Quantitative Genomic DNAs for Oncology Molecular Diagnostics

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Abstract

Introduction: Molecular tests are becoming more widely used in clinical care, especially in screening, diagnosing, and monitoring certain cancers. By detecting biomarkers relevant for personalized treatment, molecular diagnostics are increasingly relied upon to direct appropriate therapies for individual patients. To ensure the reliability and reproducibility of oncology molecular diagnostic test results, controls with known mutational allelic frequency and gene copy number variation are required. The development of standardized genomic DNA products that have been purified from characterized authenticated cell lines and contain quantified molecular genetic markers provide a reliable and sustainable alternative to variable patient tissue derived controls.

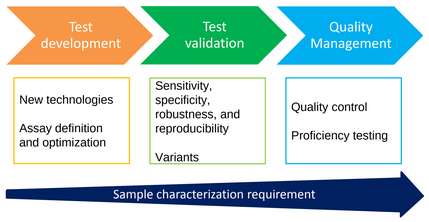
Methods: Next generation sequencing (NGS) was used to determine gene mutation status, and to quantify the mutation allelic frequency within purified genomic DNAs. Droplet digital™ PCR (ddPCR™) was used to quantify absolute copy numbers of the gene per microgram of purified genomic DNAs. The relative gene copy number variation (CNV) was further determined by comparing the gene of interest to a reference housekeeping gene.

Results: We present here a panel of quantitative genomic DNAs (shown in Table 1) isolated from ATCC® authenticated cancer cell lines that can be used as controls in oncology molecular diagnostic assays. The testing results shown in figure 1-5 are from the representative samples of 17 current available quantitative genomic DNAs. The presence of cancer gene mutations and molecular diagnostic biomarkers such as EGFR L858R, KRAS G12D, NRAS Q61R, BRAF V600E and PIK3CA H1074R were verified by NGS. The precise mutation allelic frequencies were quantified by NGS deep sequencing. Furthermore, absolute gene copy number and CNV quantified by ddPCR™ demonstrated amplifications of oncogenes such as MYC, MET, EGFR or ERBB2 within genomic DNAs isolated from a variety of tissues including gastric, lung and breast cancer cell lines.

Introduction

Reference Material	Benefit	Disadvantage
Synthetic oligonucleotides	Easy to design and synthesize	Does not resemble complexity of the whole genome
Genomic DNA from continuous cell lines	Mimics complexity of human genome; sustainable source	Genetic instability; rare mutations are difficult to obtain
Genomic DNA from patients	Mimics complexity of human genome	Not a sustainable source

Recognized need for established, fully characterized, globally accepted reference materials



Properties to consider

- Fully authenticated
- Characterized genetic alterations
- Stable molecular profiles
- Avoid contamination or misidentification
- Reproducible results

Results

Quantitative human genomic DNA from authenticated cancer cell lines containing biomarkers – mutations and copy number variations

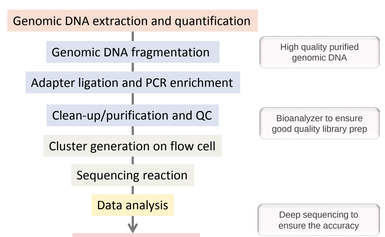
Table 1. Quantitative human genomic DNA list

ATCC® No.	Purified from cell line	Disease	Quantified oncology bio-marker	Report mutation allelic frequency *	Report absolute gene copies / ng DNA **	Report relative gene copy number **
CRL-1648DQ™	CA46	Burkitt's lymphoma	TP53 R248Q	✓	✓	✓
HTB-30DQ™	SK-BR-3	Breast adenocarcinoma	TP53 p.R175H	✓	✓	✓
HTB-122DQ™	BT-549	Breast ductal carcinoma	TP53 p.R249S	✓	✓	✓
HTB-131DQ™	MDA-MB-453	Breast carcinoma	PIK3CA p.H1047R	✓	✓	✓
CCL-225DQ™	HCT-15	Colon adenocarcinoma	KRAS p.G13D	✓	✓	✓
CCL-227DQ™	SW620	Colon adenocarcinoma	KRAS p.G12V; TP53 p.G273H	✓ ✓	✓ ✓	✓ ✓
CCL-231DQ™	SW48	Colon adenocarcinoma	EGFR p.G719S	✓	✓	✓
CL-187DQ™	LS180	Colon adenocarcinoma	KRAS p.G12D	✓	✓	✓
CRL-2158DQ™	LS1034	Colon carcinoma	TP53 p.G245S	✓	✓	✓
CRL-5973DQ™	SNU-5	Stomach undifferentiated adenocarcinoma	MET amplification	-	✓	✓
CRL-5974DQ™	SNU-16	Stomach undifferentiated adenocarcinoma	MYC amplification	-	✓	✓
HTB-111DQ™	AN3 CA	Endometrium adenocarcinoma	PTEN p.R130fs	✓	✓	✓
CRL-2868DQ™	HCC827	Lung adenocarcinoma	EGFR pELREA746del; EGFR amplification	✓ -	✓ ✓	✓ ✓
CRL-5908DQ™	NCI-H1975	Lung non-small cell carcinoma	EGFR p.T790M; EGFR p.L858R	✓	✓	✓
CRL-2177DQ™	SW 1271	Lung small cell carcinoma	NRAS p.Q61R	✓	✓	✓
CRL-5928DQ™	NCI-H2170	Lung squamous cell carcinoma	HER 2 amplification	-	✓	✓
CRL-7898DQ™	A101D	Skin malignant melanoma	BRAF p.V600E	✓	✓	✓

*Report mutation allelic frequency result using NGS (Coverage > 10,000X)
NGS result uncertainty is equal or smaller than ±5%. The reported uncertainty represents uncertainty expressed at approximately the 99% confidence level using a coverage factor of k=3.
** Report gene copy number result using ddPCR™ (Average of nine data points)
ddPCR™ uncertainty is equal or smaller than ± 30%. The reported uncertainty represents uncertainty expressed at approximately the 99% confidence level using a coverage factor of k=3.

Oncogene mutation allelic frequency quantification by using NGS

Illumina® NGS Workflow



Impact of cell passage on mutation frequency

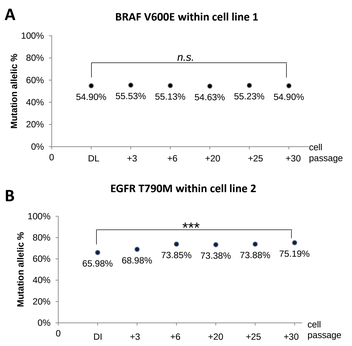


Figure 2. The impact of cell passage on mutation percentage is cell line dependent. A) Within cell line 1, the BRAFV600E mutation percentage of gDNAs purified from low passage cells and that from high passage cells were consistent by NGS test. (n.s., not significant) B) Within cell line 2, the difference between EGFR T790M mutation percentage gDNAs purified from low passage cells and that from high passage cells indicated a genetic drifting. (***, P<0.001) (DL = distribution lot of cells)

Reproducibility

Monitor the consistency in measurements across multiple runs of the NGS assay

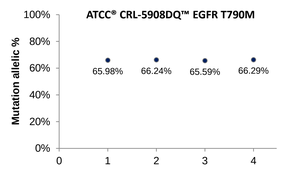


Figure 1. The same lot of CRL-5908DQ™ gDNAs were prepared and tested by four independent NGS runs with three different operators. The mutation allelic percentages of EGFR T790M from individual runs demonstrated reproducibility. Coefficient of variability (CV) from run to run =0.49%.

Lot to Lot consistency

231DQ Sample ID	Cell passage	NGS run	Freeze-thaw	NGS coverage
1	distribution lot	2nd	fresh	60322
3	plus three	1st	fresh	33057
4	plus six	2nd	fresh	34036
5	plus nine	1st	fresh	32356
6	plus twelve	2nd	fresh	71507
7	plus fifteen	1st	fresh	29380
2	distribution lot	3rd	fresh	49455
8	plus six	2nd	3 cycles	64300
9	plus six	3rd	3 cycles	18243

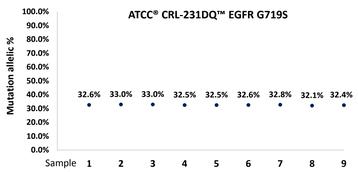


Figure 3. Multiple lots of CRL-231DQ™ gDNAs purified from different cell passage cultures or different freeze-thaw conditions were prepared for NGS. Three independent NGS runs were performed by three different operators. The mutation allelic percentages of EGFR G7219S within different lots of CRL-231DQ™ gDNAs were analyzed and compared. Coefficient of variability (CV) from lot-to-lot =0.80%

Quantitative gDNA quality attributes

Oncogene copy number quantification by using ddPCR

Gene copy number quantification

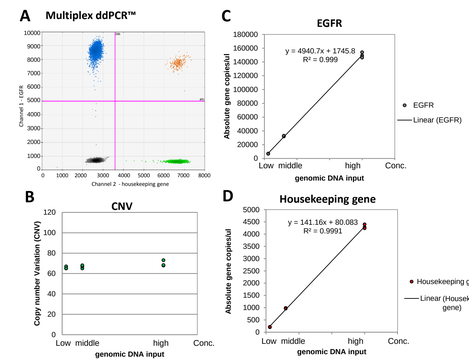


Figure 4. Quantification of absolute copies of target genes and housekeeping gene were performed by using ddPCR. A) Multiplex ddPCR was used to evaluate a target gene and housekeeping gene within the same PCR reaction. Absolute gene copies of C) EGFR, and D) housekeeping gene within CRL-231DQ™ gDNAs were determined. B) Copy number variation was calculated based on the absolute copy number quantifications of target gene EGFR and housekeeping gene.

Reproducibility

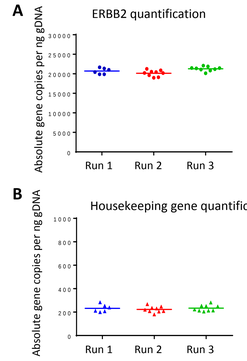


Figure 5. The same lot of CRL-5928DQ™ gDNAs were tested through three independent multiplex ddPCR runs by three different operators. Data reproducibility were demonstrated by A) quantification of absolute copies of ERBB2, and B) quantification of absolute copies of house-keeping gene.

Lot to Lot consistency

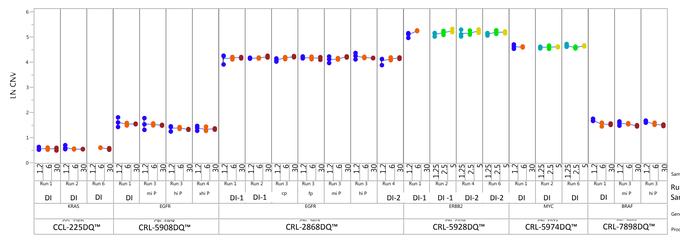


Figure 6. Lot to lot consistency was verified on multiple samples by the quantification of absolute gene copy numbers through multiple independent ddPCR runs. Copy number variation was calculated based on the absolute copy number quantifications of the indicated target genes and housekeeping gene. Three concentration dilutions in triplicate for each concentration were analyzed in each ddPCR run (n=9). Data is displayed on a logarithmic scale.

Conclusions

Overall, quantitative genomic DNAs with verified oncogene mutation allelic frequency and gene copy number isolated from fully characterized and authenticated ATCC cell lines provide useful control materials to molecular diagnostic labs for genetic testing.

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