

**CLAIMS**

1. A reaction vessel (20) for a PCR device (10) for performing PCR, the reaction vessel  
5 (20) comprising:

a reaction chamber (33) and a storage chamber (63) for receiving a liquid sample  
containing at least one target DNA;

a spacer element (42) extending into the reaction chamber (33); and

a porous membrane (51) for hybridization of the at least one target DNA within the  
10 liquid sample onto at least one specific hybridization probe immobilised on the porous  
membrane (51);

wherein the reaction chamber (33) and the storage chamber (63) are configured to be  
in fluid communication via the porous membrane (51) and a fluid channel defined by the  
spacer element (42) and

15 wherein the membrane (51) is configured such that in a dry state the porous membrane  
(51) allows the passage of air or other gases as well as liquid therethrough and in a wet state  
the porous membrane (51) still allows the passage of liquid therethrough, but blocks the  
passage of air or other gases therethrough,

wherein the lower end of the spacer element (42) extends into the reaction chamber  
20 (33), but does not reach the bottom thereof, and wherein the upper end of the spacer element  
(42) is located in close proximity of and preferably in abutting relationship with the porous  
membrane (51).

2. The reaction vessel (20) of claim 1, wherein the reaction vessel (20) is configured such  
25 that during a PCR the liquid sample remains in the reaction chamber (33) and after the PCR  
the liquid sample can be forced via the spacer element (42) through the porous membrane  
(51) for hybridization and subsequent detection of the at least one target DNA in the liquid  
sample.

30 3. The reaction vessel (20) of claim 1 or claim 2, wherein the reaction vessel is  
configured  
to provide an overpressure in the storage chamber (63) and a vacuum or an underpressure  
in the reaction chamber (33) or

to provide a vacuum or an underpressure in the storage chamber (63) and an overpressure in the reaction chamber (33),

to move at least the liquid sample at least once, preferably at least five times, and most preferably at least ten times back and forth through the porous membrane (51) while

5 remaining in contact therewith.

4. The reaction vessel (20) of any one of claims 1 to 3, in particular of claim 1, wherein the distance between the lower end of the spacer element (42) and the bottom of the reaction  
10 chamber (33) is between 0.1 and 0.5 cm.

5. The reaction vessel (20) of any one of claims 1 to 4, wherein the porous membrane (51) comprises a nylon material.

15 6. The reaction vessel (20) of any one of claims 1 to 5, wherein the reaction chamber (33) is defined by a sample vial (32) provided as part of a bottom element (30) and/or the storage chamber (63) is defined by a storage vessel (62) provided as part of a top element (60).

20 7. The reaction vessel (20) of any one of claims 1 to 6, in particular of claim 6, wherein a center element (40) is provided, which is arranged or which is configured to be arranged between the top element (60) and the bottom element (30), and wherein the center element (40) preferably comprises the spacer element (42).

25 8. The reaction vessel (20) of any one of claims 1 to 7, wherein the reaction chamber (33) is in fluid communication with a liquid supply port (34) for supplying the liquid sample containing at least one target DNA to the reaction chamber (33).

30 9. The reaction vessel (20) of any one of claims 1 to 8, in particular of claim 8, wherein the liquid supply port (34) is connected with the reaction chamber (33) by means of a first groove (37, 437).

10. The reaction vessel (20) of any one of claims 1 to 9, in particular of claim 8 or claim 9, wherein at least one guide member (47, 48) is provided, which is configured to guide the liquid sample supplied by the liquid supply port (34) into the reaction chamber (33).

5 11. The reaction vessel (20) of any one of claims 1 to 10, in particular of claim 10, wherein two guide members (47, 48) are arranged at the spacer element (42), preferably at the upper end of the spacer element, such that the liquid from the first groove (37, 437) is guided into the reaction chamber (33), and is prevented from further flowing along the upper end of the spacer element (42).

10 12. A cartridge (100) for a PCR device, comprising:  
a plurality of reaction vessels (20) according to any one of claims 1 to 11; and/or  
a plurality of individually controllable fluid channels in respective fluid  
communication with the plurality of reaction vessels (20) for supplying liquid samples to a  
15 plurality of reaction vessels (20).

13. A PCR device (10), comprising:  
at least one reaction vessel (20) according to any one of claims 1 to 11; and/or  
heating and/or cooling means (12a, 12b), such as resistive heating means and/or  
20 convective cooling means, for heating and/or cooling the reaction chamber (33) and/or the  
storage chamber (63) of the at least one reaction vessel (20); and/or  
pressure supply means (14a, 14b) for providing a pressure differential between a first  
pressure port (35) in fluid communication with the reaction chamber (33) and a second  
pressure port (36) in fluid communication with the storage chamber (63) of the at least one  
25 reaction vessel (20); and/or  
liquid supply means (16) for supplying a liquid sample and/or a reaction agent liquid  
to the reaction chamber (33) of the at least one reaction vessel (20) via a liquid supply port  
(34) thereof; and/or  
optical excitation and detection means (18), such as an appropriate light source and a  
30 CCD or CMOS detector including appropriate optical elements, for optical interrogation of  
the porous membrane (51) of the at least one reaction vessel (20), preferably by means of  
epifluorescence.

14. A method of performing PCR using a reaction vessel (20) according to any one of claims 1 to 11, comprising the steps of:

(a) performing the PCR with a liquid sample including at least one target DNA to be amplified in the reaction chamber (33) such that the liquid sample does not come into contact with the lower end of the spacer element (42) extending into the reaction chamber (33),

(b) after completing at last one cycle of the PCR, bringing the liquid sample into contact with the lower end of the spacer element (42) and forcing the liquid sample including at least one amplified target DNA via the spacer element (42) through ~~a~~ the porous membrane (51) for hybridization,

wherein during step (a) the porous membrane is kept dry and allows the passage of air or other gases as well as liquid therethrough and during step (b) the porous membrane (51) becomes wet and still allows the passage of liquid therethrough, but blocks the passage of air or other gases therethrough.

15. The method of claim 14, wherein the liquid sample is brought into contact with the lower end of the spacer element (42) by adding a hybridization buffer and/or another liquid to the liquid sample after the PCR.

16. The method of claim 14 or claim 15, wherein liquid sample is moved at least once, preferably at least five times, and most preferably at least ten times back and forth through the porous membrane (51).

17. The method of any one of claims 14 to 16, wherein pressures below 1.4 bar, more preferably in the range from 50 to 250 mbar and most preferred in the range from 100 to 200 mbar are used to force the liquid sample through the porous membrane (51).

18. The method of any one of claims 14 to 17, wherein the porous membrane (51) is heated during the PCR to a temperature of at least 80°C, preferably at least 100°C.

19. The method of any one of claims 14 to 18, wherein for optical detection of the porous membrane (51) the liquid sample is moved back into the spacer element (42).

## CLAIMS

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1. A reaction vessel for a PCR device for performing PCR, the reaction vessel comprising:

a reaction chamber and a storage chamber for receiving a liquid sample containing at least one target DNA;

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a spacer element extending into the reaction chamber; and

a porous membrane for hybridization of the at least one target DNA within the liquid sample onto at least one specific hybridization probe immobilised on the porous membrane;

15 wherein the reaction chamber and the storage chamber are configured to be in fluid communication via the porous membrane and a fluid channel defined by the spacer element and

20 wherein the membrane is configured such that in a dry state the porous membrane allows the passage of air or other gases as well as liquid therethrough and in a wet state the porous membrane still allows the passage of liquid therethrough, but blocks the passage of air or other gases therethrough,

wherein the lower end of the spacer element extends into the reaction chamber, but does not reach the bottom thereof, and wherein the upper end of the spacer element is located in close proximity of and preferably in abutting relationship with the porous membrane.

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2. The reaction vessel of claim 1, wherein the reaction vessel is configured such that during a PCR the liquid sample remains in the reaction chamber and after the PCR the liquid sample can be forced via the spacer element through the porous membrane for hybridization and subsequent detection of the at least one target DNA in the liquid sample.

30 3. The reaction vessel of claim 1 or claim 2, wherein the reaction vessel is configured to provide an overpressure in the storage chamber and a vacuum or an

underpressure in the reaction chamber or

to provide a vacuum or an underpressure in the storage chamber and an overpressure in the reaction chamber,

to move at least the liquid sample at least once, preferably at least five times,

5 and most preferably at least ten times back and forth through the porous membrane while remaining in contact therewith.

4. The reaction vessel of any one of claims 1 to 3, wherein the distance between the lower end of the spacer element and the bottom of the reaction chamber is between 0.1 and 0.5 cm.

5. The reaction vessel of any one of claims 1 to 4, wherein the porous membrane  
10 comprises a nylon material.

6. The reaction vessel of any one of claims 1 to 5, wherein the reaction chamber is defined by a sample vial provided as part of a bottom element and/or the storage chamber is defined by a storage vessel provided as part of a top element.

7. The reaction vessel of claim 6 wherein a center element is provided, which is  
15 arranged or which is configured to be arranged between the top element and the bottom element, and wherein the center element preferably comprises the spacer element.

8. The reaction vessel of any one of claims 1 to 7, wherein the reaction chamber is in fluid communication with a liquid supply port for supplying the liquid sample containing at least one target DNA to the reaction chamber.

20 9. The reaction vessel of claim 8 wherein the liquid supply port is connected with the reaction chamber by means of a first groove.

10. The reaction vessel of either one of claims 8 or 9 wherein at least one guide member is provided, which is configured to guide the liquid sample supplied by the liquid supply port into the reaction chamber .

11. The reaction vessel of claim 9 wherein two guide members are arranged at the spacer element, preferably at the upper end of the spacer element, such that the liquid from the first groove is guided into the reaction chamber, and is prevented from further flowing along the upper end of the spacer element .

5 12. A cartridge for a PCR device, comprising:  
a plurality of reaction vessels according to any one of claims 1 to 11; and/or  
a plurality of individually controllable fluid channels in respective fluid  
communication with the plurality of reaction vessels for supplying liquid samples to a  
plurality of reaction vessels.

10 13. A PCR device, comprising:  
at least one reaction vessel according to any one of claims 1 to 11; and/or  
heating and/or cooling means, such as resistive heating means and/or convective  
cooling means, for heating and/or cooling the reaction chamber and/or the storage  
chamber of the at least one reaction vessel; and/or  
15 pressure supply means for providing a pressure differential between a first  
pressure port in fluid communication with the reaction chamber and a second pressure  
port fluid communication with the storage chamber of the at least one reaction vessel;  
and/or  
liquid supply means for supplying a liquid sample and/or a reaction agent liquid  
20 to the reaction chamber of the at least one reaction vessel via a liquid supply port  
thereof; and/or  
optical excitation and detection means, such as an appropriate light source and a  
CCD or CMOS detector including appropriate optical elements, for optical  
interrogation of the porous membrane of the at least one reaction vessel, preferably by  
25 means of epifluorescence.

14. A method of performing PCR using a reaction vessel according to any one of claims  
1 to 11, comprising the steps of:

(a) performing the PCR with a liquid sample including at least one target DNA  
to be amplified in a the reaction chamber such that the liquid sample does not come into  
30 contact with the lower end of the spacer element extending into the reaction chamber,

(b) after completing at last one cycle of the PCR, bringing the liquid sample into contact with the lower end of the spacer element and forcing the liquid sample including at least one amplified target DNA via the spacer element through the porous membrane for hybridization,

5        wherein during step (a) the porous membrane is kept dry and allows the passage of air or other gases as well as liquid therethrough and during step (b) the porous membrane becomes wet and still allows the passage of liquid therethrough, but blocks the passage of air or other gases therethrough.

15        15.    The method of claim 14, wherein the liquid sample is brought into contact with the lower end of the spacer element by adding a hybridization buffer and/or another liquid to the liquid sample after the PCR.

16.    The method of claim 14 or claim 15, wherein liquid sample is moved at least once, preferably at least five times, and most preferably at least ten times back and forth through the porous membrane .

15        17.    The method of any one of claims 14 to 16, wherein pressures below 1.4 bar, more preferably in the range from 50 to 250 mbar and most preferred in the range from 100 to 200 mbar are used to force the liquid sample through the porous membrane .

18.    The method of any one of claims 14 to 17, wherein the porous membrane is heated during the PCR to a temperature of at least 80°C, preferably at least 100°C.

20        19.    The method of any one of claims 14 to 18, wherein for optical detection of the porous membrane the liquid sample is moved back into the spacer element.