Microcon DNA Fast Flow DNA Concentration and Purification		
Status:Published		Document ID: 1141
DATE EFFECTIVE 12/09/2025	APPROVED BY Nuclear DNA Technical Leader	PAGE 1 OF 6

Microcon DNA Fast Flow DNA Concentration and Purification

1 Overview

1.1 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.

1.2 Microconning can also be used to combine duplicate DNA extracts (for example, when there is a _A and _B replicate from an 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.

1.3 "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.

1.4 "Microcon to clean" – when cleaning or purifying a DNA extract, it is necessary to perform a wash step with a solution (i.e., 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.

1.5 "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.

1.6 In order to allow for duplicate amplifications, the final volume should be at least 25µL. If the starting volume is <25µL notify the lab supervisor or your supervisor before proceeding.

2 Assay Preparation

2.1 Retrieve the following reagents:

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

2.2 Retrieve samples needed for microcon from the associated refrigerator and/or freezer and scan them into your custody.

2.3 Record reagent lot numbers in LIMS.

2.4 Select which Microcon process (Clean, Concentrate, Clean & Concentrate) is being performed from the *Processed* dropdown.

2.5 The Mixture Information table contains the reagent amounts for the master mix. Click Recalculate if you updated the number of samples on the worksheet and need to recalculate the amount. Use the volumes listed in the "Total Quantity" column to prepare the master mix (LIMS will calculate 1.2x the number of samples)

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 µL 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 µL .**

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

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

2.8 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.

2.9 Spin each DNA sample briefly.

2.10 Witness Step:

2.10.1 Arrange samples in the order as they appear in the worksheet.

2.10.2 Confirm the samples by reading the LIMS label for each sample.

2.10.3 Have witness fill out the ***Tube Setup Witness*** tab in LIMS.

2.11 Measure and record the initial volume using an adjustable Micropipette to the nearest tenth of a microliter. Enter the current sample concentration in the [Starting Conc, Initial] column and the volume in the [Initial Volume] column for each sample » Click ***Save.***

2.11.1 For a sample being combined/pooled, select either of the current sample concentrations for the [Starting Conc, Initial] and the combined volume for the [Initial Volume]. The resulting sample should be re-quantified to obtain the most accurate concentration.

2.11.2 Add each sample (0.4 mL maximum volume) to the buffer in the reservoir. Don't transfer, any organic solvent! Seal with attached cap. ***Avoid touching the membrane with the pipette tip!***

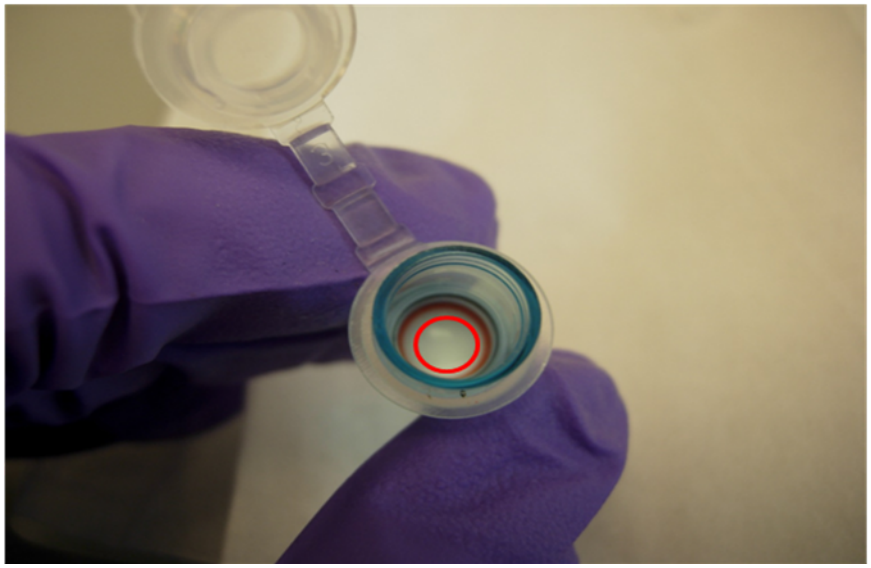
2.12 Return the original extraction tubes to their storage location. Do not discard the empty tubes.

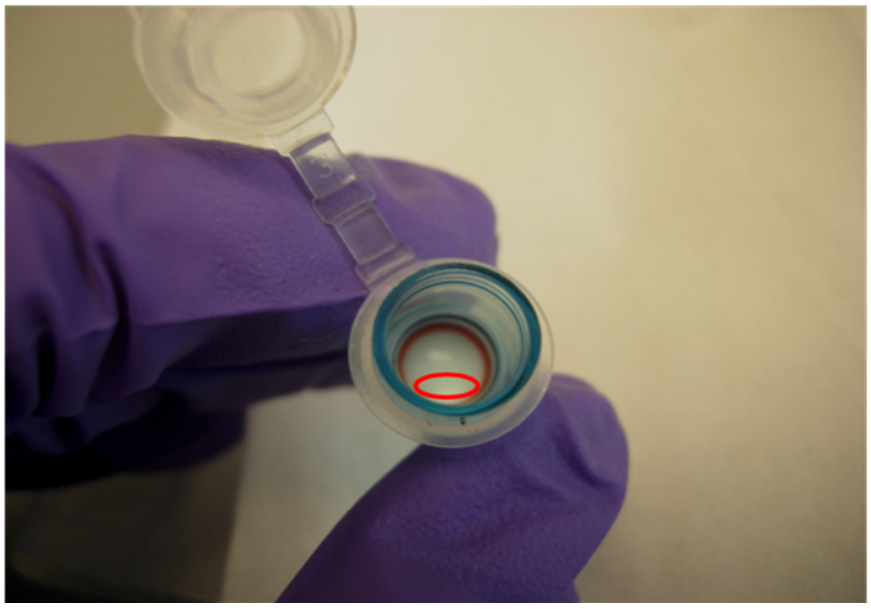
2.13 Place the Microcon assembly into a variable speed microcentrifuge. Make sure all tubes are balanced! ***To prevent failure of device, do not exceed recommended g-forces.***

2.14 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).

2.15 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).***

2.15.1 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:





2.16 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.

2.16.1 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 ***Run By review task comment*** section:

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

2.17 ***Purification Step – ONLY perform for "Microcon to clean" or "Microcon to clean and concentrate" Microcon assays*****(otherwise, skip to Step 2.18):

2.17.1 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 re-suspend the DNA into solution and repeat Steps 2.13. - 2.15.

2.17.2 Do this as often as necessary to generate a clear extract, and then continue with to Step 2.18. When performing multiple wash steps it may be necessary to empty the bottom collection tube intermittently.

2.17.3 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.

2.18 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.

2.19 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!

2.20 Remove from centrifuge and discard the sample reservoir. Measure the resulting volume to the nearest tenth of a microliter 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}.

2.20.1 **"Microcon to concentrate" assay:** low DNA concentration samples sent for microcon concentration are to be reconstituted to a minimum of 25uL and a maximum of the initial volume.

2.20.2 **"Microcon to clean" assay:** high DNA concentration samples sent for microcon clean-up are to be reconstituted to their initial volume.

2.20.3 **"Microcon to clean and concentrate" assay:** DNA samples sent for microcon clean-up and concentration are to be reconstituted to a minimum of 25uL and a maximum of the initial volume.

2.21 Record the resulting volumes and volume adjustments of each. the final elution volume in the [Final Volume] column for each sample » Click ***Save***.

2.22 Ensure that LIMS has calculated the new concentration of each sample under the [Resulting Conc.] column.

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

2.23 Fill out the 'Pass/Fail' response and complete the Run By review task.

2.24 Create the QUANTTRIO submission for the samples.

2.25 Verify the samples listed on the Trio Submission Worksheet click [Save] and close the Submission worksheet.

2.26 Assign the samples to a storage cryobox and record the transfer in LIMS. Store the extracts at 2 to 8°C or frozen.

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