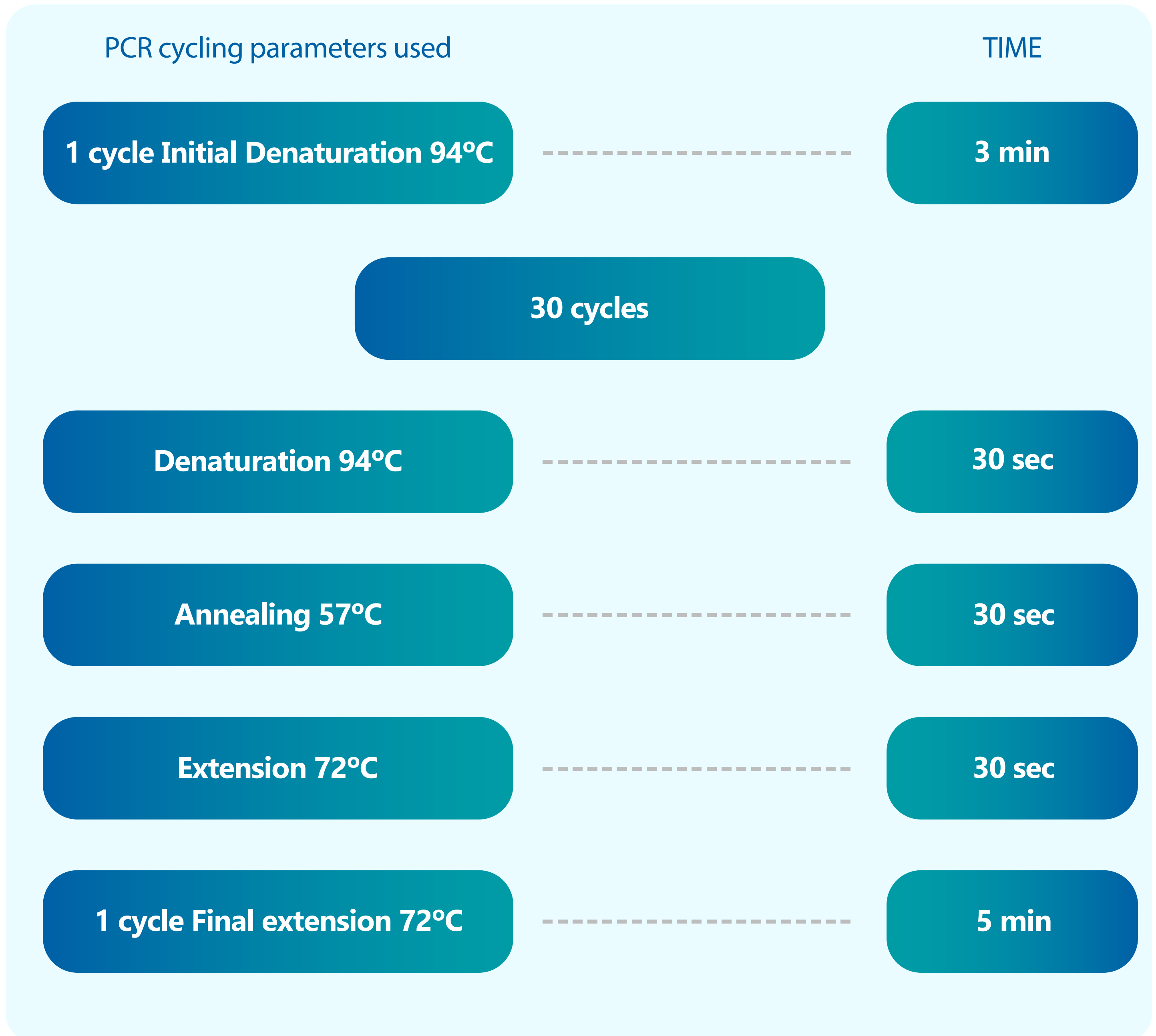
**Bioingentech Genomic DNA Purification** **Rev. Date: February 2017**

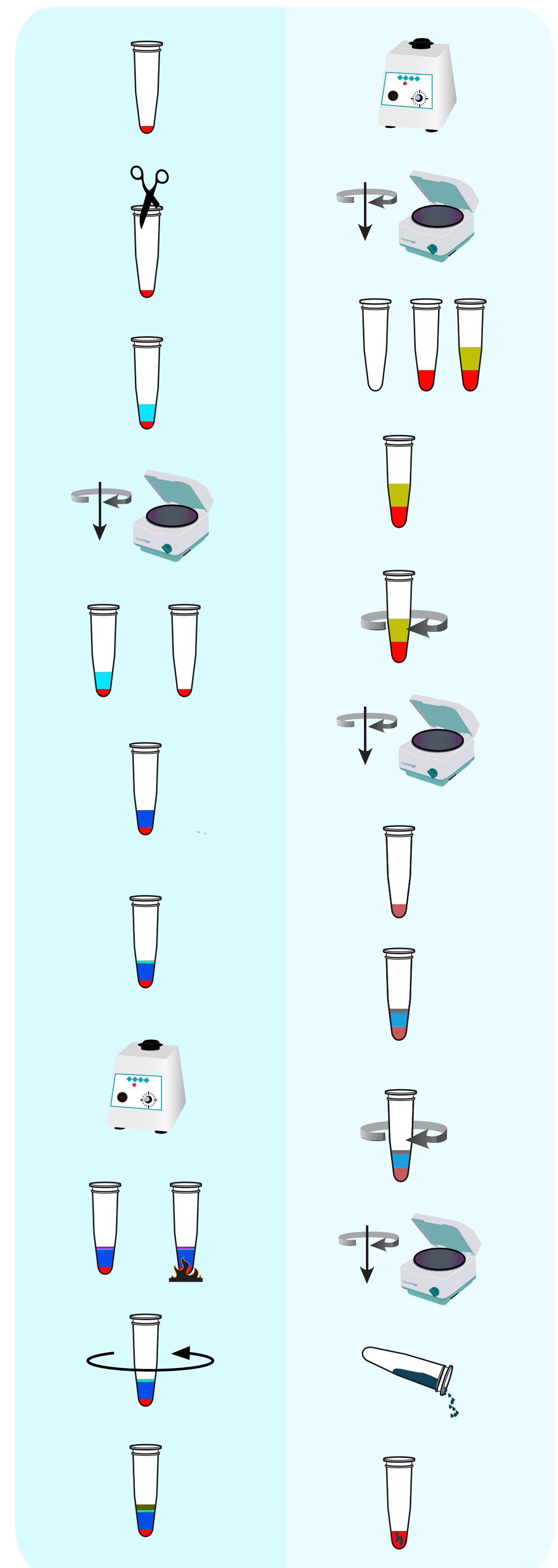
DNA pathogens detection




Note: Spin down the tubes of the Kits in a micro centrifuge before opening.
Premixes and reagents will be homogenized properly before use.
Nuclei lyses solution must be heated to 30ºC for 10 min before use. (DNA purification)

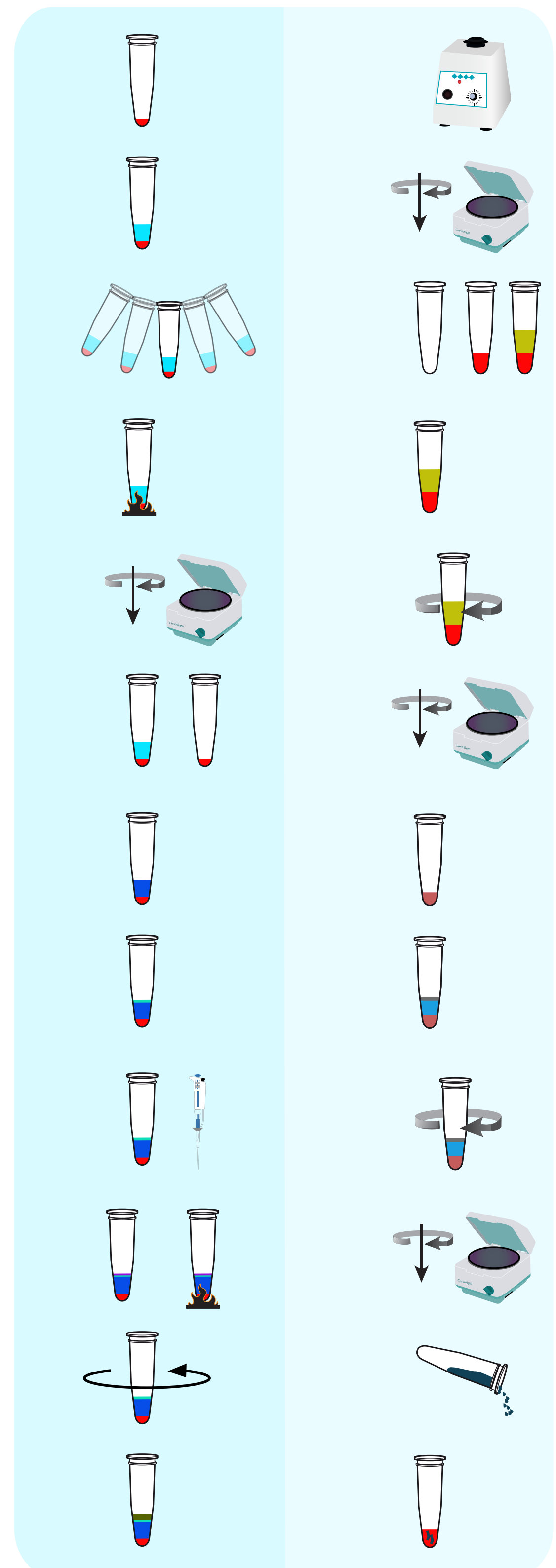
## DNA Extraction from Shrimp

Samples: Hepatopancreas, Gills and Pleopods.



1. Cut the samples and fill the tube until approximately 200 uL mark in a 2000 uL tube.
2. Macerate the sample with scissor.
3. Add 1 mL of CLS to wash the sample.
4. Centrifuge to 14.000 RPM for 3 minutes then discard supernatant.
5. Add 200-300 uL of NLS.
6. Add 2,5 - 5 uL of Proteinase K then Vortex 3 times.
7. 2 uL de RNase, Incube to 60-70°C for 60 min.
8. Spin for 5 seconds to 7.000 RPM.
9. Add 60uL of PPS (Protein Precipitation solution).
10. Vortex 3 times for 5 seconds each.
11. Centrifuge for 5 min to 13.000 RPM.
12. Take 150uL of supernatant and add to a new tube with. 150uL of Isopropanol.
13. See the white ring in the top of solution. There are the DNA.
14. Homogenize manually two times.
15. Centrifuge for 5 min to 13.000 RPM.
16. Discard supernatant but keep approximately 30-50uL in the tube bottom.
17. Add 100uL of Wash Solution (remember add 7mL (for 48 reactions) or 14mL (for 96 reactions) of Ethanol 100% depending which format you purchased.
18. Homogenize manually three times.
19. Centrifuge for 3 min to 13.000 RPM.
20. Discard all supernatant.
21. Resuspend the pellet wit 20-40uL of DNA REHYDRATION SOLUTION (Template).

## DNA extraction protocol from Birds

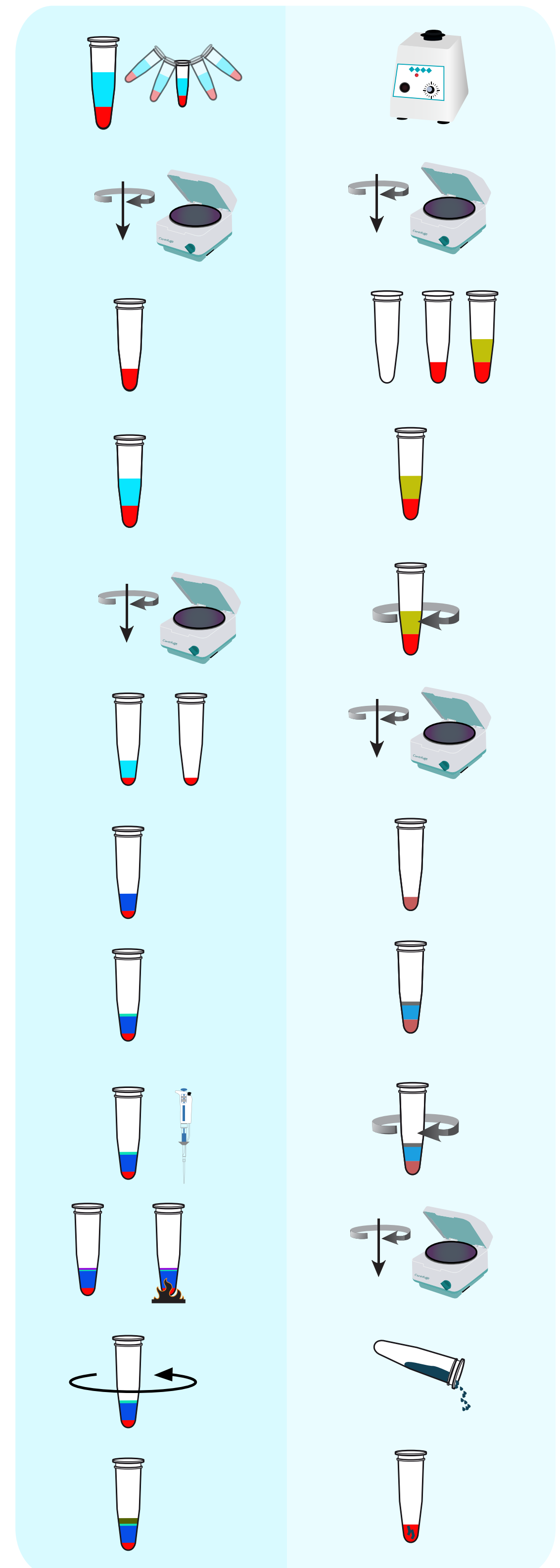


1. Take 30uL of bird total blood.
2. Add 200uL of CLS (cell lysis solution).
3. Mix manually 10 times.
4. Incube for 5 min.
5. Centrifuge for 5 min to 10.000 RPM.
6. Discard supernatant and conserve the red and white pellet.
7. Add above pellet 200uL of NLS (Nuclei lysis solution).
8. Add 2uL of Proteinase K .
9. Mix by pipetting 10 times using a 1mL filter tip.
10. 2 uL de RNase, Incube to 60-70°C for 60 min.
11. Spin for 5 seconds to 7.000 RPM.
12. Add 70uL of PPS (Protein Precipitation solution).
13. Vortex 3 times for 5 seconds each.
14. Centrifuge for 5 min to 13.000 RPM.
15. Take 150uL of supernatant and add to a new tube with 150uL of Isopropanol.
16. See the white ring in the top of solution. There are the DNA.
17. Homogenize manually two times
18. Centrifuge for 5 min to 13.000 RPM
19. Discard supernatant but keep approximately 30-50uL in the tube bottom.
20. Add 100uL of Wash Solution (remember add 7mL (for 48 reactions) or 14mL (for 96 reactions) of Ethanol 100% depending which format you purchased.
21. Homogenize manually three times
22. Centrifuge for 3 min to 13.000 RPM
23. Discard all supernatant.
24. Resuspend the pellet wit 30-40uL of DNA REHYDRATION SOLUTION (Template)

Then for PCR assay you must take 2-3uL of template and add in 11uL (5,5uL of Premix PCR (Remember homogenize your Premix PCR manually three times] + 6uL of PCR grade water) Mix well by pipetting, add one drop of Mineral Oil and put tubes in thermocycler.

Use program mentioned in quality control certificate.

## DNA extraction protocol from Blood



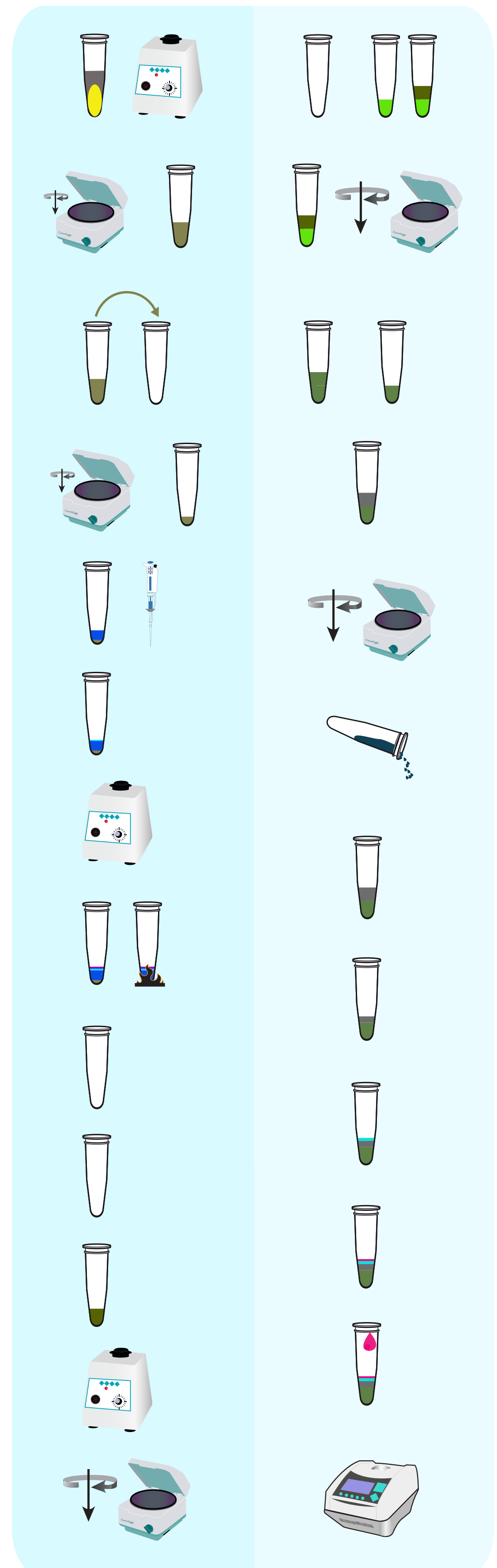
1 Take 300uL of Blood and add 1000uL of CLS then mix manually.
2 Centrifuge 5 minutes to 10.000 RPM.
3 Eliminate 1000uL of supernatant.
4 Add 700uL of CLS.
5 Centrifuge 5 minutes to 10.000 RPM.
6 Eliminate 950 uL of supernatant.
7 Add above pellet 200uL of NLS (Nuclei lysis solution).
8 Add 2uL of Proteinase K .
9 Mix by pipetting 10 times using a 1mL filter tip.
10 2 uL de RNase, Incube to 60-70°C for 60 min .
11 Spin for 5 seconds to 7.000 RPM.
12 Add 60uL of PPS (Protein Precipitation solution).
13 Vortex 3 times for 5 seconds each.
14 Centrifuge for 5 min to 13.000 RPM .
15 Take 150uL of supernatant and add to a new tube with 150uL of Isopropanol.
16 See the white ring in the top of solution. There are the DNA.
17 Homogenize manually two times.
18 Centrifuge for 5 min to 13.000 RPM.
19 Discard supernatant but keep approximately 30-50uL in the tube bottom.
20 Add 100uL of Wash Solution (remember add 7mL (for 48 reactions) or 14mL (for 96 reactions) of Ethanol 100% depending which format you purchased.
21 Homogenize manually three times .
22 Centrifuge for 3 min to 13.000 RPM .
23 Discard all supernatant.
24 Resuspend the pellet wit 30 uL of DNA REHYDRATION SOLUTION (Template).

Then for PCR assay you must take 2-3uL of template and add in 11uL (5,5uL of Premix PCR (Remember homogenize your Premix PCR manually three times] + 6uL of PCR grade water) Mix well by pipetting, add one drop of Mineral Oil and put tubes in thermocycler.

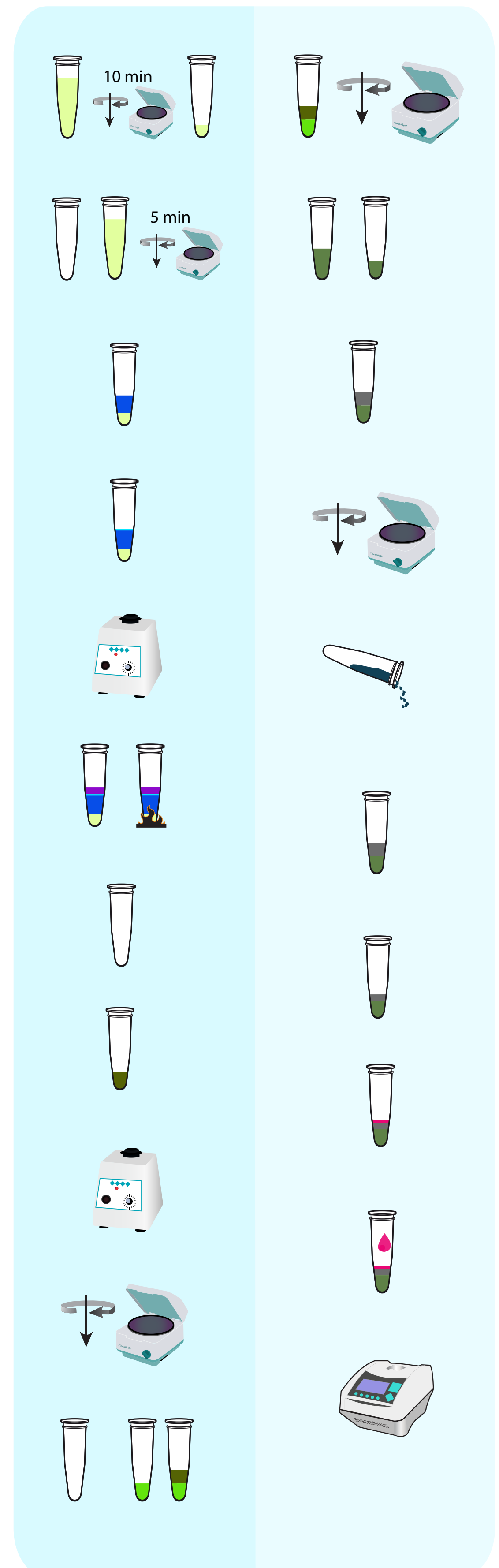
Use program mentioned in quality control certificate.

## DNA extraction protocol from Egg Yolk

1. Put 5 mL of egg yolk in a 15 ml tube. Agrege 5 mL of PBS10X and then vortexing 2 times during 10 sec.
2. Centrifuge at 4000 rpm for 5 min at Room Temperature. Remove the supernatant and leave about 2 mL in the bottom of tube.
3. Take 1.7 mL and pass to 2 mL new tube.
4. Centrifuge for 5 min at 13000 rpm then remove the supernatant and leave 200 uL in the bottom of tube.
5. Add 800 uL of NLS and Homogenize by pipeting.
6. Add 10ul Proteinase K to each tube.
7. Perform 3 Vortex cycles for 3 seconds.
8. Add 2uL RNAse and incubate 60min in thermoregulated bath between 60 and 75°C.
9. Dry thoroughly each tube and perform Spin Down to retrieve drops of water evaporated.
10. Remove the torula fragment of each tube
11. Add 280uL of Protein Precipitation solution.
12. Perform 3 Vortex cycles of 5 seconds.
13. Centrifuge at 13.000rpm for 5 min.
14. Take 500uL supernatant and move to new tube containing 500uL of Isopropanol (grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR / NO USE TECHNICAL GRADE BIOLOGY), discard the remainder. In new tube Observe DNA ring on top of the solution.
15. Homogenize manually 3 times gently (remember tightly covered tubes) and centrifuged for 3min 13.000rpm.
16. Remove supernatant and left approximately 75 ul - 100uL in the bottom of the tube.
17. Add 100uL of 70% ethanol to each tube. (Grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR BIOLOGY / NO USE TECHNICAL GRADE).
18. Centrifuge for 3min at 13.000rpm.
19. Remove all supernatant.
20. Drie all tubes in thermoplate between 60-70 °C for 1 minute.
21. When there is no ethanol in the tubes, resuspend the pellet in 30-50uL of DNA Rehydration solution.
22. Add 13uL of PCR Mix to PCR tubes for each pathogen.
23. Add 3uL of Template to each tube with PCR Mix.
24. Add to each tube a drop of mineral oil.
25. Run PCR program.

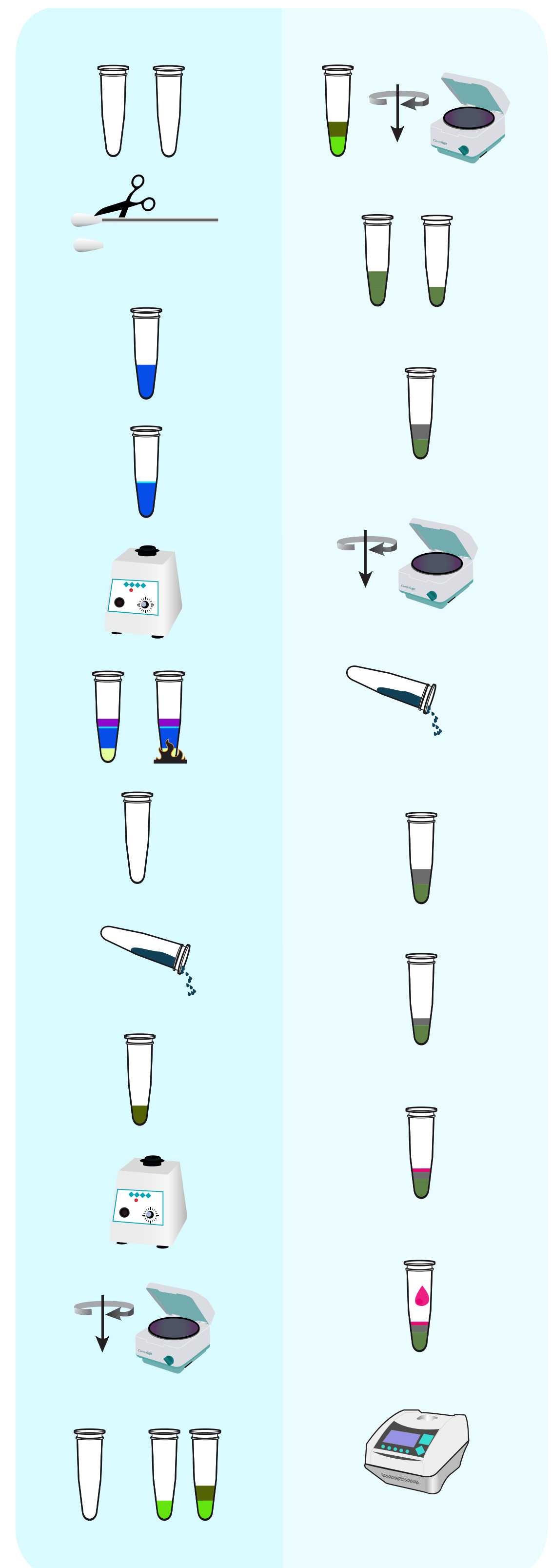


## DNA extraction protocol from milk



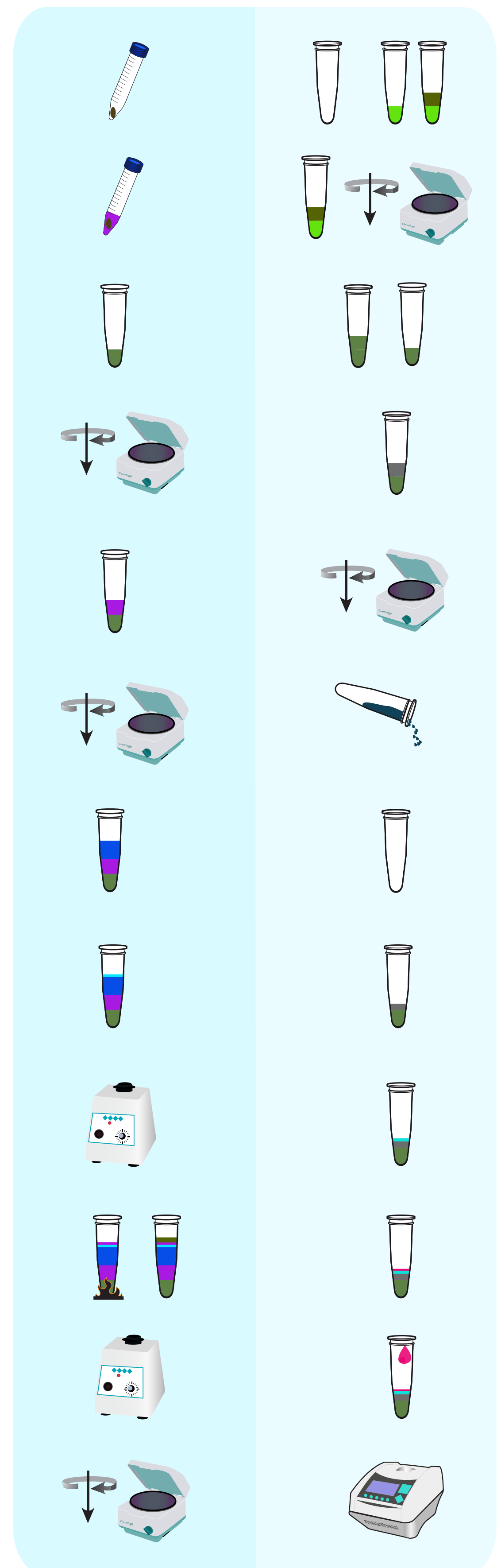

1. Put 10 mL of raw milk in 15 ml tube. Centrifuge 4000 rpm for 10 min. Remove fat layer and remaining supernatant. Leave in the bottom 2 mL.
2. Resuspend the 2 mL remaining in the same 15 mL tube. Then take 1.7 mL and place in a 2 mL new tube. Centrifuge at 13,000 rpm for 5 min and then remove the supernatant leaving only about 200 uL in the bottom.
3. Add 0,8mL of nucleolysis solution to each tube.
4. Add 10ul Proteinase K to each tube.
5. Perform 3 Vortex cycles for 10 seconds.
6. Add 2uL RNAse and incubate 60 min in thermoregulated bath between 60 and 75°C.
7. Dry thoroughly each tube and perform Spin Down to retrieve drops of water evaporated.
8. Add 350uL of Protein Precipitation solution.
9. Perform 3 Vortex cycles of 5 seconds.
10. Centrifuge at 13.000rpm for 5 min.
11. Take 500uL supernatant and move to new tube containing 500uL of Isopropanol (grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR / NO USE TECHNICAL GRADE BIOLOGY), discard the remainder. In new tube Observe DNA ring on top of the solution.
12. Homogenize manually 3 times gently (remember tightly covered tubes) and centrifuged for 3 min at 13.000rpm.
13. Remove supernatant and left approximately 75 ul - 100uL in the bottom of the tube.
14. Add 100uL of 70% ethanol to each tube. (Grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR BIOLOGY / NO USE TECHNICAL GRADE) .
15. Centrifuge for 3min at 13.000rpm.
16. Remove all supernatant.
17. Drie all tubes in thermoplate between 60-70 °C for 1 minute.
18. When there is no ethanol in the tubes, resuspend the pellet in 30-50uL of DNA Rehydration solution.
19. Add 3uL of Template to each tube with PCR Premixture (see table in procedure manual).
20. Add to each tube a drop of mineral oil.
21. Run PCR program (see program in procedure manual).

## DNA extraction protocol from swabs



1. Label 2 mL tubes with respective samples.
2. Cut the tip swabs and insert in tubes.
3. Add 0,8mL of nucleolysis solution to each tube.
4. Add 10ul Proteinase K to each tube.
5. Perform 3 Vortex cycles for 10 seconds.
6. Add 2uL RNAse and incubate 60min in thermoregulated bath between 60 and 75°C.
7. Dry thoroughly each tube and perform Spin Down. to retrieve drops of water evaporated.
8. Remove the torula fragment of each tube.
9. Add 350uL of Protein Precipitation solution.
10. Perform 3 Vortex cycles of 5 seconds.
11. Centrifuge at 13.000rpm for 5 min.
12. Take 500uL supernatant and move to new tube containing 500uL of Isopropanol (grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR / NO USE TECHNICAL GRADE BIOLOGY), discard the remainder. In new tube Observe DNA ring on top of the solution.
13. Homogenize manually 3 times gently (remember. tightly covered tubes) and centrifuged for 3min 13.000rpm.
14. Remove supernatant and left approximately. 75 ul - 100uL in the bottom of the tube.
15. Add 100uL of 70% ethanol to each tube. (Grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR BIOLOGY / NO USE TECHNICAL GRADE) .
16. Centrifuge for 3min at 13.000rpm.
17. Remove all supernatant.
18. Drie all tubes in thermoplate between 60-70 °C for 1 minute.
19. When there is no ethanol in the tubes, resuspend the pellet in 30-50uL of DNA Rehydration solution.
20. Add 3uL of Template to each tube with PCR Premixture (see table in procedure manual).
21. Add to each tube a drop of mineral oil.
22. Run PCR program (see program in procedure manual).

## DNA extraction protocol from lee



1. Add 0.5 - 1 g stool sample in a sterile 15mL tube.
2. Add 3 to 5 mL 1X PBS, homogenizes.
3. Transfer 500 uL of the mixture to a new micro tube.
4. Centrigue at 14,000 rpm for 1 minute.
   Then discard the supernatant.
5. Add 500 uL of PBS 1X and homogenize.
6. Centrigue at 14,000 rpm for 1 minute. Then discard
   the supernatant.
7. Add 0,8mL of nucleolysis solution to each tube.
8. Add 10ul Proteinase K to each tube.
9. Perform 3 cycles 3s in Vortex.
10. Add 2uL RNAse and incubate 60min in thermoregulated
    bath between 60 and 75 ° C. Add 280uL of Protein
    Precipitation solution (1/3 volume, Vol x 0.35).
11. Perform 3 cycles of 5 s at Vortex.
12. Centrifuge at 13.000rpm x 5 min.
13. Take 500uL of the supernatant and move to new tube
    containing 500uL of Isopropanol (grade
    PA - UPS / BIOTECHNOLOGICAL / MOLECULAR /
    NO USE TECHNICAL GRADE BIOLOGY), discard the
    remainder. In new tube Observe DNA ring on top of the solution.
14. homogenize manually 3 times gently (remember
    tightly covered tubes) and centrifuged for 3min 13.000rpm.
15. The supernatant is removed and a volume of 75 is
    left - 100uL in the bottom of the tube.
16. Add 100uL of 70% ethanol to each tube.
    (Grade PA - UPS / BIOTECHNOLOGICAL / MOLECULAR BIOLOGY
    / NO USE TECHNICAL GRADE)
17. Centrifuge for 3min 13.000rpm.
18. All supernatant is removed. 19 All tubes are dried to
19. 60-70 ° C in plate for 2 minute.
20. When there is no ethanol in the tubes, the pellet is
    resuspended in 30-50uL of DNA Rehydration solution.
21. Add 13uL PCR Mix in PCR tubes for each pathogen.
22. Add 3uL Template into each PCR tube.
23. Add to each tube a drop of mineral oil.
24. Run PCR with the given program.