

SUPPLEMENTARY MATERIAL FOR:

A probe-based loop-mediated isothermal amplification assay targeting IS2404 for detection of *Mycobacterium ulcerans*.

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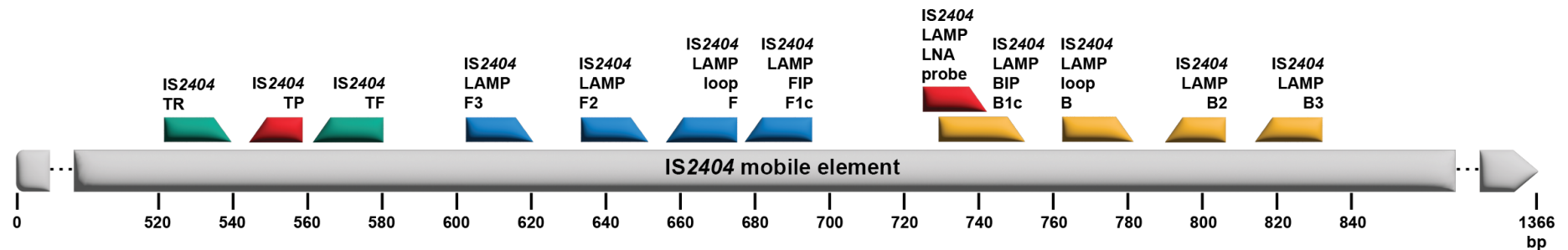
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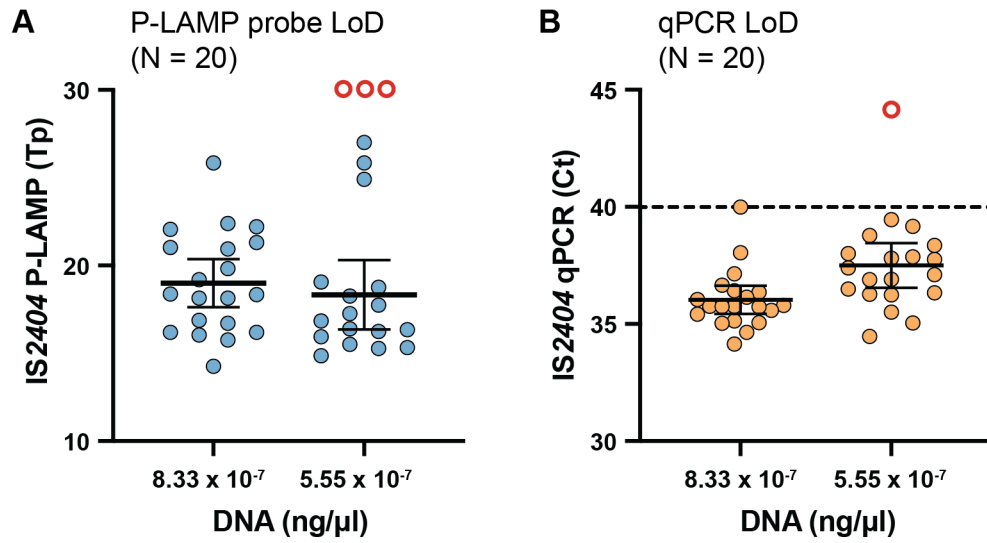
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Supplementary Table 1. Bacterial strains used in this study.

Organism (strain)	Strain reference	Sequencing Reference
<i>Mycobacterium ulcerans</i> (JKD8049, clinical, Australia)	(1)	GCA_020616615.1
<i>Mycobacterium ulcerans</i> (Agy99, clinical, Ghana)	(2)	(2)
<i>Mycobacterium ulcerans</i> (ITM 06-3844, fish, Belgium)	(3)	(4)
<i>Mycobacterium ulcerans</i> (JKD8043, clinical, Suriname)	(5)	Not published
<i>Mycobacterium ulcerans</i> (Mu_L15, fish, USA)	(6)	(7)
<i>Mycobacterium ulcerans</i> (NM20/02, clinical, Ghana)	(8)	(4)
<i>Mycobacterium abscessus</i> (TPS8830)	Not published	Not published
<i>Mycobacterium bovis</i> (Danish 1331)	(9)	(10)
<i>Mycobacterium chimera</i> (DMG160013)	(11)	(11)
<i>Mycobacterium fortuitum</i> [#]	(12)	Not published
<i>Mycobacterium marinum</i> ("M" strain)	(13)	(13)
<i>Mycobacterium smegmatis</i> (MC2155)	(14)	(15)
<i>Mycobacterium spongiae</i> (FSD4b-SM)	(16)	(16)
<i>Mycobacterium terrae</i> (NCTC 10856/ ATCC 15981)	(17)	GCA_900187145.1
<i>Mycobacterium virginiense</i> (TPS8833)	Not published	Not published
<i>Dendrosporobacter quercicolus</i> (DSM 1736)	(18, 19)	Not published
<i>Enterococcus faecium</i> (Ef_AUS0233)	(20)	(20)
<i>Escherichia coli</i> (Ec_SeRP62aI)	(21)	(21)
<i>Klebsiella pneumoniae</i> (BPH05002)	Not published	Not published
<i>Listeria monocytogenes</i> (EGDe)	(22)	(23)
<i>Nocardia brasiliensis</i> (AUSMDU00075719)	Not published	Not published
<i>Nocardia testacea</i> (AUSMDU00041268)	Not published	Not published
<i>Rouxiella chamberiensis</i> (DSM 28324)	(24)	(25)
<i>Staphylococcus aureus</i> (JE2)	(26)	(27)
<i>Staphylococcus aureus</i> (MW2)	(28)	(29)
<i>Staphylococcus epidermidis</i> (BPH0662)	(30)	(30)
<i>Staphylococcus epidermidis</i> (BPH0736)	(31)	(31)
<i>Streptomyces cacaoi</i> (AUSMDU00077794)	Not published	Not published
<i>Streptomyces</i> spp. (AUSMDU00077800)	Not published	Not published



Supplementary Figure 1. Binding sites of the IS2404 TaqMan quantitative PCR (qPCR) and IS2404 LAMP plus locked nucleic acid (LNA) probe (P-LAMP) primers and probes. Note that primers have been named according to the publications in which they were originally described, in which IS2404 TaqMan qPCR forward and reverse primers were named their reverse (32). The forwards and backward primers for the IS2404 P-LAMP were also named the reverse of convention (33).



Supplementary Figure 2. Limit-of-detection (LoD) testing comparing the (A) IS2404 probe LAMP (P-LAMP) and (B) IS2404 quantitative PCR (qPCR) for the detection of *M. ulcerans* genomic DNA. Twenty replicates of each dilution were tested by each assay. Open red circles represent replicates that were not detected. Bold horizontal bars show the mean, error bars the 95% confidence interval. Horizontal dotted line indicates the cycle threshold (Ct) ≤ 40 cut-off for the IS2404 qPCR, above which samples are considered as negative.

Supplementary Table 2. Collection site and IS2404 qPCR cycle threshold (Ct) and P-LAMP time-to-positive (Tp) values for possum excreta surveillance samples.

Sample number	Collection site	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)	Sample number	Collection site	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)
1	RES19-02287	23.37	19.45	40	RES21-03709-T	32.78	19.15
2	RES19-02289	32.53	19.47	41	RES21-03709-U	33.76	21.36
3	RES19-02292	30.60	17.59	42	RES21-03709-V	ND	ND
4	RES21-00902	24.71	13.18	43	RES21-03709-W	32.33	18.65
5	RES21-00921	17.79	11.65	44	RES21-03709-X	32.10	21.14
6	RES21-00930	36.30	21.83	45	RES21-03709-Y	32.60	17.95
7	RES21-00993	34.43	17.00	46	RES21-03841	22.76	11.65
8	RES21-00999	19.56	17.37	47	RES21-03885	28.34	12.74
9	RES21-01067-A	22.41	15.47	48	RES21-03895	31.16	16.73
10	RES21-01067-B	27.64	12.60	49	RES22-03855-A	ND	ND
11	RES21-01079	23.55	12.33	50	RES22-03855-B	ND	ND
12	RES21-01131-A	28.78	13.37	51	RES22-03855-C	ND	ND
13	RES21-01131-B	30.55	16.92	52	RES22-03855-D	ND	ND
14	RES21-02262	ND	ND	53	RES22-03856-A	ND	ND
15	RES21-02291	31.14	19.68	54	RES22-03856-B	ND	ND
16	RES21-02636	34.22	16.11	55	RES22-03856-C	ND	ND
17	RES21-02644	37.10	19.90	56	RES22-03856-D	ND	ND
18	RES21-02662 [#]	38.11	24.80	57	RES22-03856-E	ND	ND
19	RES21-03270	35.03	19.47	58	RES22-03856-E	ND	ND
20	RES21-03330	35.62	21.48	59	RES22-03856-F	ND	ND
21	RES21-03709-A	32.97	17.14	60	RES22-03856-G	ND	ND
22	RES21-03709-B	33.67	24.62	61	RES22-03856-H	ND	ND
23	RES21-03709-C	39.89	ND	62	RES22-03856-I	ND	ND
24	RES21-03709-D	28.17	16.58	63	RES22-03856-J	ND	ND
25	RES21-03709-E	32.41	18.68	64	RES22-03856-K	ND	ND
26	RES21-03709-F	33.39	18.95	65	RES22-03856-L	ND	ND
27	RES21-03709-G	32.92	21.43	66	RES22-03856-M	ND	ND
28	RES21-03709-H	32.71	17.41	67	RES22-03856-N	ND	ND
29	RES21-03709-I	32.76	20.44	68	RES22-03856-O	ND	ND
30	RES21-03709-J	38.37	ND	69	RES22-03856-P	ND	ND
31	RES21-03709-K	31.14	19.80	70	RES22-03856-Q	ND	ND
32	RES21-03709-L	33.12	23.30	71	RES22-03856-R	ND	ND
33	RES21-03709-M	34.96	18.40	72	RES22-03856-S	ND	ND
34	RES21-03709-N	32.41	21.37	73	RES22-03856-T	ND	ND
35	RES21-03709-O	31.42	20.13	74	RES22-03856-U	ND	ND
36	RES21-03709-P	32.75	24.50	75	RES22-03856-V	ND	ND
37	RES21-03709-Q	32.76	23.30	76	RES22-03856-W	ND	ND
38	RES21-03709-R	33.04	16.93	77	RES22-03856-X	ND	ND
39	RES21-03709-S	33.07	19.18	78	RES22-03856-Y	ND	ND

[#]SPRI-bead based DNA extraction tested instead of PowerSoil Pro kit extraction.

Supplementary Table 3. IS2404 qPCR cycle threshold (Ct) and P-LAMP time-to-positive (Tp) values for *M. ulcerans* DNA extracted from swabs sampled from Australian possums.

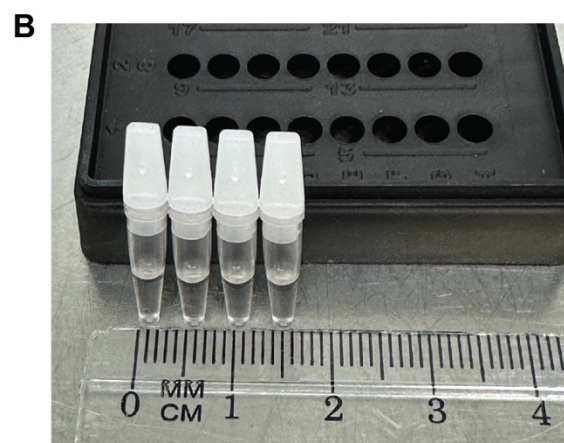
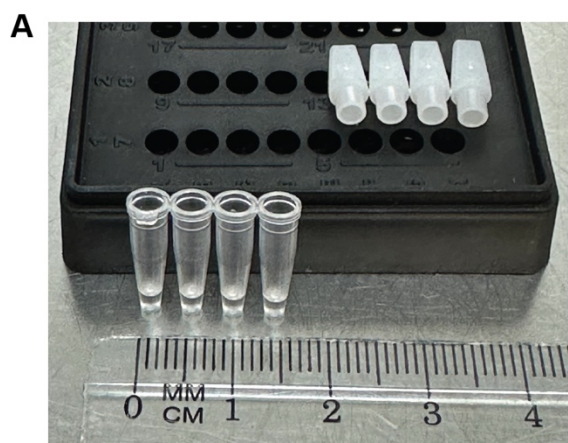
Possum number (ID)	Swabbed site	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)
1 (W379-23)	Multiple ulcers	ND	ND
2 (W381-23)	Multiple ulcers	ND	ND
3 (W386-23)	Multiple ulcers	ND	ND
4 (W387-23)	Multiple ulcers	ND	ND
5 (W380-23)	Multiple ulcers	19.81	10.33
6 (6/22)	Oral cavity	34.81	18.04
	Cloaca	27.85	13.39
7 (13/22)	Oral cavity	36.02	19.02
	Cloaca	ND	ND
8 (14/22)	Oral cavity	25.04	13.50
	Cloaca	32.58	13.50
	Pouch	33.02	16.06
9 (15/22)	Oral cavity	ND	ND
10 (20/23)	Oral cavity	32.98	19.53
	Cloaca	32.98	19.39
	Ulcer (L hind paw)	19.30	10.34

Ct: cycle threshold, ND: not detected, L: left.

Supplementary Table 4. IS2404 qPCR cycle threshold (Ct) and P-LAMP time-to-positive (Tp) values for *M. ulcerans* DNA extracted from human clinical swabs

Sample	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)	Sample	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)	Sample	IS2404 qPCR (Ct)	IS2404 P-LAMP (Tp)
1	ND	ND	51	27.09	17.10	101	ND	ND
2	ND	ND	52	27.36	12.12	102	ND	ND
3	ND	ND	53	18.06	7.71			
4	ND	ND	54	ND	ND			
5	ND	ND	55	31.66	19.53			
6	ND	ND	56	32.21	14.28			
7	ND	ND	57	ND	ND			
8	ND	ND	58	26.48	14.64			
9	ND	ND	59	20.01	9.67			
10	ND	ND	60	18.57	10.88			
11	ND	ND	61	ND	ND			
12	ND	ND	62	ND	ND			
13	ND	ND	63	ND	ND			
14	ND	ND	64	ND	ND			
15	ND	ND	65	ND	ND			
16	ND	ND	66	ND	ND			
17	ND	ND	67	ND	ND			
18	ND	ND	68	ND	ND			
19	ND	ND	69	ND	ND			
20	ND	ND	70	ND	ND			
21	ND	ND	71	ND	ND			
22	ND	ND	72	ND	ND			
23	ND	ND	73	ND	ND			
24	ND	ND	74	ND	ND			
25	ND	ND	75	ND	ND			
26	ND	ND	76	ND	ND			
27	ND	ND	77	ND	ND			
28	ND	ND	78	ND	ND			
29	ND	ND	79	ND	ND			
30	ND	ND	80	ND	ND			
31	36.42	21.96	81	ND	ND			
32	34.82	19.01	82	ND	ND			
33	31.70	18.05	83	ND	ND			
34	23.57	10.57	84	ND	ND			
35	38.45	20.84	85	ND	ND			
36	25.16	11.04	86	ND	ND			
37	26.55	13.84	87	ND	ND			
38	ND	ND	88	ND	ND			
39	28.25	16.76	89	ND	ND			
40	26.32	16.43	90	ND	ND			
41	ND	ND	91	ND	ND			
42	ND	ND	92	ND	ND			
43	24.66	10.27	93	ND	ND			
44	38.57	23.93	94	ND	ND			
45	22.53	10.63	95	ND	ND			
46	32.02	27.89	96	ND	ND			
47	28.69	14.33	97	ND	ND			
48	34.88	18.12	98	ND	ND			
49	21.77	9.77	99	ND	ND			
50	31.63	15.36	100	ND	ND			

IS2404: insertion sequence 2404, qPCR: quantitative polymerase chain reaction, LAMP: loop-mediated isothermal amplification.



Supplementary Figure 3. Mic PCR consumables. (ruler for scale).

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