MICROCON DNA FAST FLOW DNA CONCENTRATION AND PURIFICATION		
DATE EFFECTIVE 06-20-2016	APPROVED BY NUCLEAR DNA TECHNICAL LEADER	PAGE 1 OF 10

Microcon DNA Fast Flow DNA Concentration and Purification

Microconning a DNA extract is useful when attempting to concentrate it, clean it of lysate and chemical inhibitors or both. The procedure differs slightly depending on which of these results are desired.

Microconning can also be used to combine duplicate DNA extracts (for example, when there is a _A and _B replicate from an M48 Extraction that needs to be recombined). Combination microcons can be performed on any Microcon type, although a new quantitation should be performed in order to obtain the most accurate value.

"Microcon to concentrate" – bringing the total volume of the DNA extract down, therefore concentrating the DNA; initial and final volumes are recorded and the new concentration is calculated by $C_1V_1 = C_2V_2$ in the LIMS Data Entry.

"Microcon to clean" – when cleaning or purifying a DNA extract, it is necessary to perform a wash step with a solution (ie, TE^{-4} or water); the initial volume is recorded and the elution is returned to that same volume. The concentration of the DNA extract remains the same.

"Microcon to clean and concentrate" – a combination of both steps; the wash step is performed and the total volume of the DNA extract is brought down. A new quantitation should be performed in order to obtain the most accurate value, although the new concentration may be calculated in the LIMS Data Entry.

Note: When using the High Yield DNA Extraction Procedure, the Microcon procedure in Section C of that Procedure must be used.

In order to allow for duplicate amplifications, the final volume should be 25µL - 50µL.
See Table 1 for minimum sample concentration requirements.

It is recommended that swab remains fractions from differential extractions be eluted to a final volume of 50µL.

I. LIMS Pre-Processing

1. In the ***Analytical Testing*** » ***Test Batches*** tram stop, select the appropriate quantitation assay and click ***Edit.***

   Note: If you are creating a new microcon test batch use the ***New Test Batch*** tram stop followed by the ***Create New Test Batch*** wizard. In that wizard, include the following information: description, functional group, analysis, batch configuration, and test batch type (case test batch).

2. If necessary, click ***Add Unknowns*** and select any samples that need to be included on the test batch.

3. If no samples are being combined/pooled, select ***All Input Samples*** » Click ***Add Output Sample*** » 🔍 » Mcon***** » Click ***Select and Return*** » Click ***Ok*** » Click ***Create***

   * "Mcon" signifies that the sample is being microconned. This Output Sample Type will automatically add a "_mcon" suffix to all of the samples and controls (except for the Microcon negative control).

3a. For a sample being combined/pooled, select only those ***Input Samples*** » Click ***Pooled Sample***

- Create a new and consistent ***Tube Label***
- Remove the underscore from the end of the ***Sample Name***
- Select the ***Suffix*** "_mcon"
- Click ***Save***

4. Record the ***QCBatch Params*** located at the top of the screen. Select the type of microcon being performed (Clean Sample, Concentrate Sample or Clean & Concentrate Sample). Make sure to *release* and save all data stored in the ***QCBatch Params*** tab.

5. If you have created the output samples and recorded the QCBatch Params, you must fill out the ***Performed By*** tab indicating you completed *Batch Setup Review*.

- Select ***Batch Setup Review*** » Click ***Fill Perform By/Date***

- Click ***Save*** » Click ***Return to List***
- Select the test batch » Click ***Ready***

6. If you are the analyst performing the assay, generate a ***Test Batch Pick List Report*** to help locate the samples needed in the laboratory.

   - Select the desired assay in the ***Analytical Testing*** » ***Test Batches*** tram stop
   - On the side bar, click ***Choose Report » Test Batch Pick List Report***

7. If not already in the test batch, go to the ***Analytical Testing » Test Batches*** tram stop, select the appropriate assay and click ***Edit***

8. In the ***Performed By*** tab, select ***Microcon*** **»** Click ***Fill Perform By/Date*** **»** Click ***Save***

9. Using the date and time listed in the ***Performed By*** tab, update the Description in the ***main test batch*** tab (located at the top of the page) with the following format:

   MCONdate_time [e.g. MCON012115_0815]

10. Click ***Save***

II. Assay Preparation

1. Retrieve the following reagents:

0.1X TE^{-4}
Fish Sperm, 1mg/mL
UltraPure_H2O 15

2. Retrieve samples needed for microcon from the associated refrigerator and/or freezer.

3. Record lot numbers in LIMS **»** Click ***Save***

4. Calculate the Fish Sperm DNA Solution needed for the assay in the ***Reagents*** tab: Input the **Per Sample Amount** for the ***Fish Sperm, 1mg/mL*** and ***UltraPure_H2O 15*** as listed below **»** Click ***Save***. Select ***Fish Sperm, 1mg/mL*** and ***UltraPure_ H2O 15*** **»** Click ***Calculate Amount*** **»** Click ***Save***

Reagent	1 sample
UltraPure Water	199 µL
Fish Sperm DNA (1mg/mL)	1 µL

Note: **For samples with 400 µL, make a 20 µL solution of 1 uL of Fish Sperm DNA (1mg/mL). Mix well and add this solution to the membrane. Ensure that the entirety of the membrane is covered. In this manner, all of the sample may be added to the Microcon® membrane for a total volume of 420 uL.**

5. Label a sufficient number of blue Microcon® DNA Fast Flow sample reservoirs and insert each into a labeled collection tube. Print OUTPUT sample labels and label a sufficient number of 1.5mL Eppendorf tubes for elution.

6. Prepare the Fish Sperm DNA Solution as calculated in LIMS and pre-coat each Microcon® membrane with 200µL of solution. Avoid touching the membrane.

7. Process 50µL of TE^{-4} solution as a Microcon negative control. Make sure to use the same lot that will be used to dilute the samples, and don't forget to label the final negative control tube with the Microcon date and time.

8. Spin each DNA sample briefly.

9. **Witness Step:**
   a. Arrange samples in the order as they appear in the Test Batch.
   b. Confirm the sample names and order on the documentation by reading the tube-top label and complete INPUT sample ID, also read the tube-top label and complete OUTPUT sample ID for each sample.
   c. Have witness fill out the ***Witness*** tab in LIMS.

10. Measure and record the initial volume using an adjustable Micropipette to the nearest microliter. Select ***All Output Samples*** » Click ***Data Entry*** » enter the current sample concentration in the [Conc, Initial] column and the volume in the [Vol, Initial] column for each sample » Click ***Save.***

    a. For a sample being combined/pooled, chose either of the current sample concentrations for the [Conc, Initial] and the combined volume for the [Vol,

Initial]. The resulting sample should be re-quantified to obtain the most accurate concentration.

b. Add each sample (0.4 mL maximum volume) to the buffer in the reservoir. Don't transfer any Chelex beads, or in case of an organic extraction sample, any organic solvent! Seal with attached cap. ***Avoid touching the membrane with the pipette tip!***

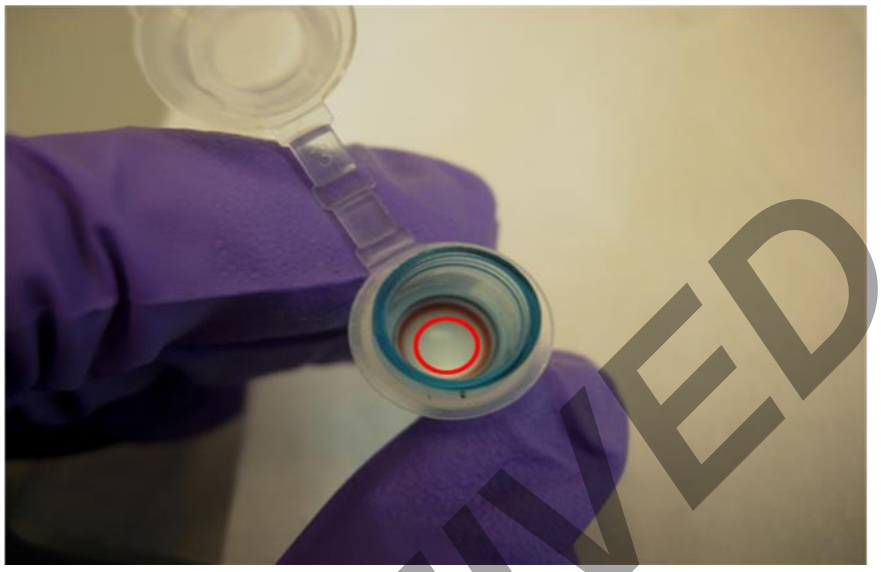
11. Return the original extraction tubes to their storage location. Do not discard the empty tubes.

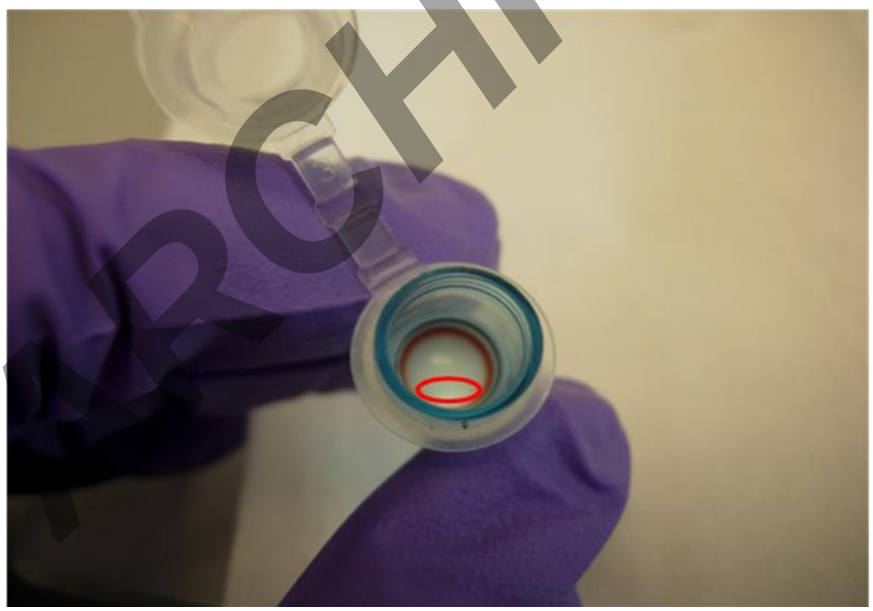
12. Place the Microcon assembly into a variable speed microcentrifuge. Make sure all tubes are balanced! ***To prevent failure of device, do not exceed recommended g-forces.***

13. Spin at 500 x g (2400 RPM, Eppendorf) for 12 minutes at room temperature. ***Do not centrifuge too long (the membrane should not be allowed to become completely dry)***.

14. Remove assembly from centrifuge. Visually inspect each Microcon® membrane tube. If it appears that more than 2µL remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm. This process may be repeated as necessary. ***Do not centrifuge too long (the membrane should not be allowed to become completely dry).***

Note: The Microcon® membrane filter should appear barely dry in the center with a faint ring of liquid visible around the edges ***BEFORE*** purification or elution. Please see the images below for clarification:





Troubleshooting:

Lint, bone dust, oils and other particles can clog the membrane. If the filtrate does not appear to be moving through the Microcon® membrane, elute the filtrate and continue centrifuging the eluant into a fresh Microcon® with a pre-coated membrane. During

transfer, pipette off the clear supernatant without disturbing any particle pellet that may have formed. Negative controls should be treated accordingly.

If the problem persists, the specific Microcon lot number might be faulty. Notify the QA Unit and try a different lot number.

Note: In this case, add the following comment in the ***Notes*** section of the ***main test batch*** tab (located at the top of the page):

"Two microcon filters were used for [Sample/Samples] FBXX-YYYYY_ sample name, etc… and [its/their] associated negative control."

15. *****Purification Step – ONLY perform for "Microcon to clean" or "Microcon to clean and concentrate" Microcon assays*****
(otherwise, skip to Step 16):
    a. Transfer the filter to a new collection tube, then add 200µL of TE^{-4} solution to the Microcon® membrane, carefully pipetting up and down in order to resuspend the DNA into solution and repeat Steps 12-14.
    b. Do this as often as necessary to generate a clear extract, and then continue with Step 16. When performing multiple wash steps it may be necessary to empty the bottom collection tube intermittently.
    c. The Microcon® membrane filter should appear barely dry in the center with a faint ring of liquid visible around the edges ***BEFORE*** elution. Please see the images above for clarification.

    Note: When purifying samples with a low DNA concentration it may be advantageous to perform a **"Microcon to clean and concentrate" assay** with several wash steps and to also reduce the volume; this leads to both a cleaner sample and an increased DNA concentration.

16. Once the sample is ready to elute, add 20µL TE^{-4} to the sample reservoir ***Avoid touching the membrane with the pipette tip!*** Separate the collection tube from the sample reservoir.

17. Place sample reservoir upside down in a new **labeled** collection tube, then spin for 3 minutes at 1000 x g (3400 RPM Eppendorf). Make sure all tubes are balanced!

18. Remove from centrifuge and discard the sample reservoir. Measure the resulting volume in the collection tube using an adjustable Micropipette and transfer to the **labeled** 1.5mL elution tube; adjust volume to desired level using TE^{-4}.
    a. **"Microcon to concentrate" assay:** low DNA concentration samples sent for microcon concentration are to be reconstituted between 25µL - 50µL. See Table 1 for minimum sample concentration requirements.
    b. **"Microcon to clean" assay:** high DNA concentration samples sent for microcon clean-up are to be reconstituted to their initial volume.
    c. **"Microcon to clean and concentrate" assay:** DNA samples sent for microcon clean-up and concentration are to be reconstituted between 25µL - 50µL. See Table 1 for minimum sample concentration.

19. Record the resulting volumes and volume adjustments of each sample in the LIMS Data Entry Screen. Select ***All Output Samples*** » Click ***Data Entry*** » enter the resulting volume in the [Vol, Result] column, any additionally added volume in the [Vol, H2O or

TE] column and the final elution volume in the [Vol, Final] column for each sample » Click ***Save***.

20. Ensure that LIMS has calculated the new concentration of each sample under the [Conc, Calc] column. Highlight that column and click ***Push Concentration***. Ensure that the new, calculated concentration is listed next to the Sample Name.

Note: The initial and calculated concentrations for samples that have not yet been quantified will be listed as "0pg/µL.

21. Assign the samples to a storage cryobox. Store the extracts at 2 to 8ºC or frozen.
- From the drop-down menu in the LIMS Data Entry screen select ***All*** » Click ***Assign Storage***
- Select ***Target SU*** » scan cryobox
- Select all ***Samples*** » Click ***Auto File***
- Click ***Save*** » Click ***Close*** » Click ***Return To List***

ATTENTION: **Do not store the DNA in the Microcon vials! The lids are not tight enough to prevent evaporation**.

TABLE 1:

	Identifiler™ 28 cycles	**Identifiler™ 31 cycles**
Minimum Desired Template	100.00 pg	^20.00 pg
Template volume for amp	5 µL	5 µL
Minimum Sample Concentration in 200 µL	20 pg/µL	^4 pg/µL
Minimum Sample Concentration in 200 µL prior to Microconning* to 50 µL	5 pg/µL	N/A
Minimum Sample Concentration in 200 µL prior to Microconning** to 25 µL	2 pg/µL	0.40 to ^0.10 pg/µL
For LCN samples: Minimum	20.00 pg/µL	**4.00 to ^1.00**

	Identifiler™ 28 cycles	Identifiler™ 31 cycles
Sample Concentration in 25 µL		pg/µL

* Sample concentration **prior** to processing with a Microcon DNA Fast Flow and elution to 50 µL

** Sample concentration **prior** to processing with a Microcon DNA Fast Flow and elution to 25 µL

^ Samples with less than 20 pg per amplification may be amplified upon referral with the LCN supervisor

III. LIMS Post Processing I

1. If not already in the test batch, go to the ***Analytical Testing » Test Batches*** tram stop, select the appropriate quantitation assay and click ***Edit***

2. In the ***Performed By*** tab, select ***Test Batch Review*** **»** Click ***Fill Perform By/Date*** **»** Click ***Save***

3. Check the remaining tabs to ensure all have been filled out properly.

4. Select the ***Output Samples*** **»** Click ***Data Entry***

5. In the ***Data Entry*** screen, ensure that the correct concentration is listed next to the Sample Names **»** Click ***Return To List***

6. Select the ***Output Samples*** **»** Click ***Review***

7. Perform the Test Batch Approval of the Microcon assay and schedule samples for the next test as necessary.