

Cross-Contamination test with capped REMP 96-900 Tube Technology™

Background

The REMP 96-900 Tube Technology™ in capped version should be used to store DNA in an automated storage system. After retrieval and automated de-capping of tube racks, aliquots of the individual DNA content will automatically be transferred into process plates for further experiments. Immediately after this step the tubes should be capped and re-stored for future purposes.

It is essential to have no cross-contamination (including aerosol appearance) during the automated de-capping and capping of the tubes .

For verification the following test including PCR-amplification has been used:

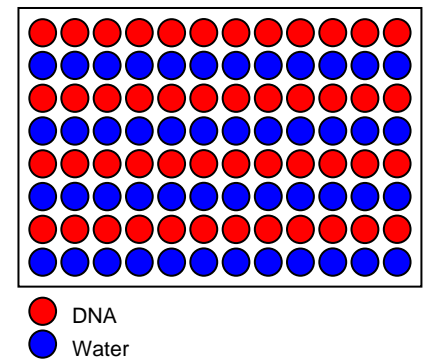
REMP Equipment and Consumables used:

- 1 empty Rack of REMP 96-900 Tube Technology™ pre-capped with blue caps (PN 30021858)
- 1 96 well REMP Automated Capper/Decapper, ACD96 (PN 30021833)

Preparation of PCR master mix

While the Tube Rack will be used as storage template for DNA respectively water (see array on the illustration), the PCR experiments are carried out on PCR-plates.

PCR master mix:	µl (well)		µl (total)
10 x buffer	2.5	} x 110	275
MgCl ₂	3.0		330
dNTPs	0.5		55
Primer F	0.4		44
Primer R	0.4		44
Taq	0.15		16.5
H ₂ O	17.05		1,875.5
<u>DNA (or water)</u>	<u>1.0</u>		<u>transferred from Tube Rack</u>
Total	25		2,640.0



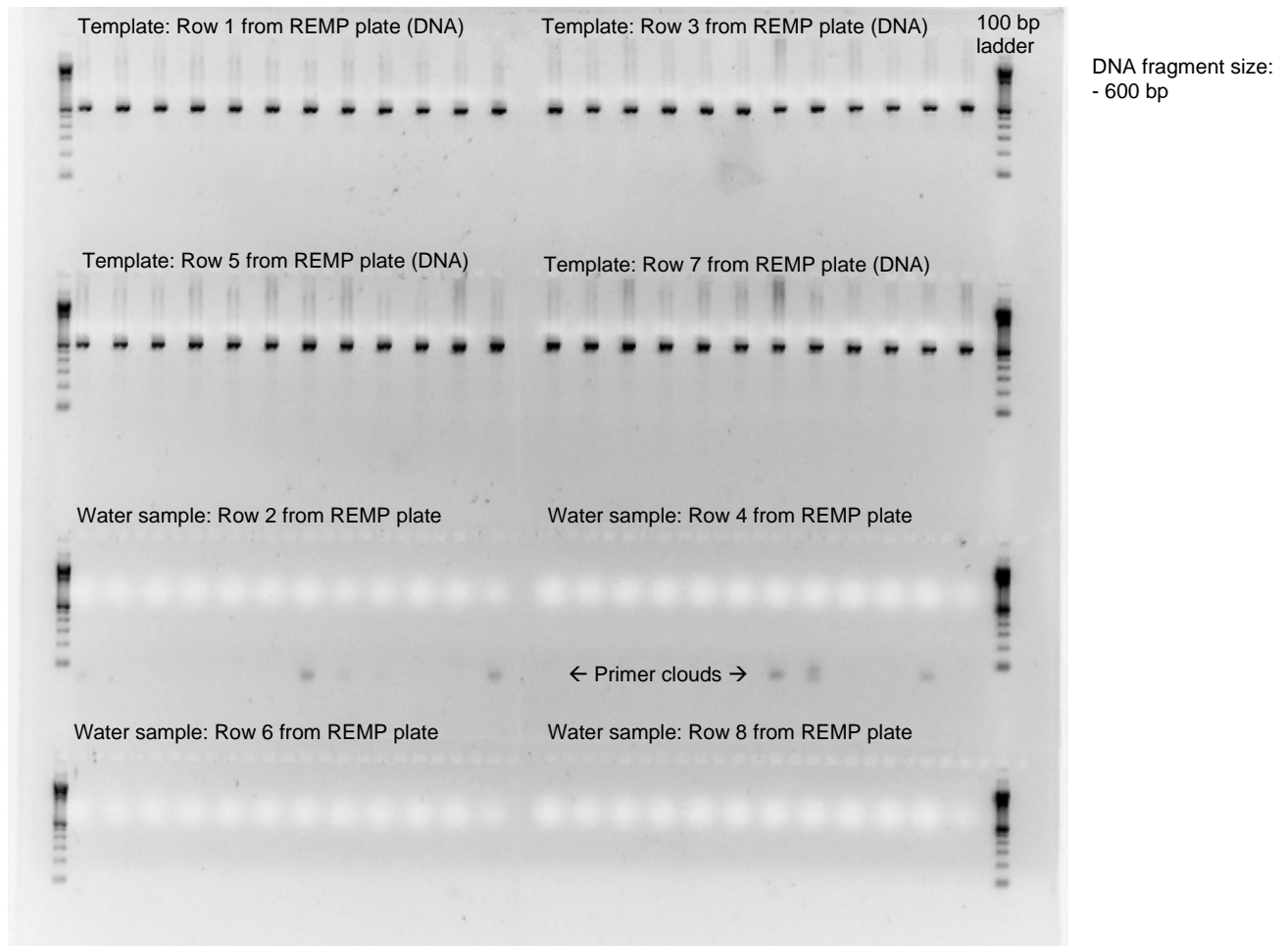
Experiment:

- The pre-capped Tube Rack was de-capped using the ACD96 (caps stayed inside the ACD96).
- 700 µl DNA (100 ng/µl) respectively 700 µl PCR water were transferred into the Tube Rack in alternating rows of 12.
- For an initial PCR verification aliquots of each tube content were transferred to the corresponding position of a prepared PCR-plate (as shown in the PCR master mix preparation list). The PCR cycling was executed as shown below.
- The Tube Rack was re-capped, turned once upside down and back and then centrifuged (approx. 1 min. at 1000 rpm) to force all liquids towards the bottom of the tubes.
- Then de-capped and re-capped the Tubes 20 times in the ACD96
- Once again, a PCR of every row was performed according to the same protocol.
- Gel electrophoresis comparison of both PCRs (rows of DNA or water) with regard to potential contamination due to the capping process.

PCR cycling:

95°	12 min	
95°	30 s	} 40 cycles
60°	30 s	
72°	1 min	
72°	10 min	

Gel after 20 x de-capping and re-capping



Result:

The picture shows the PCR amplified results of the alternating DNA or water content of each row of 12 tubes after 20x de-capping and re-capping. While the amplified DNA fragments (Row 1, 3, 5 and 7) are shown with good contrast, the water samples (blanks) of row 2, 4, 6, and 8 does not show any DNA fragments

No contamination was detected.

Special thanks to:

For making these experiments happen and grant a permit to REMP AG of using the results in this test report:

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