AAV Analytics: Automating the PicoGreen Assay
Improving Our DNA Impurity Detection Capability

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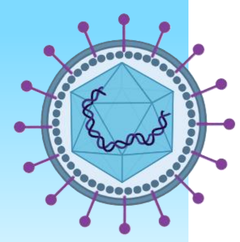
CATAPULT Cell and Gene Therapy

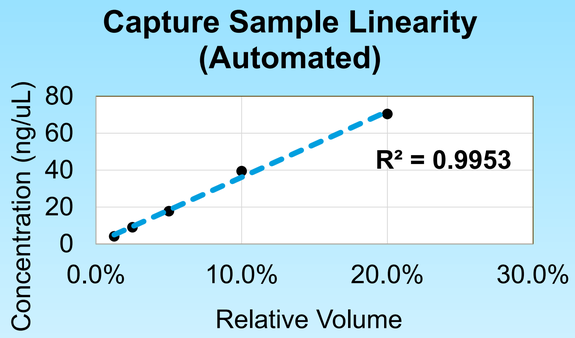
Background

- Many gene therapies use **viral vectors** (VVs) for targeted delivery of therapeutic genes
- Purification helps isolate viral vectors but may leave residual contaminants such as host cell proteins and **DNA**
- CGT Catapult uses the **Quant-iT PicoGreen dsDNA Assay Kit** to quantify double-stranded DNA (dsDNA) in viral vector samples



Development

- Required training in scripting for a Tecan Fluent liquid handler
- Development experiments investigated key analytical parameters – accuracy, precision, linearity



Development Conclusion

- Initial results suggested the automated method has equal or better analytical performance compared to manual method.

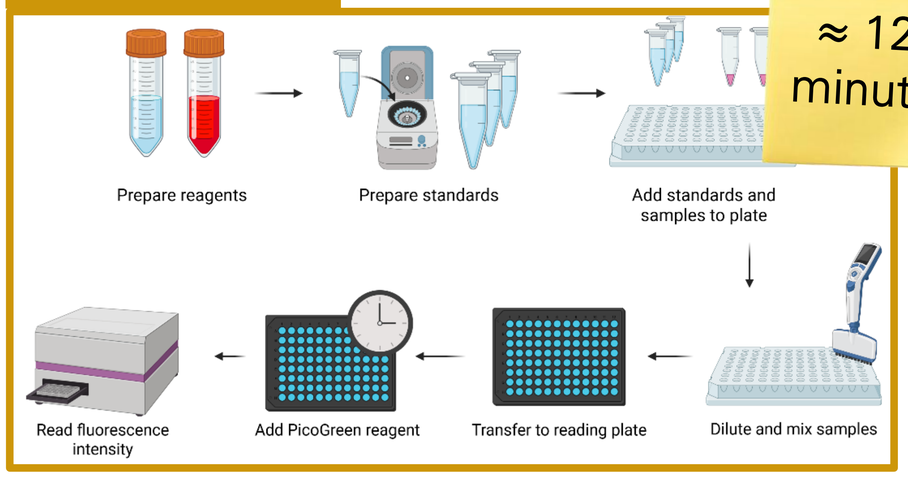
Automation & Planning

The traditional method is limited by manual handling – operator training, human error, limited throughput. The slowed data acquisition can present a bottleneck when a decision must be made in the VV production process.

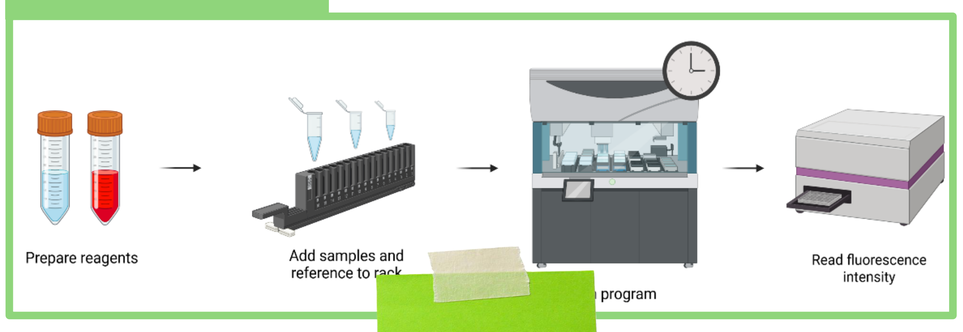
DEVELOPMENT: Program the workflow and check initial analytical performance before committing to qualification.

QUALIFICATION: Confirm assay is fit-for-purpose with extensive analysis of all performance parameters.

Manual



Automated



≈ 80 minutes

Benefits

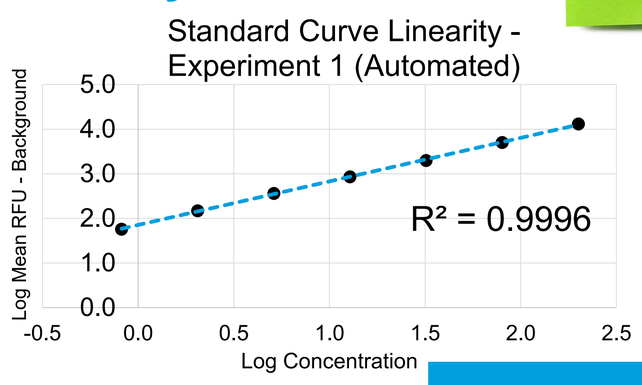
- ✓ **Faster and easier to train new staff**
- ✓ **Experiment is 30% faster start to finish**
- ✓ **70% reduction in hands-on time**
- ✓ **Analytical performance is equal to existing method**
- ✓ **Compatible with existing analysis and materials**

Qualification

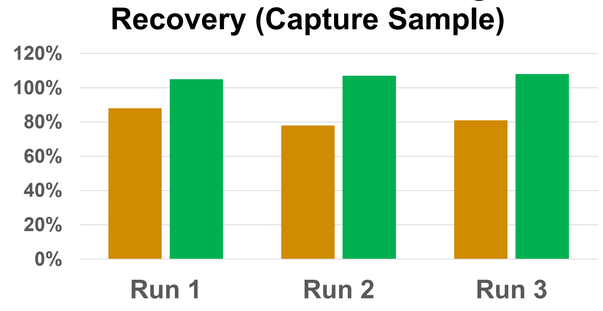
Linearity

Automated method achieved excellent linearity with an R^2 value >0.99, demonstrating a strong correlation between dsDNA concentration and fluorescence intensity.

Linearity performance was found to be consistently strong in both the standard curve and in serially diluted samples.



Accuracy



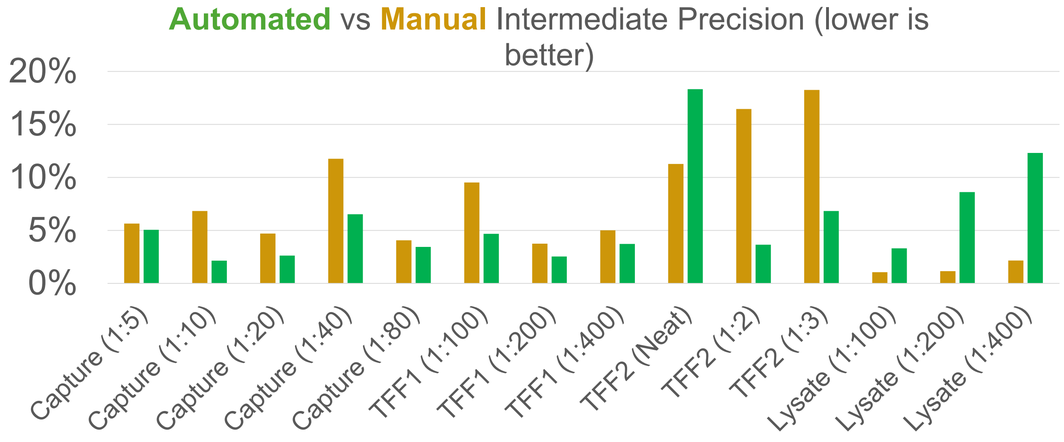
Established a reference AAV sample in development phase to use as 'baseline'.

Compares the closeness of the **measured DNA concentration** against the **reference DNA concentration.**

Automated method sees higher accuracy than manual method. This suggests it is more reliable and capable of reduced error rates.

Precision

Automated method displayed comparable **intermediate precision** within replicates (n = 3). Variation in dsDNA concentration measurement between runs is within the acceptable limits of under 25%.

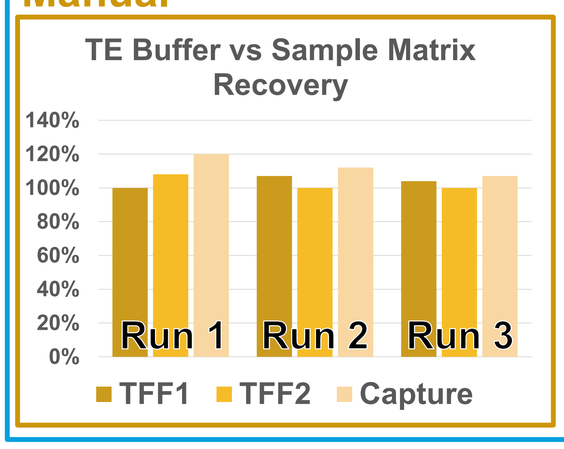


Specificity

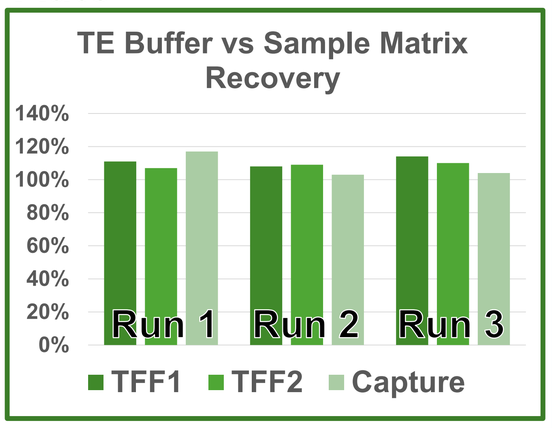
Spiking study – spike DNA standard into DSP buffers to assess whether non-specific binding occurs.

Both methods were within the acceptance criteria of 80-125% and minimally affected by matrices.

Manual



Auto



Innovate UK

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