# Wine vPCR kit

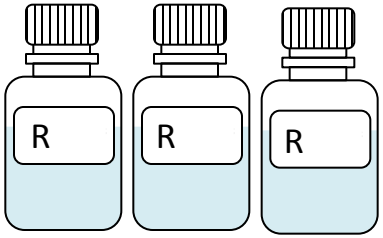
**Cat. No 4900020000**

GenIUL

# GUIDE for vPCR in wine samples

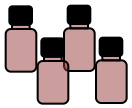
The kit is suitable for 75 samples and contains:

3 x Reaction Buffer (R), 60 mL




1 x Dilution Buffer (D), 60 mL


2 x PEMAX reagent vials of 0,5 mg
(Cat. No 4900013000)



# GUIDE for vPCR in wine samples

Not provided but necessary:

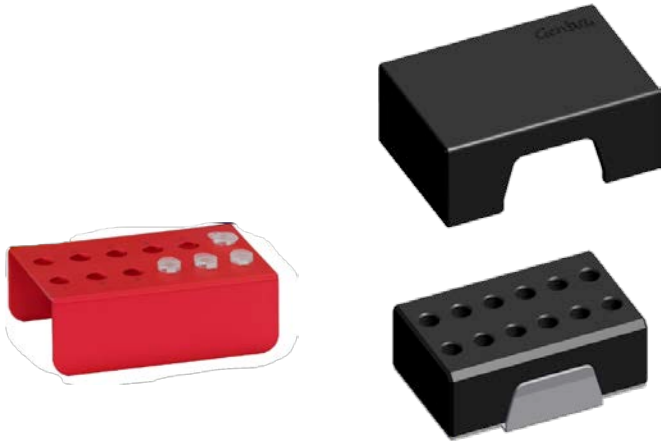
PhAST blue
PhotoActivation System for Tubes

Cat. No. 9000700

Reaction tubes
Cat. No. 4900019000

# GUIDE for vPCR in wine samples

Not provided but recommendable :

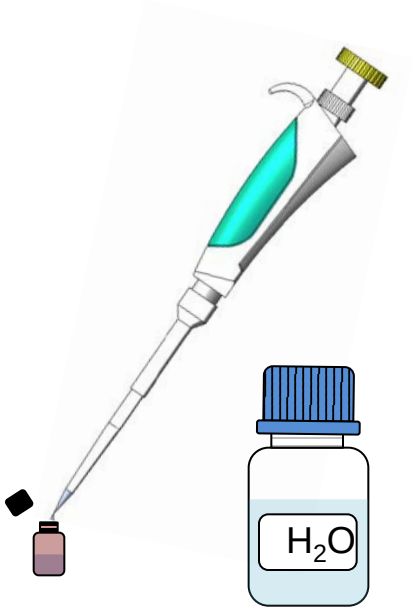


Dark Box: to protect reaction tubes from light
Cat. No. 90001200


D-Bag system: for safe removal of toxic residues
Cat. No. 900099645

# GUIDE for vPCR in wine samples

Previous Step: vPCR reagent reconstitution

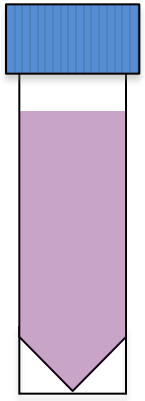



Add 500 μL of sterile bi distilled or PCR grade water (not provided) in each vial and mix thoroughly

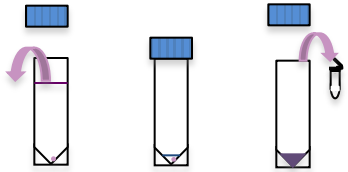
# GUIDE for vPCR in wine samples

## Step 1 : Sample concentration

Before collection, shake the sample container and allow decanting during 10 min



End or bottled samples, need one additional
and previous concentration step up to 1,7 mL



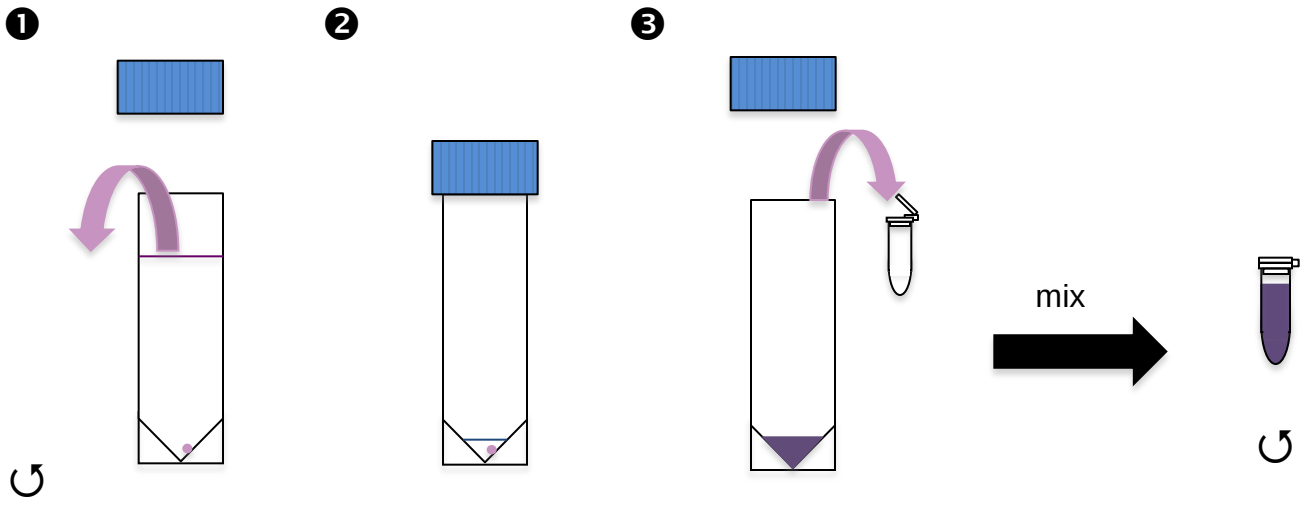
8000xg 5 min or 3500xg 30 min, <10ºC

20mL of finished product or 1,7 mL of production samples

# GUIDE for vPCR in wine samples

## Step 1 : End or bottled samples concentration

Reduce the volume from 20 mL to 1,7 mL



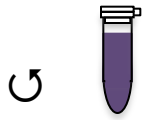

8000xg 5 min or 3500xg
30 min <10ºC
and remove the supernatant

8000xg 5 min or 3500xg 30 min
<10ºC

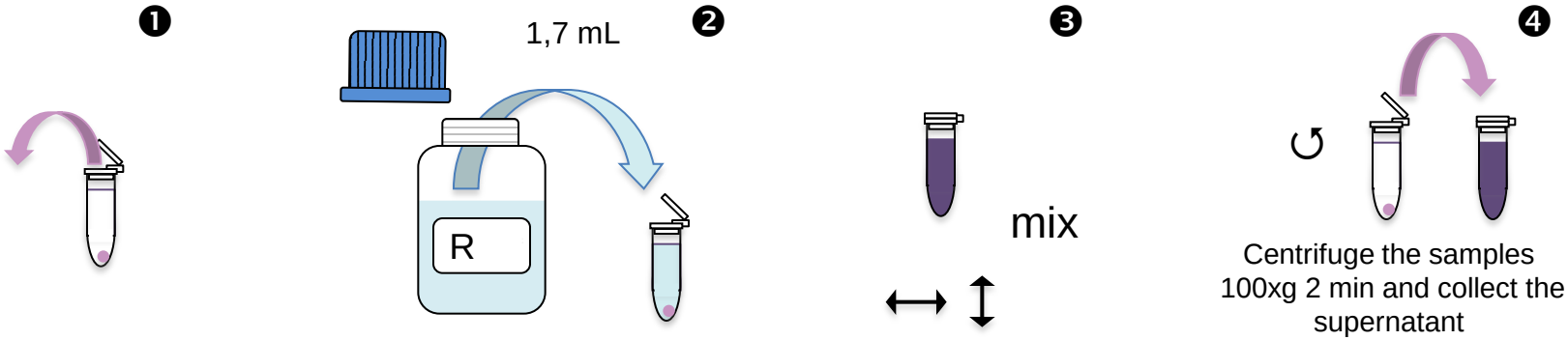
# GUIDE for vPCR in wine samples

## Step 2: Washing samples

Remove the supernatant and resuspend the pellet in 1,7 mL of Reaction Buffer (R)



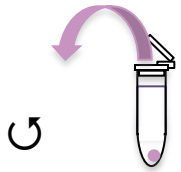
8000xg 5 min or 3500g 30 min,
<10ºC



# GUIDE for vPCR in wine samples

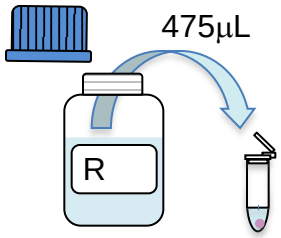
## Step 3: Sample treatment

❶



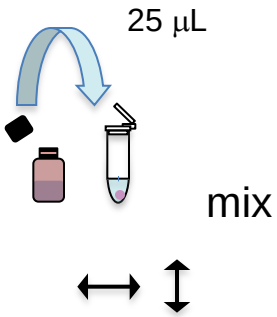
Centrifuge the sample 8000xg 5 min and remove the supernatant

❷




Resuspend the pellet with 475 µL of Reaction Buffer (R)

❸




Add 25 µL of PEMAX Reagent and mix

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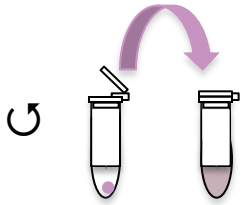
## Step 4: Incubation on darkness

20 min at room temperature

## Step 5: Sample tube change



Spin down, micropipette cell homogenization, and collect the sample to another tube

## Step 6: Photoactivation

15 min, 100% power
10 min, darkness
15 min, 100% power

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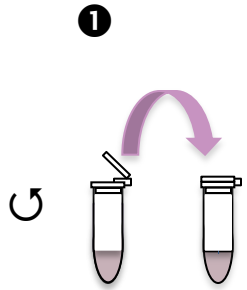
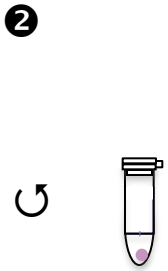
## Step 7: Removing reagents and PCR inhibitors

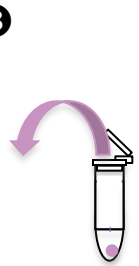
The current pH of the sample is not compatible with most of enzymatic digestions used in DNA purification procedures. Additionally, the high levels of Phenantridinium can act as PCR inhibitors. For this reason, we recommend to perform an additional centrifugation step of at least 8000xg during 5 min, remove the supernatant and resuspend the pellet with 200 µL of Dilution Buffer (D).



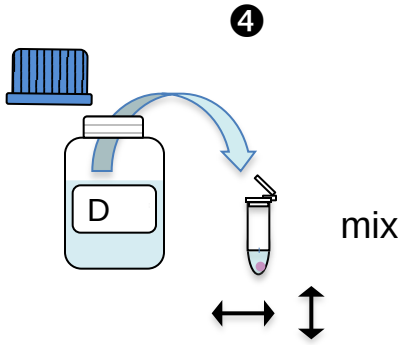

Spin down, micropipette cell homogenization, and collect the sample to another tube




Centrifuge the samples 8000xg 5 min




Remove the supernatant (store the residues in the D-Bag System)




Add 200 µL of Dilution Buffer (D) and mix

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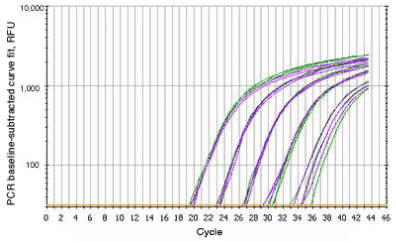
## Step 8: DNA purification and qPCR





Note: In order to increase the DNA purification yield from yeast cells, is recommended a boiling-freezing treatment (95ºC, 10 min and <-20ºC, 10 min) two times.

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Experts in vPCR