Technical Application: Alluvia System Fluidic and Optical Performance

Background

The Alluvia system facilitates 2-step nucleic acid amplification (i.e. multiplex-nested PCR) while eliminating the risk of amplification product contamination. The advantages of 2-step amplifications are improved sensitivity (as the sample is preamplified, often in a multiplex format) and specificity (i.e. 2nd step PCR primers can be "nested" relative to the 1st step primers yielding 4 primer specificity). A major problem with 2-step amplification is the necessary handling of the 1st step products, which can lead to contamination of the laboratory environment and false positive results in subsequent reactions. The Alluvia System eliminates this contamination risk by continuously containing the amplification products in plastic consumables. The attractive design and intuitive operation make the system easy to use while reducing user error compared to the pipetting steps it replaces.

Alluvia Design and Operation

The Alluvia system is comprised of two types of consumables, the Plate Loading Manifold (PLM) and the Gel Loading Manifold (GLM), and an instrument that controls operation of the PLM. The PLM will accept a single standard PCR tube containing first step PCR products, puncture the tube and dilute the products to approximately 1:100, then aliquot into a standard 8-well PCR tube strip that can be subsequently thermocycled on a qPCR instrument. If desired, the post second step PCR products contained in the tube strip can then be injected in a sealed manner into a cartridge-type disposable electrophoresis gel (Invitrogen E-Gel™, Lonza FlashGel™).

The system works as follows:

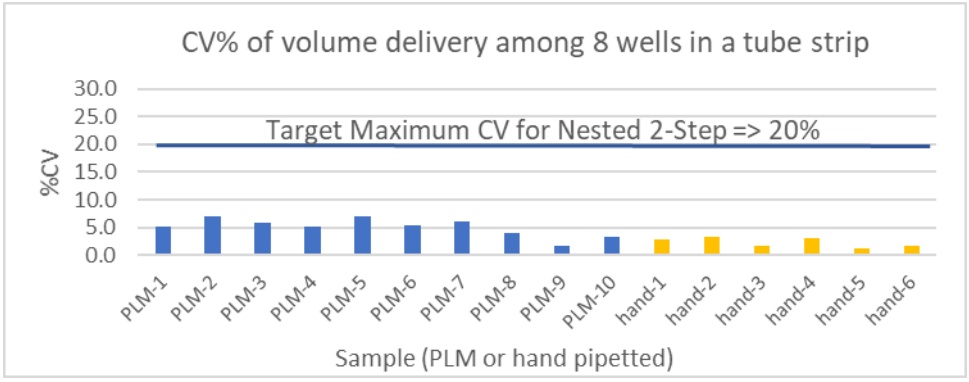
- The secondary PCR reactants (primers and master mix) are added an 8-tube PCR strip which is then attached to the PLM. The PCR reactants can also be lyophilized in the PCR tubes, the PLM attached, and provided to a user as a packaged test.
- The PLM is placed on the Alluvia instrument and the tray raised into place.
- Buffer is injected into the PLM loading port filling an expandable bladder.
- The PCR tube containing first round PCR products is inserted into the PLM loading port, thereby sealing the port, rupturing the bottom of the tube, and diluting the PCR products.
- The user presses start on the instrument, the instrument performs the following:
    - A portion of the diluted PCR products are pushed into an expandable channel located in the tube cap segment of the PLM.
    - A bubble elimination step.
    - A set of heating elements weld this channel forming small aliquots of fluid, one aliquot located above each tube of the PCR tube strip.
    - The membranes below each aliquot are ruptured allowing the liquid to flow into the associated tube strip.
    - A heating element welds the cleavage site in the PLM and degrades residual nucleic acids in this region.
- The tray is lowered, and the user cleaves the cap portion of the PLM from the remainder of the PLM using a circular blade.
- The tube strip portion is briefly centrifuged to concentrate liquid in the bottom of the tubes.

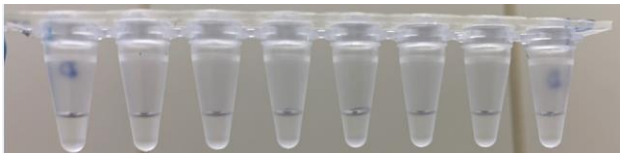
- The PLM is place in a PCR/qPCR machine for the secondary PCR reaction.
- Following PCR/qPCR, if gel electrophoresis of the secondary PCR products is desired, the GLM can be utilized.
- Two gasket release liners located on the underside of the GLM are removed, then the GLM is positioned on top of the disposable cartridge gel.
- The tube strips from the PLM are placed in the receiving slots of the GLM, then the Tube Injector positioned above the tube strip.
- Downward force is applied to the Injector, which forces the tubes into the receiving slots, ruptures the bottom of the tubes on a razor blade located at the bottom of the slots, and injects the tube contents into the electrophoresis wells.
- The Tube Injector is removed, and the gel is electrophoresed as normal.
- Following electrophoresis, the gel along with the GLM are disposed of, thereby keeping the PCR products contained and eliminating the PCR product contamination risk.

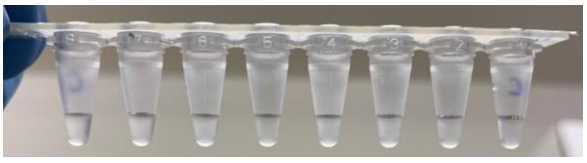
Alluvia Performance: Volume Delivery

- TET buffer (10 mM Tris, pH 8, 1 mM EDTA, 0.1% Tween-20) was delivered to an 8-tube strip cap using the PLM as described above.
- Three different individuals also hand pipetted buffer into 8-tube strips and covered with standard tube cap strips.
- The volume delivered into each well was determined by weighing the tube strip before and each time after liquid was removed from one well at a time.

➢ The results are displayed graphically and with images of two sample tube strips. Overall, the Alluvia has a slightly higher volume delivery CV than manual pipetting, but in our experience, variation of less than 20% CV has a negligible effect on qPCR results, particularly second-step qPCR results, which tend to be binary in nature.





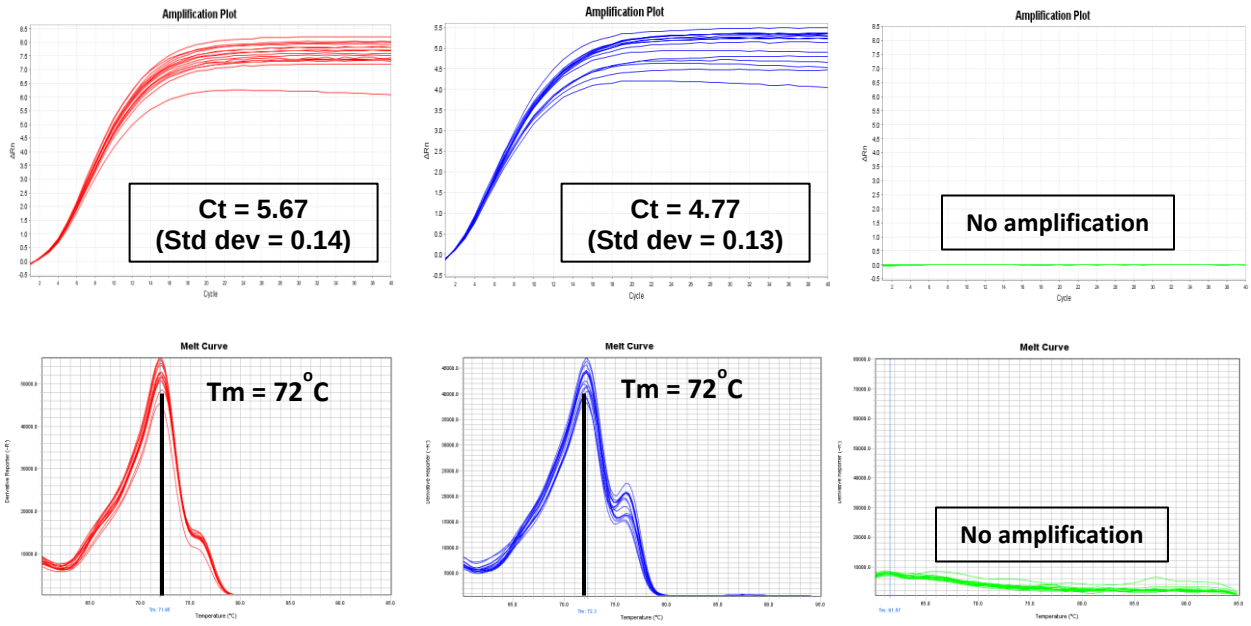


Alluvia Performance: qPCR Performance and Contamination Testing

- Two first-step qPCR reactions using a 200 bp amplicon were performed. (All qPCR reactions were performed using Applied Biosystems™ (ABI) Power SYBR™ Green PCR master mix).
- Twelve microliters of a secondary qPCR reaction mixture (master mix and primers) were dispensed into six 8-tube strips (48 wells). Two were attached to PLMs. Two were used with standard cap strips, two were used for containment testing.
- For each PLM, 25 ul drops of dilution buffer were placed at two locations on the external portion of the PLM, then 12 ul recovered and placed into two wells of the "contamination test" 8-well strips.
- The two post first-step PCR tubes were processed by the two PLMs as described above.
- Prior to performing the qPCR, 25 ul drops of dilution buffer were placed at six locations on the external portion of the PLMs including two at the cleavage zone, then 12 ul recovered from each zone and placed into wells of the containment test 8-well strips, which were then sealed with standard cap strips.
- Diluted first-step PCR products were recovered from the PLMs by removing the first-step PCR tube. 12 ul was added to each well of the standard comparison PCR strips and sealed with standard cap strips.
- qPCR including melting analysis was performed on all six strips using the ABI StepOne™ instrument using standard PCR conditions.

➢ The results show the Alluvia cap strips and standard caps strips produce similar Cts with similar standard deviations. The overall Alluvia signal is about one third less than a standard cap signal. In addition, no PCR products were detected on the external portions of the post-process PLMs

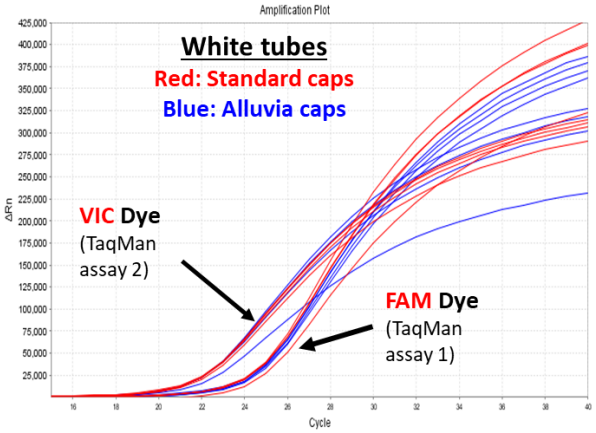
Standard Cap Strip | **Alluvia PLM Caps** | **Containment Test**

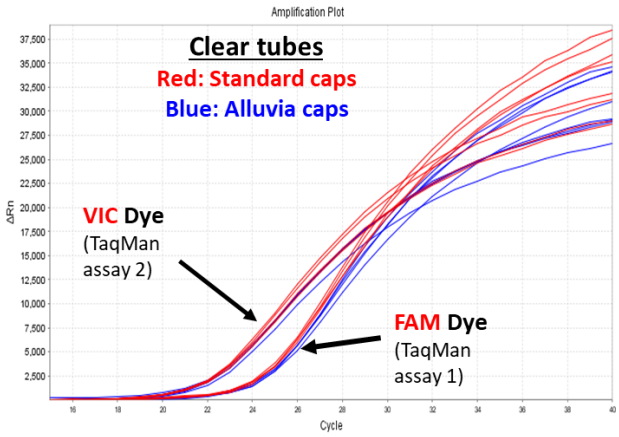


Alluvia Performance: qPCR Performance using probe-based assays (TaqMan)

- TaqMan™ probes (FAM=Pa04646123_*S. aureus*- famA gene or VIC= Ba04329501_*E*. coli gapA) and master mix were pipetted into white and clear 8-well tube strips.
- Either *S*. aureus or *E*. coli genomic DNA was pipetted into each tube, then the tube strips were caped with either an Alluvia cap strip or a standard cap strip.
- qPCR was performed using the default TaqMan protocol on the ABI StepOne instrument.

➢ The results demonstrate that Alluvia caps produce comparable qPCR data to standard caps using TaqMan chemistry, FAM and VIC dyes, and white and clear tube strips.



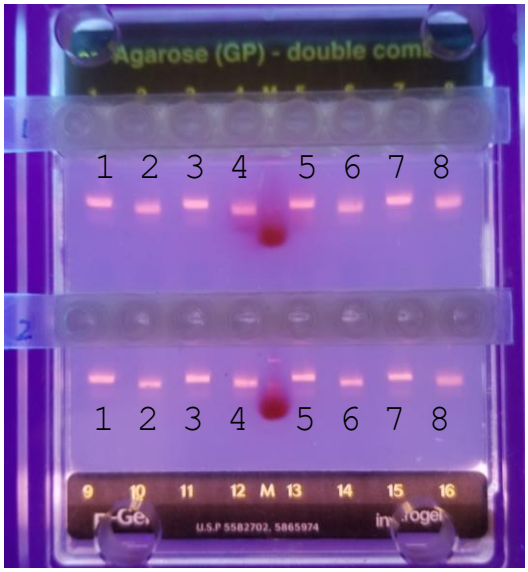


Alluvia Performance: Gel Electrophoresis using GLM system

- Different second-step PCR reactions using two different size amplicons (~ 250 and 350 bp, alternating in odd and even wells) were performed using PLMs using the methods described above.
- The resulting PLM tube strips were injected into a 2% agarose Invitrogen E-Gel™ and a 2% agarose Lonza FlashGel™ as described above.

➢ The results demonstrate that PCR products from all tubes were successfully injected using the GLM and electrophoresed in both types of cartridge gels.

A. Invitrogen E-Gel™,



B. Lonza FlashGel™