

CLAIMS

1. A device for synthesizing oligonucleotides, preferably DNA strands, in which the device comprises the following components:

- a reagent container receptacle (1) for holding a reagent container support (17) comprising multiple reagent containers (18) for reagents for the synthesis of oligonucleotides;
- an interchangeable microfluid chip (10) comprising a synthesis chamber (14) for the synthesis of oligonucleotides, fluid connectors (11) for the conveying of the reagents as well as microfluid valves (12), wherein the fluid connectors (11) are connected to the synthesis chamber (14) via a fluid connection (13) and the fluid connection (13) between a respective fluid connector (11) and the synthesis chamber (14) can be interrupted or released by means of a microfluid valve (12);
- a control device (5) for controlling the microfluid valves (12);
- fluid connecting means (2) for the transport of the reagents from the reagent containers (18) to the fluid connectors (11) of the microfluid chip (10), wherein in a loading position the device can be loaded with the microfluid chip (10), and the reagent container receptacle (1) can be loaded with the reagent container support (17),
- a chip receptacle (3) for holding the microfluid chip (10),

characterized in that

an actuator device (6) is provided, with which the reagent container receptacle (1), the microfluid chip (10), by means of the chip receptacle (3), and the fluid connecting means (2) can be brought from the loading position to an operating position, in which operating position the reagent container receptacle (1), the chip receptacle (3) and the fluid connecting means (2) are positioned relative to each other by means of the actuator device (6) in such a way, that the microfluid valves (12) of the microfluid chip (10) are connected to the control device (5), that the reagent containers (18) arranged in the reagent container receptacle (1) are connected to the fluid connectors (11) of the microfluid chip (10) via the fluid connecting means (2), so that depending on the valve position of the microfluid valves (12) reagents can be conveyed from the reagent containers (18) in the direction of the synthesis chamber (14).

2. The device according to claim 1, **characterized in that** the control device (5) has a pneumatic system for controlling the microfluid valves (12).

3. The device according to any one of the claims 1 to 2, **characterized in that** the actuator device (6) has a linear movement axis (7) and the positioning of the reagent container receptacle (1) or fluid connector pieces (2a) or chip receptacle (3) occurs along the linear movement axis (7).

4. The device according to any one of the claims 1 to 3, **characterized in that** the actuator device (6) has a rack (8), wherein the fluid connecting means (2) are attached firmly to the rack (8) and wherein the reagent container receptacle (1) and the chip receptacle (3) are shiftably or swivellably attached to the rack (8) in the direction of the fluid connecting means (2), in order for the reagent container receptacle (1) and the microfluid chip (10) to be moved from the loading position to the operating position and vice versa.

5. The device according to any one of the claims 1 to 4, **characterized in that** the chip receptacle (3) has a connector portion (3b) for the connection of the microfluid chip (10) to the control device (5), wherein the connector portion (3b) has control line channels (22) for the pneumatic system of the control device (5), which are connected to a respective microfluid valve (12) of the microfluid chip (10) in the operating position.

6. The device according to any one of the claims 1 to 5, **characterized in that** the components of the device in the operating position are positioned relative to each other in the following order, preferably as seen in the direction of the movement axis (7):

- reagent container receptacle (1)
- fluid connecting means (2)
- chip receptacle (3) with microfluid chip (10), wherein the fluid connectors (11) are arranged a side of the microfluid chip (10) facing the fluid connecting means (2) and the microfluid valves (12) are attached to the control device on a side of the microfluid chip (10) opposite to the fluid connectors (11).

7. The device according to any one of the claims 1 to 10, **characterized in that** each fluid connecting means (2) comprises a connector piece (2a) for connection with one of the fluid connectors (11) of the microfluid chip (10) and a, preferably needle-like, transport line (2b) for connection to one of the reagent containers (18) and that pneumatic connecting means (4) are provided, which are connectable to the reagent containers (18), via which pneumatic connecting means (4) in the operating position a pneumatic system is connected to the reagent containers (18) arranged in the reagent container receptacle (1), in order to convey reagents from the reagent containers (18) via the fluid connecting means (2) in the direction of the microfluid chip (10) by means of the gas pressure of the pneumatic system.

8. The device according to claim 7, **characterized in that**, in the operating position, one end of a transport line (2b) of one of the fluid connecting means (2) and one end of one of the pneumatic connecting means (4) is respectively positioned within one of the liquid reagent containers (18), in order to convey reagents from the reagent container (18) in the direction of the synthesis chamber (14) by means of gas pressure of the pneumatic system, wherein the transport line (2b) and the pneumatic connecting means (4) penetrate a sealing element (19) of the corresponding reagent container (18).

9. The device according to claim 7 or 8, **characterized in that**, in the operating position, at least one pair of corresponding reagent containers (18) is provided in the reagent container receptacle (1), wherein one of the reagent containers (18) contains a solid (B1, B2, B3, B4), preferably a phosphoramidite of the bases adenine, guanine, cytosine, thymine or uracil in powder form, and one of the reagent containers (18) contains solvent (SOL1, SOL2, SOL3, SOL4), preferably acetonitrile, and that in the operating position one end of one of the pneumatic connecting means (4) is arranged in the reagent container (18) holding the solvent (SOL1, SOL2, SOL3, SOL4), and one end of one of the transport lines (2b) is arranged in the reagent container (18) holding the solid (B1, B2, B3, B4), and that a connecting line (20) connects both of the reagent containers (18) with each other, so that via the gas pressure of the pneumatic system, solvents (SOL1, SOL2, SOL3, SOL4) are

transferred from the one reagent container (18) into the other reagent container (18), the solid (B1, B2, B3, B4) in the reagent container (18) dissolves in the solvent and the solution can be conveyed in the direction of the synthesis chamber (14).

10. The device according to any one of the claims 7 to 9, **characterized in that** the control device (5) has a pneumatic system for controlling the microfluid valves (12) and that the reagent containers (18) are connected via the pneumatic connecting means (4) with the pneumatic system of the control device (5).

11. The device according to any one of the claims 7 to 10, **characterized in that** the fluid connecting means (2) and the pneumatic connecting means (4) are fixed to a support plate (9), said support plate being firmly attached to a rack (8) in the actuator device (6), wherein the support plate (9) has gas line channels (21), via which gas line channels (21) the pneumatic system is connected to the pneumatic connecting means (4) in the operating state.

12. The device according to claims 7 to 11, **characterized in that** the reagent containers (18) are sealed by a respective sealing element (19), wherein the pneumatic connecting means (4) and/or the fluid connecting means (2) are configured in such a way that with the movement from the loading position to the operating position the sealing elements (19) of a plurality, preferably of all, of the reagent containers (18) are penetrated simultaneously.

13. The device according to any one of the claims 1 to 12, **characterized in that** the reagent container support (17) comprises at least six, preferably at least seven, reagent containers (18) and is arranged in the reagent container receptacle (1), wherein each of the reagent containers (18) contains one of the following reagents:

- one reagent containing phosphoramidites of the bases adenine, guanine, cytosine, thymine or uracil (B1, B2, B3, B4);
- one reagent for the detritylation (DEBL) of an end of an oligonucleotide, preferably containing dichloroacetic acid, trichloroacetic acid or zinc bromide;
- one reagent for the activation (ACT) of a detritylated 5'-OH group of an oligonucleotide and for the coupling of the phosphoramidite, preferably containing tetrazole;

- one reagent for the oxidation (OXI) of a phosphite triester, preferably an aqueous iodine solution comprising a weak base, in particular pyridine, lutidine or collidine, or a non-aqueous oxidation agent such as CSO;
 - preferably at least one reagent for blocking (CAP1, CAP2) non-implemented 5'-OH groups of activated oligonucleotides, preferably acetic anhydride and N-methylimidazole or DMAP;
- wherein each reagent container (18) is sealed with a sealing element (19).

14. The device according to any one of the claims 1 to 13, **characterized in that** the fluid connection (13) of the microfluid chip (10) comprises a main channel (13a) connected to the synthesis chamber (14) and a plurality of fluid connecting channels (13b), wherein each fluid connector (11) is connected to the main channel (13a) via a fluid connecting channel (13b) and a microfluid valve (12).

15. The device according to any one of the claims 1 to 14, **characterized in that** the microfluid chip (10) is configured either in one layer, wherein the microfluid valves (12) are configured as membranes and arranged on an exterior surface of the microfluid chip (10) or that the microfluid chip (10) is configured with multiple layers, wherein the microfluid chip (10) comprises a first support layer (10a) comprising the fluid connectors (11) as well as a second support layer (10b), wherein the second support layer (10b) is designed for connection to the control device (5) and the microfluid valves (12) are positioned between the first (10a) and the second support layer (10b).

16. A method for operating a device for synthesizing oligonucleotides, the device comprising

- a reagent container receptacle (1) in which a reagent container support (17) comprising multiple reagent containers (18) filled with reagents is arranged;
- a microfluid chip (10) with a synthesis chamber (14) for the synthesis of the oligonucleotide, with microfluid valves (12) and with fluid connectors (11) for the conveying of the reagents;
- a control device (5) for the control of the microfluid valves (12);
- fluid connecting means (2) for the transport of the reagents from the reagent

containers (18) to the fluid connectors (11) of the microfluid chip (10),

characterized in that

the following steps are carried out:

- positioning of the microfluid chip (10) in the device, so that the fluid connectors (11) of the microfluid chip (10) are aligned flush with the fluid connecting means (2) and the microfluid valves (12) of the microfluid chip (10) can be connected to the control device (5);
- positioning of the microfluid chip (10) by means of a chip receptacle (3) that is moveable via an actuator device (6) until a connection between the fluid connectors (11) of the microfluid chip (10) and the fluid connecting means (2) is created;
- connection of the microfluid valves (12) to the control device (5);
- positioning of the reagent container receptacle (1) by means of the actuator device (6) until a connection between the reagent containers (18) and the fluid connectors (11) of the microfluid chip (10) is created via the fluid connecting means (2), so that depending on the valve position of the microfluid valves (12) reagents can be conveyed out of the reagent containers (18) in the direction of the synthesis chamber (14).

17. The method according to claim 16, **characterized in that** the control device (5) includes a pneumatic system for the control of the microfluid valves (12) and that the microfluid valves (12) are connected to the pneumatic system of the control device (5).

18. The method according to any one of the claims 16 to 17, **characterized in that** the chip receptacle (3a) has a connector portion (3b) for the connection of the microfluid valves (12) of the microfluid chip (10) to the control device (5), in particular to the pneumatic system of the control device (5), wherein the connector portion (3b) is swiveled by means of the actuator device (6) during the movement into the operating position, in order to connect the microfluid valves (12) to the control device (5), wherein the microfluid chip (10) is preferably clamped between the fluid connecting means (2) and the connector portion (3b).

19. The method according to any one of the claims 16 to 18, **characterized in that** the reagent containers (18) are connected to a pneumatic system via pneumatic

connecting means (4) and reagents are transported out of the reagent containers (18) in the direction of the synthesis chamber (14) via gas pressure of the pneumatic system.

20. The method according to claim 19, **characterized in that** the reagent containers (18) are closed by a respective sealing element (19) and the pneumatic connecting means (4) and/or and the fluid connecting means (2) during positioning simultaneously penetrate the sealing elements (19) of a plurality, preferably of all, of the reagent containers (18).

21. The method according to claims 16 to 20, **characterized in that** before the start of the synthesis of an oligonucleotide the following steps are carried out:

- opening of a microfluid valve (12) that controls a fluid connector (11) for the conveying of reagents out of a reagent container (18);
- closing of the opened microfluid valve (12) when reagent flows through the microfluid valve (12);
- washing of the microfluid chip (10), preferably of a main channel (13a) of the microfluid chip (10), by opening a microfluid valve (12) that controls a fluid connector (11) for conveying a solvent (SOL), preferably acetonitrile;
- preferably drying of the microfluid chip (10), in particular of a main channel (13a) of the microfluid chip (10), by opening a microfluid valve (12) that controls the conveying of an inert gas (GAS), preferably argon;
- repeating the above steps until each reagent from the reagent containers (18) connected to the fluid connectors (11) of the microfluid chip (10) has been conveyed to the respective microfluid valve (12) of the corresponding fluid connector (11).

22. The method according to any one of the claims 16 to 21, **characterized in that** for the synthesis of an oligonucleotide, preferably a DNA strand, the following steps are carried out:

- opening of a microfluid valve (12) that controls a fluid connector (11) for the conveying of a reagent for the detritylation (DEBL) of one 5'-OH group of the oligonucleotide containing a dimethoxytrityl protecting group or for the detritylation of a linker molecule of a carrier medium from a reagent container (18);
- alternate opening of a microfluid valve (12), that controls a fluid connector (11) for

the conveying of a reagent for the activation of the detritylated 5'-OH group from a reagent container (18) and of a microfluid valve (12), that controls a fluid connector (11) for the conveying of a reagent containing phosphoramidites of the bases adenine or guanine or cytosine or thymine or uracil (B1, B2, B3, B4) from a reagent container (18);

- preferably opening at least one microfluid valve (12) that controls a fluid connector (11) for the conveying of a reagent for the blocking (CAP1, CAP2) of non-used 5'-OH groups from a reagent container (18);

- opening of a microfluid valve (12) that controls a fluid connector (11) for the conveying of a reagent for the oxidation (OXI) of a phosphite triester bond from a reagent container (18);

- repeating of the previous steps.