

SAMPLE PROTOCOL FOR OLIGONUCLEOTIDE LIGATION ASSAY (OLA) AND HYBRIDIZATION TO FlexMAP UNIVERSAL ARRAY MICROSPHERES

MATERIALS

- Carboxylated fluorescent microspheres with covalently attached FlexMAP anti-TAG sequences
- PCR amplification primers for each target resuspended in sterile ddH₂O. PCR primers are reconstituted to 1 mM (1 nanomole/μL).
- OLA probes with 5' TAG modification resuspended in sterile ddH₂O. OLA probes are reconstituted to 1 mM (1 nanomole/μL).
- Reporter probes with 5' phosphate and 3' biotin modifications resuspended in sterile ddH₂O. Reporter probes are reconstituted to 1 mM (1 nanomole/μL).
- Qiagen HotStarTaq 2X Master Mix (Qiagen, Cat. No. 203445) **or equivalent**
- Taq DNA Ligase, 10X Taq DNA Ligase Buffer (New England Biolabs Cat. No. M0208S)
- 2X Tm Hybridization Buffer – 0.4 M NaCl, 0.2 M Tris, 0.16% Triton X-100, pH 8.0
- 1X Tm Hybridization Buffer – 0.2 M NaCl, 0.1 M Tris, 0.08% Triton X-100, pH 8.0
- Streptavidin-R-phycoerythrin (See REAGENTS list)
- 96 well V-bottom PCR plate & cover (See CONSUMABLES list)
- Pipettors, tips, microcentrifuge tubes, etc. (See CONSUMABLES list)
- Genomic DNA samples

PROCEDURES

Multiplexed PCR Reaction – *PCR should be performed under optimized conditions. The parameters listed below are for example purposes only.*

Each final reaction contains:

1X Qiagen PCR reaction buffer
 1.5 mM MgCl₂
 200 μM each dNTP
 0.2 μM each primer
 2.5 Units Qiagen HotStarTaq polymerase
 50 ng template

PCR Cycling Parameters:

HOLD: 95°C, 15 minutes (for enzyme activation)

CYCLE: 94°C, 30 seconds

55°C, 30 seconds

72°C, 30 seconds

35 CYCLES

HOLD: 72°C, 7 minutes

HOLD: 4°C, FOREVER

Multiplex OLA ReactionEach 20 μ L final reaction contains:

1X Taq DNA Ligase Buffer (20 mM Tris-HCl, 25 mM potassium acetate, 10 mM magnesium acetate, 10 mM dithiothreitol, 1 mM NAD, 0.1% Triton X-100, pH 7.6)
 10 U Taq DNA Ligase
 5 nM each TAG-OLA probe
 250 nM each Reporter probe
 3 to 20 ng PCR target (usually 0.5 to 5 μ L)
 dH₂O (to 20 μ L)

2X OLA Master Mix (10 μ L/reaction)

10X Taq DNA Ligase Buffer	2	μ L	
Taq DNA Ligase (40,000 U/mL)	0.25	μ L	
20X TAG-OLA probe mix (100 nM each)	1	μ L	(dilute 1 mM stocks 1:10,000 for 20X mix)
20X Reporter probe mix (5 μ M each)	1	μ L	(dilute 1 mM stocks 1:200 for 20X mix)
dH ₂ O		<u>5.75</u>	<u>μL</u>
		10	μ L

OLA Cycling Parameters:

HOLD: 96°C, 2 minutes
CYCLE: 94°C, 15 seconds
 37°C, 1 minute
30 CYCLES
HOLD: 4°C, FOREVER

Hybridization to FlexMAP Microspheres

Microspheres should be protected from prolonged exposure to light throughout this procedure.

1. Select the appropriate FlexMAP microsphere sets and resuspend according to the instructions described in the Product Information Sheet provided with your microspheres.
2. Combine 2500 microspheres of each set per reaction.
3. Concentrate the FlexMAP microsphere mixture by centrifugation at $\geq 8000 \times g$ for 1-2 minutes.
4. Remove the supernatant and resuspend to 100 of each microsphere set per μL in 2X T_m Hybridization Buffer by vortex and sonication for approximately 20 seconds.
5. Aliquot 25 μL of the FlexMAP microsphere mixture to each well.
6. Add 25 μL of dH_2O to each background well.
7. Add 5 to 25 μL of each OLA reaction to the appropriate wells. (Note: 5 μL is usually sufficient.)
8. Adjust the total volume to 50 μL by adding the appropriate volume of dH_2O to each sample well.
9. Cover the plate to prevent evaporation and denature at 96°C for 90 seconds. *
10. Hybridize at 37°C for 30 minutes. *
11. Pellet the FlexMAP microspheres by centrifugation at $\geq 2250 \times g$ for 3 minutes and remove the supernatant.
12. Resuspend the pelleted FlexMAP microspheres in 75 μL of 1X T_m Hybridization Buffer.
13. Pellet the FlexMAP microspheres by centrifugation at $\geq 2250 \times g$ for 3 minutes and remove the supernatant.
14. Repeat steps 12. and 13. This is a total of two washes.
15. Resuspend microspheres in 75 μL of 1X T_m Hybridization Buffer containing 2 $\mu\text{g}/\text{mL}$ streptavidin-R-phycoerythrin.
16. Incubate at 37°C for 15 minutes.
17. Analyze 50 μL at 37°C on the Luminex analyzer according to the system manual.

*** *These steps can be performed on a thermal cycler programmed as follows –***
Hold at 96°C , 90 seconds
Hold at 37°C , FOREVER

Technical Note: Alternatively, pre-wet a 1.2 μm Millipore filter plate with 1X T_m Hybridization Buffer and filter by vacuum manifold. Transfer the reactions to the pre-wetted filter plate and remove the supernatant by vacuum filtration. Wash twice with 100 μL 1X T_m Hybridization Buffer. Resuspend the reactions in 75 μL of Reporter Mix by gently pipetting up and down several times. Proceed with step 16.