COMPARATIVE ANALYSIS OF THE EFICENCY OF POWERPLEX 16 AND POWERPLEX FUSION MULTIPLEX STR KITS IN THE ANALYSIS OF THE CHALLENGING FORENSIC SAMPLES

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Abstract

Since the introduction of the term *low copy number DNA*, also referred as *low template DNA*, *touch DNA* or *trace DNA analysis*, it has quickly become focal point of forensic DNA testing as well as other DNA based studies. Low template DNA (ltDNA) samples can be described as the samples which involve single source samples with template DNA in concentrations below 100 picograms (pg). Due to sensitivity of ltDNA samples to contamination, it is of great importance to optimize performance of the multiplex STR systems and existing protocols to increase chance of successful analysis. The main objective of this study was analysis of 20 challenging samples (skeletal remains, cigarette buts, chewing gum, poorly collected buccal swabs etc.) mostly low template DNA samples, preliminarily profiled by PowerPlex® 16 multiplex STR systems and additionally processed with new generation multiplex STR kit PowerPlex® Fusion. Sample isolation was done using a standard phenol-chloroform method for bone samples and DNeasy® Blood and Tissue Kit for other forensic samples. PowerPlex® 16 (PP16), multiplex STR system and PowerPlex® Fusion (PP Fusion) were used for co-amplification of 15 and 24 autosomal STR loci respectively. Results of this preliminary study suggest that PP Fusion primer set is better optimized for the analysis of ltDNA samples, and it is more robust regarding presence of the potential PCR inhibitors.

Short communication

Key words: *Low Template DNA, PCR inhibitors, STR, Genotyping, PowerPlex® 16, PowerPlex® Fusion*

Introduction

Forensic DNA testing also referred to as DNA fingerprinting/profiling is based on the basic principle of all living beings – molecular biodiversity (Rapley, 1998). Through history the method has undergone improvement and modern technology provides possibility to generate a DNA profile from even miniscale biological samples making it easier for low enforcement to identify suspects using the DNA found at a crime scene (Marjanović et al., 2013). DNA profiling is of vital importance in determining suspects with high percentage of correctness (Butler, 2011). STRs (Short Tandem Repeats) are highly polymorphic genetic markers, which consist of repeat units from 1-9 bp (according to some sources 2-7 bp) in length (Primorac et al., 2014). Their abundance throughout the human genome is on average one per 6-10 kb (Beckmann & Weber, 1992). The number of STR

loci used increases the discrimination value exponentially, making the possibility of finding another individual with the same STR profile extremely rare. (Butler, 2005; Butler, 2007). Extreme robustness, high level of discrimination, co-amplification of multiple STR loci as well as obtaining of good results led to production of variety of multiplex STR kits. This resulted in usage of STR systems as key tools in forensics as well as some medical researches related to genetic diseases (Harder et al., 2012). Low template DNA (ltDNA) refers to DNA samples which contain less than 100 pg which are required for generating individual complete profiles by standard PCR procedure involving 28-30 cycles. Analysis of such samples is defined as analysis of DNA samples where the results are below stochastic threshold for reliable interpretation (Grisedale & van Daal, 2012). Low template DNA results in occurrence of amplification artifacts which presents one of the main challenges in this kind of analysis. Most commonly occurring artifacts include: allele drop-out, allele drop-in, locus-drop out, increased risk of sample contamination, increased stutters and peak imbalance (Primorac et al., 2014). Frequently, ltDNA analysis results in generation of partial DNA profiles. A DNA profile is considered a full DNA profile when all allelic variants across all analyzed STR loci are clearly detected and when homozygosity and heterozygosity can be confirmed. On the other hand, profile where full detection of allelic variants is absent is called partial profile (Marjanović et al., 2013). The main objective of this study was to compare the STR profile obtained with two multiplex kits. For this purpose we analyzed 20 low template DNA samples (skeletal remains, cigarette buts, chewing gum, poorly collected buccal swabs etc.).

Materials and methods

Twenty forensic samples (skeletal remains, cigarette buts, chewing gum, poorly collected buccal swabs etc.) from different previously managed forensic cases were prepared for additional analysis. DNA isolation was done using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) (Qiagen, 2012) from samples, except bone samples. DNA was isolated from bone samples using an optimized phenol/chloroform DNA extraction procedure following a previously described phenol-chloroform DNA extraction protocol (Davoren et al., 2007). All the samples were preliminarily profiled using PowerPlex® 16 multiplex STR system - PP16 (Promega Corporation, USA) (Promega, 2016a) which resulted in partial STR profiles. Therefore, they were selected to be processed by new generation multiplex STR system PowerPlex® Fusion – PP Fusion (Promega Corporation, USA) (Promega, 2016b). The same DNA isolates were used for PP16 amplifications and PP Fusion amplification. Quantification of isolated DNA was performed using Qubit® 2.0 fluorometer. PCR amplification was done using GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) according to manufacturer's guide. Detection of PCR products was done using ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). To obtain data from analyzed samples by PowerPlex® 16 and PowerPlex® Fusion the 310 Data Collection Software and GeneMapperTM 3.2 (Applied Biosystems) were used. Concordance rate of autosomal loci shared by PowerPlex® 16 and PowerPlex® Fusion was calculated according to formula: (127 - number of concordant alleles / 600 number of tested alleles) * 100.

Results and Discussion

Quantification of isolated DNA from 20 selected samples shown that most of analyzed samples (13 out of 20) have quantity much lower than 100 pg. Seven samples have quantity higher than 1 ng. The total of 40 STR profiles were generated. In table 1 and 2 detected alleles of the 20 STR profiles, generated using PowerPlex® 16 and PowerPlex® Fusion, are shown.

All profiles generated with PowerPlex® 16 were partial profiles (Figure 1b), while amplification using PowerPlex® Fusion kit resulted in generation of complete profiles (Figure 1a). Preliminary comparison study was performed by comparing detected alleles on commonly shared loci for these two kits including: Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA,

Table 1. PowerPlex® 16 profile representation of 20 samples

No	D3S1358	TH01	D21S11	D18S51	Penta_E	D5S818	D13S317	D7S829	D16S539	CSF1PO	Penta_D	vWA	D8S1179	TPOX	FGA	Amel
1	16,17	8,9.3	No	No	No	13,14	9,12	9	12,12	10,11	11	16,18	13,15	11	21,23	X,Y
2	19	9,9.3	34	OL	No	No	No	No	No	No	No	14,17				
3	14,15	9	30,33.2				12,10		9,11			18,19				
4	15	9.3,10	29				11		9			17				
5	15,18	8,9	29			11,12	13	11	10	12	17	15,17	10,13	8	20,23	X,Y
6	16,19	7,9.3	28,30			11	11,12		11,12	12		16	12,13			
7	14,15	9				11,14	11,12	9,10	12	11	9	16	10,13	8		X,Y
8	15,16	7,9	28,29			11,12	12		8,11			15,16		11	21,23	X
9	16	6,9				9,13	11	7,9	10,11	10,12	8	14,18		811	21,25	X,Y
10	15	6,9					10,11		9			18,19				
11	14,18	8,9.3	30			11,13		8,10	11			14,16	8	8,11	22	X,Y
12		7,9	28,29			11	9,13		9,11			16,18			22	
13		6										16,17				
14		9.3	29,30.2			9,12	12,13		11,12			16,17			19,22	
15		6,9.3				9,12	11,12		10,11			16,19				
16		7,9.3	30,31.2			12	8,9	9	11,14			15,18		8,10	22,25	X,Y
17		8,9.3	30,31			11	11,12		10,11			16,17		8,11	20,22	Y
18	17,18	7,9.3	28,32.2			9,12	8,11	11,12	9,11	10,11		17	13	11	24	
19	15,17	6,7	27,28			12				11		16,19		8	23	X,Y
20	18,20	7,8	27,28				9,11		10			17				

Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. It was determined that PowerPlex® 16 profiles had many discordant alleles comparing to ones detected with PowerPlex® Fusion. It is mainly because of the amplification failures probably due to conventional primers and chemistry used by PP16 which are less suitable for successful ltDNA analysis. The same applies for the analysis of the samples with increased presence of the PCR inhibitors. Concordance rate of autosomal loci shared by PowerPlex® 16 and PowerPlex® Fusion was 21.17%. Accuracy of results varies across loci. Peaks were detected at TH01 and vWA loci for all the 20 samples and using both profiling systems. At D16S539 locus, results were obtained for 85% profiles using PowerPlex® 16 while when PowerPlex® Fusion was used detection occurred for every sample. The D13S317 locus displayed results similar to the D16S539 locus where percentage, when PowerPlex® 16 was used, was 80%. At three loci, D3S1358, D21S11 and D5S818, 70% of samples displayed results with comparison to PowerPlex® Fusion where we obtained results for 100% of samples. When PCR amplification was performed with the PowerPlex® 16 System, the success in detecting alleles on FGA and TPOX loci was 55% and 50% respectively. The detection of the alleles for locus CSF1PO was 40% and 35% for the D7S829 locus for all analyzed samples. On the other hand, for D8S1179 and Penta D loci detection was 30% and 20% respectively. However, at the D18S51 and Penta E loci no peaks were detected using PowerPlex® 16 System, but with PowerPlex® Fusion System amplification was 100% successful for all tested samples.

When considering quantity of template DNA, no pattern could be established that would suggest that the samples with higher template concentration yielded better profile.

Previous studies suggested that PP Fusion, as a representative of the new generation multiplex STR systems, is extremely efficient for the analysis of the ltDNA samples (Marjanović et al., 2013; Marjanović et al. 2015; Čakar et al., 2017). Additionally, similar results were obtained regarding analysis of the samples with potentially increased presence of PCR inhibitors, which proved that PP Fusion is very robust PCR tool (Oostdik et al, 2014).

Results of this study showed that PP Fusion, as the multiplex STR system of the new generation, is more efficient PCR tool than PP 16 kit when it comes to the analysis of the challenging forensic samples. Application of PowerPlex® Fusion kit

Table 2. PowerPlex® Fusion profile representation of 20 samples

No	D3S1358	D1S1656	D2S441	D10S1248	D13S317	PentaE	D16S539	D18S51	D2S1338	CSF1PO	PentaD	TH01
1	16,17	15.3,16	11.3,13	14,16	9,12	11,13	11,12	13,17	24,25	10,11	11,13	8,9.3
2	17,18	13,16.3	14,15	14,17	10,12	11,18	12	15,17	20,23	10,12	9,12	9,9.3
3	14,15	13,15	10,11	14,15	12	7,11	9,11	13,14	17,25	11	9,15	9
4	15,16	17.3	11,14	15	11	11,12	9,12	12	16,19	12	10,13	9.3,10
5	15,18	11,15	11,14	14,15	13	14,17	10,12	12,15	21,23	11,12	13,17	8,9
6	16,19	11,15	10,14	13,16	11,12	13,18	11,12	14,15	17	11,12	9,12	7,9.3
7	14,15	12,16	11,14	14,16	11,12	7,10	12	13,16	17,20	10,11	9,12	8,9
8	15,16	11,12	11,14	14,15	12	11	8,11	14,15	17,24	10,11	12,13	7,9
9	16	15,17.3	11,12	15,16	11	13,14	10,11	12,18	19,20	10,12	8,11	6,9
10	15	12,15	11,12	15,16	10,11	5,7	9,11	15,16	24,25	10	12,13	6,9
11	14,18	14,15	10,14	13	8,12	12,19	11	15,16	19,23	11,12	11,12	8,9.3
12	16,18	12,13	11.3,13	14	9,13	5,15	9,11	13,16	20,25	11,12	10,11	7,9
13	16	12,18.3	11,14	15	11,14	7,11	10,12	11,12	16,19	10	11,12	6,9.3
14	17,18	13,18.3	11	13,15	12,13	12,15	11,12	14,16	16,25	10,11	12,14	9.3
15	16,17	11,17.3	10,11	14	11,12	9,16	10,11	14,15	17,24	11,12	12	6,9.3
16	18	16,18.3	11,12	16	8,9	13	11,14	14,16	18,24	9,13	9,11	7,9.3
17	15,18	12,16	11,12	16,17	11,12	12,14	10,11	14,18	18	10,12	11,13	8,9.3
18	17,18	12,13	10,12	13,14	8,11	5,19	9,11	13,16	16,24	10,11	11	7,9.3
19	15,17	13,15	11,11.3	14,17	12	5,12	12,13	13,16	17,25	11	11	7,9.3
20	18	15	11,12	13	9.11	10,16	10,13	15,17	17,24	11,12	12,13	7,8

No	vWa	D21S11	D7S820	D5S818	TPOX	DYS391	D8S1179	D12S391	D19S433	FGA	D22S1045	Amel
1	16,18	30,32.2	9	13,14	11	11	13,15	18,19	13	21,23	15,17	X,Y
2	14,17	30	8,10	11,13	9,12	10	13,14	16,18	14	21,24	15,16	X,Y
3	19	30,33.2	8,11	12	8,9	11	14,15	22,23	12,14	22,24	16,17	X,Y
4	17	29,30	9,11	11	9,11		12,13	17,22	13,14	20,23	16	X,Y
5	15,17	29,31.2	11	11,12	8	11	10,13	18	12,14	20,23	15,16	X,Y
6	16	28,30	9,13	11	9,11	10	12,13	19,23	12,15	22,25	15,16	X,Y
7	16	30,32.2	9,10	11,14	8,11	10	10,13	17	13,14	20,23	15,16	X,Y
8	15,16	28,29	10,11	11,12	8,11		14	17,22	14,16	21,23	15,17	X
9	14,18	29,31.2	7,9	9,13	8,11	11	14,16	16,20	13,15.2	21,25	12,17	X,Y
10	18,19	31	10,11	12,14	8	10	12,13	16,17	13,15.2	21,26	16	X,Y
11	14,16	30,31.2	8,10	11,13	8,11	10	8,13	19,20	12,14	22	16	X,Y
12	16,18	28,29	11,13	11	8,11		14,15	18,24	14,17	22,24	15	X
13	16,17	28,31.2	8,11	11,13	11	11	10,13	17,18	14	19,21	15	X,Y
14	16,17	29,30.2	8,9	9,12	8,10	11	10,13	19,21	13,14	19,22	15	X,Y
15	16,19	28,34.2	8,12	9,12	8,9		10,14	15,25	13,15.2	19,22	14,17	X
16	15,18	30,31.2	9	12	8,10	10	10,15	19.3	13	22,25	16	X,Y
17	16,17	30,31	11,12	11	8,11	11	13,14	19,20	14	20,22	15	X,Y
18	17	28,32.2	11,12	9,12	11		13	20,23	14	24	15	X
19	18,19	29,30	10,11	12	8	10	11,13	18,19	13,14	19,23	17	X,Y
20	17	27,28	7,9	11,13	8,11	11	12,14	18,19	13,13.2	20,23	11	X,Y

resulted in generating full STR profiles for all 20 samples which prove that primers incorporated within PowerPlex® Fusion kit are more appropriate for the analysis of samples with smaller amounts of DNA and that PP Fusion chemistry is more optimized for the samples with possibly increased presence of PCR inhibitors. Similar results were obtained by previous study (Oostidik et al, 2014). Additionally, these results, together with previously published research (Čakar et al., 2017), proved that PP Fusion is suitable forensic DNA identification tool even for the human skeletal remain samples. Finally, this study showed that usage of PowerPlex® Fusion is much more informative and convenient tool for the analysis of ltDNA and challenging samples than multiplex STR systems of the previous generation.

Conclusions

Multiplex STR-PCR systems are routinely used for DNA profiling in forensic laboratories worldwide. Selection of optimal PCR system is primarily based on specific laboratory's needs but precision and consistency also need to be considered. Our study

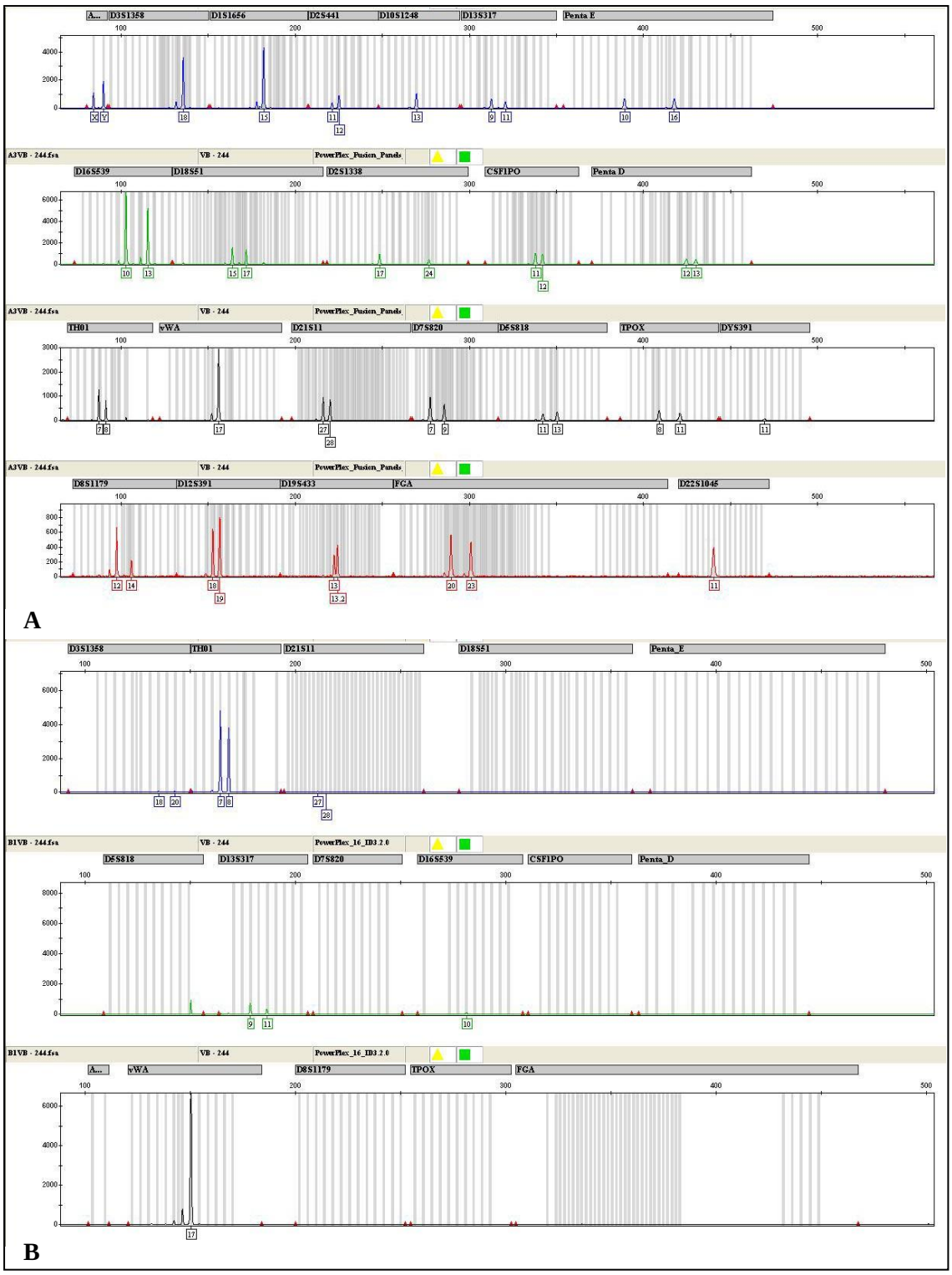

Figure 1. DNA profile of Sample 20 generated using PowerPlex® Fusion (A) DNA profile of Sample 20 generated using PowerPlex® 16 (B)

showed that PowerPlex® Fusion system provides more informative results in ltDNA analysis in comparison to previously available STR systems and therefore it represents more powerful tool for the analysis of challenging samples.

References

Beckmann JS, Weber JL (1992) Survey of human and rat microsatellites. Genomics, 12:627-631.

Butler JM (2005) Forensic DNA typing: biology, technology, and genetics of STR markers. Academic Press, San Diego.

Butler JM (2007) Short tandem repeat typing technologies used in human identity testing. Biotechniques, 43(4):2-5.

Butler JM (2011) Advanced Topics in Forensic DNA Typing: Methodology. Academic Press, San Diego.

Čakar J, Pilav A, Džehverović M, Ahatović A, Haverić S, Ramić J, Marjanović D (2017) DNA Identification of Commingled Human Remains from the Cemetery Relocated by Flooding in Central Bosnia and Herzegovina. J Forensic Sci, in press. DOI: 10.1111/1556-4029.13535

Davoren J, Vanek D, Konjhodžić R, Crews J, Huffine E, Parsons TJ (2007) Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves. Croat Med J, 48:478-485.

Grisedale KS, van Daal A (2012) Comparison of STR profiling from low template DNA extracts with and without the consensus profiling method. Investig Genet, 3(1):14.

Harder M, Renneberg R, Meyer P, Krause-Kyora B, Wurmb-Schwark NV (2012) STR-typing of ancient skeletal remains: which multiplex-PCR kit is the best?. Croat Med J, 53(5): 416-422.

Marjanović D, Primorac D (Eds) (2013) Forenzična genetika: teorija i aplikacija. Naučna i stručna knjiga Lelo doo, Sarajevo.

Marjanović D, Hadžić Metjahić N, Čakar J, Džehverović M, Dogan S, Ferić E, Džijan S, Škaro V, Projić P, Madžar T, Rod E, Primorac D (2015) Identification of human remains from the Second World War mass graves uncovered in Bosnia and Herzegovina. Croat Med J, 56(3): 257-262.

Oostdik K, Lenz K, Nye J, Schelling K, Yet D, Bruski S, Strong J, Buchanan C, Sutton J, Linner J, Frazier N, Young H, Matthies L, Sage A, Hahn J, Wells R, Williams N, Price M, Koehler J, Staples M, Swango KL, Hill C, Oyerly K, Duke W, Katzilierakis L, Ensenberger MG, Bourdeau JM, Sprecher CJ, Krenke B, Storts DR (2014) Developmental validation of the PowerPlex® Fusion System for analysis of casework and reference samples: A 24-locus multiplex for new database standards. Forensic Sci Int Genet, 12:69-76.

Primorac D, Schanfield MS, Marjanović D (2014) Basic Genetics and Human Genetic Variation. Book Chapter - Forensic DNA Applications: An Interdisciplinary Perspective Book. CRC Press, USA.

Promega Corporation (2016a) GenePrint®PowerPlex 16 System Instruction for use of Product. Promega Corporation.

Promega Corporation (2016b) GenePrint®PowerPlex Fusion System Instruction for use of Product. Promega Corporation.

Qiagen Companies (2012) QIAmp DNA Mini Kit handbook. Hilden, Germany: Qiagen,

Rapley R (1998) Molecular Biomethods Hand book (No. 1646). Springer Science & Business Media.