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(54) **DIGITAL ANALYTE ANALYSIS**

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(58) **Field of Classification Search**
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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,097,692	A	11/1937	Fiegel
2,164,172	A	6/1939	Dalton
2,656,508	A	10/1953	Coulter
2,692,800	A	10/1954	Nichols et al.
2,797,149	A	6/1957	Skeggs
2,879,141	A	3/1959	Skeggs
2,971,700	A	2/1961	Peeps
3,479,141	A	11/1969	Smythe et al.
3,608,821	A	9/1971	Simm et al.
3,698,635	A	10/1972	Sickles
3,784,471	A	1/1974	Kaiser
3,816,331	A	6/1974	Brown, Jr. et al.
3,930,061	A	12/1975	Scharfenberger
3,960,187	A	6/1976	Stock et al.
3,980,541	A	9/1976	Aine
3,982,541	A	9/1976	L'Esperance, Jr.
4,014,469	A	3/1977	Sato
4,022,575	A	5/1977	Hansen et al.
4,034,966	A	7/1977	Suh et al.
4,059,552	A	11/1977	Zweigle et al.
4,091,042	A	5/1978	Alexanderson et al.
4,117,550	A	9/1978	Folland et al.
4,130,394	A	12/1978	Negersmith
4,210,809	A	7/1980	Pelavin
4,253,846	A	3/1981	Smythe et al.
4,266,721	A	5/1981	Sickles
4,279,345	A	7/1981	Allred
4,297,345	A	10/1981	Howarth
4,315,754	A	2/1982	Ruzicka et al.
4,378,957	A	4/1983	Malkin et al.
4,383,767	A	5/1983	Jido
4,439,980	A	4/1984	Biblarz et al.
4,508,265	A	4/1985	Jido
4,533,634	A	8/1985	Maldonado et al.
4,585,209	A	4/1986	Aine et al.
4,618,476	A	10/1986	Columbus
4,675,285	A	6/1987	Clark et al.
4,676,274	A	6/1987	Brown
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,739,044	A	4/1988	Stabinsky
4,757,141	A	7/1988	Fung et al.
4,767,515	A	8/1988	Scott et al.
4,767,929	A	8/1988	Valentine
4,779,805	A	10/1988	Jackson et al.
4,801,086	A	1/1989	Noakes
4,801,529	A	1/1989	Perlman
4,829,996	A	5/1989	Noakes et al.
4,853,336	A	8/1989	Saros et al.

(Continued)

FOREIGN PATENT DOCUMENTS

AU	2004225691	B2	6/2010
CA	2520548	A1	10/2004
CH	563807	A5	7/1975
DE	4308839	C2	4/1997
DE	10015109	A1	10/2001
EP	0047130	B1	2/1985
EP	0249007	A3	3/1991
EP	0476178	A1	3/1992
EP	0718038	A2	6/1996
EP	0540281	B1	7/1996

(Continued)

OTHER PUBLICATIONS

Kiss et al. Anal. Chem. 2008, vol. 80, p. 8975-8981.*

(Continued)

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(57) **ABSTRACT**

The invention generally relates to droplet based digital PCR and methods for analyzing a target nucleic acid using the same. In certain embodiments, methods of the invention involve forming sample droplets containing, on average, a single target nucleic acid, amplifying the target in the droplets, excluding droplets containing amplicon from the target and amplicon from a variant of the target, and analyzing target amplicons.

10 Claims, 40 Drawing Sheets