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Selecting the Proper Pipetting Tools for Optimal PCR and qPCR in the Laboratory

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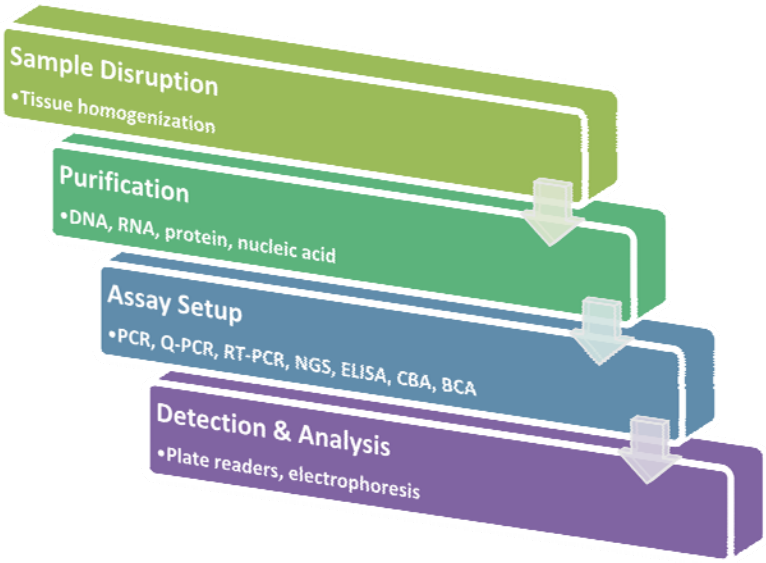
Technical Note 0212

Overview

This technical note will assist you with selecting pipetting tools for optimal PCR (Polymerase Chain Reaction) and qPCR (Quantitative Polymerase Chain Reaction) in the laboratory. Life science laboratories continue to utilize technician-friendly, basic tools to develop and optimize dedicated PCR and qPCR protocols. The typical workflow of a laboratory focused on PCR and qPCR is as follows:



Part 1: What is conventional PCR?

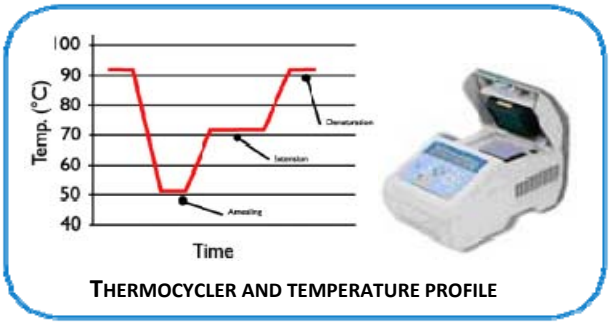
PCR is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece [(1)]. PCR technology is widely used to aid in quantifying DNA because the amplification of the target sequence allows for greater sensitivity of detection than could otherwise be achieved. This technique is widely used in molecular biology, microbiology, genetics, diagnostics, clinical laboratories, forensic science, environmental science, hereditary studies, paternity testing, and many other applications.

The following steps outline a typical PCR protocol:

1-2 HRS	Nucleotide Isolation	•Tissue and cellular disruption by physical or chemical means - separate soluble components (protein, RNA, DNA) from cellular debris (cell membrane) •Extract DNA from protein and other soluble components by Liquid Liquid Extraction (LLE), or magnetic bead cleanup
3 HRS	PCR Prep	•Creation of a Master Mix (MM) which contains all of the ingredients required for PCR reaction •Aliquot small volume of the MM to microcentrifuge tubes/plates containing DNA samples •1 to 96+ samples (each reaction volume ~10-50 µL)
3 HRS	Amplification Run	•Thermocycler machine to cycle between 3 temperatures (about 30 times)
3 HRS	Analysis	•Load PCR reactions into agarose gel •Separate by electrophoresis •Stain gel with DNA binding chemical •Reading with UV

Conventional PCR requires in addition to the chemistry (a piece of DNA - template, large quantities of the four nucleotides – dNTP's, large quantities of the primer sequences –forward/reverse end, and DNA polymerase - enzyme), some equipment, such as thermal cyclers, gel electrophoresis, UV illuminators, and consumables.

Thermal cyclers where a specific DNA sequence is amplified by Subjecting the DNA sample to cyclic temperature changes
(1- Denaturing at 94-96°C,
2- Annealing at ~65°C,
3- Elongation at 72°C.)



THERMOCYCLER AND TEMPERATURE PROFILE

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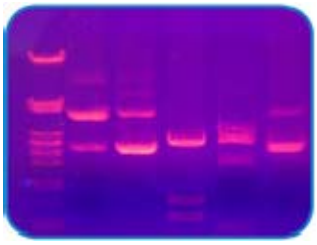
- Agarose gel electrophoresis system to isolate the amplified DNA piece

- UV illuminator to visualize the DNA bands.

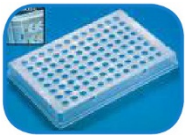


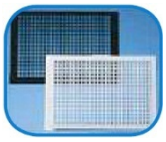
- Pipettes and tips to prepare the PCR mixes where the DNA piece will be included after extraction and purification from raw materials and prior to PCR amplification.

- Specific vessels are used to carry out the experiments (micro-tubes (0.2, 1.5, 2.0 mL), 0.2mL-tube strips, 96/384-well micro-plates, 24/48/96 PCR-well plates). Vessel ware must be free of RNAse, DNAse and pyrogens in order to prevent degradation of the starting template or inhibiting the amplification process during the cycles.

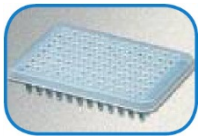
2.0, 1.5 AND 0.2 mL

0.2 mL-TUBE STRIPS (8 AND 12 TUBES)MICROTUBES





384-Well Plates



24/48-PCR Well Plates

96-PCR Well Plate

For applications to determine whether or not a target sequence of interest is present or not (qualitative studies) conventional PCR is enough.

Besides the specific laboratory safety precautions that PCR amplification requires (different rooms for extraction, PCR process and isolation, working under bio-safety cabinet with gloves, decontamination of benches, pipettes and tools, etc), the pipetting process is important because:

1. The precision required to handle the small volumes (in general, < 10 µL).

   It is important to handle small volumes reproducibly because if the DNA template volume has been dispensed excessively in the PCR mix, the efficiency of the reaction may be affected because when applying higher DNA template volumes, traces of inhibitory substances potentially present in the template DNA preparation (often diluted to a subcritical level) may increase.

2. The process requires extensive pipetting in multiple steps.

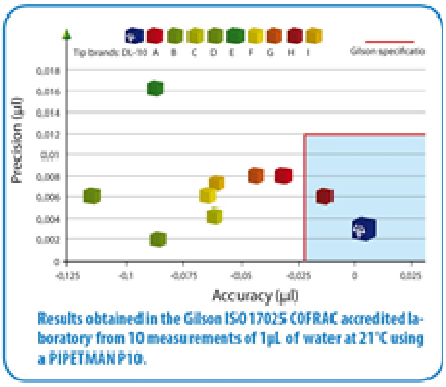
3. To prevent contamination between samples, the pipetting system (pipette & consumables) need to be protected against aerosols.

Gilson offers a large choice of pipetting systems to suit PCR and qPCR applications:

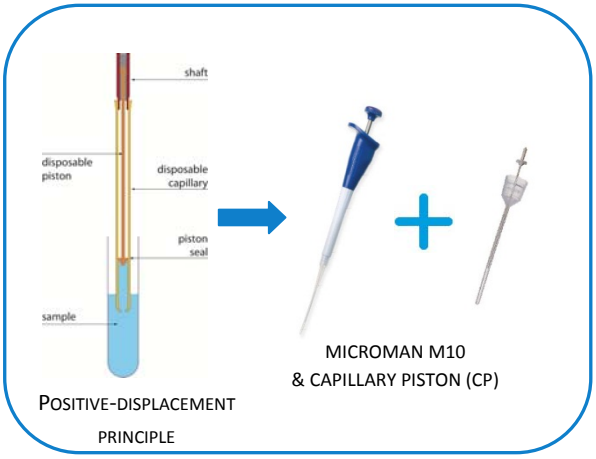
- **PIPETMAN P2, P10, P2N, P10N, P2L, P10L, P10M,** fitted with DIAMOND DF10ST, DFL10ST sterilized filter tips. The lower part of the pipette can be autoclaved if any contamination occurs. DFL10ST tips are recommended for use with 1.5/2.0 mL tubes as they permit access down to the bottom of the tube without contaminating the pipette shaft.

- **PIPETMAN P100, P100N or P100L** or may be used for dilution.

The figure below shows the best accuracy and precision the operator can expect with Gilson tip versus competition ones.



- **MICROMAN 10, 100** (fully autoclavable) based on positive-displacement dispensing can be used with sterilized capillaries and pistons CP10ST and CP100ST which offers full protection against cross-contamination. In addition, it offers more accurate pipetting of viscous samples or volatile reagents.



- **PIPETMAN Neo, PIPETMAN L MULTI 8x20L and 12sx20L, or PIPETMAN M MULTI 8x20M and 12x20M** fitted with DFL10ST or DF30ST (for diagnostic purposes) when several samples need to be processed simultaneously in PCR-tube strips, 96 or 384-well microplates or 24, 48 or 96 PCR-well plates.

- **DISTRIMAN** equipped with sterilized DistriTips micro (125 µL) for repetitive dispensing from a single tube (microtube) format into multi-well format vessels (tube strip or microplate).

Basic PCR protocol

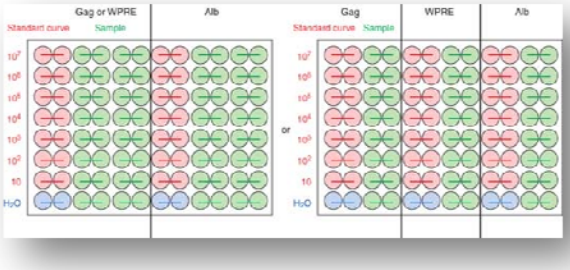
SETTING UP CONVENTIONAL PCR REACTION	INSTRUMENT
1. PCR reagents preparation	
• Thaw all frozen reagents before use.	
• Mix all reagents thoroughly.	• **Mix mode** setting on PIPETMAN Concept or PIPETMAN M.
• Briefly centrifuge them before starting the procedure to spin down the whole solution.	• **GmClab centrifuge** (0.2, 1.5/2.0 mL microtubes, 0.2 mL-tube strips).
2. PCR Master Mix preparation	
PCR Master mix is prepared in a separate room to prevent contamination of it by aerosols coming from DNA sample manipulation.	
For 10 reactions (an additional one is for control).	**Filter tips are used to prevent cross contamination of reagents during the PCR Master mix solution.**
• Dispense 36.75 µL x 11= 404.25 µL of PCR Grade water in 1.5 mL tubes on ice.	• PIPETMAN **P100, P100N, P100L, P100M** with **DF100ST** **or** • MICROMAN **M100** with **CP100 or CP100ST** (diagnostics)
• Add 5 µL x 11= 55 µL of PCR reaction buffer with $MgCl^2$.	• PIPETMAN **P20**, **P20N, P20L,** with **DF30ST** **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics)
• Add 1 µL x 11 = 11 µL of PCR Grade Nucleotide Mix.	• PIPETMAN **P2, P2N, P2L, P2M** or **P10M** with **DF10ST** or **DFL10ST** **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics)
• Add 1 µL x 11 = 11 µL of forward Primers.	• PIPETMAN **P2, P2N, P2L, P2M** or **P10M** with **DF10ST** or **DFL10ST** **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics)
• Add 1 µL x 11 = 11 µL of reverse Primers.	• PIPETMAN **P2, P2N, P2L, P2M** or **P10M** with **DF10ST** or **DFL10ST** **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics)
• Dispense 1.25 µL x 11 = 13.75 µL of Taq DNA polymerase . • Mix these components to form a homogeneous solution	• PIPETMAN **P2, P2N, P2L, P2M** or **P10M** with **DF10ST** or **DFL10ST** **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics) • Aspirate/dispense mode : ✓ **P1000, P1000N, P1000L, P1000M** with **DF1000ST** ✓ MICROMAN **M1000** with **CP1000 or CP1000ST** • **Mix mode** setting on PIPETMAN Concept or PIPETMAN M.
• Briefly centrifuge	• **GmCLab** centrifuge for spinning down solution.

SETTING UP CONVENTIONAL PCR REACTION	INSTRUMENT
3. Final PCR reaction mixture **Extraction, purification of DNA samples may lead to aerosol formation. Pipettes are fitted with filter tips and operators work under bio-safety cabinet. Positive displacement pipettes, CPs / syringes.**	
For 11 vessels (0.2, 1.5 ml microtubes or microplate wells)	
• Aliquot 45 µL of the solution into each vessel (on ice).	• PIPETMAN **P100, P100N, P100L,** with **DF100ST** (diagnostics) **or** • PIPETMAN **P8x200N, P12x200N, P8x200L, P12x200L, P8x200M or P12x200M** with **DF200ST** (diagnostics) for 96-well plates or PCR-tube strips **or** • MICROMAN **M100** with **CP100 or CP100ST** (diagnostics) **or** • DISTRIMAN with **DistriTips mini** (1.25 mL) or **ST** (diagnostics)
• Add 5 µL of DNA template in each 11 vessel (11).	• PIPETMAN **P10, P10N, P10L, P10M** with **DFL10ST** (for 1.5/2.0 mL) **or** • MICROMAN **M10** with **CP10 or CP10ST** (diagnostics).
• Gently mix each vessel.	• Aspirate/dispense mode for any vessel: ✓ **P100, P100N, P100L, P100M** with **DF200ST** (diagnostics) or ✓ MICROMAN **M100** with **CP100 or CP100ST** (diagnostics). • Or **Mix mode** setting on PIPETMAN Concept or PIPETMAN M.
• Briefly centrifuge.	• **GmCLab centrifuge** for spinning down solution in 0.2, 1.5/2.0 mL microtubes, PCR-tube strips.
• Close the tubes, PCR-tube strips or seal the microplate with adhesive foil.	
4. PCR amplification	
• Place the vessels in the thermal cyclers.	
• Set the thermal profile and start PCR immediately.	
• Run the PCR process for 30 cycles.	**The thermal cycling profile will be run for 30 cycles to amplify the original DNA material.**

Part 2: What is qPCR or RT-PCR?

qPCR or Real-time quantitative polymerase chain reaction is used to determine gene copy (transgenic work). It is the most sensitive and reproducible method for detection (qualitative) and quantification of DNA, cDNA or RNA molecules. Basically, Q-PCR measures the fluorescence signal produced by the amplification of a specific template in every PCR cycle as the amplification progresses [(2)] called "real-time PCR".

In the case of RNA templates, they must be transcribed into cDNA using a reverse transcriptase (enzyme) and then an aliquot of the reverse-transcription reaction is subsequently subjected to the real-time PCR. This method is called real-time Reverse Transcriptase PCR (QRT-PCR) [(3)].



Example Q-PCR Plate - Wiley Online Library
(http://media.wiley.com/mrw_images/cp/cpns/articles/ns0421/image_n/nns042106.jpg)

Why real-time PCR?

Conventional or endpoint PCR is not uniform or reproducible enough to be useful for the precise measurements required for quantitative analysis. Real-time PCR allows quantification of the template during the exponential phase of amplification, before limiting reagents, accumulation of inhibitors, or inactivation of the polymerase have started to have an effect on the efficiency of amplification.

Who supplies real-time PCR systems?

Real-time PCR requires an instrumentation platform that consists of a thermal cycler, a computer, optics for fluorescence excitation and emission, collection, data acquisition and analysis software. Available from several manufacturers, these machines differ in:

- Sample capacity (some are 96-well standard format, others process fewer samples or require specialized glass capillary tubes)
- Method of excitation (some use lasers, others broad spectrum light sources with tuneable filters),
- Overall sensitivity

The following steps outline a typical Q-PCR protocol:

1-2 HRS	Nucleotide Isolation	•Tissue and cellular disruption by physical or chemical means - separate soluble components (protein, RNA, DNA) from cellular debris (cell membrane) •Extract DNA from protein and other soluble components by Liquid Liquid Extraction (LLE), or magnetic bead cleanup
3 HRS	Q-PCR Prep	•Creation of a MM which contains all of the ingredients required for Q-PCR reaction, including tagged primers •Aliquot small volume of the MM to microcentrifuge tubes/plates containing DNA samples •1 to 96+ samples (each reaction volume ~10-50 µL)
3 HRS	Amplification Run	•Thermocycler (with fluorescence detector) machine to cycle between 3 temperatures (about 30 times) •Analysis occurs in real time as the PCR reaction occurs

There are platform-specific differences in how the software processes data. Real-time PCR machines are typically within budget of core facilities or labs that have the need for high throughput quantitative analysis.

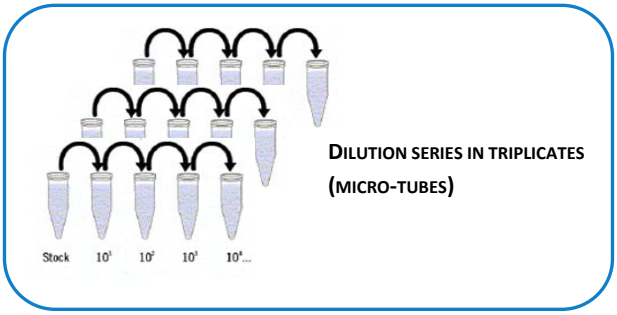
- **Roche** LightCycler® 2.0 & 480 Real-time PCR Systems .
- **Applied Biosystems** 7300 & 7900HT Fast Real-time PCR systems.
- **Agilent Technologies/Stratagene** Mx3000P® & Mx3005P QPCR systems.
- **BioRad** Real-time PCR system Family.
- **Qiagen** Rotorgene.
- And more…

Quantification using standard curve method

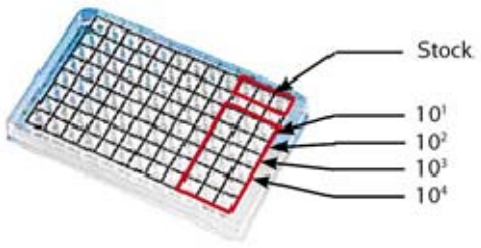
To perform DNA or RNA quantification, a standard curve is prepared from a dilution series of a template of known concentration. It is used when it is important to the experimental design and objective of the project to measure the exact level of template in the samples (e.g. monitoring the viral load in a sample). The ideal assay requires optimized primer sets, probe concentration, magnesium concentration, assay efficiency, and assay precision. Achieving optimized assay performance will allow accurate quantification of experimental samples, reliable data analysis and comparison of the experimental study.

Basic setup for standard curve quantification

1. Using a known starting concentration of template from one of a variety of existing sources, a dilution series of at least 5 different concentrations of the standards (this may be several orders of magnitude) is performed. Each concentration should be run in triplicate (or, at least, in duplicate), so that it is possible to determine the precision of pipetting, the reproducibility and the overall sensitivity of the assay. The dilution series is carried out in separate wells for micro-plates or separate micro-tubes or tube strips.

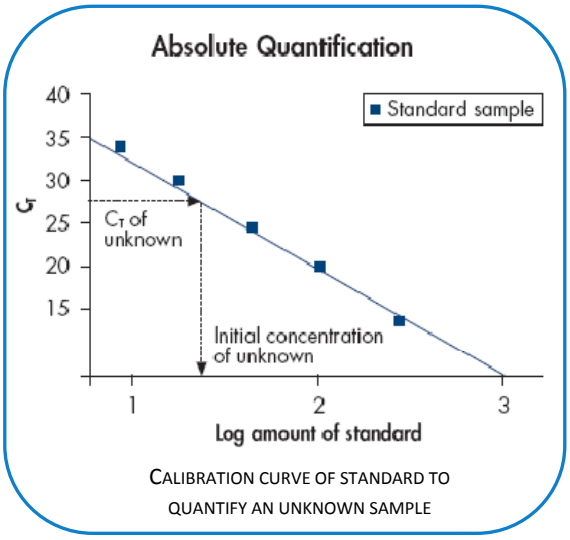


DILUTION SERIES IN TRIPLICATES (MICRO-TUBES)



DILUTION SERIES IN TRIPLICATES (MICROPLATE FORMAT)

2. Following amplification of the standard dilution series, the standard curve is generated by plotting the logarithm (log) of the initial template copy number against the C_t values generated for each dilution.



CALIBRATION CURVE OF STANDARD TO QUANTIFY AN UNKNOWN SAMPLE

The amount of unknown target should fall within the range tested.

Quantitative or Real-Time PCR Protocol

SETTING UP FOR THE REAL-TIME PCR REACTION OF DNA TEMPLATES	INSTRUMENT
1. PCR reagents preparation	
• Thaw all frozen reagents before use.	
• Mix all reagents thoroughly.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter
• Briefly centrifuge them before starting the procedure to spin down the whole solution (kept on ice).	• **GmClab centrifuge** (0.2, 1.5/2.0 mL microtubes, 0.2 mL-tube strips)
Reagents are kept on ice to prevent their degradation that may occur at room temperature.	
2. Making serial dilution of DNA standard template in 1.5 mL tubes (keep on ice) **The operator prepares the serial dilution of the DNA sample in various micro-tubes in a dedicated room under bio-safety cabinet, using filter tips. Electronic pipettes are used because a maximum of precision is required for the serial dilution of standards.**	
• Thaw DNA standard tube.	
• Vortex for 30 sec.	• **GVLab Vortex mixer** for tubes up to 30 mm diameter.
• Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• Add 18 µL of PCR Grade water in 7 microtubes of 1.5 mL.	• PIPETMAN **C100,** with **DF200 or DF100ST** (diagnostics) • PIPETMAN **P20M** with **DF30ST** (diagnostics).
• Add 2 µl of standard DNA in the second one (1.5 mL).	• PIPETMAN **C10, P10M** with **DFL10 or DFL10ST** (for 1.5/2.0 mL).
• Vortex for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter.
• Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• Add 2 µl of second tube to the third one(1.5 mL).	• PIPETMAN **C10, P10M** with **DFL10 or DFL10ST** (for 1.5/2.0 mL).
• Vortex for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** tubes up to 30 mm diameter.
• Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• Repeat step h to step j with the other tubes (3 to 7).	

3. PCR reagents preparation	
• Thaw all frozen reagents before use.	
• Mix all reagents thoroughly.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter
• Briefly centrifuge them before starting the procedure to spin down the whole solution (kept on ice).	• **GmClab centrifuge** (0.2, 1.5/2.0 mL microtubes, 0.2 mL-tube strips)
Reagents are kept on ice to prevent their degradation that may occur at room temperature.	
4. Making serial dilution of DNA standard template in 1.5 mL tubes (keep on ice) **The operator prepares the serial dilution of the DNA sample in various micro-tubes in a dedicated room under bio-safety cabinet, using filter tips. Electronic pipettes are used because a maximum of precision is required for the serial dilution of standards.**	
• **a)** Thaw DNA standard tube.	
• **b)** Vortex for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter.
• **c)** Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• **d)** Add 18 µL of PCR Grade water in 7 microtubes of 1.5 mL.	• PIPETMAN **C100,** with **DF200ST or DF100ST** OR **P20M** with DF200ST or DF30ST.
• **e)** Add 2 µl of standard DNA in the second one (1.5 mL).	• PIPETMAN **C10, P10M** with **DFL10 or DFL10ST** (for 1.5/2.0 mL).
• **f)** Vortex for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter.
• **g)** Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• **h)** Add 2 µl of second tube to the third one(1.5 mL).	• PIPETMAN **C10, P10M** with **DFL10 or DFL10ST** (for 1.5/2.0 mL).
• **i)** Vortex for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter.
• **j)** Spin down the tube for 15 sec.	• **GmClab centrifuge**.
• Repeat step h to step j with the other tubes (3 to 7).	

5. PCR Master Mix preparation for 30 reactions in 1.5 mL tube (keep on ice)	**PCR Master mix is prepared in a separated room to prevent contamination of it by aerosols coming from manipulation of DNA samples. Electronic pipettes are used because a maximum of precision is required for so many pipetting steps and volume settings.**
• Dispense 300 µL of Taqman Master mix (10 µL, 1 reaction).	• PIPETMAN **C300** with **DF300ST** (diagnostics). or • PIPETMAN **P1000/N/L/M** with **DF1000ST** (diagnostics).
• Add 2.25 µL of 100 µM Forward primer (0.075 µL).	• PIPETMAN **C10, P10M** with **DFL10ST** (for 1.5/2.0 mL).
• Add 2.25 µL of 100 µM Reverse primer (0.075 µL).	• PIPETMAN **C10, P10M** with **DFL10ST** (for 1.5/2.0 mL).
• Add 3.75 µL of 20 µM Probe (0.125 µL).	• PIPETMAN **C10, P10M** with **DFL10ST** (for 1.5/2.0 mL).
• Add 231.8 µL of PCR Grade water (7.725 µL).	• PIPETMAN **C300** with **DF300ST** (diagnostics) or • PIPETMAN **P1000M** with **DF1000ST** (diagnostics).
• Vortex to form a homogeneous solution for 30 sec.	• **PIPETMAN M or PIPETMAN Concept in mix mode** for tubes up to 30 mm diameter.
• Spin down briefly for 15 sec.	• **GmClab centrifuge.**
6. Fill up the 96-well PCR plate (triplicates)	
Each DNA standard is dispensed in its dedicated well (triplicates).	**The calibration curve is setup (Standard DNA templates) into the same 96-PCR well plates as for the unknown samples. The operator works under a bio-safety cabinet with pipettes fitted with filter tips. Triplicates are made for checking pipetting precision.**
• Dispense 2 µL of first diluted DNA standard into B1 – 3.	• PIPETMAN **C10, P10M** with **DFL10ST** (for 1.5/2.0 mL).
• Continue with the other diluted DNA standards into C1 – 3 and until H1 – 3 and mix by aspiration & dispensing mode as for the previous step.	• PIPETMAN **C10**, **P10M** with **DFL10ST** (for 1.5/2.0 mL).
Each unknown DNA sample is dispensed into its dedicated well	

SETTING UP FOR THE REAL-TIME PCR REACTION OF DNA TEMPLATES	INSTRUMENT
• Dispense 2 µL of the six unknown DNA samples µL into A5 to F5.	• PIPETMAN **C10, P10M** with **DFL10ST** (for 1.5/2.0 mL).
PCR Master mix is added into each prepared wells (Triplicates)	**Electronic pipettes are used for their high precision and comfort for repetitive process of long periods of pipetting.**
Dispense 18 µL of PCR Master Mix in wells (A1-3 until H1-3 and A5 to F5) and gently mix by aspiration & dispensing mode to minimize aerosol formation.	• PIPETMAN **C8x100** with **DF100ST** (diagnostics) • PIPETMAN **P8X20M** with **DF30ST**. • PIPETMAN **C100,** with **DF100ST** (diagnostics) • PIPETMAN **P8X20M** with **DF30ST**.
Centrifugation is used to spin down all the reaction solution prior to the PCR process.	
• Cover the plate with adhesive foil and spin it in plate centrifuge.	
• Leave it on ice until starting the PCR.	
7. PCR Amplification **The PCR process is run (standards & samples amplification) while the data are collected through the q-PCR system software for further calibration curve building and unknown DNA sample quantification.**	
• Place the vessels in the Q-PCR machine	
• Set the thermal profile and start PCR immediately	
• Run the PCR process for 30 cycles	**The thermal cycling profile will be run for 30 cycles to amplify the starting DNA material.**
8. PCR Data analysis	
• Analyze Q-PCR results with the real-time PCR software	

Part 3: Considerations for PCR and qPCR Applications

Precision of Pipetting Samples and Reagents

Because the standard curves are based on dilution series, PIPETMAN Concept C10 and C100 (depending on the volume to be pipetted) and PIPETMAN M P10M, P20M and P200M provide higher precision (≤ ± 0.8 %) than mechanical pipettes (≥ ± 1.5 %).

PIPETMAN M (left)
PIPETMAN *Concept*® C10 (0,2 – 10 µL)

Moreover, poor serial dilution preparation may lead to deviations in reaction efficiency, resulting in concentrations insufficient for the sensitivity of the assay.

The precision of the pipetting gesture is paramount to the outcome of the serial dilutions. When using the PIPETMAN Concept motorized pipette, the pipette motor controls the speed of the piston guaranteeing the reproducibility of the manipulation especially during the intensive pipetting for assay optimization and running replicates in qPCR. Use of a motorized pipette relieves the operator of thumb fatigue possible during long repetitive manual pipetting sequences (variation of aspiration and dispensing speed).

So not only is it useful to avoid variations in rhythm of the same operator, it also reduces the inter-operator variability in the preparation of multiple templates used for the assays of various samples (intra or inter-lab quantifications) [(4)]. Errors in pipetting will skew the variability of results.

Higher throughput quantitative real-time PCR is carried out with specific vessel formats such as 96 or 384-well micro-plates, 96-PCR plates, PCR-tube strips**. PIPETMAN Concept multichannel C8x10 or C12x10** or **PIPETMAN M multichannel P8x20M or P12x20M** may be used to prepare PCR mixes and **PIPETMAN Concept C10, C100 or PIPETMAN P10M, P20M or P200M** to dispense standard and unknown samples.

PIPETMAN *Concept*® C10
AND C8x10 (0,2 – 10 µL)

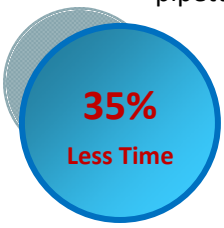
PIPETMAN *M*
Single and Multi-Channel

Productivity

Real-time PCR is perceived as:

- Less labor-intensive (especially when performing assay optimization): it may take a few hours instead of days /months for conventional PCR (8).
- Allowing higher throughput: fast-cycling times possible with new thermal cyclers, having faster ramping times or through innovative PCR chemistries permitting reduced cycling times than conventional PCR (End point quantitative PCR).

PIPETMAN Concept and M pipettes allow the operator to speed up the dispensing of reagents and samples by **adjusting the pipetting speed** and selecting **the repetitive mode** to get reliable and reproducible results for dilution series (standard curves) and triplicate dispensing. PIPETMAN Concept & PIPETMAN M multichannels (C8x10, C12x10, C8x100, C12x100, P8x20M, P8x200M, P12x20M, P12X200M) permit a faster pipetting process when microplate or tube strip formats are used.

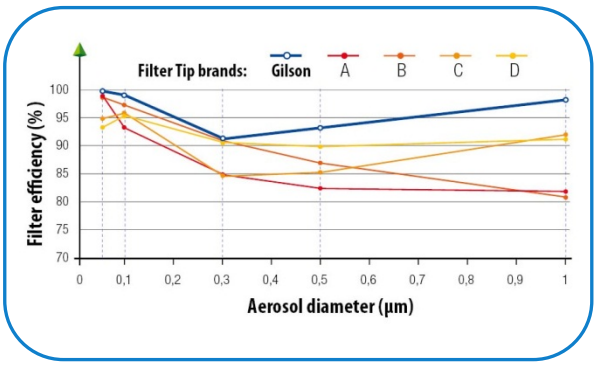


It takes on average, 35% less time to carry out a sample run with a PIPETMAN Concept or a PIPETMAN M pipette than with a mechanical pipette using repetitive mode for dispensing.

Large Choice of Consumables

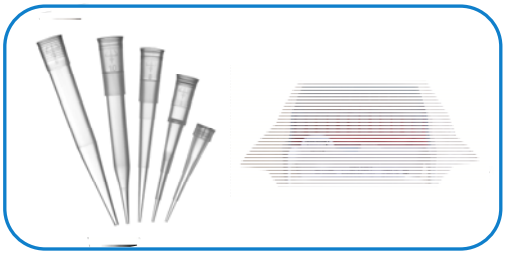
Consistent, stable formulation of low copy number targets requires stringent control of the laboratory environment to prevent contamination by exogenous RNAse or DNAse. This justifies the use of **Gilson DIAMOND filter tips (DFL10ST for PIPETMAN Concept C10** and **P10M, DF100ST for PIPETMAN Concept C100, DF30ST for P20M and DF200ST for P200M) to reduce cross-contamination by aerosol between samples.**

The graph below shows that Gilson DIAMOND filter tips are more efficient than other brands whatever the size of the aerosol; a comparison of filter tip efficiency.



Because of the price of PCR reagents and the time spent for assay optimization and sample runs (standards and unknowns), the operator tends to minimize the volumes used to prepare the PCR mixes.

Quality of the tips is a must to carry out experiments with the maximum accuracy. In addition, sterilized filter tips may be used to minimize the risk of contamination of the PCR mix (additional enzymes may inhibit the PCR reaction for RNA) especially for diagnostics where patients' samples require reliable results so that physicians may confidently propose the right therapeutic protocol.



Cost Effective for a Limited Budget

- Minimizing manual pipetting errors prevents the operator from having to run the same sample again (if sufficient quantity is available) reducing the requirements of expensive reagents, costly tips and consequently, reducing the reagents and tips costs.

Higher Comfort and Less Stressful Working Environment

- Because you prevent repeat PCR runs for the same sample (in case of manual pipetting errors) and benefit from the assistance of the motor for pipetting, making the operator less subject to Repetitive Strain Injuries (RSI), the work environment is improved.

- Another bottleneck in real-time PCR is the template preparation where contamination is a key issue. It may occur easily at any step of the process (assay optimization, standard /samples run, etc) modifying the results (efficiency, sensitivity and specificity) and consequently, provide inaccurate quantification. Because the operator is fully confident in the pipetting performance, more attention can be devoted to the template preparation rather than the operator's pipetting technique.

Efficient Sample Handling and Mixing

In many PCR protocols, very small volumes of reagents and samples are pipetted to minimize the cost of experiments. Most of the time, pipette users may touch the vessel wall to get rid of the last drop of liquid which may stick there or drops of liquid stick on the wall while dispensing (purging), preventing an optimum PCR mix.

In order to use the total amount of liquid, centrifugation is required to spin down the liquids to the bottom of the vessel. **Gilson GmCLab minicentrifuge** offers the possibility to quickly do the job for vessel formats such 1.5/2.0 mL micro-tubes, individual PCR tubes or PCR-tube strips.

Usually, when you prepare the PCR mix and add the sample to it, optimum mixing is required. The **PIPETMAN M** mix mode allows for single-point control directly after pipetting.

GILSON PIPETMAN M – PIPET, MIX, REPETITIVE, REVERSE MODES

GILSON GMCLAB MINICENTRIFUGE

Technical Summary & References

Technical Summary

- In the case of **RT-PCR**, the RNA sample is converted into cDNA (complementary DNA) sample using the **Reverse Transcription** method.
- In the **one-step RT-PCR** method both reverse transcription and PCR amplification processes are carried out in the same vessel successively.
- In the **two-step RT-PCR** method, the RT process (Conversion of RNA into cDNA) is carried out first and then the cDNA sample is added to the PCR reaction in a separate vessel.

References

1. PCR, A practical approach, M.J. Mc Pherson, P. Quirke and G.R. Taylor, 1991.

2. Orlando C., Pinzani, P. & Pazzagli M., Development in quantitative PCR, Clinical Chemistry Laboratory Medecine, 36, 255-269.

3. Integrated Solutions – Real-Time PCR Applications : Critical Factors for Successful Real-Time PCR, Qiagen, 02/2006.

4. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR):

Questions? Please Contact Us

If you have application questions related to this technical note or if you are interested in further information on the Gilson system configurations discussed, please feel free to contact us:

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