

Materials List for:**Multiplex PCR and Reverse Line Blot Hybridization Assay (mPCR/RLB)**Matthew V. N. O'Sullivan¹, Fei Zhou¹, Vitali Sintchenko¹, Fanrong Kong¹, Gwendolyn L. Gilbert¹¹Centre for Infectious Diseases and Microbiology, University of SydneyCorrespondence to: Matthew V. N. O'Sullivan at matthew.osullivan@swahs.health.nsw.gov.auURL: <https://www.jove.com/video/2781>DOI: [doi:10.3791/2781](https://doi.org/10.3791/2781)**Materials**

Name	Company	Catalog Number	Comments
5' amine C6 modified oligonucleotide probes	Sigma-Aldrich		
NaHCO ₃	Sigma-Aldrich	S-8875	0.5 M solution made up to pH 8.4
NaOH	Sigma-Aldrich	S5881	0.1M solution
20xSSPE buffer	Amresco	0810-4L	
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich	L-4390	Make up 10% stock solution, do not autoclave, should be kept for 1 week maximum before use
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich	E9884	Make up 0.5 M solution adjusted to pH 8.0 and autoclave
N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC)	Sigma-Aldrich	E7750	Make up a 20 ml 16% solution just prior to use using 3.2 g EDAC and 18 ml Millipore water
BiodyneC 0.45 µm nylon Membrane 20x20 cm	Pall Corporation	74480C	
Deionised, purified water			
Liquid Pyroneg detergent	Johnson Diversey	HH12291	
Streptavidin-peroxidase conjugate	Roche Group	11 089 153 001	
Amersham ECL detection reagents	GE Healthcare	RPN2105	
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OHP Transparency film	Corporate Express	EXP 504 OHP	
Amersham hyperfilm ECL high performance chemiluminescent film 18x24 cm	GE Healthcare	28906837	
Ice			

Table 1. Consumables used in the mPCR/RLB assay.

Hybaid Shake'n'Stack Ovens (2) rolling bottle and nylon separating mesh	Thermo Fisher Scientific, Inc.	HBSNSRS220	Method can be performed with a single oven as long as there is facility for maintaining solutions at 42°C and 60°C prior to use
The Belly Dancer rocking platform	Stovall Life Sciences, IBI Sciences	Euro BDbo	
Miniblotter	Immunetics	MN100-45	
Water bath			
Suction			
Hot plate			
Exposure cartridge	Sigma-Aldrich	Z36,009-0	
X-ray film developer			Can also use developer, fixer and water in trays in dark room instead of automated developer. Lumino-

		imager may also be used instead of Xray film
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Table 2. Equipment required for the mPCR/RLB assay.