

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,

20 wherein the lower end of the spacer element (42) extends into the reaction chamber
(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, wherein the lower end of the spacer element (42) extends into the reaction chamber (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).~~
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54. The reaction vessel (20) of any one of claims 1 to 43, in particular of claim 41, wherein the distance between the lower end of the spacer element (42) and the bottom of the reaction chamber (33) is between 0.1 and 0.5 cm.

65. The reaction vessel (20) of any one of claims 1 to 54, wherein the porous membrane (51) comprises a nylon material.

76. The reaction vessel (20) of any one of claims 1 to 65, 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).
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87. The reaction vessel (20) of any one of claims 1 to 76, in particular of claim 76, 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).
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98. The reaction vessel (20) of any one of claims 1 to 87, 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).
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1409. The reaction vessel (20) of any one of claims 1 to 98, in particular of claim 98, wherein the liquid supply port (34) is connected with the reaction chamber (33) by means of a first groove (37, 437).

1410. The reaction vessel (20) of any one of claims 1 to 1409, in particular of claim 98 or claim 1409, 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).

1411. The reaction vessel (20) of any one of claims 1 to 1410, in particular of claim 1410, 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).

1412. A cartridge (100) for a PCR device, comprising:
a plurality of reaction vessels (20) according to any one of claims 1 to 1411; 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 plurality of reaction vessels (20).

1413. A PCR device (10), comprising:
at least one reaction vessel (20) according to any one of claims 1 to 1411; and/or
heating and/or cooling means (12a, 12b), such as resistive heating means and/or 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 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 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.

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1514. A method of performing PCR using a reaction vessel (20) according to any one of claims 1 to 11, comprising the steps of:

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

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(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,

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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.

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1615. The method of claim 1514, 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.

1716. The method of claim 1514 or claim 1615, 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).

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1817. The method of any one of claims ~~15 to 17~~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).

~~19~~18. The method of any one of claims ~~15 to 18~~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.

5 ~~20~~19. The method of any one of claims ~~15 to 19~~14 to 18, wherein for optical detection of the porous membrane (51) the liquid sample is moved back into the spacer element (42).