Protocol for isolation of DNA from silica & RNA later preserved faeces
Vigilant Lab March 2011

When you open a new Qiagen QIAamp DNA Stool Kit:

1. Clean the bench and pipettes with bleach and ethanol

2. Add 25ml ethanol to AW1 and 30ml ethanol to AW2

3. Make aliquots of all the reagents depending on the size of the extractions you will be undertaking. For example, for extractions of 22 samples + 2 negative controls (24 tubes total) you should make the following aliquots:

ASL buffer*:	45 ml aliquot	* *Make sure the ASL and AL buffers have not precipitated before you make your aliquots, if they have, dissolve the precipitate by swirling the bottle of buffer in a ~70° C water bath (microwave is in extraction room, beakers can be found in hallway)*
AL buffer*:	15 ml aliquot	
Ethanol:	15 ml aliquot	
AW1:	13 ml aliquot	
AW2:	13 ml aliquot	
AE:	5 ml aliquot	

Extraction materials set-up:

1. Clean the bench and pipettes with bleach and ethanol

2. UV the necessary number of tubes for an extraction of 24 (22 samples + 2 negatives) you will need:

   - 24 X 2ml tubes (Eppendorf) (To weigh your samples into)
   - 24 X 2ml tubes (Eppendorf) (Inhibidex tablet step)
   - 24 X 2ml tubes (Eppendorf) (Proteinase K step)
   - 24 X 1.5ml tubes (Eppendorf) (Supernatant step 8, day 2)
   - ***24 X1.5 ml tubes (Eppendorf) (RNAlater extraction eluate ***for RNAlater extraction only!)*
   - **24 X 1.5ml SILICONIZED tubes**. The DNA eluate (in the final step of the protocol) should be put into siliconized tubes :
     stocks: 1.7ml microcentrifuge tubes art. T3406-250EA from Sigma
     aliquots: 0.65ml microcentrifuges tubes art. T3281-500EA from Sigma

     ***** IF THERE ARE NO SILICONIZED TUBES** then dilute the whole bottle AE buffer with Tween20 10% (the final concentration should be 0.05% Tween20) before aliquoting the AE. i.e. 60µl Tween20 10% for a full bottle of AE buffer.

3. Each set of tubes should be in a separate rack and Ued in that rack

4. Label all tubes, the first and last tube should be negative controls where all conditions are the same for the extraction except that no faeces is added to the tube.

5. Make sure you have enough gloves, pipette tips, and kimwipes

6. Make sure that the aliquots of buffers ASL and AL have not precipitated, if so dissolve in a 70°C water bath as described above

Day 1

(i) UV hood weighing of samples (numbers in paratheses are for a 24 tube extraction)

1. Turn on UV light for 5-10 minutes before weighing out feces in hood

2. Have the appropriate number of 2ml tubes in a rack for weighing samples into

3. Check list:
   * Latex gloves (switch between EACH sample),
   * Waste bag (from the autoclave room),
   * 1000 µl tips if using RNA later samples and pipetteman,
   * Scalpel blades + scalpel waste box,
   * Plastic tweezers in autoclaved beaker with bleach
   * Kim-wipes & bleach (wipe hood area with bleach between each sample)

(A) For RNAlater preserved faeces:

1. Shake faeces sample in RNAlater well

2. Cut tip of 1000µl pipetteman tip off by piercing plastic with scalpel blade and then cutting through.

3. Transfer 1000µl of liquid to a 2ml safelock-tube.

4. Centrifuge sample for 15 min @ **7 rcf**
(if pellet has not settled or becomes disturbed, centrifuge @ 13 rcf for 10 minutes, if still not settled then add 200µl 1XTE/0.9% NaCl buffer and centrifuge @ 13 rcf for 10 minutes, then take away most of the supernatant and proceed to step 3)

5. Remove ~800µl of the supernatant, place into 1.5 ml tube, reserve and freeze supernatant.

6. Add 500µl of 1XTE/0.9% NaCl buffer to "pellet" and gently mix by inverting

7. Centrifuge for 10 min @ **7 rcf**

8. Discard supernatant

9. Add 1.6 ml ASL to pellet, vortex and incubate for ~10 min R.T. Go to Day2 of protocol.
(Can also leave overnight in shaker @23°C shaking intermittently as in step 7 for silica samples, see below)

(B) For silica preserved faeces:

1. Wipe bleach from tweezers, place on fresh Kim-wipe (after handling feces, replace tweezers in bleach, repeat)

2. Tare scale with small weighing boat, place fresh Kim-wipe in hood, place large weighing boat on Kim-wipe, and transfer sample from silica (in tube) to large boat, cut off a chunk of feces and weigh

3. Aim for ~0.1g faeces, place in labelled 2ml safelock-tube

4. Clean up (bleach entire workspace, wipe bleach off all tweezers and place upside down in beaker, pleace a new Kleenex under weighing boats) and put UV on in hood minimum 10 minutes.

5. Waste should go into the autoclave waste bucket in the lab kitchen

6. Add 1.7ml ASL to each sample (1.4ml for negative controls), vortex very well, place in thermoblock

7. Samples soak over night @ 23°C in thermoblock mixer cycle program: 5min shake (500rpm), 30 min no shake OR leave at 500rpm shaking (no pause). Samples can soak in ASL for up to 3 days.

8. Make sure to shut iff the UV in hood before you leave.

Day2

(iii) Extraction for both RNAlater & silica preserved faeces:

Check list:
* Set thermoblock mixer to 70°C
* Latex gloves (switch between EACH sample)
* Separate pipettemen for DNA and DNA-free reagents
* Pipette tips -1000µl, several boxes

1. Clean the bench and pipettes with bleach and ethanol

2. Place an InhibitEx tablet in the appropriate number of the 2ml tubes. Close tubes.

3. Add 25ul proteinase-K in the appropriate number of 2ml tubes. Close tubes. tube (Proteinase K provided from Qiagen, 20 mg/ml)

4. Centrifuge samples for 3 min full speed to pellet feces

5. Transfer all of the supernatant into a 2ml tube containing the InhibitEX tablet and vortex vigorously until the tablet is completely suspended. Discard pelleted faeces.

6. Incubate suspension for few minutes at room temperature

7. Centrifuge at full speed for 10 min

8. Transfer ALL the supernatant into a new 1,5ml-tube

9. Centrifuge the pellet AGAIN at full speed for 3 min

10. Transfer ALL the supernatant into the tube from step 6, discard the pellet (you need 600 µl supernatant for step 11) steps 6 and 7 may be repeated

11. Centrifuge supernatant at full speed for 6 min

12. **1 TUBE AT A TIME:** Transfer 600µl supernatant from step 9 to the 2ml-tube containing proteinase K, add 600µl buffer AL and IMMEDIATELY mix by vortexing for 15 sec, quick spin and incubate at 70°C for 10 min

13. Add 600µl of 100% ethanol to the lysate and mix by vortexing. Once ethanol is added to all tubes, quick spin tubes down in centrifuge. (Samples can sit in ethanol for up to 1 hour)

14. Carefully apply 600 µl of the mix from step 12 to a QIAamp spin column without moistening the rim.

15. Centrifuge at full speed for 2 min, place the spin column in a new 2ml-collection tube, discard tube ϖ filtrate

16. Apply a second aliquot of the mix from step 12 to the column and centrifuge at full speed for 2 min, place the spin column in a new 2ml-collection tube and discard tube ϖ filtrate

17. Apply the last aliquot of filtrate (600µl) and centrifuge at full speed for 2 min, place the spin column in a new 2ml-collection tube and discard tube ϖ filtrate

18. Wash the column with 500µl AW1, centrifuge at full speed for 2 min, place the spin column in a new 2ml-collection tube, discard tube ϖ filtrate

19. Wash the column with 500µl buffer AW2, centrifuge at full speed for **6 min** discard tube with filtrate

20. Transfer spin column into a new labelled 1,5ml **SILICONIZED** tube and pipette 200µl buffer AE directly onto the membrane

    ***** IF THERE ARE NO SILICONIZED TUBES** then dilute the whole bottle AE buffer with Tween20 10% (the final concentration should be 0.05% Tween20) before aliquoting the AE. i.e. 60µl Tween20 10% for a full bottle of AE buffer.

21. Incubate for at least 30 min (and up to 2 hours) at room temperature then centrifuge at full speed for 2 min to elute DNA into the siliconized tube.

(iv) After the extraction:

1. Organize the bench

2. Clean the bench and pipettes with ethanol and bleach

3. Tie up and remove small plastic bags of trash (bring to autoclave room), replace plastic bags

4. If big trash can is full, put it outside the door and bring the can outside the room into the room

5. Re-stock gloves, pipettes, and kimwipes

6. UV or wash racks