

Supplementary Protocol

Pipetting and Reaction Setup Guide for Custom dPCR LNA[®] Assays

These protocols enable seamless integration of the Custom dPCR LNA Assay components into the *Quick-Start Protocol for Custom dPCR LNA Assays designed by QIAGEN[®] Genomic Services* (qiagen.com/HB-2937).

Protocol: Assay Option 1 (Individual Oligos)

1. Create Individual 10x Primer Probe Mix (PPM) as indicated in the following table.

Table 1. Volume and concentration of components for the preparation of Individual 10x PPM

Component	Volume (µL)	Concentration in mix (µM)
Water	80	–
Fw Primer	8	8
Rev Primer	8	8
Probe	4	4
Total	100	–

2. Set up the reaction as specified in Table 2.

Table 2. Concentration and volume of components for the reaction setup of Option 1

Component	Volume (26k Nanoplate) (µL)	Volume (8.5k Nanoplate) (µL)	Concentration in mix
4x Probe PCR MasterMix	10	3	1x
10x Primer Probe Mix 1	4	1.2	0.8 µM primer (each), 0.4 µM probe
10x Primer Probe Mix 2, ...	4	1.2	0.8 µM primer (each), 0.4 µM probe
Restriction Enzyme (optional)	Up to 1	Up to 1	1–10 units/reaction
RNase-Free Water	Variable	Variable	–
Template DNA	Variable	Variable	–
Total	40	12	–

For Multiplex Setup of individual components (Option 1) with the same primer pair for each probe, alternative setup with less primer input can be created as follows.

1. Create Multiplex Primer Probe Mix by using Table 3 (next page) as a guide.

Table 3. Concentration and volume of components for the preparation of Multiplex PPM

Component	Volume (µL)	Concentration in mix (µM)
Water	Variable	–
Fw Primer	8	8
Rev Primer	8	8
Probe 1	4	4
Probe 2	4	4
Probe 3	4	4
...
Total	100	–

2. Set up the reaction as detailed in Table 4.

Table 4. Concentration and volume of components for the alternative setup for Option 1

Component	Volume (26k Nanoplate) (µL)	Volume (8.5k Nanoplate) (µL)	Concentration in mix
4x Probe PCR MasterMix	10	3	1x
10x Primer Probe Mix	4	1.2	0.8 µM primer (each), 0.4 µM probe
Restriction Enzyme (optional)	Up to 1	Up to 1	1–10 units/reaction
RNase-Free Water	Variable	Variable	–
Template DNA	Variable	Variable	–
Total	40	12	–

Protocol: Assay Option 2 (Primer Pool + Probe)

1. Prepare Individual 10x Primer Probe Mix (PPM) according to Table 5.

Table 5. Volume and concentration of components for the preparation of Individual 10x PPM

Component	Volume (µL)	Concentration in mix
Water	88	–
Primer Pool (Fw + Rev)	8	8 µM primer (each)
Probe	4	4 µM
Total	100	–

2. Setup the reaction as specified in Table 6.

Table 6. Concentration and volume of components for the reaction setup of Option 2

Component	Volume (26k Nanoplate) (µL)	Volume (8.5k Nanoplate) (µL)	Concentration in mix
4x Probe PCR MasterMix	10	3	1x
10x Primer Probe Mix 1	4	1.2	0.8 µM primer (each), 0.4 µM probe
10x Primer Probe Mix 2	4	1.2	0.8 µM primer (each), 0.4 µM probe
Restriction Enzyme (optional)	Up to 1	Up to 1	1–10 units/reaction
RNase-Free Water	Variable	Variable	–
Template DNA	Variable	Variable	–
Total	40	12	–

For Multiplex Setup of primer pool + probe (Option 2) with the same primer pair for each probe, alternative setup with less primer input can be created as follows.

1. Create Multiplex Primer Probe as follows.

Table 7. Concentration and volume of components for the preparation of Multiplex Primer Probe for Option 2

Component	Volume (µL)	Concentration in mix
Water	Variable	–
Primer Pool (Fw + Rev)	8	8 µM primer (each)
Probe 1	4	4 µM
Probe 2	4	4
Probe 3	4	4
...
Total	100	–

2. Set up the reaction according to the specifications in Table 8.

Table 8. Concentration and volume of components for the alternative setup for Option 2

Component	Volume (26k Nanoplate) (µL)	Volume (8.5k Nanoplate) (µL)	Concentration in mix
4x Probe PCR MasterMix	10	3	1x
10x Primer Probe Mix	4	1.2	0.8 µM primer (each), 0.4 µM probe
Restriction Enzyme (optional)	Up to 1	Up to 1	1–10 units/reaction
RNase-Free Water	Variable	Variable	–
Template DNA	Variable	Variable	–
Total	40	12	–

Protocol: Assay Option 3 (Lyophilized Assay Bundle)

Resuspension of lyophilized product

Before opening the tube, briefly centrifuge the tube prior to resuspension to minimize risk of loss and contamination.

Dissolve the lyophilized product in 320 μL TE to obtain a Primer-Probe Mix (PPM) stock. Vortex thoroughly to resuspend the (potentially invisible) pellet and spin to collect. The resulting PPM stock now has a concentration of 100 μM Primer (each) and 50 μM Probe.

1. Prepare 10x Primer Probe Mix with Table 9 as a guide.

Table 9. Volume and concentration of components for the preparation of 10x PPM

Component	Volume (μL)	Concentration in mix
Water	92	–
Resuspended Assay	8	8 μM primer (each), 4 μM probe
Total	100	–

Alternatively, higher or lower concentrated PPM can be set up as follows when needed.

- For 2x (double) concentrated 10x PPM, dilute 16 μL PM stock + 84 μL water to obtain a stock with 16 μM Primer (each), 8 μM Probe concentration.
- For 3x (triple) concentrated 10x PPM, dilute 24 μL PPM stock + 76 μL water to obtain a stock with 24 μM Primer (each), 12 μM Probe concentration.

2. Set up the reaction according to the specifications in Table 10.

Table 10. Reaction setup for Option 3

Component	Volume (26k Nanoplate) (μL)	Volume (8.5k Nanoplate) (μL)	Concentration in mix
4x Probe PCR MasterMix	10	3	1x
10x Primer Probe Mix	4	1.2	0.8 μM primer (each), 0.4 μM probe
Restriction Enzyme (optional)	Up to 1	Up to 1	1–10 units/reaction
RNase-Free Water	Variable	Variable	–
Template DNA	Variable	Variable	–
Total	40	12	–

Document Revision History

Date	Changes
12/2023	Initial release

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