# Basehunter Kit Contents

1. Mastermix labelled Tube A (54ul)
2. Tube B (4ul)
3. Tube C (4ul)

# Additional Equipment required

## For the Detector Reaction;

1. PCR Machine or alternatlively two waterbaths, one at 70 degrees celsius and a second at 10 degrees celsius.
2. Variety of calibrated pipettes and appropriate tips (p10, p20, p100 or p200 and a p1000)

# Additional Equipment required

## For the transformation;

1. 6 mls of LB liquid media
2. Slushy Ice with box
3. 540ul of *E. coli* competent cells
4. Seven 2ml eppendorf tubes
5. Waterbath that reaches 42 degrees celsius.
6. Hockey stick or glass beads (at least 35).
7. Bunsen burner and lighter.
8. Variety of calibrated pipettes and appropriate tips (p10, p20, p100 or p200 and a p1000)
9. Timer (in seconds)
10. 6 chloramphenicol plates

# Additional Equipment required

## For Results Analysis (Post Transformation)

1. UV light source eg, UV light box
2. Marker
3. Optional Tally Counter.

# Protocol 1. Basehunter protocol for Detector Reaction

Approximate runtime: 20 minutes.

1. Vortex Tube A briefly (less than 7 seconds)
2. Pipette 9ul from Tube A into each of the smaller tubes numbered 1 to 6.
3. Pipette 1ul from Tube B into the smaller tubes 1,2,3.
4. Pipette 1ul from Tube C into the smaller tubes 4,5,6.
5. Then mix each of smaller tubes (1-6) by vortexing individually for 2 seconds.
6. Incubate tubes 1-6 at 70 degrees celsius for 10 minutes and then 10 degrees Celsius for 10 minutes. (You may use a PCR machine or two waterbaths for this step).
7. Commence steps 1-3 of protocol 2 while tubes are being incubated during step 6 of protocol 1.

Continue to this step immediately

# Protocol 2. Basehunter Transformation Protocol

Approximate runtime 90 minutes

1. Place the 6 chloramphenicol plates, inverted in an incubator at $37^0$C (until protocol 3). Do not stack the plates on top of each other
2. Put 540ul of *E. coli* competent cells into an Eppendorf (labelled cells), while on slushy ice.
3. Pipette 90ul of *E. coli* competent cells into each of the 2ml eppendorfs labelled 1 to 6, while on slushy ice.
4. Pipette 5ul from each of the coloured tubes labelled 1-6 into 90ul of competent cells into the corresponding 2ml eppendorfs (labelled 1-6).
5. Incubate on slushy ice for 5-10 minutes (5 minutes minimum). (Record time for feedback)
6. Heat shock each of the tubes at $42^0$C for 45 to 60 seconds. (record time for feedback).
7. Place the Eppendorfs on slushy ice immediately for 5 minutes.
8. Remove from the slushy ice.
9. Add 1ml of LB liquid media to each Eppendorf.
10. Ensure the eppendorfs are closed tightly and tape the eppendorfs horizontally to the bottom of a beaker (beaker to fit shaking incubator).
11. Incubate shaking at $37^0$C for 60-90 mins. (record time for feedback)

# Protocol 3. Basehunter Plating Protocol

Approximate runtime; 10 minutes. Perform next to a lighted bunsen burner

1. Centrifuge tubes 1 to 6 at 4000 rpm for 3 minutes.
2. Remove the plates l-6 from the incubator.
3. Without disturbing the pellet of cells, remove 980ul of the supernatant.
4. Resuspend the pellet (less than 120ul present).
5. Plate the remaining solution in the tube onto the corresponding plates. Spread the solution either using glass beads or a hockey stick.
6. Incubate the plates, inverted and singly (not stacked) at $37^0$C overnight.

# Protocol 4. Analysis of results

1. Under the UV light box count the green colonies.
2. Under the UV light box (or natural light), count the red colonies.
3. Fill out the feedback form & table and email it to corkigem@gmail.com

Note: if the red is not an intense colour, it will brighten if left on the bench at room temperature for over an hour.

## Results

| Plate | GFP | RFP |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |

## Feedback

Any feedback is valuable to us. If you could comment on, for example, the protocols, level of difficulty of the testing of the system, range of equipment required, validity of performing this test in a developing world setting.