, 3	1.3	1.13	0.20
8ME, B, Ws, #4, 3	2.2	2.20	0.02
8MH, B, s, #5, 3	1.8	-4.90	0.01
8MH, C, Ws, #5, 3	141.6	1.12	2.48
8ME, C, s, #5, 3	4.8	2.44	0.01
8ME, C, s, #5, 3	0.5	-0.75	0.01
8ME, D, Ws, #5, 3	159.6	1.10	1.88

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

17-Apr-2021: Extraction from pre-treatment #3, 3

Procedure:

For protocol #3, 1000µL of 1x PBS on the shaker for 30 mins [Kantor protocol: **DBS_Elution_Protocols.docx]**

1. Moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
2. Then followed: **DBS_Elution_Protocols.docx**
3. Used 80uL for Elution Buffer
    1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
    2. Samples sat for ~5min at RT to remove solutions on the lid

Sample Name	Concentration (ng/uL)	260/280	260/230
1ME, E, s, #3, 3	1.1	5.74	0.00
8ME, E, Ws, #3, 3	5.3	2.02	0.01
8MH, D, s, #3, 3	-1.2	1.01	-0.04
8MH, E, Ws, #3, 3	0.6	-2.81	0.05

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

9-Apr-2021: Bioanalyzer analysis of RNA from 2 Whole spot samples & qPCR of the 1s and 2s of the pre-treatment trials

Goal: Do qPCR of the 1s and 2s of the pre-treatment trials. Test RNA Bioanalyzer results

Procedure:

1. Bioanalyzer: used RNA Pico kit to analyze the 2 samples spilled on 2-Apr-2021: 4ME, D, Ws, #2, 1 ***and*** 4MH, D, Ws, #2, 1.
    1. RIN for MH was around 2 and RIN for ME was N/A mostly...
        1. Results: **4ME_H_DWs_2_x_2100_expert_Eukaryote_Total_RNA_Pico_DE23101932_2021-04-09_14-08-39.xad**
    2. RNA Nanodrop results:

Sample Name	Concentration (ng/uL)	260/280	260/230
4ME, D, Ws, #2, 1 ***X***	5.5	1.87	0.01
4MH, D, Ws, #2, 1 ***X***	10.5	1.59	0.04

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**
    1. **Catalog#:** 4369016
       Lot #: 00799270
    2. Using SRY probe
        1. **Catalog#:** 4331182
           ID#: Hs00243216_s1
            1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].
    3. Results... Raw: **20210409_182655_CT047233_GENEEXP.zpcr,** Csv file: **20210409_182655_CT047233_GENEEXP.csv**
    4. Analyzed results: **qPCR1_of_pre-treatments.xlsx**

THIS QPCR RUN IS NOT GOOD DUE TO CONTAMINATION OF THE NTCs.

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4ME_H_DWs_2_x_2100_expert_Eukaryote_Total_RNA_Pico_DE23101932_2021-04-09_14-08-39.xad (1.27 MB)

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20210409_182655_CT047233_GENEEXP.csv (12.1 kB)

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20210409_182655_CT047233_GENEEXP.zpcr (205 kB)

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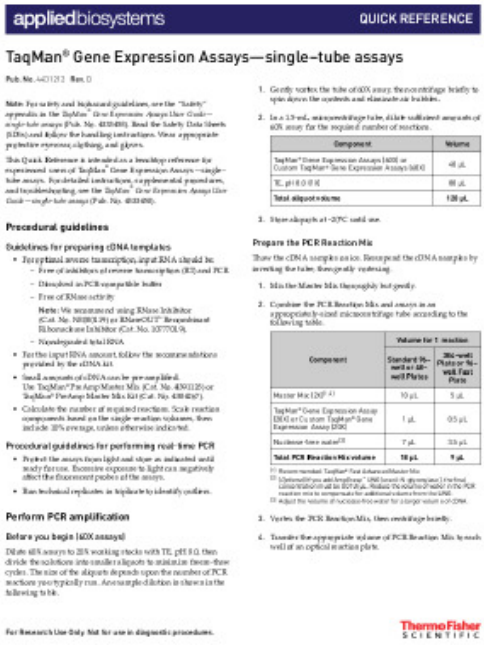

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TaqMan® Gene Expression Assays—single-tube assays

Procedural guidelines

Perform PCR amplification

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GEx_Quick_Reference_Card_4401212D_2_.pdf (215 kB)

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Overview

Sheet 1: Sheet1

Sheet 2: Data

Download

qPCR1_of_pre-treatments.xlsx (29 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

17-Apr-2021: qPCR re-do of replicates 1 & 2 for pre-treatments #2, #3, #4, #5

For the qPCR, used correct SRY probe this time (may have mixed up the probes from last time). Did qPCR of replicates 1s and 2s for protocols #2, #3, #4, & #5. Also tested the FTA samples that were in elution buffer.

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**
    1. **Catalog#:** 4369016
       Lot #: 00799270
    2. Using SRY probe
        1. **Catalog#:** 4331182
           ID#: Hs00243216_s1
            1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].
3. Results... Raw: **20210417_161736_CT047233_GENEEXP.pcrd,** Csv file: **20210417_161736_CT047233_GENEEXP_1_.csv**
4. Analyzed results: **qPCR1_of_pre-treatments_with_CORRECT_probe.xlsx**

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20210417_161736_CT047233_GENEEXP_1_.csv (13.6 kB)

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20210417_161736_CT047233_GENEEXP.pcrd (185 kB)

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qPCR1_of_pre-treatments_with_CORRECT_probe.xlsx (33 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:30 PM EST

22-Apr-2021: prep of #1:

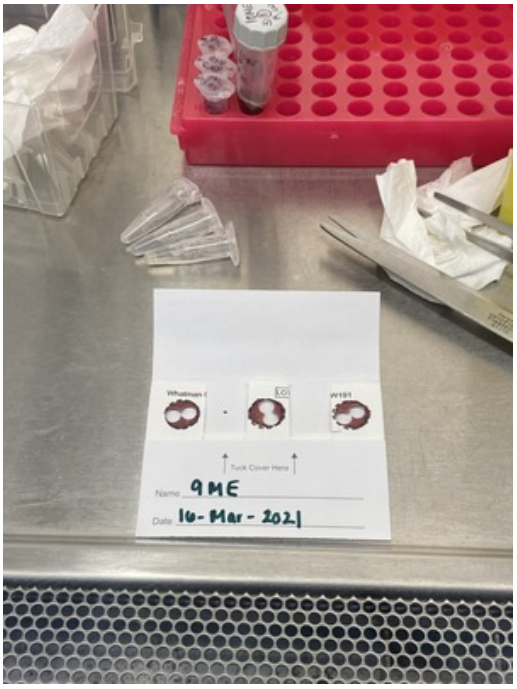
Goals: Start the pre-treatment of #1 to shake overnight

Procedure:

To prep #1s:

1. Made 14mL of the PBS/0.05% Tween/0.08% Sodium Azide solution
    1. For Tween, added 50* 14 = 700uL into 13.3mL of PBS
    2. For sodium azide, added 0.148mg * 14 = 2,072mg to 13.3mL of the PBS
2. Set up 12 samples (3 reps for both blood types with a 2 x 6mm spot & whole spot)
3. Shook overnight

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT



Download

IMG_8778.jpg (1.91 MB) Image of the spots after they're removed from the DBS, to show how much area differs between the 2x6mm spots and the whole spots

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:30 PM EST

23-Apr-2021: Extraction of #1, reps 1 & 3

Goals: Do the extraction of 2 replicates of protocol #1 (reps. 1 & 3); do reverse transcriptase of Heparin replicate 1s & of protocol #1 replicates 1 for Heparin & EDTA

Procedure:

For the #1 extractions....

1. Removed from shaker
2. Moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
    1. Messed up 2 of them (they spilled) so will have to re-do the 2 x 6mm spots for both. Will re-setup for extraction tomorrow (these are rep 2)
    2. Also lysed the Ws, rep 2 and stored at -80 for extraction tomorrow
3. Used 80uL for Elution Buffer
    1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]
    2. Samples sat for ~5min at RT to remove evaporated liquid on the lid

Nanodrop:

Sample Name	Concentration (ng/uL)	260/280	260/230
9ME, C, s, #1, 1	20	1.34	0.09
9ME, B, Ws, #1, 1	1.0	1.97	0.10
9MH, B, s, #1, 1	0.5	3.5	0.04
9MH, A, Ws, #1, 1	1.1	3.36	0.03
9ME, E, s, #1, 3	0.7	1.49	0.02
10ME, A, Ws, #1, 3	25.5	1.58	0.22
9MH, C, s, #1, 3	6.8	1.95	0.01
9MH, D, Ws, #1, 3	1.8	2.55	0.01

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

24-Apr-2021: Extraction from pre-treatment #1, 2

Procedure:

For the #1 extractions,

1. For s, moved the 1000uL into a lysis buffer tube and followed: **Nuclisens_Lysis_Buffer_200292_89130-532.pdf** AND **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
2. For Ws (already lysed), warmed them up at 37degC for ~30 minutes and followed **Biomerieux_NucliSENS_Full_Kit_Protocol.pdf**
3. Used 80uL for Elution Buffer
    1. Eluted at 60degC for 10min with vortexing every 3min [Kantor protocol: **DBS_Elution_Protocols.docx**]

Sample Name	Concentration (ng/uL)	260/280	260/230
10ME, B, s, #1, 2	0.7	2.02	0.01
9ME, D, Ws, #1, 2	1.9	1.97	0.08
1MH, E, s, #1, 2	0.8	-26.86	0.03
9MH, E, Ws, #1, 2	1.4	1.51	0.09

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 5:25 PM EST

27-Apr-2021: qPCR of replicate 3 for pre-treatments #2, #3, #4, #5 and all of pre-treatment #1

Done by A. Did qPCR of replicates 3 for protocols #2, #3, #4, & #5, and all replicates for protocol #1

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**
    1. **Catalog#:** 4369016
       Lot #: 00799270
    2. Using SRY probe
        1. **Catalog#:** 4331182
           ID#: Hs00243216_s1
            1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].
    3. Results... Raw: **20210427_152035_CT047233_GENEEXP.pcrd ,** Csv file: **20210427_152035_CT047233_GENEEXP.csv**
    4. Analyzed results: **qPCR_of_pre-treatments_all_of_1_and_rep_3_for_rest.xlsx**

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

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20210427_152035_CT047233_GENEEXP.pcrd (187 kB)

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qPCR_of_pre-treatments_all_of_1_and_rep_3_for_rest.xlsx (30.1 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 5:25 PM EST

29-Apr-2021: qPCR re-do of pretreatment #5, rep 3, AND testing of pre-treatment #2s solution with pure gDNA to see if DNA will amplify in it

Done by A. Did re-did qPCR of replicate 3 for protocols #5, and tested pre-treatment #2 solution with pure gDNA to see if DNA will amplify in it

1. For qPCR: Using Gene Expression Mastermix (over Fast Advanced, a newer mastermix) b/c it is inhibitor tolerant and I am looking for inhibition of the PCR. **GEx_Quick_Reference_Card_4401212D_2_.pdf**
    1. **Catalog#:** 4369016
       Lot #: 00799270
    2. Using SRY probe
        1. **Catalog#:** 4331182
           ID#: Hs00243216_s1
            1. Hs00243216_s1 is the Taqman probe ID. [_s1 means it is best for gDNA. m1 means best for cDNA].
    3. Results... Raw: **20210429_172840_CT047233_GENEEXP.pcrd ,** Csv file: **20210429_172840_CT047233_GENEEXP.csv**
    4. Analyzed results:**qPCR_0429.xlsx**

- DNA didn't amplify with pre-treatment #2... so this is a problem in terms of using this solution to do qPCR
- No difference b/t this data set and the last one for #5, so those 2 samples didn't amplify in general

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20210429_172840_CT047233_GENEEXP.pcrd (177 kB)

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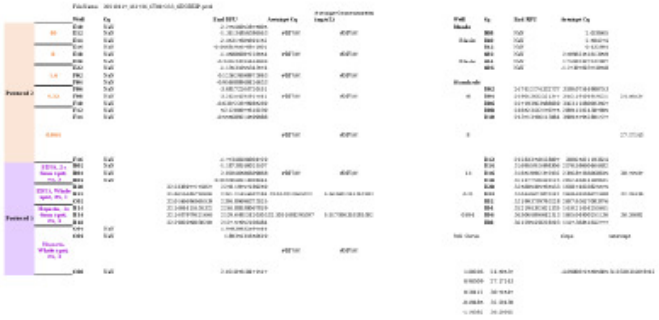
20210429_172840_CT047233_GENEEXP.csv (10.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT



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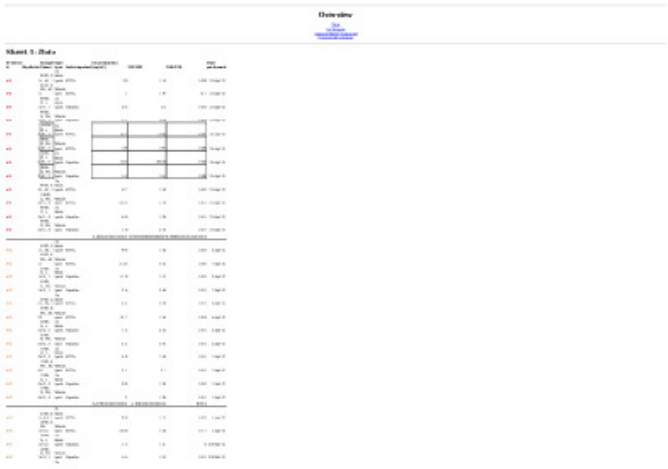
qPCR_0429.xlsx (22.7 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Nanodrop results of all pre-treatments

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT



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Nanodrop_comparisons_of_5_pre-treatments_1__2_.xlsx (30.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

qPCR results for everything:

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

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qPCR_for_ALL_pre-treatments.xlsx (68.5 kB)

Figure 2 Data with %CV

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 10:15 PM EST

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qPCR_for_ALL_pre-treatments_2_.xlsx (62.9 kB)

Figure 3 Experiments: Protocol 6 qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:47 AM EST

11-Feb-2022: KOH Controls with correct volumes (1 punch) w/ Feb blood

Goal: Perform KOH controls with correct volumes of KOH/Tris buffer

Procedure:

1. Followed the KOH/Tris protocol for 1 x 6mm spot and 2 x 6mm spots for EDTA and Heparin:
    1. did 2x the 3mm volumes for 1 spot and 4x the 3mm volumes for 2 spots
        1. BUT the 4x volume for the elution step for the 2 spots protocol over filled and spilled, so will re-do with a lower volume.
    2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

For qPCR standards:

- Made new 40, 8, 1.6, 0.32, 0.064ng/uL
- 183ng/uL was the conc. of stock solution
- "40ng/uL" -> measured at 35ng/uL and "8ng/uL" -> measured at 15.6ng/uL

Used in Fig 3

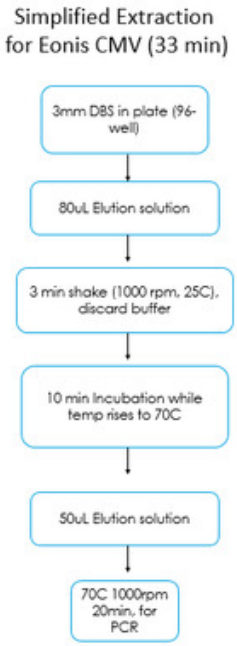
20220211_184602_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_KOH_controls_1_spot.xlsx

Picogreen_of_KOH_controls_1_spot.xlsx

Nanodrop_of_KOH_contorls_1_spot.txt

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:24 PM EST



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koh_tris_control.png (15.3 kB)

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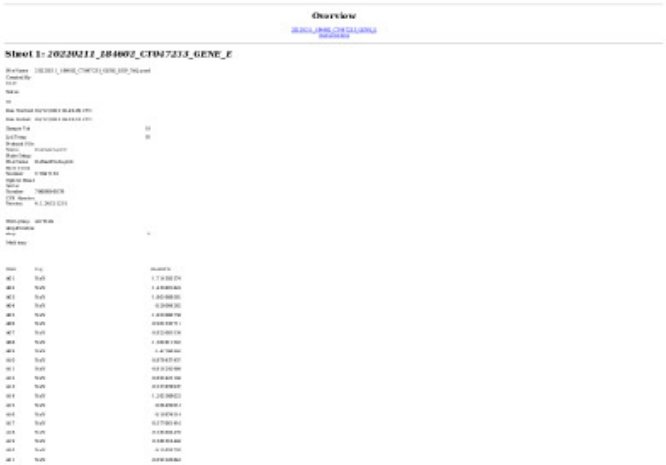

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qPCR_of_KOH_controls_1_spot.xlsx (34.6 kB)

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Picogreen_of_KOH_controls_1_spot.xlsx (21.9 kB)

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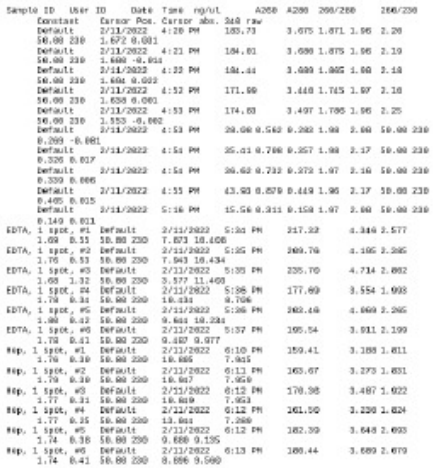

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Nanodrop_of_KOH_contorls_1_spot.txt (2.04 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:24 PM EST

15 and 16-Feb-2022: 2 spot controls

Goal: Do 2 spot controls

Procedure:

1. Followed the KOH/Tris protocol for 2 x 6mm spots for EDTA and Heparin:
    1. did 4x the 3mm volumes for 2 spots
        1. Some spilled so re-did 4 for EDTA and 4 for Heparin on 16-Feb (only 2 were used. 2 of the Hep spilled but all of the EDTA were OK)
    2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate
7.
8. Used in Fig 1

Nanodrop_of_KOH_controls_2_spots_1-6.txt

20220215_174736_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_KOH_controls_2_1-4.xlsx

20220216_144954_CT047233_GENE_EXP_TAQ.zpcr ; qPCR_of_KOH_2_spot_controls_5-6.xlsx

Picogreen_of_2_spots_and_variations.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 9:24 PM EST

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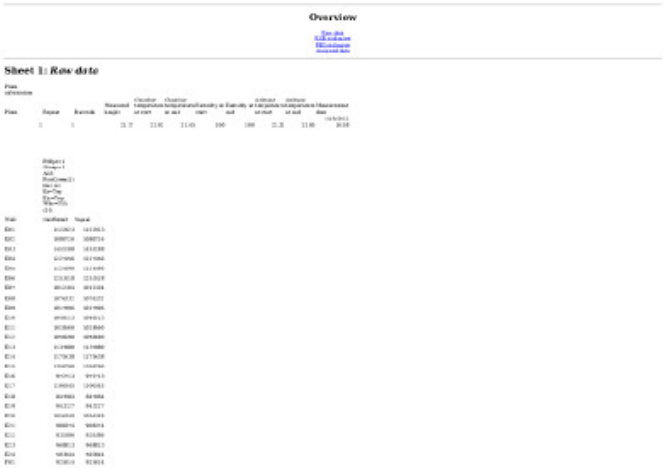

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Picogreen_of_2_spots_and_variations.xlsx (31 kB) Picogreen of 2 spots, all in 1 sheet

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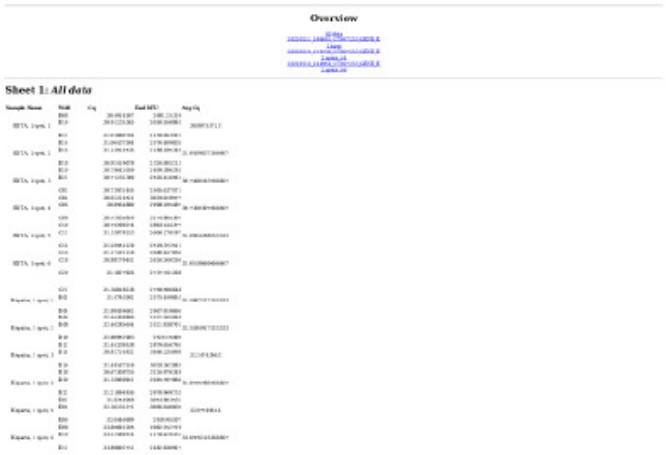

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qPCR_for_KOH_controls_1_spot_and_2_spots.xlsx (68.4 kB) All data in one sheet

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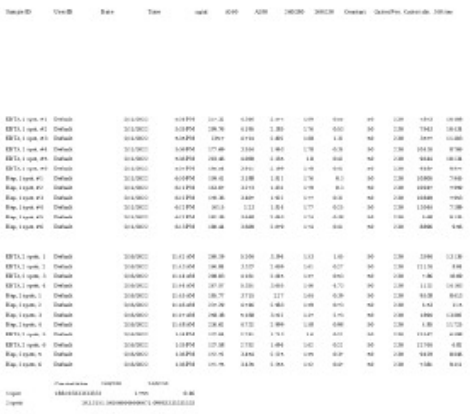

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Nanodrop_of_Protocol_6_1_spot_and_2_spots.xlsx (12.8 kB) Nanodrop of all in one data sheet

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nd-1000-v3.8-users-manual-8_5x11-2.pdf (3.96 MB)

Figure 3 Data with % CV

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 10:25 PM EST

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qPCR_KOH_1_punch_vs_2_punches.xlsx (50.2 kB)

DBS preparations for Figure 3 and 5

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:38 PM EST

9-Feb-2022: Preparation of Dried Blood Spots (Feb)

1. Made more 903 cards
    1. 75uL of blood**,** EDTA & Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Donor ID	Age	Gender	Ethnicity	Blood Type
KP63782	33	Male	African American	B+

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:38 PM EST

8-Mar-2022: Preparation of Dried Blood Spots (Mar)

1. Made more 903 cards
    1. 75uL of blood**,** EDTA & Heparin
2. Let sit overnight
3. Put each into a bag with a desiccant and into the -20degC freezer

Donor ID	Age (yrs.)	Gender	Ethnicity	Blood Type
KP63983 (EDTA)	43	Male	Caucasian	A+
KP63984 (Heparin)	45	Male	Caucasian	O-

Figure 4A-C: WBC blood fractions DBS 2

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT

29-Apr-2022:Making DBS blood fractions

Received blood on 29-Apr-2022 and did the preparation of the spots on 29-Apr-2022

Blood name: KP 64306

Goal: make dried blood spot fractions with same protocol as before.

Procedure:

For all, used 75uL of the solution on the DBS circle

- 2mL whole blood volumes was centrifuged at 2500rpm for 15min then I would pipette out the plasma first (150uL) and then the RBCs (135uL)
- After this would take the RBC lysis protocol and do it on the remaining volume (usually like 500-1000uL) and keep the WBCs
  - resuspended WBCs in 1400uL of 1X PBS to mix with plasma or RBCs
    - 350uL of this solution with 150uL of plasma or 365uL with 135uL of RBCs to bring solution to 500uL
  - OR resuspended WBCs in 2000uL of 1X PBS to be WBCs only
  - and mixed)
- Also did whole blood ones
- Also did plasma only and RBC only to test for background DNA presence
  - RBC only was odd.. won't use

Card counts:

Type	EDTA	Heparin
Pure WBCs	6	6
WBCs + plasma	6	5
WBCs + RBCs	6	6
Plasma only	3	3
Whole blood	7	7

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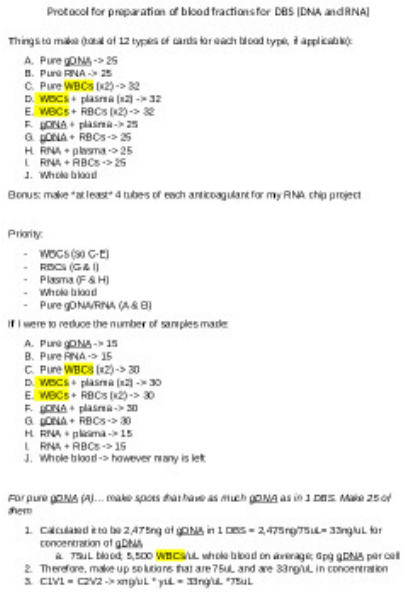

Protocol for preparation of blood fractions for DBS [DNA and RNA]

Things to make (total of 12 types of cards for each blood type, if applicable):

A. Pure gDNA -> 25
B. Pure RNA -> 25
C. Pure WBCs (x2) -> 32
D. WBCs + plasma (x2) -> 32
E. WBCs + RBCs (x2) -> 32
F. gDNA + plasma -> 25
G. gDNA + RBCs -> 25
H. RNA + plasma -> 25
I. RNA + RBCs -> 25
J. Whole blood

Bonus: make *at least* 4 tubes of each anticoagulant for my RNA chip project

Priority:

- WBCs (30 C-E)
- RBCs (G & I)
- Plasma (F & H)
- Whole blood
- Pure gDNA/RNA (A & B)

If I were to reduce the number of samples made:

A. Pure gDNA -> 15
B. Pure RNA -> 15
C. Pure WBCs (x2) -> 30
D. WBCs + plasma (x2) -> 30
E. WBCs + RBCs (x2) -> 30
F. gDNA + plasma -> 30
G. gDNA + RBCs -> 30
H. RNA + plasma -> 15
I. RNA + RBCs -> 15
J. Whole blood -> however many is left

For pure gDNA (A)... make spots that have as much gDNA as in 1 DBS. Make 25 of them

1. Calculated it to be 2,475ng of gDNA in 1 DBS = 2,475ng/75uL= 33ng/uL for concentration of gDNA
    a. 75uL blood; 5,500 WBCs/uL whole blood on average; 6pg gDNA per cell
2. Therefore, make up solutions that are 75uL and are 33ng/uL in concentration
3. C1V1 = C2V2 -> xng/uL * yuL = 33ng/uL *75uL

Download

Protocol_for_DBS_fraction_experiments_and_general_day_of_protocol.docx (25.5 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:05 PM EST

30-Apr-2022: Do DBS extraction and Picogreen

Goal: Do DBS extraction with KOH/Trizma protocol & then do Picogreen

Procedure:

1. Followed KOH/Trizma protocol to do DBS extraction
    1. Used 1 punch for all and correct protocol volumes
    2. Did EDTA fractions & Heparin ones
2. Did Picogreen...Procedure for Picogreen
    1. Perform Picogreen with the usual protocol (see protocols tab)
    2. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
        1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
    3. Still used 20uL volume onto the Picogreen plate (though it could hold 40uL)
    4. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
        1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
3. Measured the "Background" that is the plasma without anything added into it. Will not include this in the analysis because the "background" may differ based on the punch and also including it for all samples would yield the same results anyway since results are presented as % yield. The lower actual yield would be lower due to the "background". Also the "background" DNA would be present in whole blood too so that is not exactly relevant to the experiments as this is just looking generally at DNA yield which with DBSs can come from cfDNA or something else.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 1:34 PM EDT

Plate information
Plate Repeat Barcode Measured height Chamber temperature at start Chamber temperature at end Humidity at start Humidity at end Ambient temperature at start Ambient temperature at end Measurement date
1 1 14.98 23.38 22.5 100 100 23.39 23.39 4/30/2022 11:40:07 AM

Curve information - Curve quality data
Name Formula Slope at 50% binding Intercept ED-20 ED-50 ED-80 Correlation coefficient (R) Forced through origo Fitting status
Standard Curve Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1 18713689.0233 27526.75 0.02066768719 0.07515873419 0.1282398212 0.99802301292 No OK

PltRpt=1 Group=1 AGS PicoGreen(1) Ctn=1st Ex=Top Em=Top Wdw=N/A (14)
PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1 PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1

Well	CalcResult	Signal	CalcResult	StdConc
D01	1165681	1165681	0.06188711689	
D02	1202886	1202886	0.06281722782	
D03	1148585	1148585	0.05990471215	
D04	1198266	1198266	0.06256392376	
D05	1194986	1194986	0.06238864163	
D06	1221366	1221366	0.06379858023	
D07	1037680	1037680	0.05398274131	
D08	376880	376880	0.01867166773	
D09	1178513	1178513	0.06180081219	
D10	441762	441762	0.02213659569	
D11	1258264	1258264	0.06514802289	
D12	367299	367299	0.01815731825	
D13	1432210	1432210	0.07452082139	
D14	310599	310599	0.01512728574	
D15	1344884	1344884	0.07039807637	
D16	368582	368582	0.01822108617	
D17	1320020	1320020	0.06852872981	
D18	354832	354832	0.01749188583	
D19	1257585	1257585	0.06572902461	
D20	418251	418251	0.02088017651	
D21	1125398	1125398	0.0588089818	
D22	325688	325688	0.01593363907	
D23	1330615	1330615	0.06995190524	
D24	374279	374279	0.01852567131	
E01	184700	184700	0.00816829066	
E02	585215	585215	0.02553294898	
E03	339369	339369	0.01666474815	
E04	504837	504837	0.02578247883	
E05	422365	422365	0.02110002733	
E06	626855	626855	0.03200110641	
E07	423555	423555	0.02116362084	
E08	567120	567120	0.02885171983	
E09	319870	319870	0.01562272786	
E10	560852	560852	0.02860172786	
E11	347770	347770	0.01711369485	
E12	666281	666281	0.03529281231	
E13	507198	507198	0.02563347519	
E14	627897	627897	0.03204883865	
E15	541693	541693	0.02747697674	
E16	587179	587179	0.02860762275	

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Overview

Sheet 1: Raw data

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Figure_4_Data_Picogreen_of_WBC_fractions_2.xlsx (53.1 kB)

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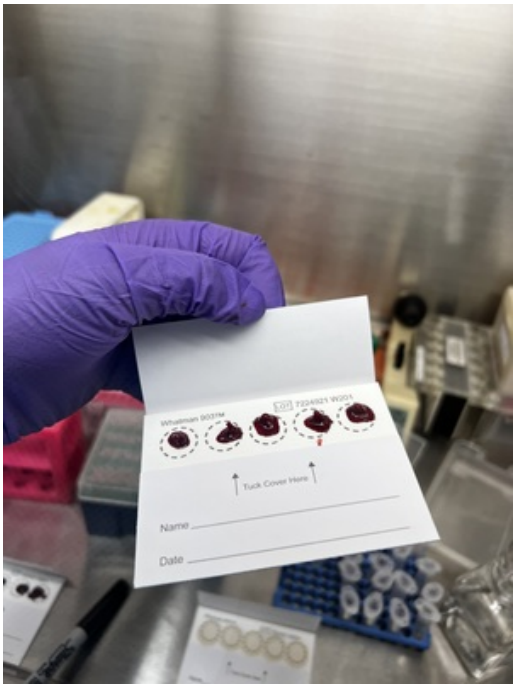

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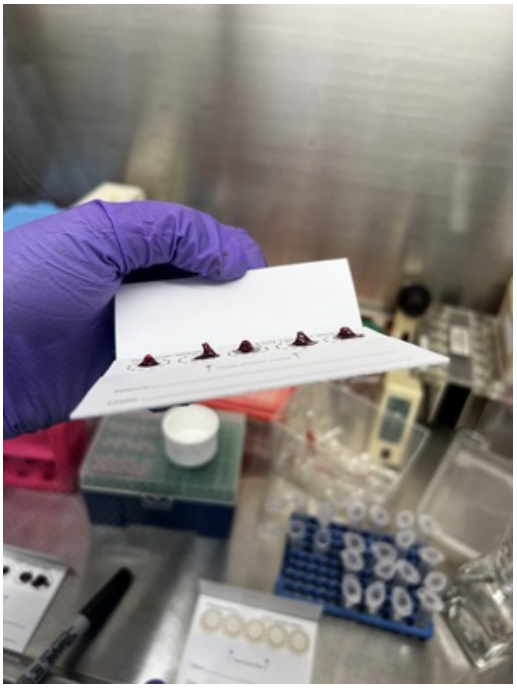

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Figure 5: Positive controls with Feb and Mar blood

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:40 PM EST

11-Feb-2022: KOH Controls with correct volumes (1 spot) w/ Feb blood

Goal: Perform KOH controls with correct volumes of KOH/Tris buffer

Procedure:

1. Followed the KOH/Tris protocol for 1 x 6mm spot and 2 x 6mm spots for EDTA and Heparin:
    1. did 2x the 3mm volumes for 1 spot and 4x the 3mm volumes for 2 spots
        1. BUT the 4x volume for the elution step for the 2 spots protocol over filled and spilled, so will re-do with a lower volume.
    2. Used the Feb blood
2. Did Picogreen, qPCR, Nanodrop
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

For qPCR standards:

- Made new 40, 8, 1.6, 0.32, 0.064ng/uL
- 183ng/uL was the conc. of stock solution
- "40ng/uL" -> measured at 35ng/uL and "8ng/uL" -> measured at 15.6ng/uL

Positive Control for Fig. 5 (EDTA, KOH)

20220211_184602_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_KOH_controls_1_spot.xlsx

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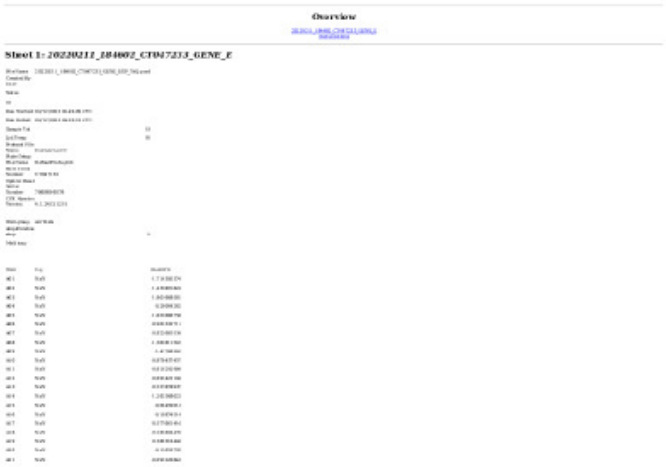

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qPCR_of_KOH_controls_1_spot.xlsx (34.6 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:16 AM EST

16-Feb-2022: Protocol variations: PBS, 1 spot

Goal: do PBS, 1 spot again (Feb samples)

Procedure:

1. PBS
    1. Used 1 spot and Heparin for PBS
    2. Did KOH protocol with 1X PBS as the buffer

PBS Feb qPCR Fig 5

Data: **20220216_144954_CT047233_GENE_EXP_TAQ.zpcr** ;

qPCR_of_Variations_3_PBS_1s.xlsx

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20220216_144954_CT047233_GENE_EXP_TAQ.zpcr (202 kB) re-doing the PBS ones

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qPCR_of_Variations_3_PBS_1s.xlsx (28.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:13 AM EST

9-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
    1. did 2x the 3mm volumes for 1 spot
    2. Used Heparin for PBS and EDTA for KOH

20220309_121148_CT047233_GENE_EXP_TAQ.zpcr

Mar_KOH_and_PBS_1s_pos_controls.xlsx

Use this for KOH EDTA March qPCR for Fig. 5 and supplementary

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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Mar_KOH_and_PBS_1s_pos_controls.xlsx (29.1 kB)

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28-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
    1. did 2x the 3mm volumes for 1 spot
    2. Used KOH for Heparin and PBS for EDTA
2. I think that the PBS needs to be replaced... so will use a fresh one to repeat the EDTA and Heparin PBS ones.

20220328_154654_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx

Use this for KOH PBS March qPCR

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qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx (27.5 kB)

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30-Mar-2022: PBS March controls, EDTA & Heparin

Goal: See yield from fresher PBS aliquot for EDTA and Heparin and use these as controls.

Followed the KOH/Tris protocol for 1 x 6mm spot

1. did 2x the 3mm volumes for 1 spot
2. Used Heparin and EDTA
3. Did qPCR and Picogreen

For Picogreen:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

qPCR: **qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx**

Picogreen: **Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx**

Use this for PBS Heparin & EDTA March qPCR and Picogreen

Use this for KOH Heparin March Picogreen

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20220330_135758_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

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Overview

Sheet 1: *20220330_135758_CT047233_GENE_E*

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qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx (30.4 kB)

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Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx (36.1 kB)

Figure 5 & Table 2: Protocol 6 variations

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:35 AM EST

11-Feb-2022: No shaking with PBS & KOH

Goal: See if shaking is important for the protocol (with 1 spot)

Procedure:

1. Did room temp part outside of the plate shaker and then put the plate in the plate shaker and it sat at ~70degC for 15min during the incubation period.
2. PBS was done with Heparin and KOH was done with EDTA - Feb samples
    1. To preserve samples
3. Did qPCR and Picogreen
    1. Saw that the PG concentrations are higher for the PBS samples but those Cq values are higher.
4. Picogreen:
    - Perform Picogreen with the usual protocol (see protocols tab)
    - Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
        1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
    - Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
        1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
    - Used 20uL volume onto the Picogreen plate
    -
    - USED FOR FIGURE 5

Results:

20220211_184602_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_KOH_and_PBS_no_shaking.xlsx

Picogreen_of_No_shaking_PBS_and_KOH.xlsx

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qPCR_of_KOH_and_PBS_no_shaking.xlsx (26 kB)

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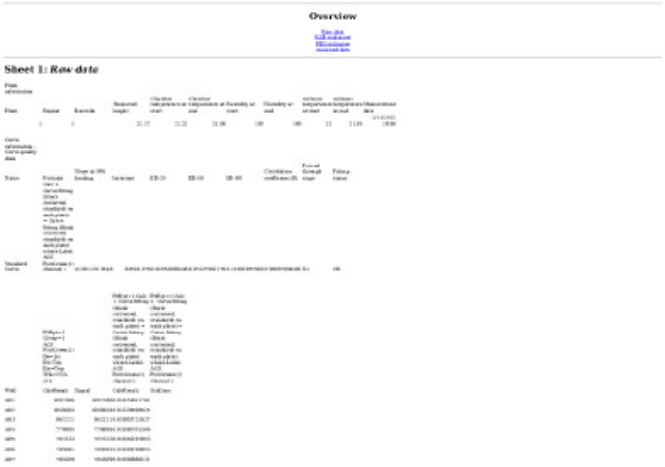

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Picogreen_of_No_shaking_PBS_and_KOH.xlsx (22.5 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:35 AM EST

15-Feb-2022: Protocol variations: No discard (KOH& PBS) and Room temp (KOH & PBS)

Goal: do the no discard and do the room temp

Procedure: All with Feb samples

1. No discard
    1. Used 1 spot with EDTA for KOH and Heparin for PBS
    2. Did KOH protocol but didn't discard the PBS or KOH after putting it in in the beginning
        1. So final elution volume was 160uL instead of 100uL
2. Room temp
    1. Used 1 spot with EDTA for KOH and Heparin for PBS
    2. Did entire KOH protocol @ room temperature
    3. Did 10min incubation (instead of 15min)
3. Did the time trials and the PBS only but had a slight issue with the temperature
    1. for time trials/PBS and no discard the temp was set to 25degC so it was cooling for the first 5 min of the shaking but that is fine for the no discard b/c it only went down to 60. Then turned it back up
    2. The PBS ones were in the "splash zone" from the 2 spot ones on this plate
    3. Will re-do PBS and time trials

USED FOR FIGURE 5

Data:

20220215_174736_CT047233_GENE_EXP_TAQ.zpcr ; **qPCR_of_Variations_2_No_discard_room_temp.xlsx**

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220215_174736_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:10 AM EST

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qPCR_of_Variations_2_No_discard_room_temp.xlsx (32.7 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:34 AM EST

18-Feb-2022: Picogreen of all variations

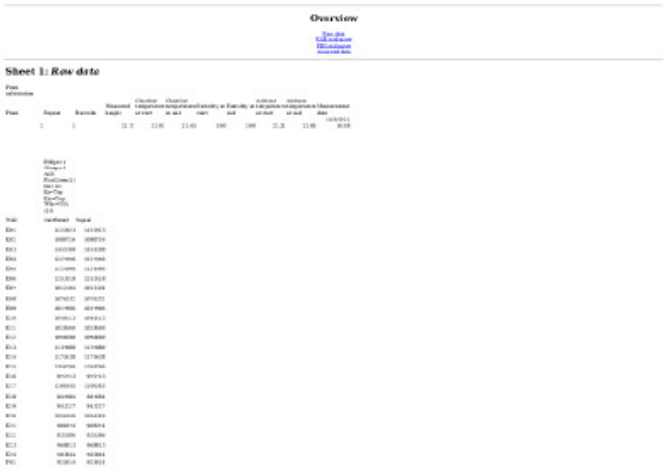
Goal: do picogreen of the variations; also did RT of the PBS variations for the RNA DBS project

Procedure:

1. Did Picogreen of all variations except no shaking
2. Perform Picogreen with the usual protocol (see protocols tab)
3. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
4. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
5. Used 20uL volume onto the Picogreen plate

Picogreen_of_2_spots_and_variations.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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Picogreen_of_2_spots_and_variations.xlsx (30.3 kB) PIcogreen of variations

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:42 AM EST

1-Mar-2022: PBS 5min shake; No discard + no shake + room temp KOH & PBS

Goal: Test all of the variations at once for PBS to see if it would work without all the variations; do 5min shake with PBS

Procedure: All with Feb samples

1. No discard + no shake + room temp, PBS
    1. Used Feb Heparin samples
    2. Did no discard, so elution volume is 160uL
    3. Did no shaking & room temp and used the plate shaker to incubate it so was at 26-27degC instead of 25degC
    4. Did a 10min incubation
2. Shake in PBS for 5min
    1. Used Feb Heparin samples
    2. Kept the discard and such
    3. Shook for 5min instead of 20min.

qPCR_of_PBS_no_anything_and_5min_PBS.xlsx

20220301_150700_CT047233_GENE_EXP_TAQ.zpcr

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220301_150700_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:41 AM EST

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qPCR_of_5min_PBS_and_PBS_no_anything.xlsx (31.3 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 10:34 AM EST

3-Mar-2022: PBS, 10min shake

(without removing it multiple times from the shaker)

Goal: Do 10min shake for PBS again without removing it from heat.

Procedure:

1. Use 1 spot from Heparin Feb sample
2. Follow protocol, except make the final shake time 10min

Data: **20220303_131851_CT047233_GENE_EXP_TAQ.zpcr**

qPCR_of_10min_PBS_shaking.xlsx

USED FOR FIGURE 5

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220303_131851_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:27 AM EST

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qPCR_of_10min_PBS_shaking.xlsx (30 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

4-Mar-2022: Picogreen of "No anything" PBS and KOH; 5, 10, 15min shake for PBS

Goal: do Picogreen of the variations listed in the title.

Procedure:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

3.4.22_KL.txt ; **Picogreen_of_PBS_time_trials_and_no_anything.xlsx**

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

Plate information
Plate Repeat Barcode Measured height Chamber temperature at start Chamber temperature at end Humidity at start Humidity at end Ambient temperature at start Ambient temperature at end Measurement date
1 1 14.88 21.02 21.06 133.7 133.7 22 21.95 3/4/2022 2:37:45 PM

Curve information - Curve quality data
Name Formula Slope at 50% binding Intercept ED-20 ED-50 ED-80 Correlation coefficient (R) Forced through origo Fitting status
Standard Curve Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1 20061001.7204 -12560.605 0.0216010044 0.07597357451 0.11998798257 0.99716646228 No OK

PltRpt=1 Group=1 AGS PicoGreen(1) Ce=ist Ex=Top Em=Top Wdw=N/A (1s)
PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1 PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate) = Curve fitting (blank corrected, standards on each plate) where Label: AGS PicoGreen(1) channel 1

Well	CalcResult	Signal	CalcResult	StdConc
L01	274100	274100	0.00980392016[illegible]	
L02	322227	322227	0.01155470773	
L03	294830	294830	0.01057723900	
L04	339880	339880	0.01212738366	
L05	288518	288518	0.01035316488	
L06	353080	353080	0.01259048122	
L07	305425	305425	0.01094100000	
L08	310004	310004	0.01137670775	
L09	292075	292075	0.01054427000	
L10	288798	288798	0.01036965141	
L11	307461	307461	0.01101186158	
L12	329127	329127	0.01175757927	
L13	302219	302219	0.01082419013	
L14	426558	426558	0.01510992433	
L15	296000	296000	0.01064118200	
L16	358082	358082	0.01271510572	
L17	329414	329414	0.01176725477	
L18	372508	372508	0.01325002588	
L19	341572	341572	0.01218560461	
L20	280629	280629	0.01009827564	
L21	288130	288130	0.01027787705	
L22	292816	292816	0.01048041006	
L23	311581	311581	0.01115191096	
L24	370000	370000	0.01314124231	
M01	286497	286497	0.01029056511	
M02	1387874	1387874	0.04818825000	
M03	264244	264244	0.00952479229	
M04	1770863	1770865	0.06130670047	
M05	229149	229149	0.00831719318	
M06	1220064	1220064	0.04240425060	
M07	256811	256811	0.00926902701	
M08	1532280	1532280	0.05315740215	
M09	294808	294808	0.01057827137	
M10	700020	700020	0.02452014613	
M11	234979	234979	0.00851780035	
M12	641762	641762	0.02302780415	
M13	1019929	1019929	0.03552748137	
M14	1061529	1061529	0.03695891381	
M15	1486273	1486273	0.05157411226	
M16	601277	601277	0.02122219400	

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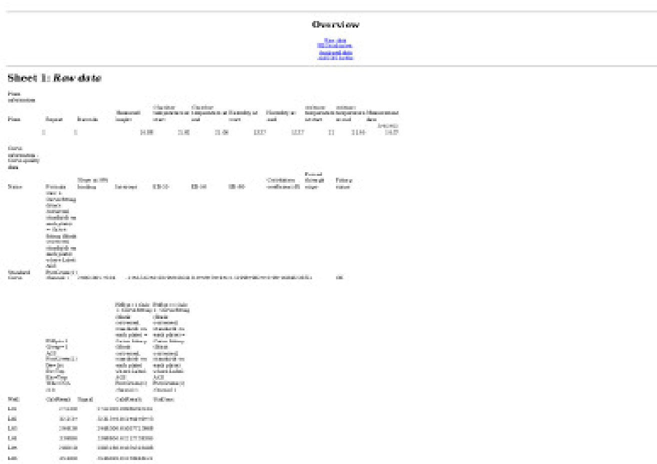
3.4.22_KL.txt (4.65 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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Picogreen_of_PBS_time_trials_and_no_anything.xlsx (23.3 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:28 AM EST

8-Mar-2022: PBS, 15min shake

(without removing it multiple times from the shaker)

Goal: Do 15min shake for PBS again without removing it from heat.

Procedure:

1. Use 1 spot from Heparin Feb sample
2. Follow protocol, except make the final shake time 15min
3. Do qPCR

USED FOR FIGURE 5

20220308_150315_CT047233_GENE_EXP_TAQ.zpcr ; 15min_PBS.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220308_150315_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:30 AM EST

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15min_PBS.xlsx (28.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:14 AM EST

10-Mar-2022: 5min, 10min, 15min shake with new KOH buffer and EDTA; no anything EDTA with new KOH buffer + test some voltages with new device

Goal: Do time trials and no anything with new KOH buffer

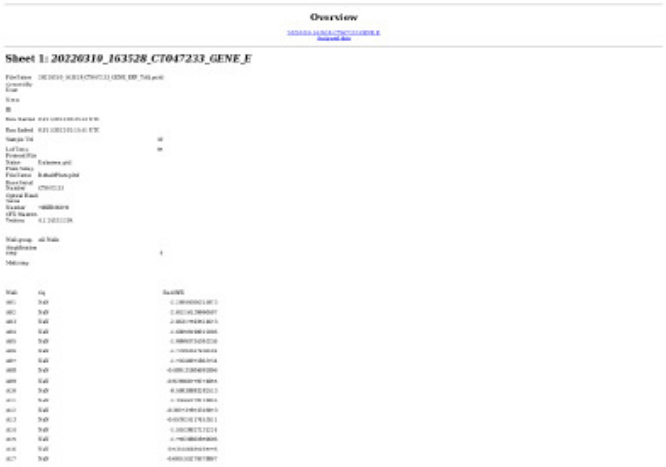
Procedure:

1. Use 1 spot from EDTA March samples
2. Follow protocol, except make the final shake time 5, 10, or 15min on 3 different plates so that they aren't being moved back and forth out of heat and in heat on one plate
3. Did "no anything" -> no discard, no shake, room temp.
    1. Did 3min at RT
    2. Shortened the "rise to 70degC" step to 5min
    3. Took 100uL of the plate and not 160uL to have same volume as the other samples.
4. Tested some voltages
    1. Tested KOH @ 6V and PBS @ 3, 4, 5V

USED THESE FOR FIGURE 5

20220310_163528_CT047233_GENE_EXP_TAQ.zpcr ; **qPCR_of_KOH_time_trials_and_test_of_voltages.xlsx**

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:23 AM EST



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qPCR_of_KOH_time_trials_and_test_of_voltages.xlsx (33.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

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20220310_163528_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

11-Mar-2022: Picogreen of KOH time trials & "No anything" & some voltages

Goal: do Picogreen of KOH time trials & KOH "no anything" and some voltages tested briefly

Procedure:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

3.11.22_KL.txt ; Picogreen_of_KOH_time_trials_and_15min_PBS_time_trial_and_KOH_no_anything.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT

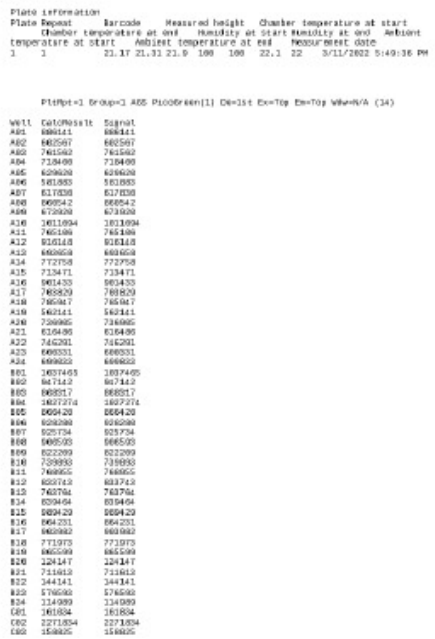

Plate Information
Plate Repeat Barcode Measured height Chamber temperature at start
Chamber temperature at end Humidity at start Humidity at end Ambient
temperature at start Ambient temperature at end Measurement date
1 1 21.17 21.33 21.9 100 100 22.1 22 3/11/2022 5:49:36 PM

PltRpt=1 Group=1 A05 PicoGreen[1] Dil=1st Ex=Top Em=Top Wav=N/A (14)

Well	CalcMassIt	Signal
A01	880141	880141
A02	682567	682567
A03	761582	761582
A04	718498	718498
A05	629620	629620
A06	581883	581883
A07	617838	617838
A08	900542	900542
A09	673020	673020
A10	1011094	1011094
A11	765186	765186
A12	916148	916148
A13	660658	660658
A14	772758	772758
A15	713471	713471
A16	901433	901433
A17	788829	788829
A18	765947	765947
A19	562141	562141
A20	726985	726985
A21	636496	636496
A22	746291	746291
A23	600331	600331
A24	600823	600823
B01	1037469	1037469
B02	867142	867142
B03	888817	888817
B04	1027274	1027274
B05	869428	869428
B06	826288	826288
B07	925734	925734
B08	906593	906593
B09	822299	822299
B10	739898	739898
B11	768855	768855
B12	833743	833743
B13	763784	763784
B14	839464	839464
B15	869429	869429
B16	864231	864231
B17	803882	803882
B18	771973	771973
B19	865599	865599
B20	124147	124147
B21	711613	711613
B22	144141	144141
B23	576598	576598
B24	134989	134989
C01	161834	161834
C02	1271854	1271854
C03	158825	158825

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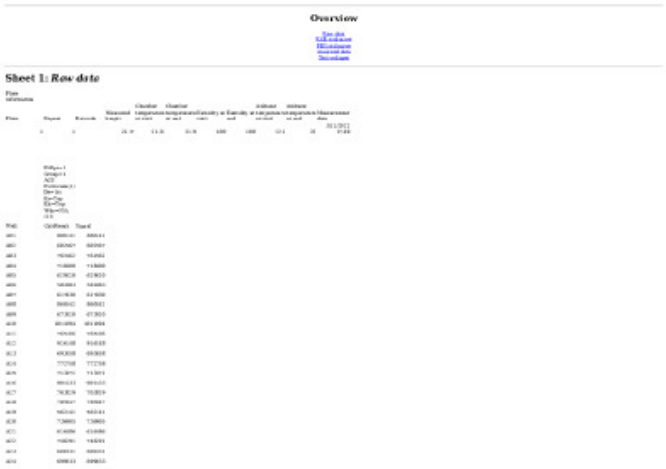
3.11.22_KL.txt (4.24 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 2:59 PM EDT



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Picogreen_of_KOH_time_trials_and_15min_PBS_time_trial_and_KOH_no_anything.xlsx (28.7 kB)

Table 2: Buffer compatibilty with DNA

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

27-Jan-2022: Test of Buffer qPCR Compatibility

Goal: see what DBS buffers (tested previously in "DBS elution pre-treatment variation") are compatible with qPCR directly

Procedure:

1. Made solutions that were 90% buffer + 10% human male gDNA (Promega, 242ng/uL)
    1. 2uL DNA + 18uL buffer
2. Used PBS, NucliSENS Lysis buffer, NucliSENS Lysis buffer/PBS, Tween/sodium azide/PBS, KOH/Tris, and the gDNA diluted in PicoGreen TE buffer as a control
    1. 1X PBS for all
3. Did qPCR
4. Conclusions:
    1. The results were mixed in the sense that the Cq value varied a lot. This could be due to the nature of gDNA pipetting, so I will do more replicates tomorrow with PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer
    2. Anything with the lysis buffer did not amplify

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr

Buffer_compatability_DNA_trial_1.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

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20220127_154629_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

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Buffer_compatability_DNA_trial_1.xlsx (27 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

28-Jan-2022: More replicates of buffer qPCR compatibility_

Goal: do more replicates of the buffer compatibility experiment (see 27-Jan)

Procedure:

1. Diluted the gDNA stock (242ng/uL) 1:10 in TE buffer (Picogreen) first
    1. Dilutions measured at:

Concentration (ng/uL)	**260/280**	**260/230**
150.3	1.95	2.04
171.1	1.96	2.25
77	1.95	2.11

1. Then, added 2uL of the diluted gDNA to 18uL of buffer (PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer)
2. Also measured the qPCR standards
    1. qPCR standards measured at:
3.

Sample name	**Concentration (ng/uL)**	**260/280**	**260/230**
"40ng/uL"	20.5	1.97	1.40
"8ng/uL"	4.3	2.17	0.61

20220128_165240_CT047233_GENE_EXP_TAQ.zpcr

qPCR_buffer_compatibility_9_replicates.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

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20220128_165240_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

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qPCR_buffer_compatibility_9_replicates.xlsx (27.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 02, 2022, 12:31 AM EDT

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qPCR_buffer_compatibility_all.xlsx (43.6 kB)

Supp. Fig 1A-B: gDNA in WBC-free blood

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Math and planning...

1) Measure gDNA stock

2) Make it into 40ng/uL stock

3) Want to start with the same concentrations and do 1:1 serial dilutions. Need at least 75 * 5 *3 = 1,125 per conc. = 1500 per conc.

1. Started by diluting 400uL of the stock into 2020uL of TE buffer from Picogreen
2. Did 1:1 dilutions by having each dilution have 1210uL of TE buffer and added 1200uL of the previous solution into the new one.
    1. Took 1200uL of 40ng/uL and addded it to 1210uL of TE buffer, etc.
        1. used 1200 b/c was easiest to pipette
        2.
        3.

4) Make 40, 10, 2.5uL solutions (1:3 dilutions)... 600uL into 1800uL

5) 150uL plasma + 135uL RBCs (45%) + 215uL gDNA for 1 card... (5 spots)? [to keep ratio of plasma:RBC and better control the gdna]

OR 270uL plasma (54%) + 60.75uL RBCs (45%) +5uL of gDNA to keep the volumes accurate... [closer to the viscosity]

Can try to make it so that same amount of gDNA is on spots as in gDNA fraction experiments.... which would be for 40: 40ng/uL*75uL= 3000ng. for 3 cards = 45000ng so to add 45000ng into a 500uL solution... need to use 500uL of a 90ng/uL.

45000ng into a 215uL solution? 209ng/uL (impossible)

Could attempt to make a 80ng/uL solution?? either way will need 1800uL per sample

Then, using 40ng/uL with 215uL = 1720ng DNA for each spot. So this is closer to the 20ng/uL... so just make 40ng/uL (same amount of gDNA as 20ng/uL) and 10ng/uL conc. (same amount of gDNA as 5ng/uL) and be done.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

17-Jan-2022: gDNA + WBC-free blood creation

Goal: make cards of WBC-free blood + spiked gDNA

Procedure:

1. gDNA stock was (on Nanodrop):

Sample Name	Concentration (ng/uL)	260/280	260/230
gDNA stock	206.8	1.95	2.24
	179.9	1.95	2.24
	179.6	1.93	2.21

2. Made dilutions for 40, 20, and 10ng/uL:

- Did 1:1 serial dilutions based on gDNA being at 190ng/uL... mixed 400uL of stock into 1500uL of 1X PBS

Sample Name	Concentration (ng/uL)	260/280	260/230
"40ng/uL"	36.12	1.85	1.65
	32.17	1.87	1.68
"20ng/uL"	18.53	1.72	1.64
	17.67	1.83	1.53
"10ng/uL"	9.81	1.66	1.68
	9.16	1.64	1.79

3. Centrifuged blood for 15min at 2500rpm (used 1mL or 3mL)

4. Took 150uL of plasma and 135uL of RBCs and mixed them with 215uL of "40", "20" or "10"ng/uL or a blank of just PBS

5. Made 1 set of cards for EDTA. Made 3 cards for each concentration for Heparin except 40, made 2 cards

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

18-Jan-2022: KOH/Tris protocol controls of the gDNA/blood samples

Goal: Do extractions from the gDNA/WBC-free blood samples

Procedure:

1. Used the Heparin ones, 40, 20, 10, and blank
2. Used 2 spots & doubled the extraction volume
    1. Used circles A, C, E
3. Perform Picogreen with the usual protocol (see protocols tab)
4. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
    2. Did 1:99 for the stocks and 1:10000-ish for the gDNA
5. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
6. Used 20uL volume onto the Picogreen plate

Raw data: KL_1.18.22

Analzyed data: gDNA_blood_fractions.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

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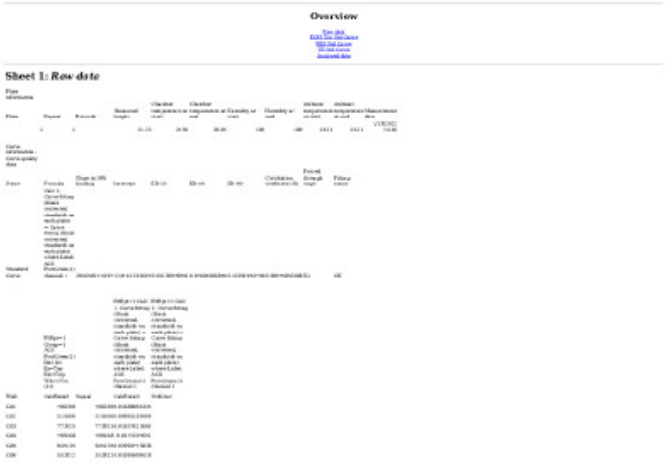
KL_1.18.22 (4.69 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT



Download

gDNA_blood_fractions.xlsx (28.6 kB)

Supp. Fig 1C: gDNA only DBS experiments

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

14-Dec-2021: Prep of gDNA DBS at different concentrations for extraction

Goal: Make gDNA solutions and put them on DBSs for extraction

Procedure:

1. gDNA stock is at 242ng/uL and there is 100ug
    1. it is about 419uL
    2. Before I used 66.2uL into 334uL of PBS which = ~40ng/uL
    3. Want to start with the same concentrations and do 1:1 serial dilutions
        1. Started by diluting 400uL of the stock into 2020uL of TE buffer from Picogreen (=2420)
        2. Did 1:1 dilutions by having each dilution have 1210uL of TE buffer and added 1200uL of the previous solution into the new one.
            1. Took 1200uL of 40ng/uL and addded it to 1210uL of TE buffer, etc.
                1. used 1200 b/c was easiest to pipette
2. Measured the concentrations on Nanodrop
    1.

Sample Name	Concentration (ng/uL)	260/280	260/230
"40ng/uL"	28.4	1.89	2.86
	27.9	1.82	1.89
"20ng/uL"	14.1	1.8	3.45
	13.6	1.89	3.92
"10ng/uL"	7.5	1.81	5.35
	7.6	1.63	8.68
"5ng/uL"	2.8	2.36	2.41
	2.5	2.95	8.27
"2.5ng/uL"	1.4	1.63	2.15
	1.2	1.29	1.79
gDNA stock	171	1.93	2.42

3. Put 75uL of each onto a dried blood spot spot, made 3 cards for each concentration and left over night to dry.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

15-Dec-2021: Extraction of gDNA of different concentrations from DBSs

Goal: extract gDNA of different concentrations from DBSs

Procedure:

1. Followed KOH/Tris protocol (control protocol)
2. Did 3 replicates of 1 spot and 3 replicates of 2 spots for each
    1. Used spots from circles A, C, and E for 1 spot and B, D, A for 2 spots
    2. For 2 spots, doubled the volume of KOH/Tris buffer (take this into account for Picogreen)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

16-Dec-2021: Picogreen of gDNA DBS from 15-Dec-2021

Goal: measure 15-Dec-2021 samples with Picogreen

Picogreen procedure:

1. Perform Picogreen with the usual protocol (see protocols tab)
2. Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
3. Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
4. Used 20uL volume onto the Picogreen plate

gDNA_release_exp.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

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gDNA_release_exp.xlsx (35.4 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

```
Plate information
Plate Repeat     Barcode     Measured height   Chamber temperature at start
      Chamber temperature at end    Humidity at start Humidity at end   Ambient
temperature at start    Ambient temperature at end    Measurement date
1     1          21.23 23.38 23.28 100   100   24.14 24.14 1/20/2022 1:34:31 PM

Curve information - Curve quality data
Name  Formula     Slope at 50% binding    Intercept   ED-20 ED-50 ED-80
      Correlation coefficient (R)   Forced through origo    Fitting status
Standard Curve    Calc 1: Curve fitting (blank corrected, standards on each
plate) = Curve fitting  (blank corrected, standards on each plate) where Label:
AGS PicoGreen(1) channel 1   24867682.2333    175868.222223    0.01451210861
      0.08839434643    0.12847658333    0.99998108567    No    OK

      PltRpt=1 Group=1 AGS PicoGreen(1) Ce=1st Ex=Top Em=Top Wdw=N/A (14)
      PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate)
= Curve fitting  (blank corrected, standards on each plate) where Label: AGS
PicoGreen(1) channel 1  PltRpt=1 Calc 1: Curve fitting (blank corrected,
standards on each plate) = Curve fitting  (blank corrected, standards on each
plate) where Label: AGS PicoGreen(1) channel 1
Well  CalcResult  Signal      CalcResult  StdConc
K01   637038      637038      0.01322612903
K02   907828      907828      0.0200964017
K03   657829      657829      0.01362271239
K04   958221      958221      0.02243791573
K05   656094      656094      0.01377295282
K06   830031      830031      0.01878725515
K07   798082      798082      0.01778721678
K08   374922      374922      0.00804101881
K09   724000      724000      0.01600185141
K10   272201      272201      0.0027629517
K11   844059      844059      0.01016276148
K12   281939      281939      0.00804229527
K13   619751      619751      0.01373084883
K14   878242      878242      0.02014412845
K15   665391      665391      0.01461219665
K16   927943      927943      0.02155233872
K17   684522      684522      0.01159855065
K18   767540      767540      0.01698928965
K19   472580      472580      0.00650970536
K20   363598      363598      0.00531842857 3
K21   467421      467421      0.00838189581
K22   322613      322613      0.00420876128
K23   570594      570594      0.01132882398
K24   398224      398224      0.00834875658
L01   377065      377065      0.00290872970
L02   5200072     5200072     0.11079907 16      0.15
L03   446997      446997      0.00777697816
L04   2802377     2802377     0.07512800283      0.075
L05   434461      434461      0.00741054732
L06   1498777     1498777     0.03700023149      0.0375
L07   255491      255491      0.00228371108
L08   815410      815410      0.01823158335      0.01875
L09   232939      232939      0.00163692287
L10   180565      180565      0.00028272154      0.009375
L11   235563      235563      0.00171217865
L12   364462      364462      0.00541080128 3
L13   195314      195314      0.00055784258
L14   5118399     5118399     0.1417584172
L15   170832      170832      0.00006219786
L16   1441093     1441093     0.03620002164
```

Download

KL_1.20.22.txt (3.82 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Overview

Sheet 1: *Raw data*

Download

gDNA_electrical_trials_1.xlsx (23.1 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

27-Jan-2022: Test of gDNA only on DBS in electrical device, replicates 2 & 3

Goal: finish the electrical trials of gDNA only DBS.

Procedure:

1. Took 2 of each of the gDNA only concentrations I made (3 for 40ng/uL) and tested them on the electrical device.
    1. 250uL of KOH/Tris at 30V for 5min, 1 spot
    2. Did them in this order... for replicates 2 (10, 5, 2.5, 40, 20) and then replicates 3 (40, 20, 10, 5, 2.5) and then replicate 1 of 40
        1. Noticed solution turning blue after replicate 2 of 2.5, and got worse from there
2. Then did PicoGreen
    - Perform Picogreen with the usual protocol (see protocols tab)
    - Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
        1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
    - Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
        1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
    - Used 20uL volume onto the Picogreen plate
3. Conclusions:
    1. need to re-do. the corrosion of the positive electrode ONLY caused issues. It turned completely blue and disintigrated upon touch
        1. This well had a spray on it to protect it, but that did not work
    2. John put tape on another well for future experiments

1.27.22_KL.txt ; PicoGreen_of_gDNA_DBS_electrical_trials_2.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

```
Plate information
Plate Repeat    Barcode   Measured height   Chamber temperature at start
     Chamber temperature at end   Humidity at start Humidity at end   Ambient
temperature at start   Ambient temperature at end   Measurement date
1    1          21.17 23.84 24.45 134   133.8 24.92 24.75 1/27/2022 5:39:46 PM

Curve information - Curve quality data
Name  Formula    Slope at 50% binding   Intercept   ED-20 ED-50 ED-80
     Correlation coefficient (R)   Forced through origo   Fitting status
Standard Curve   Calc 1: Curve fitting (blank corrected, standards on each
plate) = Curve fitting (blank corrected, standards on each plate) where Label:
A65 PicoGreen(1) channel 1   24731125.0031   91023.0022223   0.0004726563
     0.07488876445   0.11938080965   0.99947448794   No   OK

     PltRpt=1 Group=1 A65 PicoGreen(1) Dens=1st Ex=Top Em=Top Wdw=N/A (11)
     PltRpt=1 Calc 1: Curve fitting (blank corrected, standards on each plate)
= Curve fitting (blank corrected, standards on each plate) where Label: A65
PicoGreen(1) channel 1 PltRpt=1 Calc 1: Curve fitting (blank corrected,
standards on each plate) = Curve fitting (blank corrected, standards on each
plate) where Label: A65 PicoGreen(1) channel 1
Well  CalcResult  Signal      CalcResult  StdConc
A01   439175      439175      0.0102890085
A02   220579      220579      0.00025121277
A03   368889      368889      0.00084115497
A04   202387      202387      0.00047132069
A05   345254      345254      0.00758479986
A06   171926      171926      0.00259427636
A07   200918      200918      0.00025081993
A08   184242      184242      0.00095179211
A09   166295      166295      0.00001798699
A10   203726      203726      0.00350957366
A11   158805      158805      0.00797755668
A12   90725  90725  0.00051541856
A13   317271      317271      0.00679190237
A14   374193      374193      0.00041882233
A15   276051      276051      0.00559228171
A16   157246      157246      0.0021718044
A17   835199      835199      0.01609720411
A18   185124      185124      0.00297427804
A19   316720      316720      0.00674022851
A20   145798      145798      0.00184175903
A21   654822      654822      0.01649799588
A22   157588      157588      0.00218145139
A23   295227      295227      0.00614448417
A24   174277      174277      0.00260190723
B01   222191      222191      0.00104974736
B02   133190      133190      0.00147911141
B03   190185      190185      0.00311999622
B04   210780      210780      0.00371297504
B05   143542      143542      0.00177703378
B06   81279  81279  0.00027225601
B07   158539      158539      0.00220883293
B08   182272      182272      0.00659999153
B09   179004      179004      0.00279806571
B10   110072      110072      0.00001228201
B11   158681      158681      0.00221292145
B12   165222      165222      0.00097700060
B13   122937      122937      0.00118376703
B14   75968  75968  -0.0001089257
B15   147904      147904      0.00190435363
B16   85600  85600  0.00011151011
```

Download

1.27.22_KL.txt (7.99 kB) 1.27

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Overview

Sheet 1: *Raw data*

Download

PicoGreen_of_gDNA_DBS_electrical_trials_2.xlsx (36.9 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

1-Feb-2022: Preparation of gDNA only DBSs #4-6

Goal: Prepare gDNA only DBSs

Procedure:

gDNA stock is at 242ng/uL and there is 100ug

1. it is about 419uL
2. Before I used 66.2uL into 334uL of PBS which = ~40ng/uL
3. Want to start with the similar concentrations and do 1:1 serial dilutions
    1. Started by diluting 400uL of the stock into 2000uL of TE buffer from Picogreen (=2400)
    2. Did 1:1 dilutions by having each dilution have 1000uL of TE buffer and added 1000uL of the previous solution into the new one.
        1. Took 1000uL of 20ng/uL and addded it to 1000uL of TE buffer for 10ng/uL, etc. for serial dilution
            1. used 1000 b/c was easiest to pipette
        2.
4. Let sit overnight
5. Nanodrop values: **Nanodrop_of_gDNA_only_DBSs.txt**

Kiara kiara_lee@brown.edu (klee104@brown.edu) - May 03, 2022, 6:31 PM EDT

Sample ID	User ID	Date	Time	ng/uL	A260	A280	260/280	260/230
	Constant	Cursor Pos.	Cursor abs.	340 raw				
40	Default	2/1/2022	3:45 PM	27.64	0.553	0.286	1.93	1.64	10.00	230
	0.337	0.003								
40	Default	2/1/2022	3:45 PM	28.33	0.563	0.274	2.05	1.31	10.00	230
	0.430	0.004								
30	Default	3/1/2022	3:46 PM	13.63	0.273	0.155	1.76	0.87	10.00	230
	0.308	2.672								
20	Default	2/1/2022	3:47 PM	14.38	0.288	0.136	2.11	1.64	10.00	230
	0.175	0.010								
10	Default	2/1/2022	3:52 PM	5.92	0.118	0.050	2.38	1.78	10.00	230
	0.070	0.007								
10	Default	3/1/2022	3:53 PM	0.45	0.139	0.088	1.90	1.59	10.00	230
	0.082	0.008								
5	Default	3/1/2022	3:55 PM	2.56	0.051	0.017	3.05	1.10	10.00	230
	0.034	0.022								
5	Default	3/1/2022	3:55 PM	3.56	0.071	0.022	3.24	1.66	10.00	230
	0.036	0.010								
2.5	Default	2/1/2022	3:55 PM	1.95	0.039	0.007	5.36	2.07	10.00	230
	0.019	0.001								
2.5	Default	3/1/2022	3:56 PM	2.02	0.040	0.016	2.46	1.47	10.00	230
	0.028	0.044								

Download

Nanodrop_of_gDNA_only_DBSs.txt (894 B)

Buffer compatibilty with DNA - Copy

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

27-Jan-2022: Test of Buffer qPCR Compatibility

Goal: see what DBS buffers (tested previously in "DBS elution pre-treatment variation") are compatible with qPCR directly

Procedure:

1. Made solutions that were 90% buffer + 10% human male gDNA (Promega, 242ng/uL)
    1. 2uL DNA + 18uL buffer
2. Used PBS, NucliSENS Lysis buffer, NucliSENS Lysis buffer/PBS, Tween/sodium azide/PBS, KOH/Tris, and the gDNA diluted in PicoGreen TE buffer as a control
    1. 1X PBS for all
3. Did qPCR
4. Conclusions:
    1. The results were mixed in the sense that the Cq value varied a lot. This could be due to the nature of gDNA pipetting, so I will do more replicates tomorrow with PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer
    2. Anything with the lysis buffer did not amplify

20220127_154629_CT047233_GENE_EXP_TAQ.zpcr

Buffer_compatability_DNA_trial_1.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

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20220127_154629_CT047233_GENE_EXP_TAQ.zpcr (203 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

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Buffer_compatability_DNA_trial_1.xlsx (27 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

28-Jan-2022: More replicates of buffer qPCR compatibility_

Goal: do more replicates of the buffer compatibility experiment (see 27-Jan)

Procedure:

1. Diluted the gDNA stock (242ng/uL) 1:10 in TE buffer (Picogreen) first
    1. Dilutions measured at:

Concentration (ng/uL)	**260/280**	**260/230**
150.3	1.95	2.04
171.1	1.96	2.25
77	1.95	2.11

1. Then, added 2uL of the diluted gDNA to 18uL of buffer (PBS, Tween/sodium azide/PBS, KOH/Tris, and gDNA diluted in PicoGreen TE buffer)
2. Also measured the qPCR standards
    1. qPCR standards measured at:
3.

Sample name	**Concentration (ng/uL)**	**260/280**	**260/230**
"40ng/uL"	20.5	1.97	1.40
"8ng/uL"	4.3	2.17	0.61

20220128_165240_CT047233_GENE_EXP_TAQ.zpcr

qPCR_buffer_compatibility_9_replicates.xlsx

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

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20220128_165240_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

Sheet 1: *20220128_165240_CT047233_GENE_E*

Download

qPCR_buffer_compatibility_9_replicates.xlsx (27.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:39 PM EDT

Overview

Sheet 1: *20220127_154629_CT047233_GENE_E*

Download

qPCR_buffer_compatibility_all.xlsx (43.5 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:40 PM EDT

28-Jun-2022: Test of inhibition of Cq values with the buffers

Goal: See how 1:10 dilutions affect the Cq value with the various buffers used, a test to see if the assay is inhibited.

Procedure:

1. Make a solution of gDNA that is constant
    1. gDNA was ~160ng/uL, diluted it 1:10
    2. Measured to be ~12ng/uL
2. Took the above solution and diluted 1:10 into the buffer the first sample and then serially diluted that 1:10 to test if the difference in Cq value was around 3.2.

This could be a better assay for the paper to show what inhibited qPCR the most.

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:40 PM EDT

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Buffer_inhibition_experiment.xlsx (29.4 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Aug 30, 2022, 11:40 PM EDT

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20220628_172129_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Supp. Fig. S2: Comparison of 2 donors

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST

9-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
    1. did 2x the 3mm volumes for 1 spot
    2. Used Heparin for PBS and EDTA for KOH

20220309_121148_CT047233_GENE_EXP_TAQ.zpcr

Mar_KOH_and_PBS_1s_pos_controls.xlsx

Use this for KOH EDTA March qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:38 AM EST

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Mar_KOH_and_PBS_1s_pos_controls.xlsx (29.7 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST

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20220309_121148_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:36 AM EST

28-Mar-2022: Test of new 1 spot positive controls with Mar blood + new KOH buffer

Goal: Have positive controls for the Mar blood for future experiments.

1. Followed the KOH/Tris protocol for 1 x 6mm spot
    1. did 2x the 3mm volumes for 1 spot
    2. Used KOH for Heparin and PBS for EDTA
2. I think that the PBS needs to be replaced... so will use a fresh one to repeat the EDTA and Heparin PBS ones.

20220328_154654_CT047233_GENE_EXP_TAQ.zpcr

qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx

Use this for Heparin KOH March qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST

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20220328_154654_CT047233_GENE_EXP_TAQ.zpcr (204 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:42 AM EST

Overview

Sheet 1: *20220328_154654_CT047233_GENE_E*

Download

qPCR_of_Hep_KOH_and_EDTA_PBS_March_controls.xlsx (27.8 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:35 AM EST

30-Mar-2022: PBS March controls, EDTA & Heparin

Goal: See yield from fresher PBS aliquot for EDTA and Heparin and use these as controls.

Followed the KOH/Tris protocol for 1 x 6mm spot

1. did 2x the 3mm volumes for 1 spot
2. Used Heparin and EDTA
3. Did qPCR and Picogreen

For Picogreen:

- Perform Picogreen with the usual protocol (see protocols tab)
- Used 1:2 dilutions for the samples.... 6uL of sample + 12uL of TE
    1. Did using multichannel pipette, though this was below its threshold of 10uL (6uL was probably as low as it can accurately go)
- Standard curve: 0.3, 0.15, 0.075, 0.0375... etc. 6-point curve 1:1 dilutions (before diluting them in Picogreen dye sln)
    1. 4uL of lambda DNA stock into 36uL of 1X TE buffer to make 10ng/uL solution. then take 12uL of that solution and add it to 388uL of 1X TE buffer to make 0.3ng/uL for standard curve
- Used 20uL volume onto the Picogreen plate

qPCR: **qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx**

Picogreen: **Picogreen_of_Mar_controls_KOH_Heparin_and_old_PBS_and_new_PBS_EDTA_and_Hep.xlsx**

Used this for PBS Heparin (Donor 2) & EDTA PBS (Donor 1) qPCR

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 05, 2023, 11:22 PM EST

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20220330_135758_CT047233_GENE_EXP_TAQ.zpcr (202 kB)

Kiara kiara_lee@brown.edu (klee104@brown.edu) - Jan 06, 2023, 12:44 AM EST

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qPCR_of_New_PBS_March_controls_EDTA_and_Hep.xlsx (30.8 kB)