LABORATORY OF MOLECULAR PATHOLOGY

qPCR library quantification

Using KAPA Library Quant Kit

Prep:

☐ 01 Thaw the KAPA SYBR FAST qPCR Master Mix. Keep it on ice.
Make sure the primers were added, or add the primers to the master mix yourself.

☐ 02 Thaw Dilution Buffer and **dilute it 10×** with water to get a working solution.

☐ 03 Thaw the six qPCR standards, keep them on ice.

☐ 04 Dillute your samples **3 000×, 9 000× and 27 000×** with the prepared 1× dilution buffer. *Do not use water for diluting samples. Do not pipette less than 2 μL for accuracy.*

☐ 05 Calculate how much you need and then prepare the master mix for the qPCR. Don't forget the six DNA standards and a negative control:

Master mix	**1×**	**×**
KAPA SYBR MM 2×	9,0 μL	
DEPC-H_2O	3,0 μL	
Diluted DNA sample	3,0 μL	–

☐ 06 Pipette **12 μL of the master mix** in each of the wells in the white strips or white plates for the Roche LightCycler.

☐ 07 Add **3 μL of the diluted samples** or the standards or water.
Pipette the standards from the lowest dilution to the highest (From 6 to 1).

☐ 08 Seal the plate or the strips. Quick vortex and quick spin.

☐ 09 Run the qPCR programme, don't forget to set the data acquisition during the annealing step:

Amplification programme	**Temperature**	**Time**	
Initial denaturation	95 °C	5 min	
Denaturation	95 °C	30 s	**40×**
Annealing + Extension	60 °C	45 s	
Melting curve	65–95 °C		