VALIDATION OF APPLIED BIOSYSTEM'S QUANTIFILER® DUO DNA QUANTIFICATION KIT

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Abstract

The determination of the quantity of human DNA within a forensic sample is an important step in the development of a DNA profile. The Quantifiler® Duo DNA Quantification kit has advantages in a forensic casework laboratory over the Quantifiler® Human DNA Quantification kit. Not only will this system simultaneously amplify the total human and total male DNA within a sample, but it will also give a more accurate representation of the amount of amplifiable DNA. The Duo kit enables the DNA analyst to evaluate the quality of a sample prior to short tandem repeat (STR) analysis along with whether autosomal or Y-STR analysis will be more beneficial. Following the guidelines set forth by the Quality Assurance Standards for Forensic DNA Testing Laboratories, the Quantifiler® Duo DNA Quantification kit was shown to be a reliable, robust, and reproducible quantification system.

Introduction

The determination of the quantity of human DNA within a forensic sample is an important step in the development of a DNA profile. Multiplex short tandem repeat (STR) amplification kits produce optimal results at a narrow concentration range. Real-time PCR is used to determine the amount of amplifiable DNA within a sample. The laboratory currently utilizes the Quantifiler® Human DNA Quantification kit. Although this kit has provided useful quantification results, the Quantifiler® Duo DNA Quantification kit has features that are more advantageous for this laboratory such as:

- Detects total human and total male DNA concentrations simultaneously
- Aids in decision to proceed with autosomal or Y-STR analysis
- Target amplicon length has been increased
- More accurate representation of amplifiable DNA

https://products.appliedbiosystems.com/ab/en/US/htdocs/productMgr/images/Quant_Duo_Large.jpg

Materials and Methods

- WVSP Forensic Laboratory DNA Analysis Procedures Manual, revision 19
- Phenol/Chloroform or DNA IQ™ (Promega Corporation, Madison, WI) extraction
- Quantification on the ABI Prism® 7500 Real-Time PCR System using either Quantifiler® Human or Quantifiler® Duo Quantification Kits. Analyzed with SDS software v1.2.3 (Applied Biosystems, Foster City, CA)
- Amplifications conducted using the PowerPlex® 16 or PowerPlex® Y systems (Promega Corporation) on a GeneAmp® PCR System 9700 (Applied Biosystems)
- Applied Biosystems 3130 or 3130*xl* genetic analyzers at 3 and 5 second injection times unless otherwise stated
- GeneMapper™ *ID* analysis software v3.1 (Applied Biosystems)
- Sources of DNA
  - 9947A Female DNA and 9948 Male DNA (Promega Corporation)
  - Standard Reference Material ® 2372 Human DNA Quantitation Standard (National Institute of Standards and Technology, Gaithersburg, MD)
  - DNA Control 1, Heterozygote Standard R671 (Serological Research Institute, Richmond, CA)
  - Blood stains and buccal swabs from laboratory volunteers
  - Mock casework samples obtained at the laboratory

NIST SRM® 2372

- Human Quantitation Standard
- Aid in determination of Duo DNA standard concentration
  - Duo DNA standard should be 200ng/µl
  - Variation exhibited in different kit lot numbers
- Component A used as a standard curve
- Component B dilutions used as quality control measure
- Duo DNA standard dilutions set as unknowns
- Quantity of Duo DNA standard dilutions determined and adjusted as needed

Results and Discussion

- **Buffer Study**
  - Tris EDTA pH 8.0 (TE^{-4}) with the addition of Glycogen versus Duo Dilution Buffer
  - Standard curves prepared with each diluent
  - Each curve fell within manufacturer's specifications
    - Slope between -3.0 and -3.6
    - $R^2 \geq 0.99$
  - Prolonged storage of standards from recommended 2 weeks to 4 weeks at 2 to 8°C
- **Precision Study**
  - Precision of real-time PCR assay is determined by the variation of cycle threshold (C_T) values
  - Evaluated C_T of standard curves from buffer study
  - Greatest C_T variation observed at low end of curve

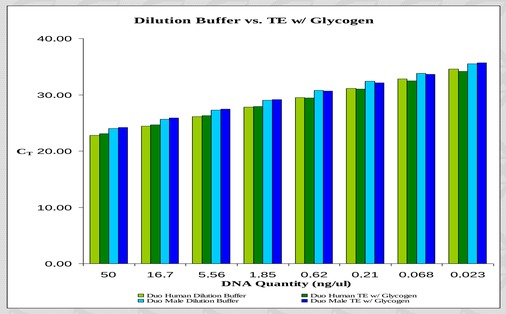

Figure 1. Precision Study average C_T values for both standard curves

- **Optimal Signal Study**
  - Combined with the NIST SRM® 2372 Evaluation
  - 9947A diluted to a target of 0.1ng/µl
  - Amplified 5µl (0.5ng) and 10µl (1.0ng)
  - Target relative fluorescent unit (RFU) range 500 to 1500

PowerPlex® 16 Positive Control		0.5ng RFU Values		1.0ng RFU Values	
Locus	**Allele**	**3 sec.**	**5 sec.**	**3 sec.**	**5 sec.**
D3S1358	14	1006	1677	1954	2414
	15	933	1519	1925	2397
TH01	8	871	1410	1825	2258
	9.3	848	1357	1980	2438
D21S11	30	1345	2198	2892	3457
D18S51	15	730	1179	1386	1596
	19	586	979	1045	1213
Penta E	12	810	1312	1329	1616
	13	807	1327	1053	1296
D5S818	11	1710	2758	3029	3826
D13S317	11	1395	2248	2791	3394
D7S820	10	1023	1676	1832	2218
	11	821	1345	1995	2421
D16S539	11	1697	2687	3325	4019
	12	1520	2479	2663	3225
CSF1PO	10	1180	1898	2300	2721
	12	1230	2026	2185	2553
Penta D	12	3193	5136	4440	5131
Amelogenin	XX	2536	4235	3154	4841
vWA	17	1714	2863	2595	4068
	18	1374	2312	2453	3850
D8S1179	13	1750	2927	3916	6031
TPOX	8	3312	5470	4035	6158
FGA	23	1567	2659	2320	3627
	24	1638	2805	1842	2885
Average RFU Values=		**1424**	**2339**	**2411**	**3186**

- **Reproducibility Study**

	Human				Male			
Sample	Average C_T	Std. Dev. C_T	Average Quantity	Std. Dev. Quantity	Average C_T	Std. Dev. C_T	Average Quantity	Std. Dev. Quantity
9947A	28.04	0.063	0.909	0.073	Undet.	Undet.	Undet.	Undet.
9948	27.63	0.123	1.25	0.162	28.86	0.290	1.202	0.172
Seri	31.37	0.183	0.0949	0.014	32.53	0.256	0.1038	0.031
2372A	22.26	0.028	45.51	2.154	23.45	0.51	48.61	3.475

Results and Discussion continued

- **Sensitivity Study**
  - Show capabilities of the Duo system below 0.023ng/µl with both PowerPlex® 16 and PowerPlex® Y systems
  - Laboratory's amplification target is 0.5 to 1.0ng
  - PowerPlex® 16: <0.1ng of template DNA stochastic effects observed
  - PowerPlex® Y: <0.3ng of template DNA alleles below threshold observed

Figure 2. PowerPlex® 16 Sensitivity Study (0.01ng/µl); Quant = 0.00375ng/µl; Amplified 15µl; Analyzed 100 RFU

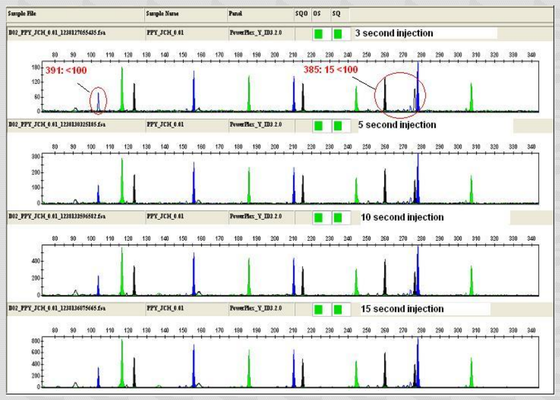

Figure 3. PowerPlex® Y Sensitivity Study (0.01ng/µl; Quant = 0.0181ng/µl; Amplified 15µl; Analyzed 100 RFU

- **Known/Non-Probative Comparison Study**
  - Quantifiler® Human versus Quantifiler® Duo
    - 4 samples chosen for amplification based on human quantification results from both kits
    - Average quantity decrease of 54% from Human to Duo
    - Average RFU increase of 70% from Human to Duo

Sample	Quant Human Results	Quant Duo Human Results	Quantity % Difference	RFU % Difference
KS M1	0.514	0.254	-51	102
KS F2	0.495	0.333	-33	47
KS M1	0.766	0.421	-45	62
QB1	0.400	0.252	-37	69

  - Quantifiler® Duo versus Quantifiler® Duo
    - Reproducible results achieved by NIST SRM® 2372 adjustment
    - 10 samples analyzed with 3 Duo kits: 0803002, 0901005, 0903007
    - On average no more than ±15% difference in DNA quantity
    - 3 samples amplified based on kit 0903007 results

Sample	Kit (1) 0803002 Results	Kit (2) 0901005 Results	Kit (3) 0903007 Results	% Diff. Kits 1 & 3	Average RFU (0903007)	Average RFU (0803002)
KB F2	0.259	0.198	0.191	-26	1647	n/a
QB1	0.252	0.367	0.299	19	1343	1259
QB2	1.24	1.08	1.22	-2	1293	n/a

Results and Discussion continued

- **Mixture Study**
  - Male to Female ratio where autosomal STR testing no longer provides useful information about male contributor
  - Male portion remained constant
  - Male:Female Ratios used 1:0, 1:1, 1:3, 1:10, 1:30, 0:1
  - <1:10 mixture, autosomal STR prove beneficial
  - >1:10 mixture, Y-STR prove beneficial

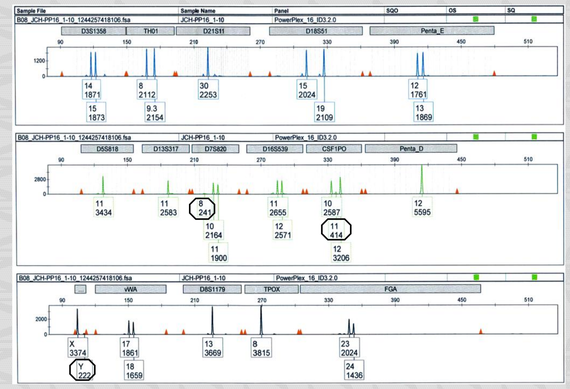

Figure 4. 1:10 Male to Female mixture; 5 second injection, Analyzed at 200 RFU

Conclusions

The validation of the Quantifiler® Duo Quantification system in conjunction with the 7500 Real-Time PCR instrument proves to be a reliable, robust, and reproducible quantification system. This study showed:

- Diluent used to prepare the standard curve had no impact on the curve parameters, and each showed good precision.
- The standard curve fell within the manufacturer's specified parameters for up to a month when stored at 2 to 8ºC.
- The NIST SRM® 2372 human quantitation standard proved to be a valuable tool for measuring the concentration of the Duo DNA standard and producing repeatable sample results using different kit lot numbers.
- The optimal sample concentration of 0.1ng/µl will produce the desired RFU values with an amplification target of 0.5ng of template DNA.
- Samples having DNA concentrations within the parameters of the standard curve could be reproduced over a period of time.
- The system was sensitive beyond the 0.023ng/µl standard, with stochastic effects around an amplification target of 0.1ng for autosomal STR analysis, and alleles below threshold at an amplification target of 0.3ng for Y-STR analysis. A microcon filter was not used and could prove to be beneficial.
- For mixture samples the following was determined:
  - Ratio < 1:10, autosomal STR analysis should prove useful information on the minor male contributor.
  - Ratio > 1:30, Y-STR analysis should be carried out.
  - Ratios between 1:10 and 1:30 should be evaluated on a case to case basis.

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References

- Quantifiler® Duo DNA Quantification Kit User's Manual. Part Number 4391294 Rev. B. 2008. Applied Biosystems.
- Butler, J. M. Forensic DNA typing: biology, technology, and genetics of STR markers. 2nd ed. 2005. Elsevier, London.
- Locascio, L. E., and R. L. Watters, Jr. National Institute of Standards and Technology: Certificate of Analysis Standard Reference Material® 2372 Human DNA Quantitation Standard. 2007 September 18. Gaithersburg, MD.
- Quantifiler™ Kits: Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual. Part Number 4344790 Rev. C. 2005. Applied Biosystems
- Barbisin, M., R. Fang, C. E. O`Shea, L. M. Calandro, M. R. Furtado, and J. G. Shewale. Developmental validation of the Quantifiler® Duo DNA Quantification Kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. Journal of Forensic Sciences 2009;54(2):305-19.
- Cupples, C. M., J. R. Champagne, K. E. Lewis, and T. D. Cruz. STR profiles from DNA Samples with "undetected" or low Quantifiler™ results. Journal of Forensic Sciences 2009;54(1):103-7.