# Manual MPS Library Amplification of the Mitochondrial DNA Control Region with the PowerSeq® CRM Kit

## 1 Purpose

1.1 To increase the amount of available mtDNA for the purposes of DNA sequencing, by performing an *in vitro* replication of template DNA in ten amplicons. Adaptors are incorporated to facilitate sequencing-by-synthesis.

## 2 Preparation

2.1 Retrieve the following reagents and thaw if necessary. After thawing, the components should be vortexed for 5 seconds.

| |
|---|
| PowerSeq® 5x Master Mix |
| PowerSeq® CRM Nested 10x Primer Pair Mix |
| 10x Index D5 Primer (All relevant tubes) |
| 10x Index D7 Primer (All relevant tubes) |
| 2800M control DNA (10 ng/µL) |
| Amplification-grade Water |

2.2 Log all reagent lot numbers in LIMS as appropriate.

2.3 Retrieve a 96-well Eppendorf semi-skirted PCR plate and label it with the run name followed by "amplified libraries."

2.4 Locate the Powerseq® Sample Sheet for your run.

2.5 Preparing Sample Dilutions:

2.5.1 Referring to the PowerSeq® Sample Sheet and/or LIMS, determine if the samples for your run require a dilution prior to amplification**.** The dilution factor is based on the nuclear DNA concentration for each sample as presented in Table 1.

| Table 1: Dilution Factors | |
|---|---|
| **Template Concentration (pg/µL)** | **Dilution Factor** |
| No nuclear conc. available | No dilution needed |
| 1-500 | No dilution needed |
| 501-5000 | 0.1 |
| 5001-50000 | 0.01 |
| 50000-99000 | 0.001 |

2.5.2 If dilutions are needed, retrieve and label 1.5 mL microcentrifuge tubes.

2.5.3 Perform the sample dilutions by adding template DNA and Promega amplification grade water in the volumes indicated in Table 2 below. Briefly vortex and centrifuge samples prior to aliquoting for dilution.

| Table 2: Dilutions | | |
|---|---|---|
| **Dilution** | **Volume of DNA Template (µL)** | **Volume of Water, Amplification Grade (µL)** |
| 0.1 | 2 | 18 |
| 0.01 | 2 | 198 |
| 0.001 | * | * |

*Perform a 0.1 dilution followed by a 0.01 dilution.

2.6 Preparing Positive Control:

2.6.1 Retrieve and label a 1.5 mL microcentrifuge tube.

2.6.2 Vortex and briefly centrifuge the stock 2800M Control DNA (10 ng/µL).

2.6.3 Dilute the control DNA to 0.1 ng/µL by adding the 2800M Control DNA and Promega amplification grade water in the volumes indicated in Table 3 below.

| Table 3: Preparing Positive Control | |
|---|---|
| **Volume of 2800M Control DNA (µL)** | **Volume of Water, Amplification Grade (µL)** |
| 2 | 198 |

2.7 Preparing master mix:

2.7.1 Retrieve and label a 1.5 mL microcentrifuge tube.

2.7.2 Referring to the PowerSeq® Sample Sheet or LIMS, create the master mix by adding PowerSeq® 5x Master Mix and PowerSeq® CRM Nested 10x Primer Pair Mix in the

calculated volumes. (The volumes are indicated in cells I42 and I43 of the completed PowerSeq Sample Sheet.)

| Reagent | Per Reaction |
|---|---|
| PowerSeq® 5x Master Mix | 5.0 µL |
| PowerSeq® CRM Nested 10x Primer Pair Mix | 2.5 µL |

2.7.3 Vortex and centrifuge briefly.

2.8 Arrange Your Samples and Reagents:

2.8.1 Samples should be arranged to begin in well A2. Up to 32 samples can be batched to form an amplification set. Thus, samples should be loaded in wells A2-H5.

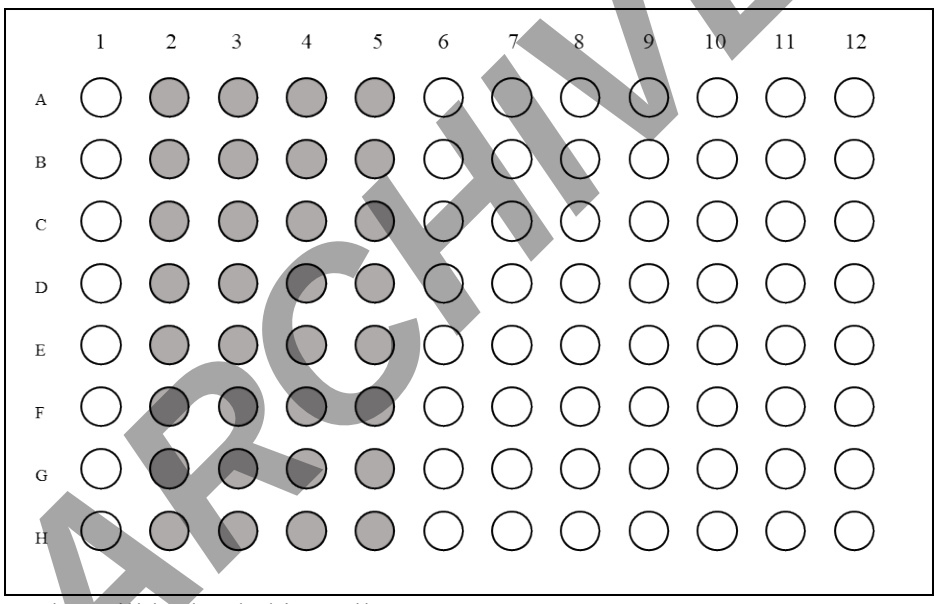


**Figure 1**: Samples will be loaded in wells A2-H5.

2.8.2 Vortex and centrifuge the samples briefly. Arrange your samples in the order indicated on the PowerSeq® Sample Sheet and/or LIMS.

2.8.3 Arrange the index primers for your run in the order indicated on the PowerSeq® Sample Sheet and/or LIMS. The arrangement of the index primers should result in a unique combination of index primers for each sample.

2.8.3.1 A different D5 index primer will be loaded in each row of the 96-well plate. The specific index primers due to be used for your run will be indicated on the PowerSeq® Sample Sheet and/or LIMS.

2.8.3.2 A different D7 index primer will be loaded in each column of the 96-well plate. The specific index primers due to be used for your run will be indicated on the PowerSeq® Sample Sheet and/or LIMS.

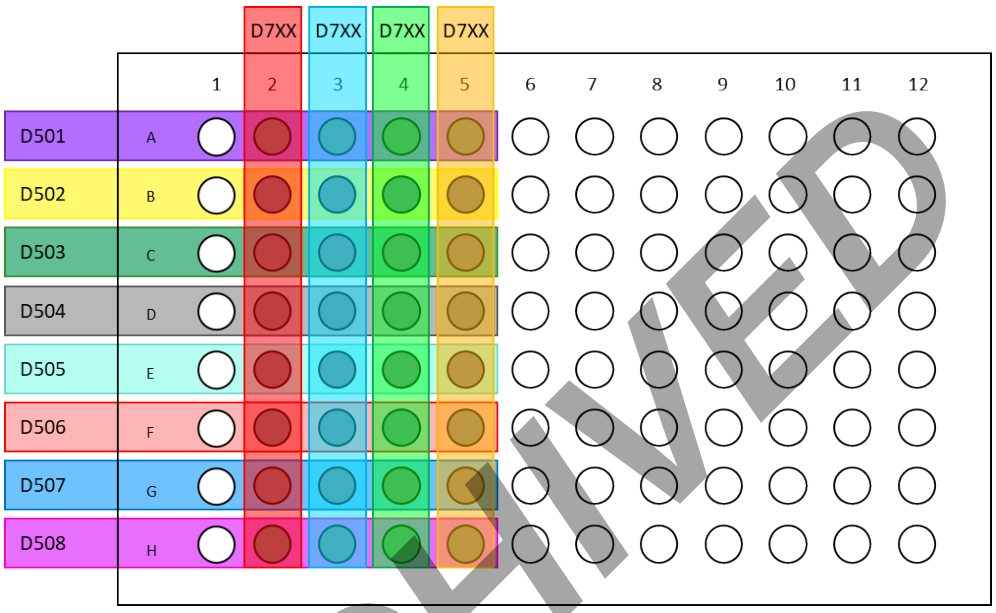


**Figure 2**: Generic Plate Layout for PowerSeq® CRM Library Amplification

2.9 WITNESS STEP

2.9.1 Before proceeding, ensure that your controls and extracts are in the proper order, and that your array of index primers is properly laid out. Check all sample orders against your sample sheet and the layout in LIMS, as appropriate. Have your setup witnessed and logged in LIMS.

# 3 Procedure

3.1 Load 7.5 µL of master mix into each well in use.

3.2 Load 2.5 µL of the index primers to each well in the appropriate arrangement.

3.2.1 A multichannel pipet may be used to load the index primers. If using a multichannel pipet, follow steps 3.2.1.1-3.2.1.5 below.

3.2.1.1 Retrieve an unused 96-well plate.

3.2.1.2 Into column 1 of the new plate load 12.5 µL of each D5 primer in the appropriate order.

3.2.1.3 Beginning with well A7, load 22.5 µL of each D7 primer to the new plate horizontally from well A7-A10. Make sure the index primers are loaded in the appropriate order.

3.2.1.4 Using a multichannel pipet, transfer 2.5 µL of D5 primer to each well of the amplified library plate that will be in use. Change pipet tips as each column is loaded.

3.2.1.5 Using a multichannel pipet, transfer 2.5 µL of D7 primer to each well of the amplified library plate that will be in use. Change pipet tips as each row is loaded.

3.3 Consult the sample sheet and load the indicated volume of each sample or control to the appropriate wells of the plate.

3.4 Consult the sample sheet and load the indicated volume of Promega amplification grade water to each well of the plate to bring the total sample volume up to 25 µL.

3.5 Seal the plate using Bio-Rad film Microseal A.

3.6 Vortex the plate on a plate mixer at 1000 rpm for 1 minute. Centrifuge the plate at 1000 rpm for 1 minute.

# 4 PowerSeq® CRM on the Applied Biosystems GeneAmp PCR System 9700

4.1 Turn on the ABI 9700 Thermal Cycler.

4.2 Place the plate on the thermal cycler and close the lid.

4.3 PowerSeq® CRM Conditions for the Applied Biosystems GeneAmp PCR System 9700

| | |
|---|---|
| 9700<br><br>**PowerSeq® CRM**<br><br>user: **casework**<br>file: (TBD) | The PowerSeq® CRM file is as follows:<br><br>Soak at 96°C for 10 minutes<br><br><u>30 cycles:</u><br>Denature at 96°C for 5 seconds<br>Anneal at 60°C for 35 seconds<br>Extend at 72°C for 5 seconds<br><br>2 minute incubation at 60°C.<br><br>Storage soak indefinitely at 4°C. |

4.4 Record instrument in LIMS

4.5 The run will start when the heated cover reaches temperature. The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed, hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

4.6 Upon completion of the amplification

4.6.1 Remove sample-plate and press the STOP button repeatedly until the “End of Run” screen is displayed.

4.6.2 Select the EXIT option (F5)

4.6.3 Wipe any condensation from the heat block with a lint free wipe and pull the lid closed to prevent dust from collecting on the heat block.

4.6.4 Turn the instrument off

4.7 After completion of the thermal cycling protocol, if not proceeding immediately with purification, centrifuge the plate and store the amplified product at -20 °C.