## **Transferring Sapphire Chip ESR1 assay** to Ruby Chip for cfDNA and FFPE



- ESR1 assay with Ruby Chip compared to validated 3plex assay with Sapphire Chip for FFPE and cfDNA samples
- 3 FFPE samples with concentration between 0.4 and 0.7ng/μL
- 24 cfDNA samples with concentration between 0.5 and 3ng/μL
- Some samples carry multiple mutations



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Sample			ESR1 Assays (CEM)		ESR1 12Plex (STILLA)				
Sample	Sample concentration in the chamber (ng/µL)	Sample type	MUT	MAF(%)	MUT3	Maf 1chb	MAF 2 chbs	MAF 3 chbs	
12	1,34	cfDNA	D538G	0,13	D538G	0,18	0,22	0,21	
10	1,08	cfDNA	E380Q	0,14	E380Q	0,29	0,32	0,27	
22	1,36	cfDNA	Y537N	0,22	Y537N	0,44	0,30	0,25	
23	3,23	cfDNA	Y537C	0,24	Y537C	0,24	0,24	0,25	
7	1,05	cfDNA	Y537S	0,28	Y537S	0,16	0,17	0,27	
19	1,05	cfDNA	D538G	0,47	D538G	0,46	0,43	0,42	
5	1,05	cfDNA	D538G	0,49	D538G	0,27	0,36	0,41	
22	1,36	cfDNA	Y537D	0,71	Y537D	1,16	0,84	0,91	
22	1,36	cfDNA	Y537C	1,19	Y537C	1,38	1,28	1,96	
22	1,36	cfDNA	D538G	1,23	D538G	1,52	1,21	1,16	
22	1,36	cfDNA	L536P	1,32	L536P	1,88	1,58	1,62	
11	0,64	cfDNA	Y537S	2,34	Y537S	1,71	1,78	2,21	
11	0,64	cfDNA	D538G	2,73	D538G	1,96	2,04	2,30	
21	1,02	cfDNA	D538G	3,02	D538G	2,54	2,23	2,35	
22	1,36	cfDNA	L536R	3,28	L536R	4,63	4,37	4,41	
20	0,53	cfDNA	Y537S	3,68	Y537S	3,09	2,99	3,39	
19	1,10	cfDNA	E380Q	5,55	E380Q	4,17	4	3,91	
5	1,10	cfDNA	Y537S	6,59	Y537S	6,36	6,22	6,33	
22	1,36	cfDNA	L536H	6,83	L536H	7,66	7,73	8,02	
7	1,05	cfDNA	Y537N	11,58	Y537N	11,32	11,32	11,72	
17	1,32	cfDNA	Y537S	13,40	Y537S	12,81	13,69	14,32	
30	0,47	FFPE DNA	Y537N	20,00	Y537N	19,88	15,34	16,01	
1	1,09	cfDNA	Y537S	21,30	Y537S	18,3	19,74	19,54	
23	3,23	cfDNA	L536H	25,63	L536H	25,25	25,62	25,47	
13	1,01	cfDNA	L536R	28,68	L536R	36,86	39,52	40,46	
29	0,73	FFPE DNA	Y537N	32,46	Y537N	29,22	29,33	27,94	
27	0,38	FFPE DNA	D538G	63,69	D538G	60,25	53,44	53,41	





A 5µL of Ruby chip with highplexing, allows to have a concordance of 0.98 with the 3 colors Drop-off assay and specific assays developed by the CEM on a 25µL Sapphire chamber

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23	3,23	cfDNA	Y537C	0,24	Y537C	0,24	0,24	0,25	
7	1,05	cfDNA	Y537S	0,28	Y537S	0,16	0,17	0,27	
19	1,05	cfDNA	D538G	0,47	D538G	0,46	0,43	0,42	
5	1,05	cfDNA	D538G	0,49	D538G	0,27	0,36	0,41	
22	1,36	cfDNA	Y537D	0,71	Y537D	1,16	0,84	0,91	
22	1,36	cfDNA	Y537C	1,19	Y537C	1,38	1,28	1,96	
22	1,36	cfDNA	D538G	1,23	D538G	1,52	1,21	1,16	
22	1,36	cfDNA	L536P	1,32	L536P	1,88	1,58	1,62	
11	0,64				Y537S	1,71	1,78	2,21	
11	0,64	22		1,36	D538G	1,96	2,04	2,30	
21	1,02				D538G	2,54	2,23	2,35	
22	1,36	11		0.64	L536R	4,63	4,37	4,41	
20	0,53					3,09	2,99	3,39	
19	1,10	11		0.64	E380Q	4,17	4	3,91	
5	1,10	<u> </u>		0,04	Y537S	6,36	6,22	6,33	
22	1,36	21		1.02	L536H	7,66	7,73	8,02	
7	1,05				Y537N	11,32	11,32	11,72	
17	1,32	22		1,36	Y537S	12,81	13,69	14,32	
30	0,47				Y537N	19,88	15,34	16,01	
1	09	20		0,53	Y537S	18,3	19,74	19,54	
23	3,23	10		1 10	L536H	25,25	25,62	25,47	
13	1,01	19		1,10	L536R	36,86	39,52	40,46	
29	0,73	FFPE DNA	Y537N	32,46	Y537N	29,22	29,33	27,94	
27	0,38	FFPE DNA	D538G	63,69	D538G	60,25	53,44	53,41	





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# ESR1 Liquid biopsy testing with Ruby Chip

#### Challenges

Evaluate mutation detection and quantification robustness and sensitivity between Crysal Digital PCR® and droplet digital PCR (ddPCR)

#### Method

As it's routinely validated to run samples in triplicates (60µL) with ddPCR to achieve good sensitivity using a duplex Drop-off assay. 15µL Ruby Chip chambers format was used for each sample with 18plex ESR1 assay



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## ESR1 Liquid biopsy testing with Ruby Chip



- Same samples with same initial concentration tested in:
  - $~\circ~~$  15µL with 18plex assay on Ruby Chip
  - $~\circ~~60\mu L$  with duplex assay on ddPCR Cartridge

	Dupl	ex ddPCR da	ita	18plex cdPCR STILLA			
	Mutant copies/µL	WT copies/µ L	VAF %	Mutant copies/µ L	WT copies/µ L	VAF %	
Ctle WT	0	78,4	0,00	0	115,1	0,00	
Ctl Mut Ex5 (E380Q)	0,6	72,6	0,82	1,01	137,3	0,74	
Ctle Mut Ex8 (D538G)	0,9	91,1	0,98	2,46	185,7	1,32	
Sample 1 Mut Ex8	0,23	54,6	0,42	0,4	207,9	0,19	
Sample 2 Mut Ex8	5,7	60,9	8,56	14,8	186,3	7,94	
Sample 3 mut	NA	NA	0,42	1,58	256,8	0,62	



High concordance of data obtained with 15µL on Ruby Chip compared to 60µL with very higher plex, 4 times less input of precious sample and competitive price per reaction for a 18plex