

# Userguide

epMotion® | No 015

## Normalization of DNA/RNA Samples using the DNA/RNA Normalization Calculator

Renate Fröndt, Eppendorf AG; Barbara Schmidt, Eppendorf Instrumente GmbH, Hamburg, Germany

### Abstract

One of the most time consuming methods in a molecular biology laboratory is normalizing raw DNA and RNA samples to a specified concentration in the samples.

Manually, this is a very complicated process because each sample must be calculated separately for the amount of DNA or RNA and for the required diluent.

To facilitate this process Eppendorf has introduced a new calculating tool for normalization of samples (DNA/RNA) for the automated pipetting system epMotion. This tool can calculate the required sample and diluent volumes automatically. The generated list is uploadable to the epMotion via epBlue or epMotion Editor.

### Introduction

After purification of DNA or RNA, different samples can have very different amounts and concentrations of nucleic acids. However, for a downstream PCR-reaction, all samples should have the same amount of DNA or RNA as starting material.

The new DNA/RNA Normalization Calculator calculates all required samples and diluent volumes. The results can be uploaded to epMotion software by a CSV file. Then, the epMotion dispenses the correct volume of DNA/RNA samples and the correct volume of diluent and mastermix automatically into each well of a plate or in single tubes. This program works with up to 500 samples.

In this Userguide, we will show how to use this tool utilizing a generic example.

### Materials and Methods

- epMotion 5070 or epMotion 5075
- epMotion Editor
- epMotion PC-Version
- Normalization Calculator
- DNA or RNA samples
- Mastermix
- Diluent

### Working process

In this example, the DNA concentration in each well after purification of gDNA from blood is quite different (Table 1).

Tab. 1: List of DNA samples after purification

Well	Concentration ng/µl	Well	Concentration ng/µl	Well	Concentration ng/µl	Well	Concentration ng/µl
A1	103	B2	71	A3	71	B4	56
C1	200	D2	71	C3	84		etc.
E1	93	F2	67	E3	76		
G1	68	H2	70	G3	70		

In this case, the samples are too concentrated for a PCR or qPCR reaction. For the normalization process, the epMotion dilutes the samples from the elution plate to another plate, e.g., by 1:20. For using the epMotion, it is necessary to have a volume bigger than 1 µl in each well. Afterwards, the samples have the correct concentration range and the calculation of the amount of each sample can be started.

## Using the normalization list of epMotion

	A	B	C	D	E	F	G	H
1	Sample	Concentration	Dilution ratio	DNA-amount /	Template	Total volume	Mastermix	Dilution
2		of		Reaction		in PCR tube	volume	volume
3		of DNA-samples						(water)
4								
5	Name,		Dilute: 1 to	Template amount:		Volume:	Volume:	
6	ID, No		20	10		40	18	
7		ng/µl	ng/µl	ng	µl	µl	µl	µl
8	1	103	5.2	10	1.9	40	18.0	20.1
9	2	200	10.0	10	1.0	40	18.0	21.0
10	3	93	4.7	10	2.2	40	18.0	19.8
11	4	68	3.4	10	2.9	40	18.0	19.1
12	5	71	3.6	10	2.8	40	18.0	19.2
13	6	71	3.6	10	2.8	40	18.0	19.2

Fig. 1: Normalization Calculator worksheet

First, the results of the concentrated samples are entered into the DNA/RNA Normalization Calculator worksheet 1 (normalization list). The DNA/RNA Normalization Calculator is an Excel worksheet (Figure 1).

In the second step, the dilution rate, the template amount, the total volume of the PCR reaction, and the volume of the mastermix are entered into the blue fields.

It is also possible to enter a sample name or sample ID. For each sample, the volume of the template and the diluent (water) for the whole reaction will automatically be calculated.

The CSV file is on worksheet 2. It is automatically connected to worksheet 1. The columns A to F must be sorted as follows: "Rack" (source rack), "Source" (source position),

"Rack" (destination rack), "Destination" (destination position), "Volume" (transfer volume in µl), and "Tool" (dispensing tool).

To illustrate the required file structure, the following example (Fig. 2) shows the first few rows of the CSV file as displayed in working sheet 2.

Remark: If you use Microsoft Excel to create or edit a CSV file for import, make sure that the Regional Settings in the Windows Control Panel on your PC are set to "English", i.e. that the decimal point is used for decimal numbers, and that the comma is used as the standard separator for lists. Save your edited table in CSV format before closing Excel (you do not need to save it as an Excel file as well).

	A	B	C	D	E	F
1	Rack	Source	Rack	Destination	Volume	Tool
2						
3	1	A1	1	A1	1.9	1
4	1	C1	1	A5	1.0	1
5	1	E1	1	C6	2.2	1
6	1	G1	1	D6	2.9	1
7	1	B2	1	D7	2.8	1
8	1	A6	1	A6	2.8	1

Fig. 2: CSV file

“Volume” is the volume of the template from working sheet 1 (normalization list), which is needed for PCR reactions.

Finally, the working sheet 2 must be saved as a CSV file.

With this CSV file, it is possible to create a new application on the epMotion (epBlue) or create a new application via epMotion Editor. The new application must start with the “Number of Samples” command as the first step.

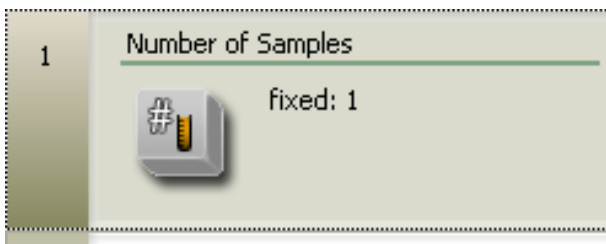


Fig. 3: Command “No of Samples”

Activate the option “Fix number of samples” and set the number of samples to 1.

The number of samples for the following steps has now been limited so that each Sample Transfer command which follows will only be carried out once, i.e. for one sample. As the second step in the procedure, add a Sample Transfer command.

This first Sample Transfer command and its source and destination locations on the worktable will serve as the master setting for the entire sequence of commands which will be imported from the CSV file. Only the source and destination locations defined manually in this first Sample Transfer command will be available for use during the sequence of imported commands.

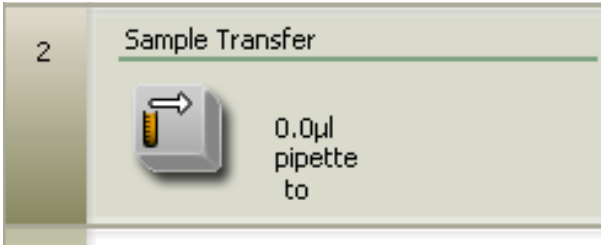


Fig. 4: Command “Sample Transfer”

To import the sequence of commands from the file, click on the Sample Transfer command in the program list to make sure it is selected.

Select **Edit – Import from CSV** from the main menu.

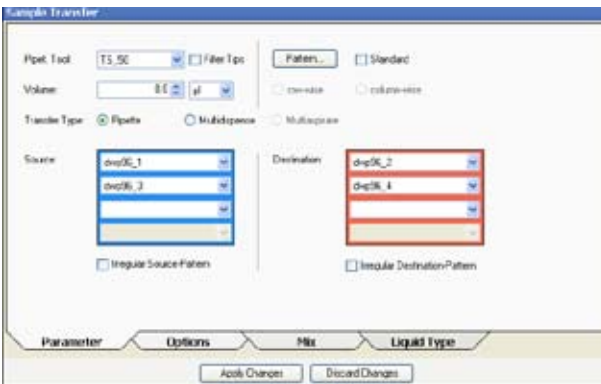


Fig. 5: Sample Transfer

Select the CSV file you want to import, and click **Open**. The CSV file is imported. Each row defined in the CSV file is added to the procedure as a Sample Transfer command with settings for source, destination, volume and tool defined in the file. The imported sequence of commands is displayed in the program list.

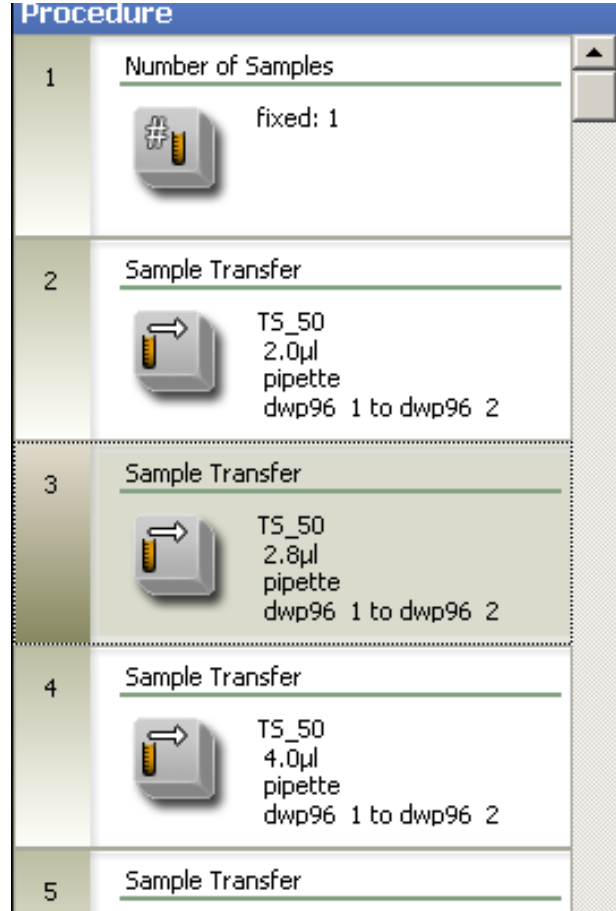


Fig. 6: Application with loaded CSV file

The epMotion works with this program and automatically fills all sample wells which are defined in the normalization list for the downstream reaction.

## Conclusion

Working with the DNA/RNA Normalization Calculator is a real simplification for calculating the amounts of DNA/RNA samples needed after an elution step. This is of great help, e.g., when setting up PCR or qPCR reactions.

## References

Operation manual epMotion® 5070  
 Operation manual epMotion 5070 with integrated PC and epBlue  
 Operation manual epMotion 5075  
 Operation manual epMotion 5075 with integrated PC and epBlue

## Ordering information

Description	International Order no.	North American Order no.
epMotion® 5070	5070 000.000	960000005
epMotion® 5070 with integrated PC	5070 000.140	960000111
epMotion® 5075 LH	5075 000.008	960020006
epMotion® 5075 LH with integrated PC	5075 000.750	960020111
epMotion® 5075 VAC	5075 000.016	960020014
epMotion® 5075 VAC with integrated PC	5075 000.768	960020222
epMotion® 5075 MC	5075 000.032	960020022
epMotion® 5075 MC with integrated PC	5075 000.776	960020333
epBlue – epMotion® PC software	5075 016.001	
epMotion® Editor	5075 014.009	960000269



**Your local distributor: [www.eppendorf.com/worldwide](http://www.eppendorf.com/worldwide)**

Eppendorf AG · 22331 Hamburg · Germany · Tel. +49 40 538 01-0 · Fax +49 40 538 01-556 · E-Mail: [eppendorf@eppendorf.com](mailto:eppendorf@eppendorf.com)

Eppendorf North America, Inc. · One Cantiague Road, P.O. Box 1019 · Westbury, N.Y. 11590-0207 USA · Tel. +1 516 334 7500

Toll free phone 800 645 3050 · Fax +1 516 334 7506 · E-Mail: [info@eppendorf.com](mailto:info@eppendorf.com)

## Application Support

Europe, International: Tel. +49 1803 666 789 · E-Mail: [support@eppendorf.com](mailto:support@eppendorf.com)

North America: Tel. 800 645 3050 ext. 2258 · E-Mail: [support\\_NA@eppendorf.com](mailto:support_NA@eppendorf.com)

Asia, Pacific: Tel. +603 8023 2769 · E-Mail: [support\\_AsiaPacific@eppendorf.com](mailto:support_AsiaPacific@eppendorf.com)