

Vector Copy Number Assays: Digital Drop PCR & Benzonase Treatment

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Introduction

- ddPCR, or Digital Drop PCR, is a relatively recent technology (2011) and method for digital PCR
- Like qPCR, ddPCR can be used to determine the Vector Copy Number (VCN) of gene modified cell therapy products¹
- Benzonase is a product that removes excess nucleic acids from transduced cells³
- This project aims to explore the use of ddPCR for determination of the VCN for cell therapy products and whether Benzonase treatment is necessary before DNA extraction and ddPCR

Digital Droplet PCR (ddPCR)

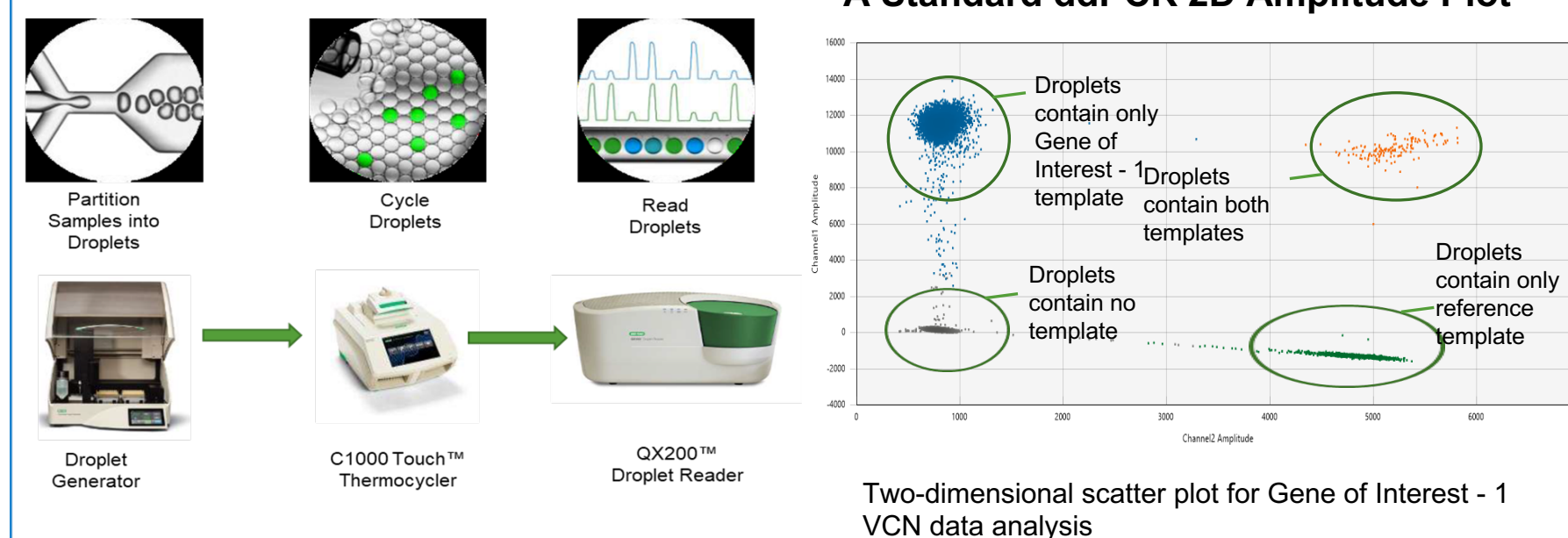


Figure 1: ddPCR workflow & 2-D scatter plot for ddPCR

Benzonase

- Genetically engineered product form *Serratia marcescens* that hydrolyzes nucleic acids
- Degrades nucleic acids to 5'-monophosphate terminated oligonucleotides (2-5 bases) and requires Mg²⁺ and Ca²⁺ for activity
- Removes the residual DNA during virus production
- Current cells samples are shared with VCN and RCR. RCR samples need to be treated by Benzonase, so the VCN cell samples are also treated with Benzonase.
- A search of published literature on VCN assay found that samples were not Benzonase treated³

Vector Copy Number (VCN)

- The FDA recommends that the VCN should be less than 5 copies per genome in gene modified cell therapy products
 - The vector is integrated into the genomic DNA
 - Increased risk of oncogenesis when VCN is high
- ddPCR can be used to find the Vector Copy Number²

$$\text{VCN} = \frac{\text{Gene of interest DNA}}{\text{Reference DNA (RNase P/RPP30)}} \times 2$$

Experimental Design/Methods

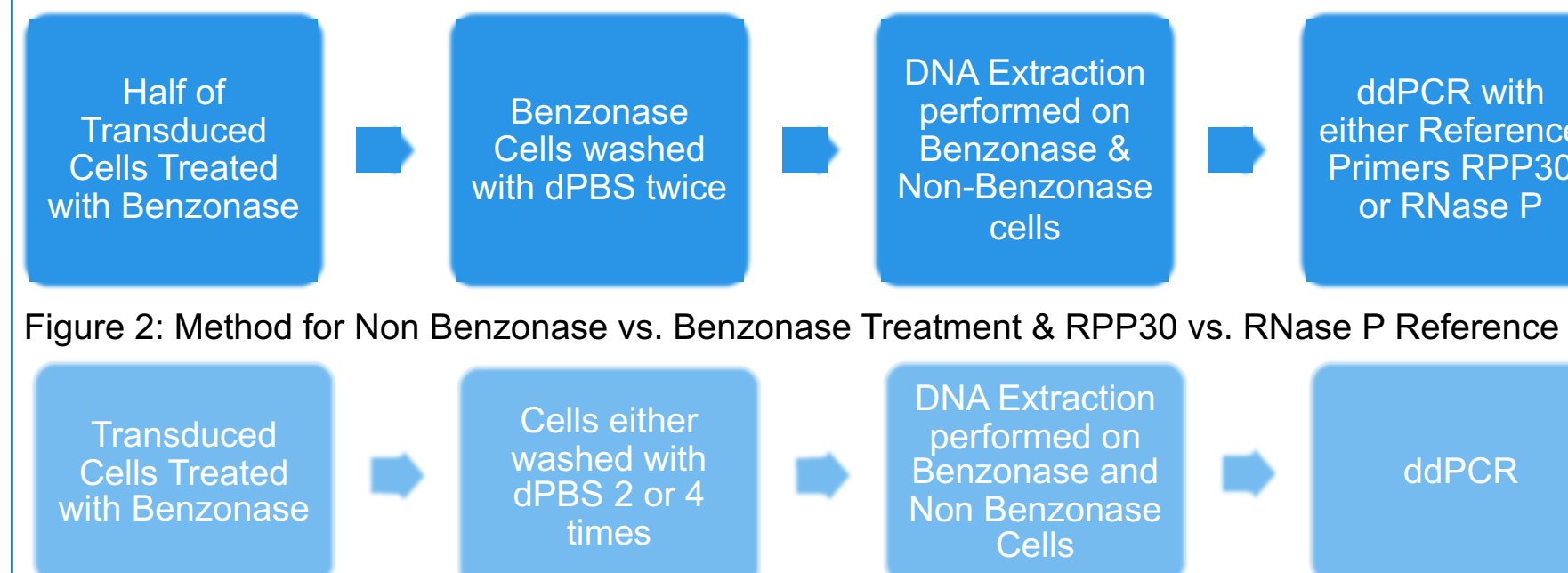


Figure 2: Method for Non Benzonase vs. Benzonase Treatment & RPP30 vs. RNase P Reference

Figure 3: Method for different wash times after Benzonase Treatments

Results

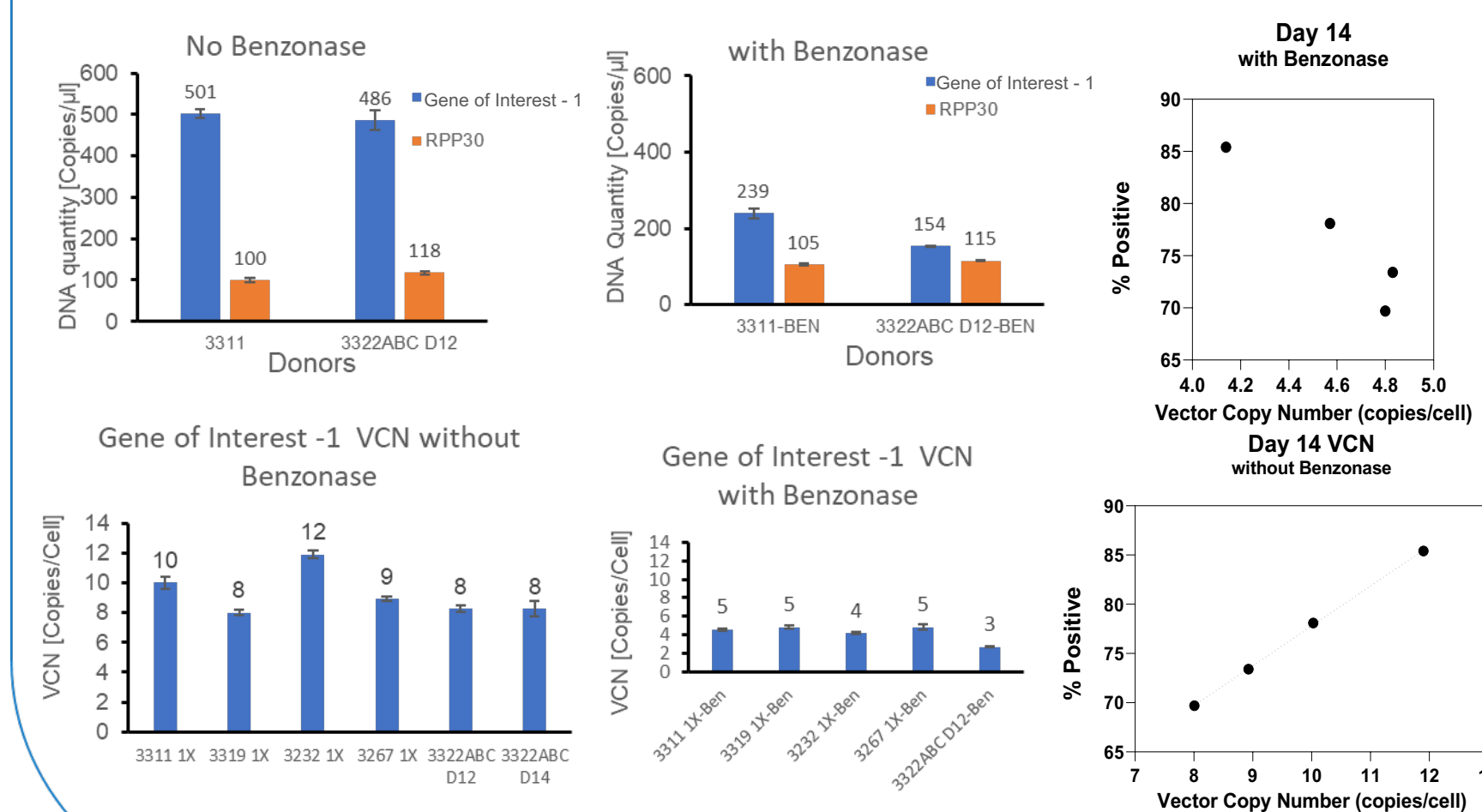


Figure 4: Comparison of Benzonase and Non Benzonase VCN and DNA Quantity

Results

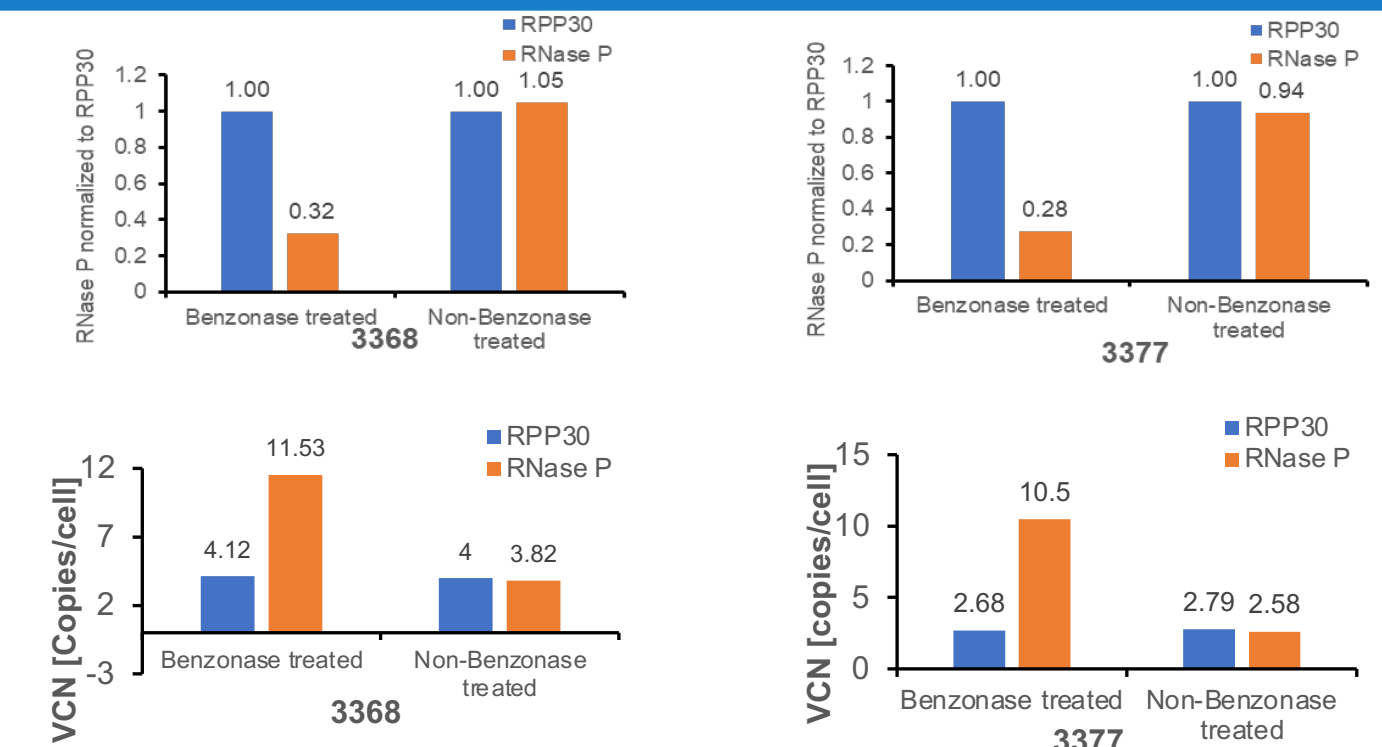


Figure 5: Benzonase Treatment with RNase P & RPP30 Reference Genes (using Gene of Interest 2)

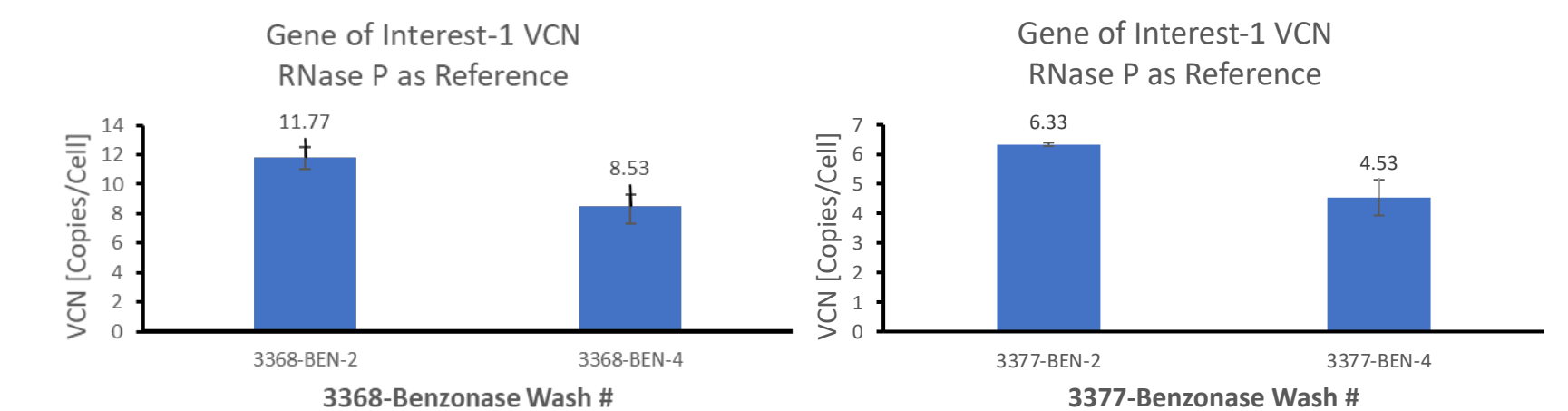


Figure 6: Comparison of different wash times after Benzonase Treatments

Conclusions

- Benzonase treatment reduced RNase P concentration in the final ddPCR products but did not affect Gene of Interest-1 and RPP30.
- Benzonase treatment did not affect DNA of Interest-2 VCN with RPP30 as a reference but did affect Gene of Interest-1 VCN with RPP30.
- Residual Benzonase or Impurity of the Product affected RNase P PCR Product
- Based on the results, the fact that Benzonase treatment is not a requirement for VCN samples, and published literature of VCN samples not treated with Benzonase, it is suggested that Benzonase treatment should not be included in VCN sample preparation.

References

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2. Lu, A., Liu, H., Shi, R. *et al.* Application of droplet digital PCR for the detection of vector copy number in clinical CAR/TCR T cell products. *J Transl Med* 18, 191 (2020). <https://doi.org/10.1186/s12967-020-02358-0>
3. Yang, S., Karne, N. K., Goff, S. L., Black, M. A., Xu, H., Bischof, D., Cornetta, K., Rosenberg, S. A., Morgan, R. A., & Feldman, S. A. (2012). A simple and effective method to generate lentiviral vectors for ex vivo gene delivery to mature human peripheral blood lymphocytes. *Human gene therapy methods*, 23(2), 73–83. <https://doi.org/10.1089/hgtb.2011.199>