LeishDETECTR-001

TUESDAY, 10/15/2019

Objective

To test all the Leishmania guides on each of the three strains

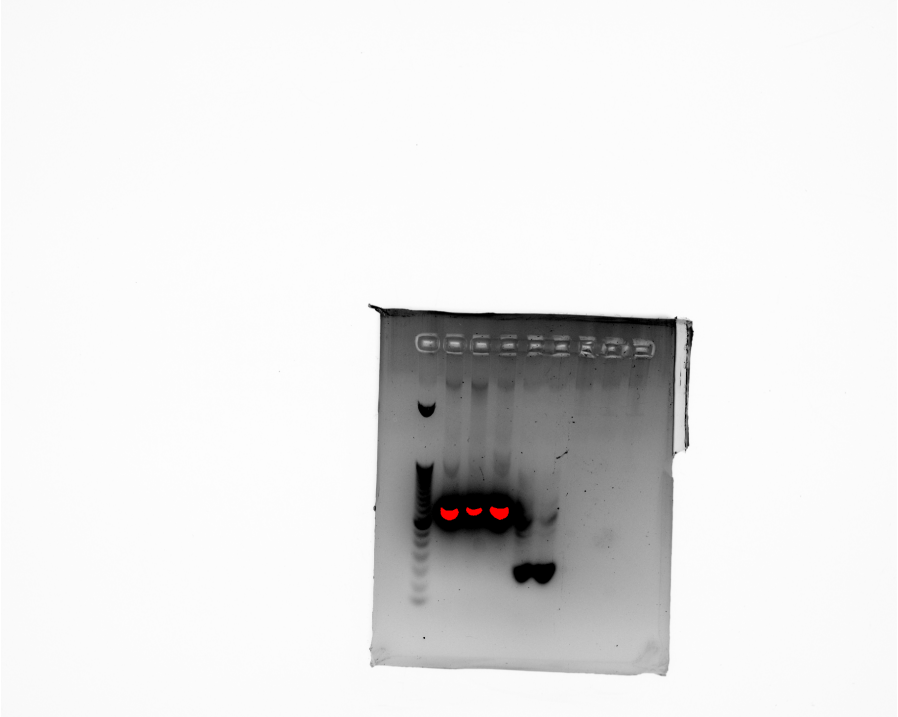
Sample List

	A	B
1	Major	1_Major
2	Major	2_Major
3	Major	3_Major
4	Major	1_Infantum
5	Major	2_Infantum
6	Major	3_Infantum
7	Major	4_Infantum
8	Major	5_Infantum
9	Major	1_Tropica
10	Major	2_Tropica
11	Major	3_Tropica
12	Infantum	1_Major
13	Infantum	2_Major
14	Infantum	3_Major
15	Infantum	1_Infantum
16	Infantum	2_Infantum
17	Infantum	3_Infantum
18	Infantum	4_Infantum
19	Infantum	5_Infantum
20	Infantum	1_Tropica
21	Infantum	2_Tropica
22	Infantum	3_Tropica
23	Tropica	1_Major
24	Tropica	2_Major
25	Tropica	3_Major
26	Tropica	1_Infantum
27	Tropica	2_Infantum
28	Tropica	3_Infantum
29	Tropica	4_Infantum
30	Tropica	5_Infantum
31	Tropica	1_Tropica
32	Tropica	2_Tropica
33	Tropica	3_Tropica
34	no target	1_Major
35	no target	2_Major
36	no target	3_Major

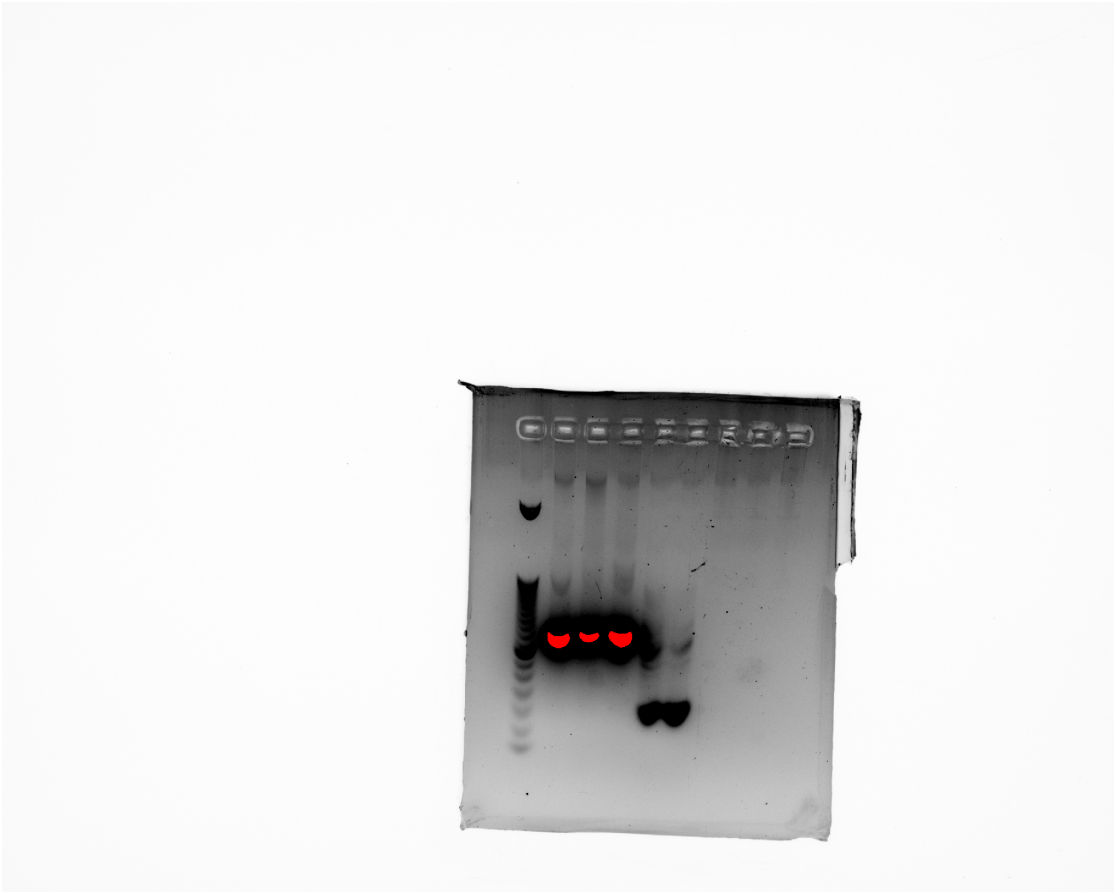
37	no target	1_Infantum
38	no target	2_Infantum
39	no target	3_Infantum
40	no target	4_Infantum
41	no target	5_Infantum
42	no target	1_Tropica
43	no target	2_Tropica
44	no target	3_Tropica

384 Plate:
1-11 went in row A, even columns 2-22
12-22 went in row B, even columns 2-22
23-33 went in row C, even columns 2-22
34-44 went in row D, even columns 2-22

RPA_saturated_lab_user 2019-10-16 13h10m51s(SYBR Gold) (1).tif



RPA_saturated_lab_user 2019-10-16 13h10m51s(SYBR Gold) (1).tif



LeishDETECTR-001_oct162019.xls

DETECTR Assay V3

Introduction

DETECTR with RPA and DNA-FAM probe

Materials

- Lbcas12a, from NEB, 100uM
- NEB Buffer 2.1
- Synthetic DNA or target
- crRNA
  - transcribed from template with T7
- Florescent reporter
  - λex: 485 nm; λem: 535 nm
  - /56-FAM/TT ATT /3IABkFQ/
- Corning 384 flat bottom, black, clear bottom plates

Procedure

Pre-assembly of cas12a-crRNA-targetDNA

1. Thaw buffer, crRNA and cas12a to room temperature. Find buffer and cas12a in DETECTR reagents box in -20C, find crRNA in -80C.
2. Dilute crRNA to 35ng/uL if needed
3. Thaw your unpurified RPA product
4. Dilute cas12a 1:100 in 1X NEB 2.1 to a concentration of 1uM by adding 99uL of 1X NEB 2.1 to 1uL of cas12a.
5. The final reaction will be as follows. If you are changing the concentrations of target DNA, human gDNA, or gRNA, be sure to adjust your stock concentration appropriately.

Preassembly

	Stock concentration	Per reaction volume	Units	Final Concentration in 10uL	Reagent	45X MM
1	1uM	1	uL	100nM	cas12a	45
2	2X	1	uL	1X	10X NEB 2.1 Buffer	45
3	2.66uM (35ng/uL)	1.5	uL	400nM	crRNA	
4		6	uL		H20	270
5	unknown	1	uL	unknown	target dsDNA	
6		10.5	10uL reaction			

- [ ] 6. Make a master mix of cas12a, buffer, and water for each gRNA. Add 8uL to each tube.
- [ ] 7. Add 1.5uL of the correct crRNA
- [ ] 8. Add 1uL of target dsDNA
- [ ] 9. Mix by pipetting or tapping sides of tubes
- [ ] 10. Incubate at 37C for 30 minutes with heated lid at 40C. Leave at 10C after incubation.

Adding Reporter

- [ ] 11. Thaw the reporter stored at 1μM in the DETECTR reagents box.

    Note: protect the reporter from light when possible

- [ ] 12. The final reaction will be as follows:

Reporter

	Stock concentration	Per reaction volume	Units	Final Concentration in 20uL	Reagent
1	100nM:400nM:50nM	10	uL	50nM:200nM:25nM	cas12a:crRNA:targetDNA
2	1uM	5	uL	250nM	reporter
3	10X	1	uL	1X	1X NEB 2.1 buffer
4		4	uL		H20
5		20	uL	reaction	

- [ ] 13. Combine the reporter, water and buffer together.
- [ ] 14. Add the 10uL from MM to your 384 low volume plate, skipping columns.
- [ ] 15. Transfer the appropriate 10uL of assembled cas12a:crRNA to your plate and mix by pipetting. Spin down plate.

Reading the results

- [ ] 16. Set the temperature on the SpectraMax M3 (the older, brown model) to 37C.
- [ ] 17. Save an old DETECTR experiment as a new file.

18. Under Experiments in the left hand tab, select the plate you will run (usually Plate 1). Click settings to change the plate area, and any variables you would like. You can either do a kinetic experiment and take a number of readings over time, or an endpoint, where you take a single reading. If taking an endpoint, be sure to incubate plate at 37C for at least 2 hours.

    Check: λex: 485 nm; λem: 535 nm

19. In the workflow tab, press 'Run'. Be careful not to overwrite data you haven't saved anywhere else.