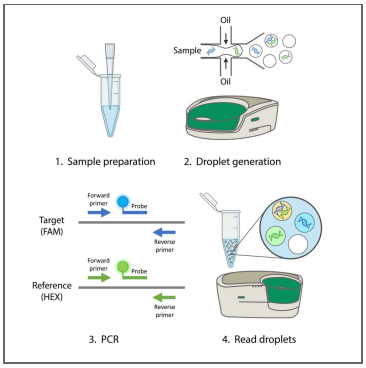

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Copy number variation analysis by ddPCR

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ABSTRACT

This protocol describes copy number variation analysis using Bio-Rad Droplet Digital PCR ddPCR and QuantaSoft Software (with modifications).

MATERIALS

	A	B	C
	Reagent	Manufacturer	Catalog No.
	ddPCR Copy Number Assay: RPP30, Human, Homo sapiens	Bio-Rad	dHsaCP1000485
	ddPCR Supermix for Probes (No dUTP)	Bio-Rad	1863024
	Droplet Generation Oil for Probes	Bio-Rad	1863005

	A	B	C
	Equipment/Consumable	Manufacturer	Catalog No.
	C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module	Bio-Rad	1851197
	ddPCR 96-Well Plates	Bio-Rad	12001925
	DG8 Cartridges	Bio-Rad	1864008
	DG8 Cartridge Holder	Bio-Rad	1863051
	DG8 Gaskets	Bio-Rad	1863009
	PCR Plate Heat Seal, foil, pierceable	Bio-Rad	1814040
	PX1 PCR Plate Sealer	Bio-Rad	1814000
	QX200 Droplet Digital PCR System	Bio-Rad	

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Keywords: Bio-Rad, ddPCR

ddPCR workflow

1

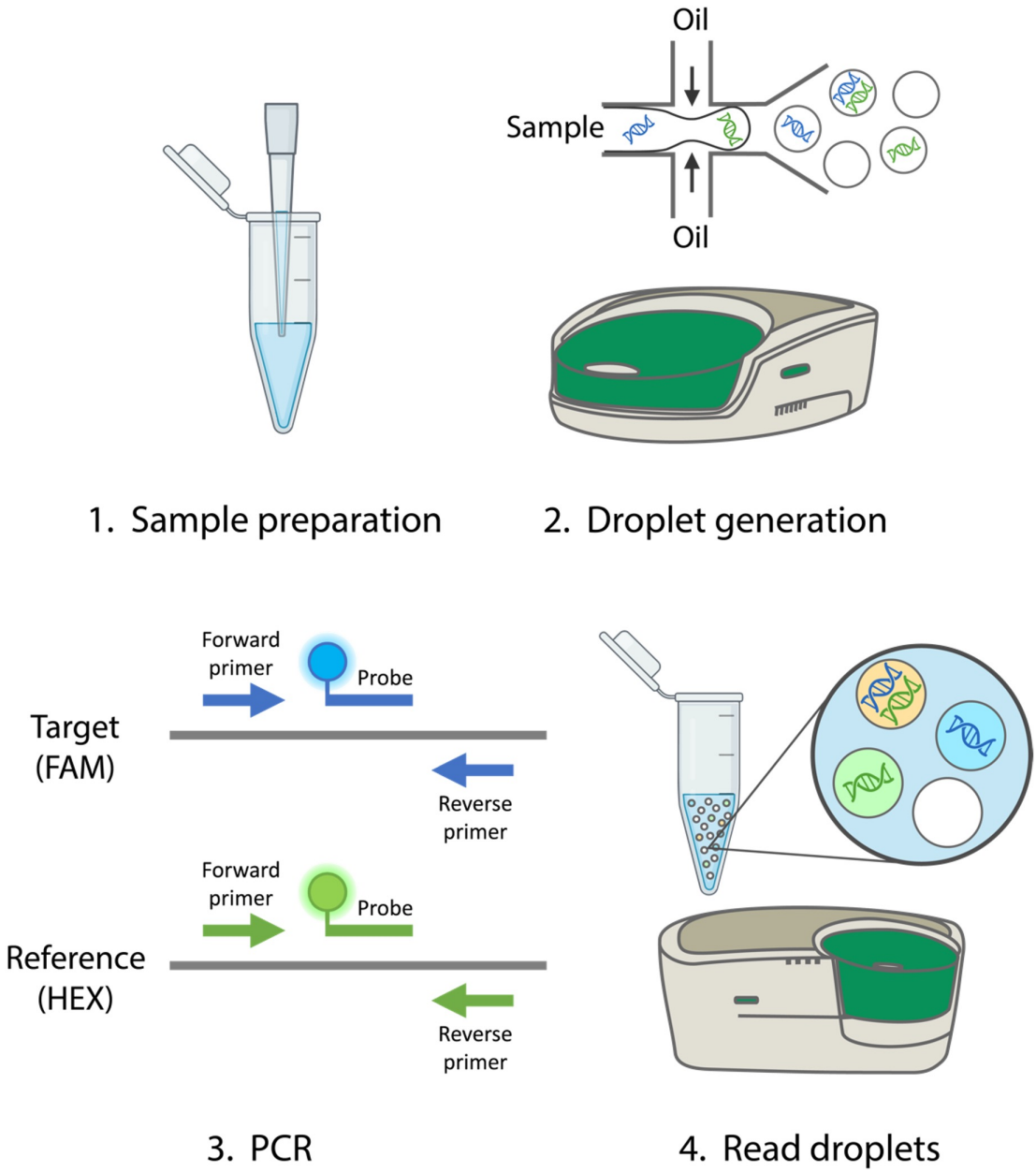

Figure 1. *ddPCR workflow* [1]

ddPCR reaction set up

2 Thaw all components to room temperature

3 Prepare 20X target primers/probe

	A	B
	Forward primer (100 µM)	18 µL
	Reverse primer (100 µM)	18 µL
	Target probe (FAM) *light sensitive	5 µL
	RNase/DNase-free H2O	59 µL

Note: Store at -20°C

4 Prepare Master Mix – Number of reactions + 1 (as extra)

	A	B
	2X ddPCR Supermix for Probes (No dUTPs)	11 µL
	20X target primers/probe (FAM)	1.1 µL
	20X reference primers/probe (HEX/VIC)	1.1 µL
	RNase/DNase-free H2O	Up to 22 µL (including DNA sample)

Note: RRP30 is used as the reference primer/probe in this protocol

5 For each PCR tube: DNA sample (Up to 350 ng) + Master Mix = 22 µL

Droplet generation

6 Insert DG8™ Cartridge into cartridge holder

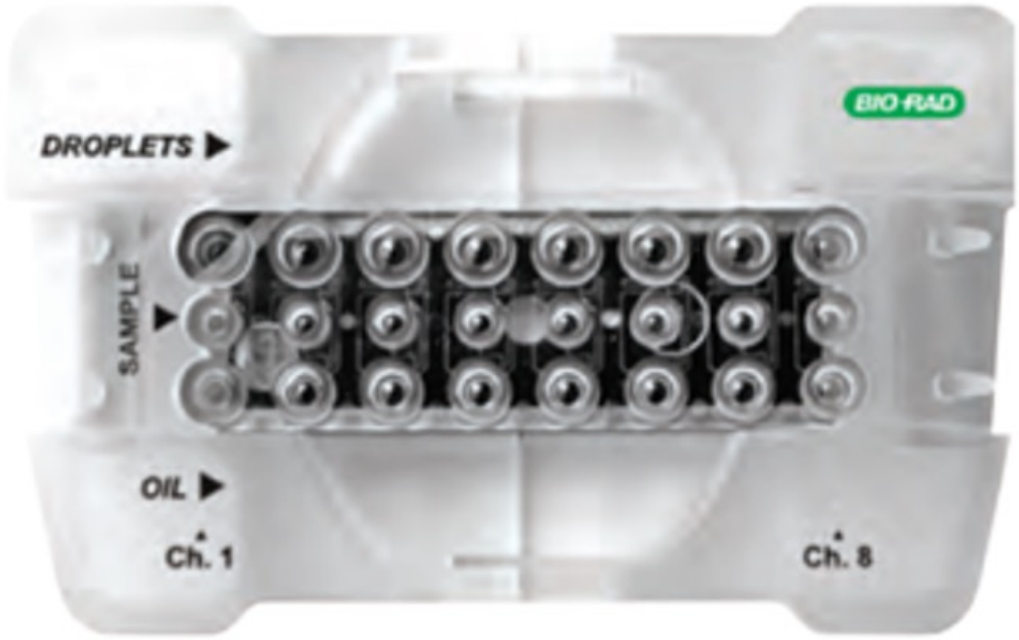

Figure 2. Loaded DG8 Cartridge [2]

7 FIRST - Load >20µL sample into wells

Note:

- Add a 50:50 mix of Supermix & H_2O in empty wells
- All 8 wells must be loaded with samples or Supermix + H_2O
- Make sure there are no bubbles

8 Load 70 µL of generation oil into all 8 wells

Note:

- Make sure samples are loaded before generation oil
- Generation oil is one-time use/can only be used within a day once opened

9 Apply a DG8 Gasket on cartridge

10 Load cartridge into QX200™ Droplet Generator

Figure 3. Loaded GD8 Cartridge in QX200™ Droplet Generator [3]

Transfer Droplets

11 Droplets should be transferred to 96-well plate within 5 mins of generation

12 Transfer 40 µL droplets to a ddPCR 96-Well Plate
Note: Pipette **SLOWLY** for droplet suction and dispensing

13 Cover 96w plate with a foil seal sheet
Note: The side with red line should be facing up

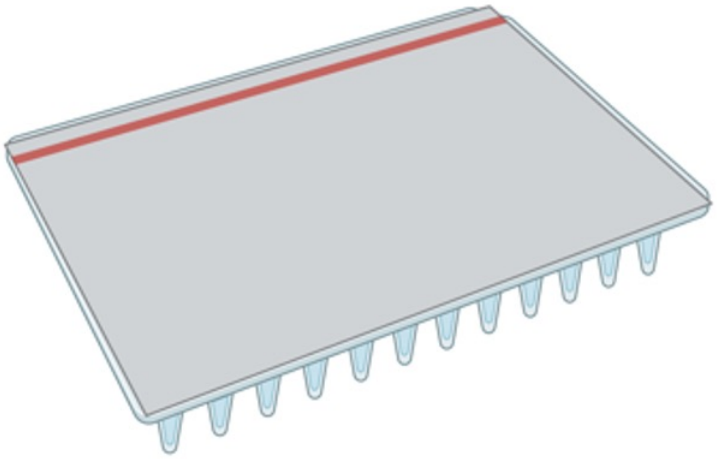

Figure 4. 96-well plate covered with foil seal sheet [1]

14 Seal plate in a PX1 PCR Plate Sealer at 180°C for 5 secs

Figure 5. Bio-Rad PX1 PCR Plate Sealer [4]

ddPCR cycle

15 ddPCR cycle should be started within 40 mins after sealing

16 Place plate into Bio-Rad Thermal Cycler with 96-Deep Well C1000 block

	A	B	C	D
	Step	Temperature	Time	
	Enzyme Activation	94°C	10 mins	Ramp rate
	Denaturation	94°C	30 sec	2°C/sec
	Annealing/Extension	*50-65°C	1 min	
	Repeat from step 2 (39x)			
	Enzyme Deativation	98°C	10 mins	
	Hold	4°C	∞	

Note: Optimized annealing temperature when using primers/probe set for the first time by using gradient temperatures

Reading the plate

17 Set plate in room temperature for 1-2mins

Note: The plate can be stored at 4°C for ≤3 days before reading

18 Place plate in Bio-Rad QX200™ Droplet Reader

Figure 6. Bio-Rad QX200™ Droplet Reader [5]

19 Open QuantaSoft Software & set up

19.1 Select a well

19.2 E.g. Sample
- Enter sample name
- Experiment: **CNV2**
- Supermix: **ddPCR Supermix for Probes (no dUTP)**

19.3 E.g. Target 1 (FAM)
- Enter name
- Type: **Ch 1 Unknown**

19.4 E.g. Target 2 (HEX/VIC)
- Enter name (RPP30)
- Type: **Ch 2 Reference**

19.5 Repeat (copy & paste) for every well used in the plate

20 Save template & RUN

Data analysis

21 Check if there are >10k events for each sample

22 Set threshold manually (pink line) - if necessary

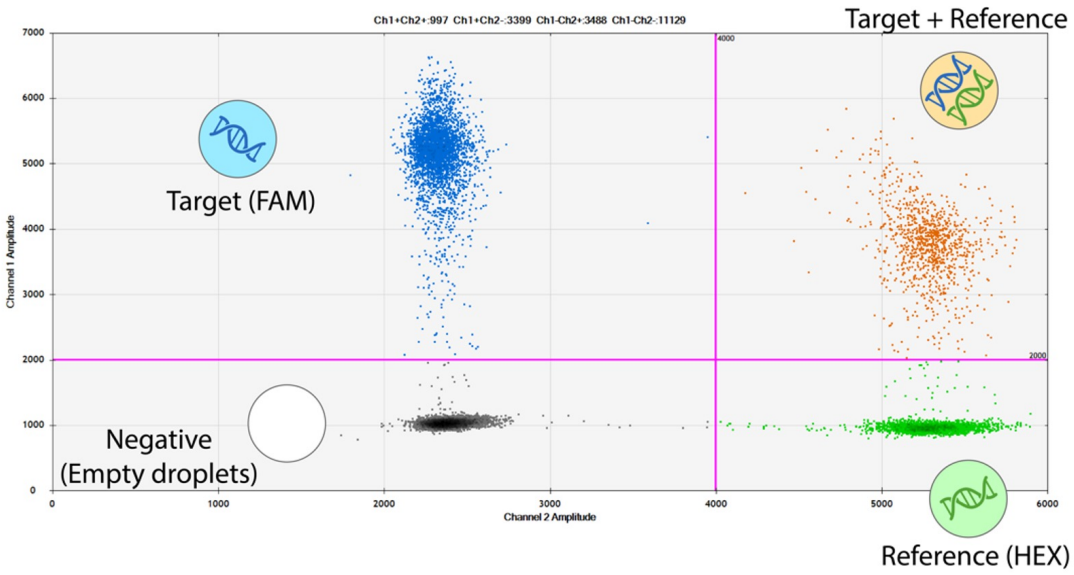

Figure 7. *ddPCR 2-D plot* [1,6]

Note: Make sure the positive droplets (blue/green) are separated from the negative droplets (grey)