

3399. Detection of rare mutations in blood samples by droplet digital PCR (ddPCR)

BIO-RAD

Austin So¹, Benjamin Hindson¹, Ryan Koehler¹, Serge Saxonov¹, George Karlin-Neumann¹, Helen White². ¹Bio-Rad Laboratories, Pleasanton, CA; ²National Genetics Reference Laboratory (Wessex), Salisbury, United Kingdom

Life Science Group
2000 Alfred Nobel Drive
Hercules, CA 94547 USA

Abstract

Detection and quantitation of specific mutations in circulating plasma holds promise for earlier and less invasive diagnosis of disease. This presents significant analytical challenges, particularly as the biomarker may differ from its highly abundant wildtype by only a single nucleotide. Conventional methods have poor selectivity and fail to detect mutant sequence below 1 in 100 wildtype sequences. Here we present a simple strategy using droplet digital™ PCR (ddPCR™) for the detection of somatic mutations with high selectivity and sensitivity. Based on the simple principle of sample partitioning into water-in-oil microdroplets, this ddPCR method increases the abundance of a mutant DNA sequence up to 20,000 times compared to an equivalent bulk PCR reaction. Using conventional TaqMan chemistries and workflow, selectivity of up to 1/100,000 can readily be achieved in any laboratory. We evaluated ddPCR for the detection and quantitation of several clinically important mutations in the KRAS locus from clinical samples derived from normal and tumor samples. We also demonstrate the feasibility of multiplexing of Kras assays to improve sample processing efficiency.

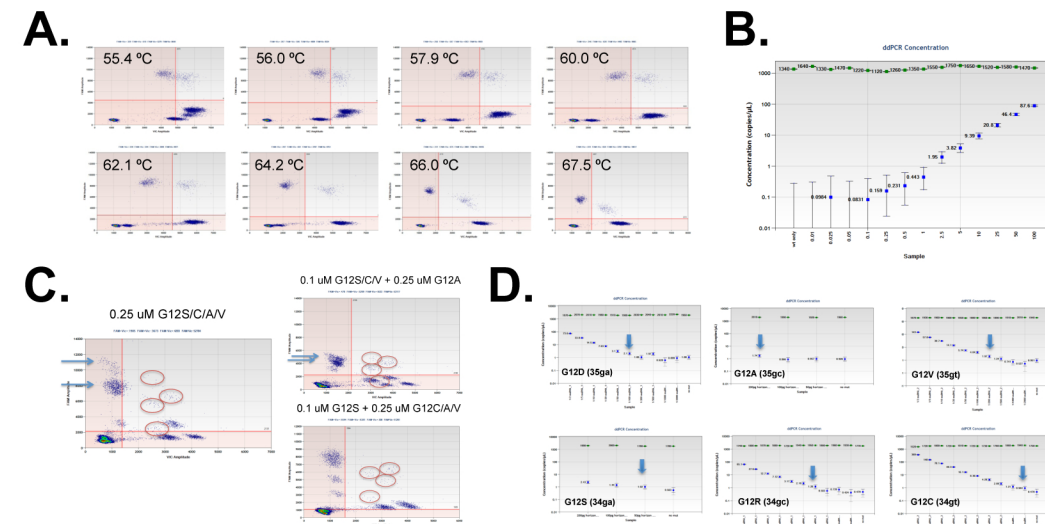
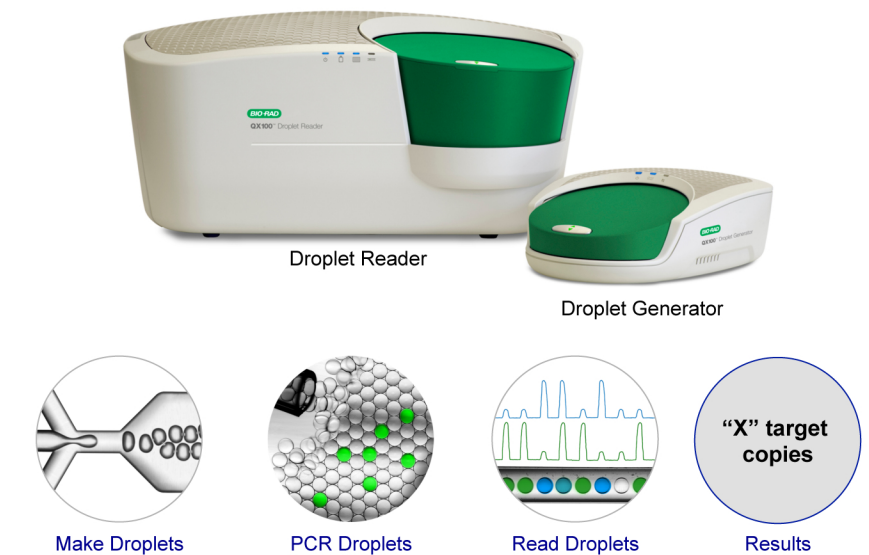


Figure 2. Optimization of KRAS codon 12 mutation duplex and multiplex assays via ddPCR system. A. Temperature gradient is used to define optimal annealing temperature (64–66°C) for KRAS G12A assay that permit maximal discrimination of mutant and wild-type assays and minimize non-specific amplification products. B. A spike-in of mutant DNA (NCI-H1573) into a background of ~70 ng wild-type genomic DNA (Promega female DNA) demonstrates a limit of quantification/detection (LOQ/LOD) of ~0.01%. C. Strategies for multiplexing defined by consistency in fluorescence amplitudes of wild-type assay to enable unambiguous separation from mutant assay signal. D. Preliminary LOQ/LOD (<0.1%) for KRAS codon 12 6-plex + wild-type using spike-in of mutant cell line DNA into wild-type DNA as in B.



Conclusions

- Partitioning of PCR reactions via droplet digital PCR enables the sensitive detection of KRAS mutations (<0.01%) within a high background of wild-type (60–80 ng) DNA.
- Assays for all KRAS exon 2 mis-sense mutations (G12A/C/D/R/S/V) can be multiplexed to provide an initial screen of KRAS mutational status for further investigation.
- Application of KRAS codon 12 multiplex assays revealed low-frequency KRAS mutations in 5/50 blood samples from JAK2 V617F positive PBMCs
- Further analysis revealed that 1 of 2 JAK2 V617F positive samples harbored a G12D conversion in the KRAS locus, underlying the complex phenotype of myo-proliferative disorders

References

1. Gormally, E., Vineis, P., Matullo, G., Veglia, F., Caboux, E., Le Roux, E., Peluso, M., et al. (2006). *Cancer research*, 66(13), 6871–6876.
2. Scott, L. M. (2011). *American journal of hematology*, 86(8), 668–676.
3. Benlloch, S., Payá, A., Alenda, C., Bessa, X., Andreu, M., Jover, R., Castells, A., et al. (2006). *The Journal of molecular diagnostics : JMD*, 8(5), 540–543.
4. Whitehall, V., Tran, K., Umapathy, A., Grieu, F., Hewitt, C., Evans, T.-J., Ismail, T., et al. (2010). *The Journal of molecular diagnostics : JMD*, 11(6), 543–552.
5. Herreros-Villanueva, M., & Aggarwal, G. (2011). *Molecular Biology Reports*.
6. Julien Rocquain, Nadine Carbuccia, Virginie Trouplin, Stéphane Raynaud, Anne Murati, Meyer Nezri, Zoulika Tadrist, Sylviane Olschwang, Norbert Vey, Daniel Birnbaum, Véronique Gelsi-Boyer, and Marie-Joelle Mozziconacci. (2010) *BMC Cancer* 10, 401-407.

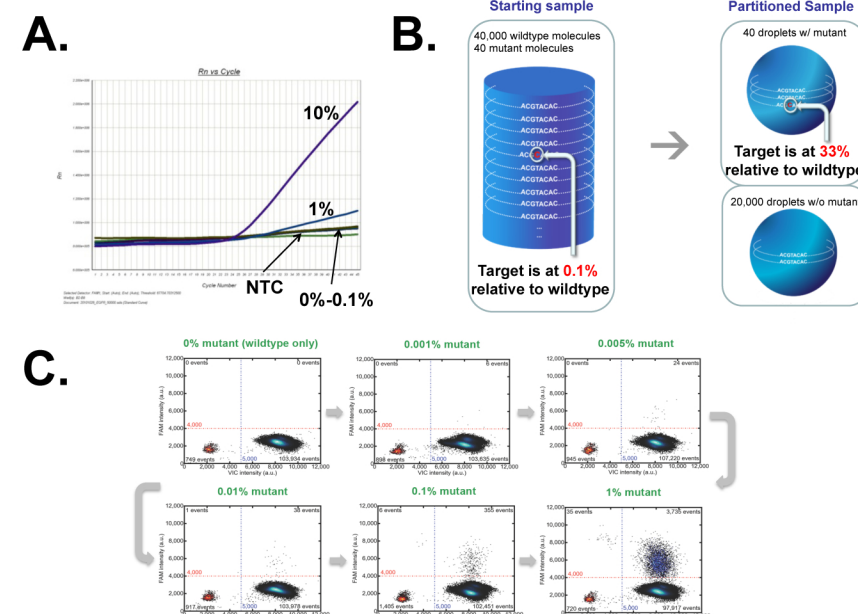


Figure 1. Partitioning of templates via droplets improves the sensitivity of Taqman assays. A. qPCR traces of a dilution series of genomic DNA harboring the BRAF V600E mutation in a background of 2.6 ug of wild-type DNA showing a limit of detection of ~1%. B. Partitioning the PCR improves sensitivity by reducing the relative amount of wild-type DNA >1000-fold. C. Under ddPCR, detection limit of 1/100,000 can be readily achieved.

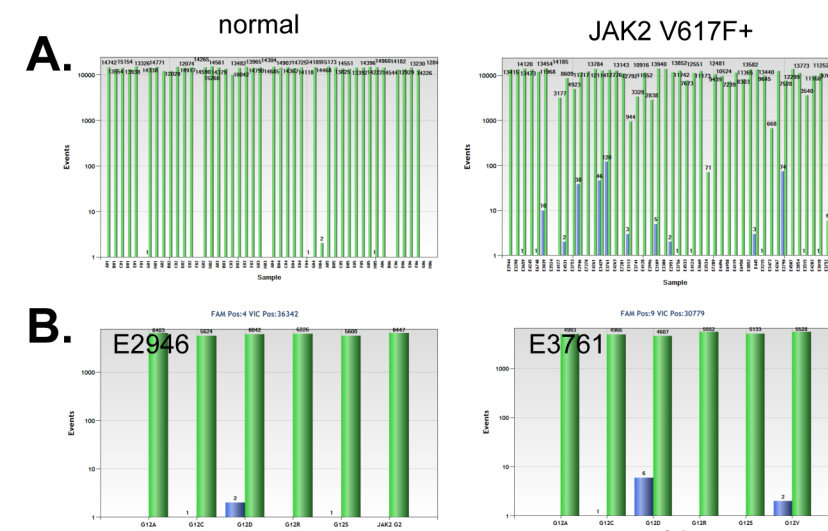


Figure 3. KRAS mutation screen of blood samples reveals low level KRAS mutations in 5/50 individuals harboring JAK2 V617F mutations. A. Screen of 50 JAK2 617F positive versus 50 normal controls reveals the presence of a low fraction of KRAS mutation in 10% of the samples analyzed. B. Two representative samples (E2946, E3761) with detectable KRAS mutations were further screened with individual KRAS duplex assays (G12A, G12C, G12D, G12R, G12S, and G12V). suggesting that one individual may harbor a G12D mutation at a frequency of <0.1%. Sample dilution precluded unambiguous confirmation of KRAS mutation status.