AmplideX®
mPCR *FMR1* Kit

A streamlined PCR approach for the detection and quantification of methylation status in the *FMR1* gene

Sensitive, allele-specific methylation quantification is rapidly delivered in this easy-to-use assay. The innovative design improves turnaround time by fivefold compared to Southern blot (<8 hours vs. 1 week). This assay was designed to complement the AmplideX PCR/CE *FMR1* Reagents*.

REDUCED COMPLEXITY

- Eliminates the need for Southern blot
- Easily quantify allele-specific methylation with digestion, PCR, and CE
- Includes spike-in digestion and amplification controls to ensure high performance with every sample

OPTIMIZED WORKFLOW

- PCR and CE design empowers simple implementation
- Results in under a day
- Flexible use on widely installed CE equipment

QUALITY RESULTS

- Provides allele-specific methylation quantification consistent with Southern blot analysis
- Detects lower abundance methylation and size mosaics
- High quality controls available from a single vendor ensure sensitive, reliable testing

AmplideX mPCR *FMR1** Workflow – <1 day from DNA to data

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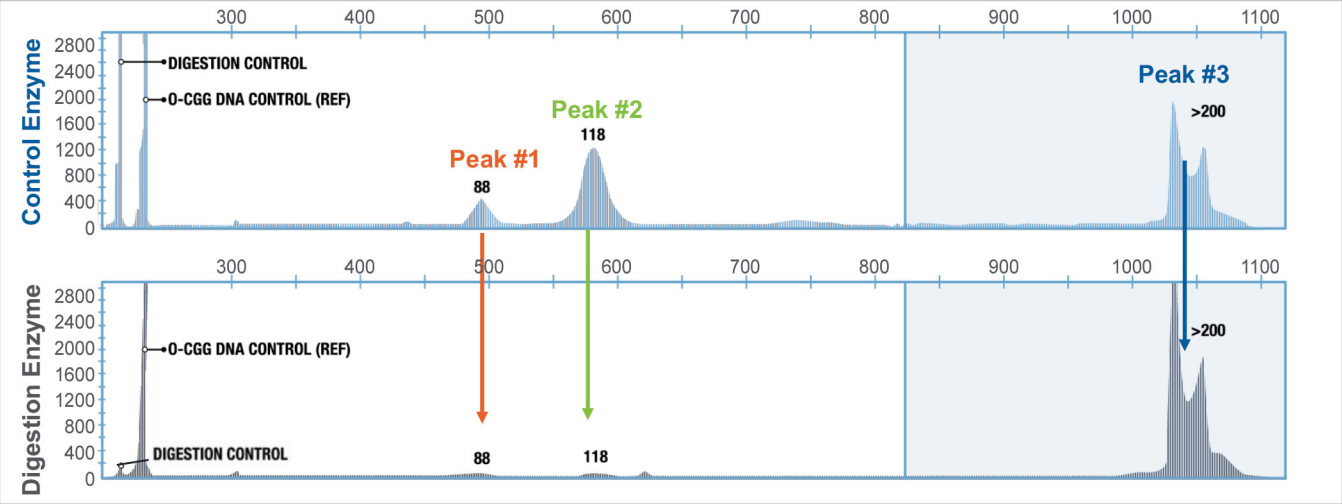
SIMPLER ALLELE-SPECIFIC METHYLATION QUANTIFICATION



Digestion control confirms complete digestion of unmethylated alleles

	Calculated Methylation		
	Peak #1	Peak #2	Peak #3
CGG repeats	88	118	>200, >200
% Methylated	1%	0%	100%,100%

- Control and methylation-sensitive digestions allow comparison of peak heights to determine percent methylation
- Methylation protects from digestion, ensuring simple detection of unmethylated peaks

KIT RESULTS CORRELATE WELL WITH OBSERVED PHENOTYPE

Phenotype†

- Male in his 50s with normal cognitive function and severe anxiety. Self-referred because brother and sister have FXS. Daughter has a premutation.

Outcomes: Methylation mosaicism

- Lack of severe intellectual disability (here, normal intelligence) was consistent with methylation mosaicism, which included an unmethylated full mutation as the primary allele.

	Methylation Results		
	Peak #1	Peak #2	Peak #3
CGG repeats	**53**	**153**	**>200**
% Methylated	3%	96%	**4%**

ORDERING INFORMATION

Part Number	Product Description	Number of Reactions
49442	AmplideX mPCR *FMR1* Kit*	24 reactions
49513	AmplideX PCR/CE *FMR1* Control*	24 ul
49514	AmplideX mPCR *FMR1* Control*	24 ul

For more information, please contact asuragen.com

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†Case courtesy of Rush University Medical Center

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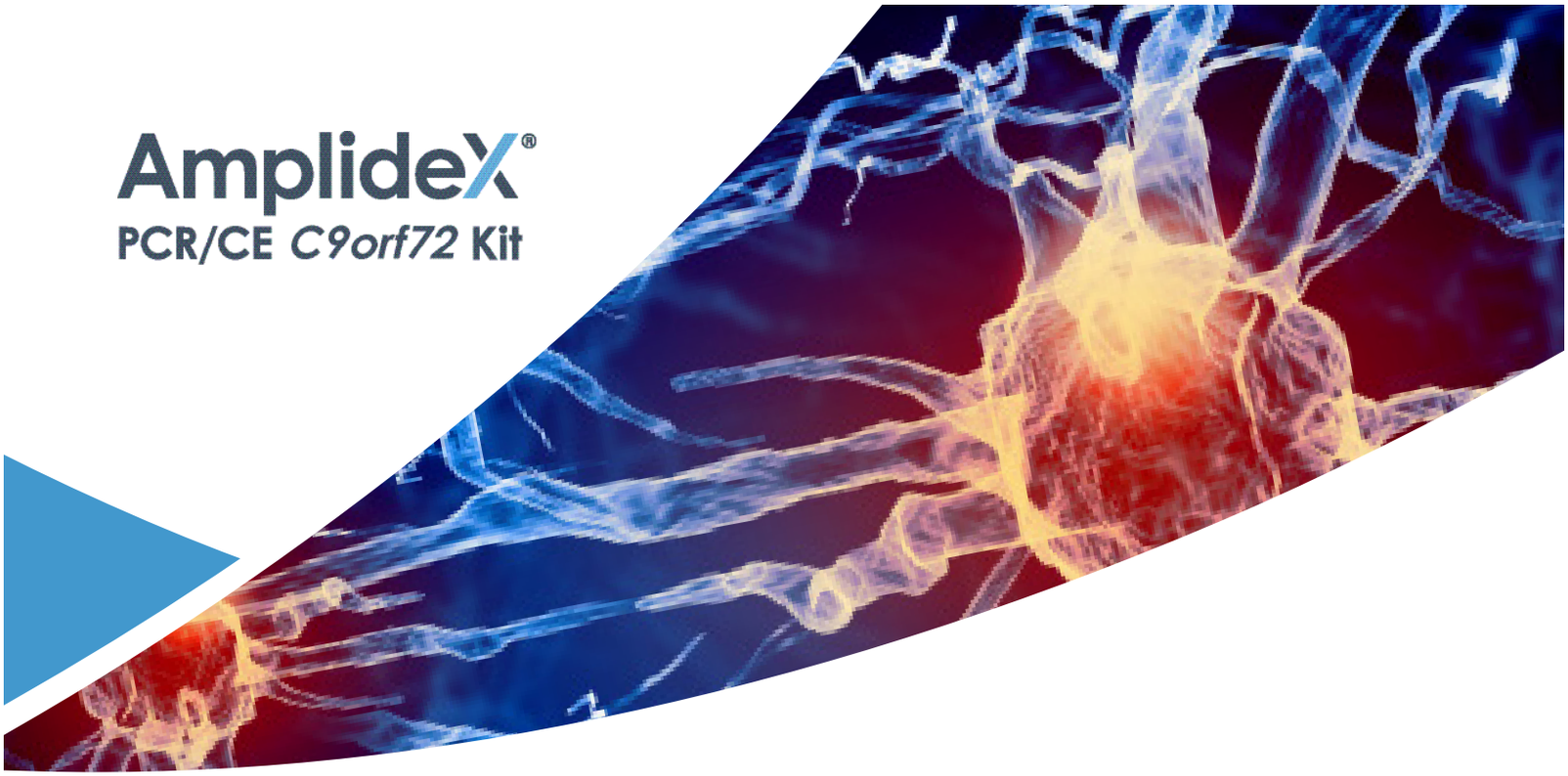
2000-0216
1/22

AmplideX®
PCR/CE *C9orf72* Kit



A better understanding of hexanucleotide repeat expansions in the *C9orf72* gene requires a robust PCR amplification assay. The AmplideX® PCR/CE *C9orf72* Kit is a research tool for the reliable amplification of pathogenic hexanucleotide repeats (GGGGCC) in the *C9orf72* gene that are associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Using our proprietary GC-rich PCR chemistry and building on the success of our AmplideX *FMR1* product, this kit provides a simple, single-tube PCR workflow to profile *C9orf72* repeat sequences.

Figure 1: AmplideX PCR/CE *C9orf72* Kit components

SIMPLE WORKFLOW

- Single PCR reaction for both sizing and screening
- A single sourced kit consisting of all PCR reagents needed for *C9orf72* repeat amplification
- Streamlined workflow with minimal hands-on time
- Decreased need for Southern blot analysis

IMPROVED PERFORMANCE

- Five-fold improvement in length of alleles sized: accurate sizing up to 145 repeats
- Detection of alleles greater than 145 repeats
- Reveal low-level mosaicism and minor alleles
- Identifies sequence variability near the repeat region

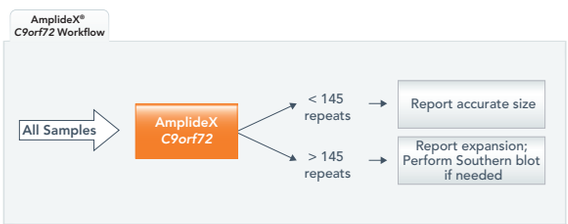


Figure 2: AmplideX PCR/CE *C9orf72* Kit workflow

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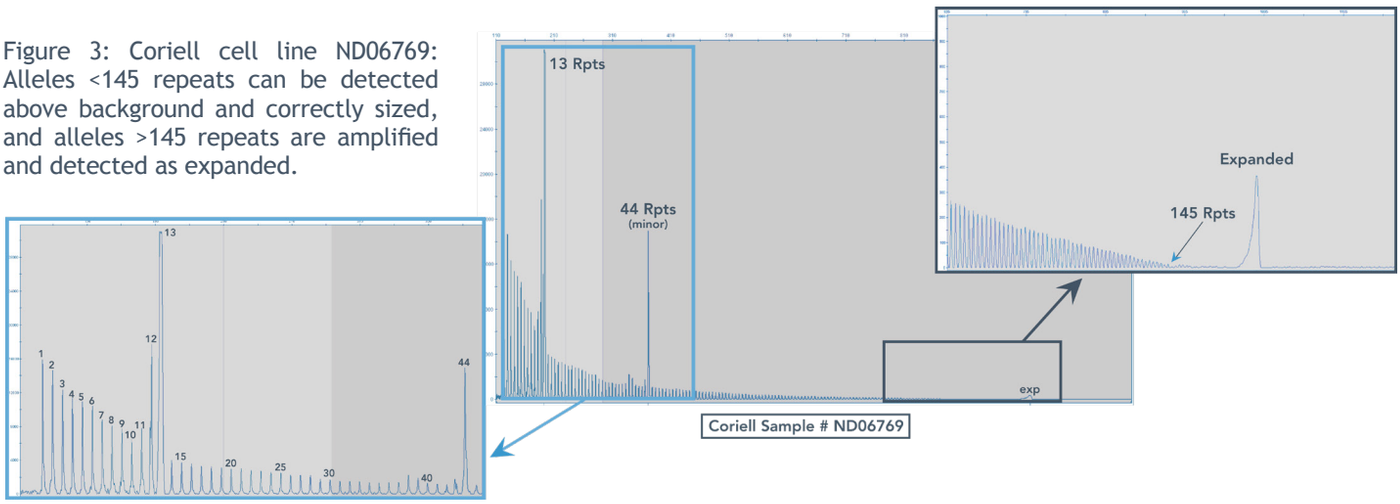
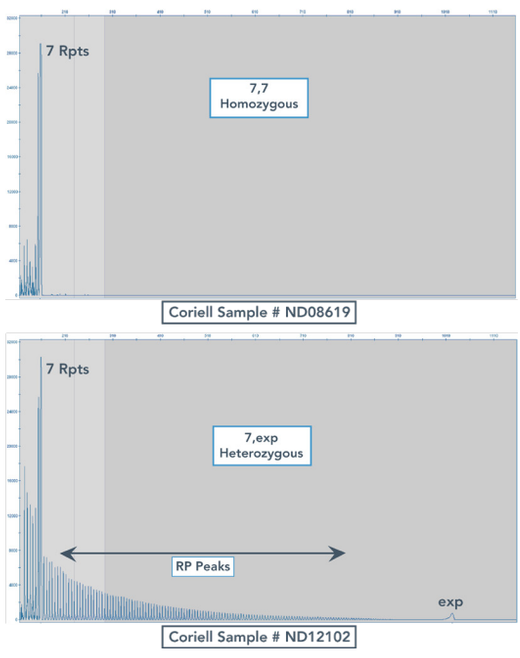
AmplideX®
PCR/CE *C9orf72* Kit

ACCURATELY SIZE UP TO 145 REPEATS AND DETECT ALLELES GREATER THAN 145 REPEATS

Figure 3: Coriell cell line ND06769: Alleles <145 repeats can be detected above background and correctly sized, and alleles >145 repeats are amplified and detected as expanded.





CLEAR ZYGOSITY RESOLUTION THROUGH DISTINCT REPEAT-PRIMED PATTERN

Figure 4: Side by side comparison of 2 Coriell cell lines: ND08619 (7,7 homozygous) and ND12102 (7,expanded heterozygous): Extension of the repeat-primed peak pattern beyond the shorter allele clearly differentiates the heterozygous sample from homozygous sample.

KIT ORDERING INFORMATION	
AmplideX PCR/CE *C9orf72* Kit*	[P/N 49581] 50 REACTIONS

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2500-624 Rev A 2017

AmplideX®
PCR/CE *CFTR* Kit*

Providing the broadest coverage of the U.S. population† of any commercially available, targeted *CFTR* testing kit

The AmplideX® PCR/CE *CFTR* Kit* brings the simplicity and scalability of AmplideX technology to *CFTR* variant detection. Offering the streamlined detection of 67 pathogenic variants, and covering approximately 93%† of the U.S. population, the assay provides the broadest coverage of any commercially available, targeted testing kit. The assay is optimized for use on widely established laboratory equipment and delivers genotype results from DNA in under five hours.

REDUCED COMPLEXITY

- Broad coverage† provided using only PCR/CE workflow
- Similar testing process to AmplideX PCR/CE *FMR1** and *SMN1/2* Plus* kits eases implementation
- Streamlined data analysis via AmplideX Reporter software

OPTIMIZED WORKFLOW

- Easy-to-use workflow with fewer hands on steps than most competitor assays
- Flexible use on widely installed CE equipment
- <5 hrs from DNA to data

QUALITY RESULTS

- Built on the latest prevalence data to provide the best coverage† for all U.S. ethnicities
- Detects complex yet key *CFTR* mutations (CNVs, STRs, SNPs, INDELs) and resolves zygosity
- Excellent concordance with other methods

Figure 1. AmplideX PCR/CE *CFTR* Kit* workflow.

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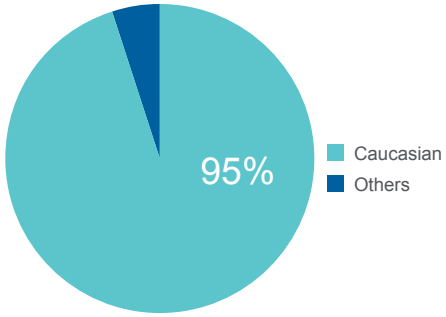

Figure 2A. Ethnic diversity of 89K patients in CFTR2 database.

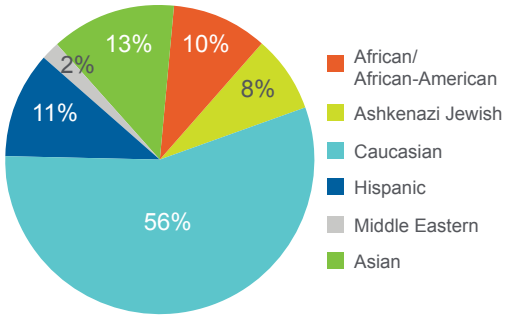

Figure 2B. Ethnic diversity of 115K subjects in U.S. study.†

Commercial Kit Manufacturer/Panel	Full Product Name	# of Variants Detected	% Coverage, CFTR2	% Coverage, U.S.	% Coverage Difference, U.S.
Asuragen	AmplideX PCR/CE *CFTR* Kit	67	92.1%	93.0%	-
Luminex 97	xTAG Cystic Fibrosis (*CFTR*) 97 kit (Custom)	97	92.2.%	88.2%	4.8%
Illumina	MiSeqDx Cystic Fibrosis 139 Variant Assay	139	94.3%	87.7%	5.3%
Agena	iPLEXPro *CFTR* Panel	74	91.5%	86.9%	6.1%
Elucigene	CF-EU2v1	50	90.5%	86.2%	6.8%
Luminex 60	xTAG Cystic Fibrosis (*CFTR*) 60 kit v2	60	91.2%	86.1%	6.9%
Luminex 39	xTAG Cystic Fibrosis (*CFTR*) 39 kit v2	39	89.1%	80.7%	12.3%

Table 1. Percent coverage of variants detected by commercially available kits based on frequencies observed in the CFTR2 database** and the U.S. population.

76 Cell Lines from Coriell Repository

Sample Agreement with Reference Assay		Sanger Sequencing			Overall Sample Agreement
		WT/WT	MUT/MUT	MUT/WT	
CFTR PCR/CE Assay	WT/WT	22	0	0	22/22 (100%)
	MUT/MUT	0	24	0	24/24 (100%)
	MUT/WT	0	0	27	27/27 (100%)

Table 2A. Mutations in Cell Lines Detected by Prototype PCR/CE *CFTR* Kit and Sanger Sequencing.

102 Clinical Samples Across 3 Sites

Sample Agreement with Reference Assay		xTAG® Cystic Fibrosis (*CFTR*) 60 kit v2 or Sanger Sequencing			Overall Sample Agreement
		WT/WT	MUT/MUT	MUT/WT	
CFTR PCR/CE Assay	WT/WT	34	0	0	34/34 (100%)
	MUT/MUT	0	19	0	19/19 (100%)
	MUT/WT	0	0	49	49/49 (100%)

Table 2B. Mutations in Clinical Samples Detected by Prototype PCR/CE *CFTR* Kit and Either xTAG® Cystic Fibrosis (*CFTR*) 60 Kit v2 or Sanger Sequencing.

ORDERING INFORMATION

Part Number	Product Description	Number of Reactions
A00076	AmplideX PCR/CE *CFTR* Kit	50
A00077	AmplideX PCR/CE *CFTR* Kit	100

For more information please contact asuragen.com

*For Research Use Only. Not for use in diagnostic procedures.
**The Clinical and Functional Translation of CFTR (CFTR2); available at http://cftr2.org
†Beauchamp, et al. Genet Med. 2019 Nov;21(11):2569-2576.

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2000-0187
10/21

AmplideX®
PCR/CE *DMPK* Kit*

Simply and Reliably Size *DMPK* Repeat Expansions within a Single Day

The AmplideX® PCR/CE *DMPK* Kit* introduces a groundbreaking alternative for the detection and sizing of CTG repeats within the *DMPK* gene. Challenged by very large trinucleotide expansions, *DMPK* analysis has required the use of Southern blot technology, thereby limiting many laboratories' ability to resolve these repeat sequences. By introducing a simple, streamlined, PCR-only workflow, the AmplideX PCR/CE *DMPK* Kit* can replace Southern blot and put *DMPK* analysis within the reach of laboratories everywhere.

REDUCED COMPLEXITY

- Proprietary PCR solutions for GC-rich amplification and detection
- May eliminate need for Southern blot
- Resolves zygosity and detects mosaicism

OPTIMIZED WORKFLOW

- Direct injection of PCR products into CE instruments
- Fully kitted, end-to-end solutions that significantly reduce hands-on-time
- Sample-to-result possible within a single shift

QUALITY PERFORMANCE

- Accurate sizing of alleles ≤200 CTG repeats and detection of all alleles >200 repeats using CE
- Optional AGE protocol allows sizing of alleles up to 1000 repeats
- Analysis tool converts raw base pair data into repeat number

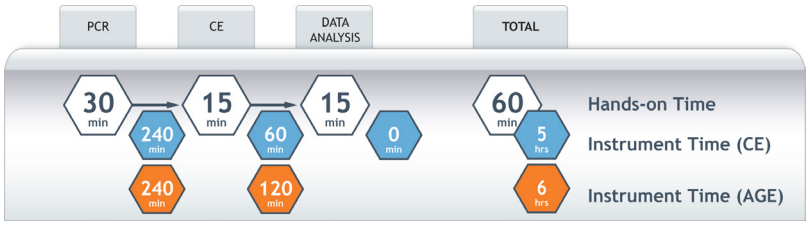

Figure 1. AmplideX PCR/CE *DMPK* Workflow, including optional AGE workflow for larger expansions.

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AmplideX®
PCR/CE *DMPK* Kit*

Sample Name	External Lab *PCR + Southern*	Asuragen *PCR/CE*	Asuragen *PCR/AGE (allele 2)*
S1	10 , 300-500	11 , >200	Smear , High Mosaic 300-500
S2	4 , 1200-1350	5 , >200	~1100
S3	4 , 1100-1500	5 , >200	Smear , High Mosaic >500
S4	11 , 200-300	12 , >200	~260-350
S5	4 , 800-900	5 , >200	~650-700
S6	25 , 1050	26 , >200	~1000
S7	12 , 100-150	13 , 71 , 129	70 , 130
S8	11 , 1900-2220	12 , >200	700 , 1900
S9	21 , 300	22 , >200	Mosaic , 261
S10	4 , 50-250	5 , >200	150 , Smear >200

*AmplideX detects mosaicism down to 5%

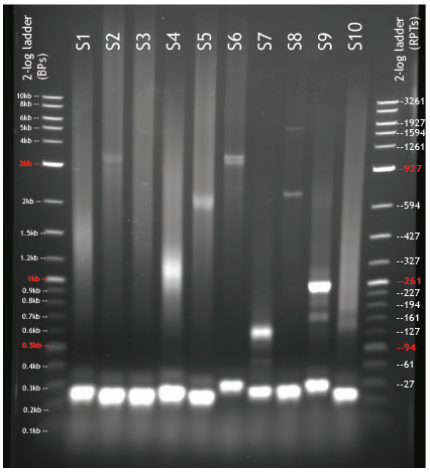

Figure 2. Comparison of residual clinical sample results between Asuragen (AmplideX Kit) and partner lab (PCR + Southern blot).

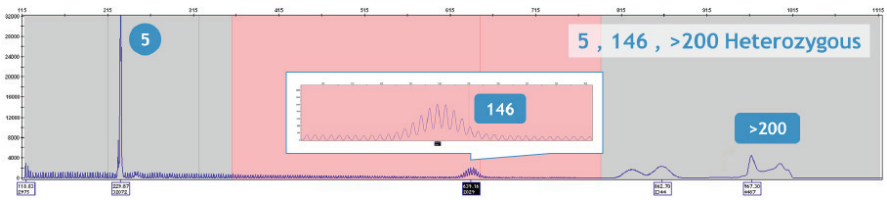

Figure 3. Sensitive and accurate sizing of *DMPK* expansions up to 200 repeats.

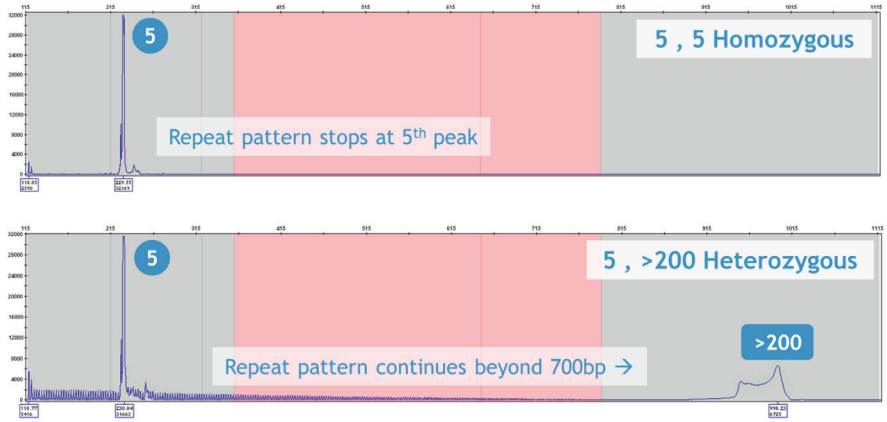

Figure 4. Clear resolution of zygosity.

KIT ORDERING INFORMATION	
AmplideX PCR/CE *DMPK* Kit*	[P/N: 49655] 32 REACTIONS

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2000-027 Rev B 08/2018

AmplideX®

PCR/CE *SMN1/2* Plus Kit*

SMN1 and *SMN2* Copy Number, Silent Carrier, and Disease Modifier Genotyping—in Less Than Four Hours

Copy number variations in *SMN1* and *SMN2* are, respectively, associated with the onset and severity of spinal muscular atrophy (SMA), a debilitating and life-threatening illness of the central nervous system. Recent studies have demonstrated that transmission risk and disease severity may be impacted by the presence of additional variants, such as *SMN1* gene duplication events and disease modifier in *SMN2*.

The AmplideX® PCR/CE *SMN1/2* Plus Kit* revolutionizes the analysis of these two genes by delivering comprehensive results in less than four hours. Powered by AmplideX technology, the assay accurately quantifies *SMN1* and *SMN2* exon 7 copy number and also detects *SMN1* gene duplication and *SMN2* disease modifier variants – all from a single reaction. The assay shares a common workflow with other assays in the AmplideX product portfolio and is optimized for use on widely established laboratory equipment.

REDUCED COMPLEXITY

- Similar workflow to AmplideX PCR/CE *FMR1*[*†] kit eases implementation and training
- Multiplexed, scalable design allows analysis of single-nucleotide variants, small indels, and copy-number changes from a single PCR reaction
- Assay-specific software automates variant calls and streamlines data analysis

OPTIMIZED WORKFLOW

- DNA-to-data in less than four hours with only 60 minutes of hands-on-time
- Optimized for use on commonly installed CE equipment
- Fully-kitted solution sourced from a single vendor

QUALITY PERFORMANCE

- Ability to differentiate between 0, 1, 2, 3 and ≥4 copies for both *SMN1* and *SMN2*
- Automated variant and copy-number genotyping
- Accuracy demonstrated through comparisons with multiple orthogonal methods

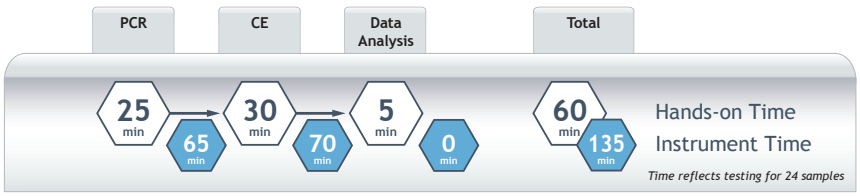

Figure 1. Assay workflow for AmplideX PCR/CE *SMN1/2* Plus Kit*

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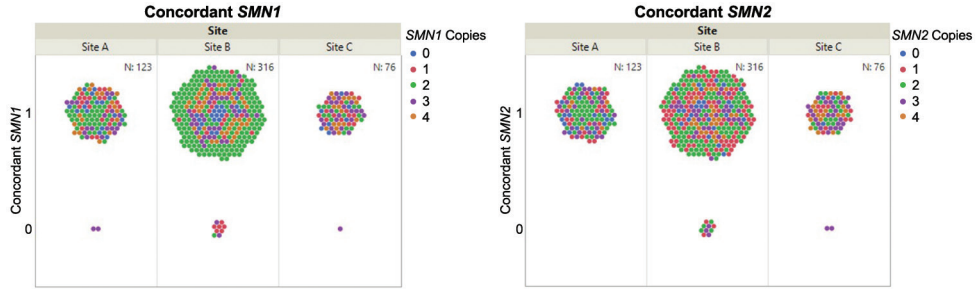
AmplideX®

PCR/CE *SMN1/2* Plus Kit*



SMN1 Exon7 Copy Number

SMN1 Copies	Count	0	1	2	3	4	Total
	0	41	0	0	0	0	41
	1	0	52	6	0	0	58
	2	0	1	257	0	0	258
	3	0	0	5	79	0	84
	4	0	0	0	0	74	74
	Total	41	53	268	79	74	515

503/515, 97.7% Concordant
(excluding QC failures)

SMN2 Exon7 Copy Number

SMN2 Copies	Count	0	1	2	3	4	Total
	0	45	0	0	0	0	45
	1	0	133	3	0	0	136
	2	0	0	197	4	0	201
	3	1	0	0	78	4	83
	4	0	0	0	0	50	50
	Total	46	133	200	82	54	515

503/515, 97.7% Concordant
(excluding QC failures)

Figure 2. Excellent concordance of *SMN1* and *SMN2* copy number across sites for 500+ measurements using the AmplideX PCR/CE *SMN1/2* Plus Kit*

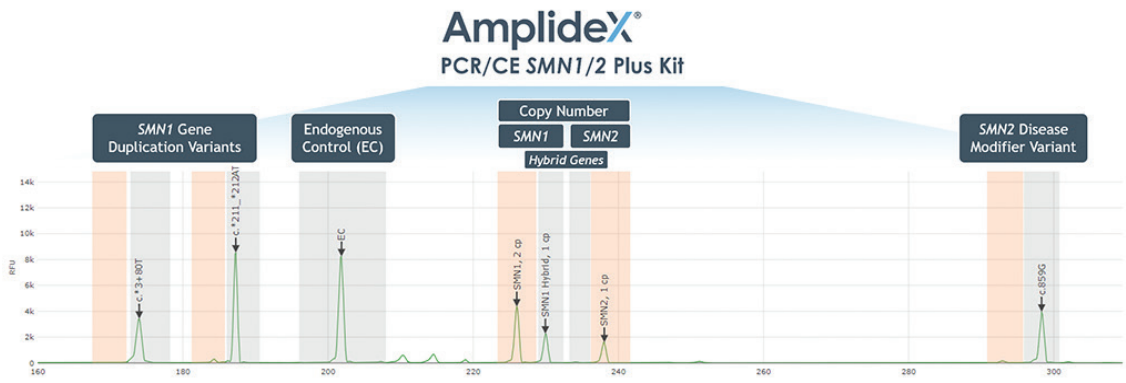


Figure 3. Example Electropherogram Output - One reaction provides information on *SMN1* and *SMN2* copy number, disease modifier variant and gene duplication variants

Product Name	Number of Reactions	Catalog Number
AmplideX® PCR/CE *SMN1/2* Plus Kit (RUO)	50	A00050
AmplideX® PCR/CE *SMN1/2* Plus Kit (RUO)	100	A00054

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2000-098 rev. B

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AmplideX®

PCR/CE *HTT**

Sensitive and Accurate Sizing of CAG Repeats in the *HTT* Gene

The AmplideX® PCR/CE *HTT* Kit makes the detection and sizing of CAG trinucleotide repeats in the *HTT* gene easier than ever. Accurate sizing of the CAG expansions in *HTT* is challenged by the presence of adjacent SNPs and variable CCG repeats, which can complicate primer binding and cause allele dropouts. With its unique, two-primer design, the AmplideX PCR/CE *HTT* Kit overcomes these challenges to accurately and reliably report the number of repeats in one day, from as little as 10ng gDNA.

REDUCED COMPLEXITY

- Proprietary PCR solutions for GC-rich amplification and detection
- Eliminates need for multiple PCRs – one result, straightforward analysis
- Resolves zygosity and detects large expansions

OPTIMIZED WORKFLOW

- Fully kitted, end-to-end solutions that significantly reduce hands-on-time
- Sample-to-result possible within a single shift
- Identical PCR and CE conditions as the AmplideX® PCR/CE *DMPK* Kit

QUALITY PERFORMANCE

- Reliable, unambiguous results and a robust stutter peak pattern
- Accurate sizing across the entire CAG repeat range
- Software converts raw base pair data into number of repeats

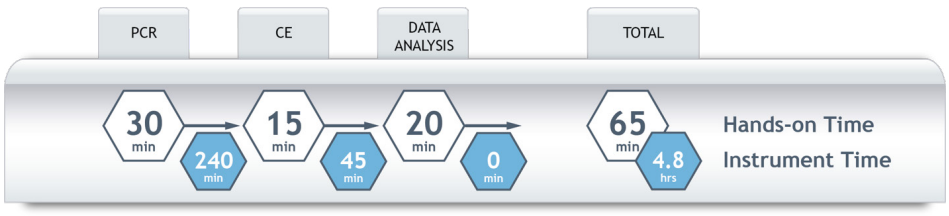

Figure 1. AmplideX PCR/CE *HTT* Kit Workflow.

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AmplideX®

PCR/CE *HTT**

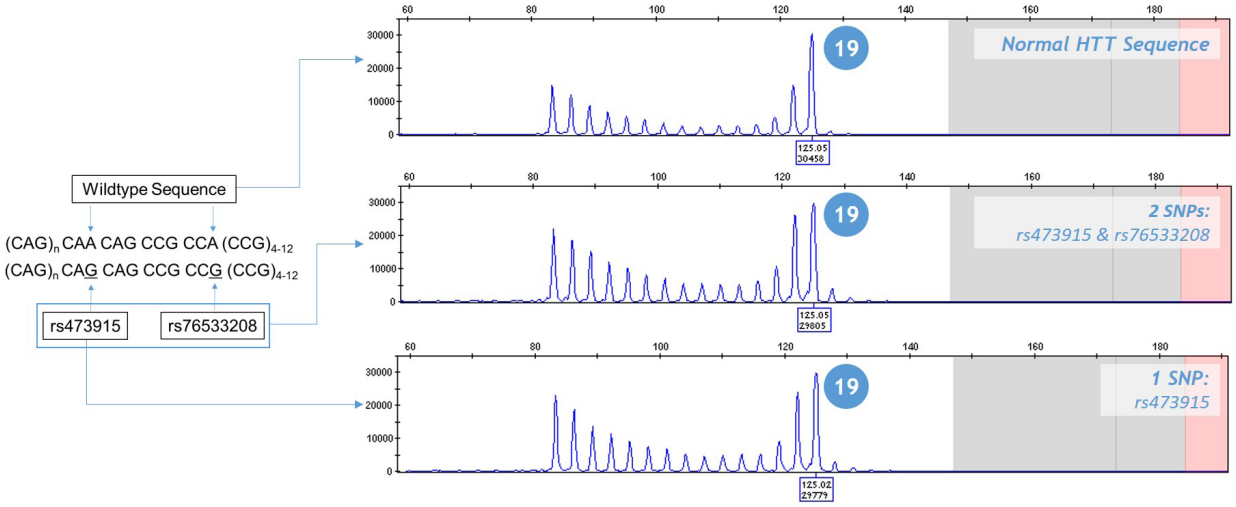

Figure 2. Repeat profile is conserved across known SNPs

Sample ID Number	Reported Genotype	Reported Alleles	Observed Alleles
NA20206	NORMAL	17, 18	18, 18
NA20207	NORMAL	19, 21	19, 21
NA20208	EXPANDED	35, 45	35, 45
NA20209	EXPANDED	45, 47	45, 47
NA20210	EXPANDED	17, 74 or 75	17, 75
NA20245	NORMAL	15, 15	15, 15
NA20246	NORMAL	15, 24	15, 24
NA20247	INTERMEDIATE	15, 29	15, 29
NA20248	REDUCED PENETRANCE	17, 36	17, 36
NA20249	REDUCED PENETRANCE	22, 39	22, 39
NA20250	EXPANDED	15, 40	15, 40
NA20251	EXPANDED	39, 50	39, 50
NA20252	EXPANDED	22, 65 or 66	22, 63*, 66
NA20253	EXPANDED	22, 101	22, 100, 128*
SRM 2393 A	INTERMEDIATE	15, 29	15, 29
SRM 2393 B	REDUCED PENETRANCE	17, 36	17, 36
SRM 2393 C	EXPANDED	15, 40	15, 40
SRM 2393 D	EXPANDED	35, 45	35, 45
SRM 2393 E	EXPANDED	39, 50	39, 50
SRM 2393 F	EXPANDED	17, 75	17, 75

*Additional minor peak consistently identified with Asuragen assay

Figure 3. 100% Concordance (±1 repeat) observed with previously characterized samples, as reported by Kalman et al. (2007)

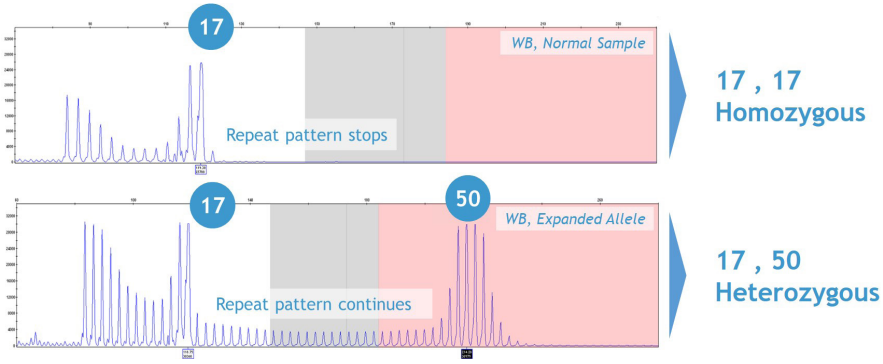

Figure 4. Clear resolution of zygosity.

KIT ORDERING INFORMATION	
AmplideX PCR/CE *HTT* Kit*	[P/N: 49657] 32 REACTIONS

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2000-064 Rev A 12/2018

Product Name	Number of Reactions	Catalog Number
QuantideX® qPCR BCR-ABL IS Kit†	60	86003
QuantideX® qPCR BCR-ABL minor Kit†	60	49640

† CE-marked for US export only.

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3000-001

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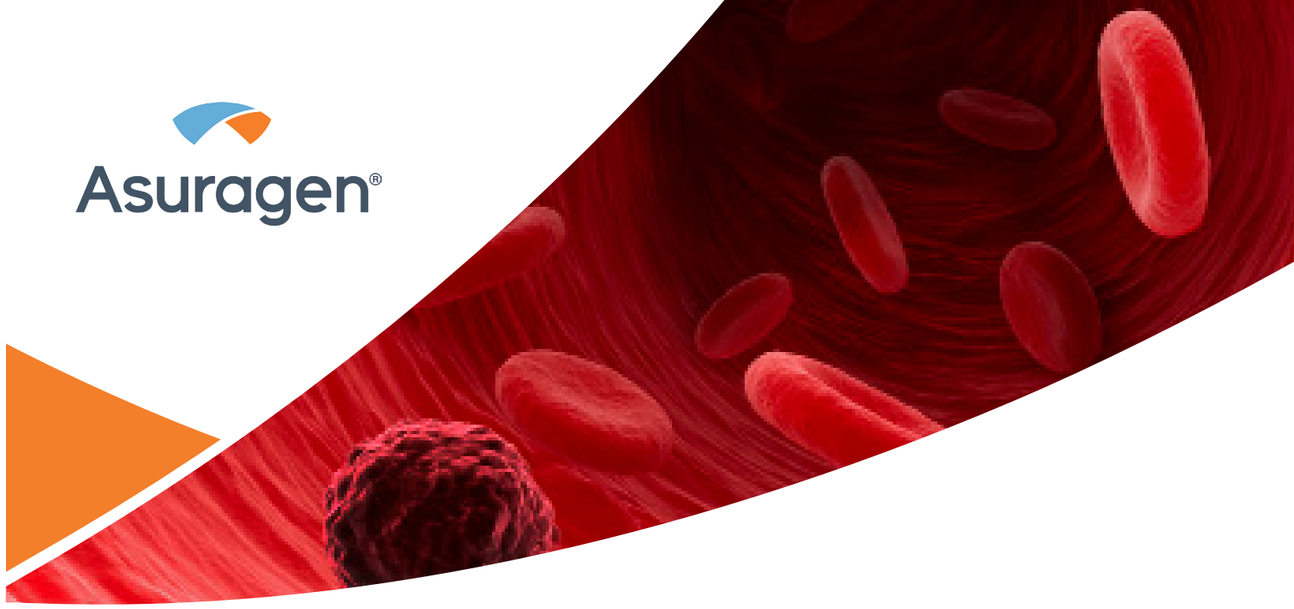

QuantideX®
qPCR BCR-ABL IS Kit & qPCR BCR-ABL minor Kit†

Complete solution for ultra-sensitive quantitation of *BCR-ABL1* fusions for molecular response monitoring in Chronic Myeloid Leukemia

Sensitive | Reliable | Simple

† CE-marked for US export only.

QuantideX®

qPCR BCR-ABL IS Kit & qPCR BCR-ABL minor Kit†

Advances in TKI therapy have driven 5-year survival rates in CML patients to nearly 90%[1] and are helping to make treatment-free remission a reality for a significant number of patients[2]. However, to accurately assess response to treatment and determine eligibility for treatment cessation, a rapid, accurate and highly sensitive assay for measurement of disease burden is required.

The QuantideX® BCR-ABL Portfolio offers simple, reliable, ultra-sensitive quantitation of both the Major and minor breakpoints, allowing any molecular laboratory to assess the deepest molecular response with unprecedented ease and deliver the results physicians and patients rely on.

Reduced Complexity

- **Direct reporting on the International Scale (IS)*:**
  Multi-point standard curve reduces variability and removes need for costly, complex sample exchange
- **Single method, comprehensive reporting:**
  QuantideX® Reporter Software† provides automated calculation of %IS*, *BCR-ABL1*/*ABL1* %ratio and *ABL1* copy number**

Streamlined: Multiplexed design yields workflow and cost efficiencies

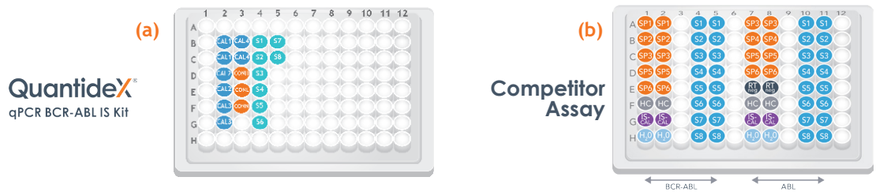

Figure 1: Comparison of plate layout for an 8 sample run between the (a) Asuragen assay, which features a multiplexed design and samples run in singlicate, resulting in only 19 reactions; and (b) a competitor assay, which features a singleplex design and samples run in duplicate, resulting in 60-64 reactions.

Optimized Workflow

- **Reduced hands-on time:**
  Multiplexed design amplifies and detects both fusion and control genes in the same reaction
- **Simplified inventory & quality management:**
  All necessary RT and qPCR reagents and controls in a single, vendor-sourced kit to reduce QC burden
- **Common workflows:**
  Major & minor kits share common workflows to streamline testing and reduce risk of error

Simple: Two Kits. One Workflow.

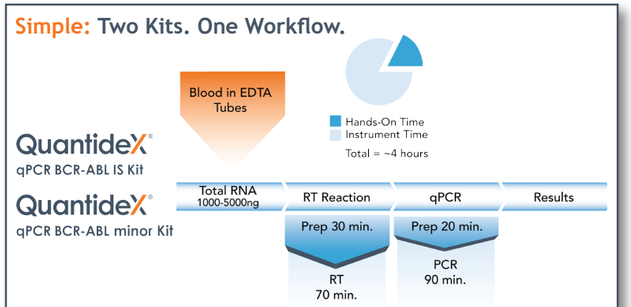

Figure 2: Both kits share a common workflow with limited hands-on-time, maximizing laboratory efficiency and sample throughput.

† CE-marked for US export only. *QuantideX® qPCR BCR-ABL IS Kit only. **QuantideX® qPCR BCR-ABL minor Kit only.

[1] American Cancer Society [2] Saußele S, et al. Leukemia (2016) 30, 1638–1647.

Quality Performance

- **Performance established using human RNA:**
  Limits of Detection (LOD) of MR4.7 (0.002% IS) and LR4.61 (0.0025% ratio) confirmed in human RNA, not cell lines
- **Multi-point Armored RNA®-based standards:**
  Provide reproducible, traceable RNA quantitation values
- **Increased analytical sensitivity without compromising analytical specificity:**
  Unique Limit of Blank (LOB) approach used to minimize miscalling of non-leukemic low positives

Reproducible: Proven sensitivity based on rigorous testing criterion

QuantideX® qPCR BCR-ABL IS Kit

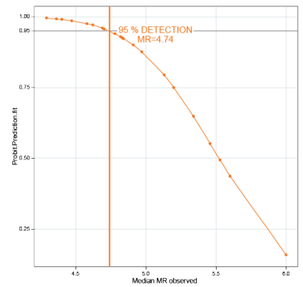

Figure 3: LOD of the QuantideX qPCR BCR-ABL IS Kit as determined by CLSI EP17-A2 guidelines by testing Human RNA dilutions ranging from MR4.4 to MR6: 60 replicates of each dilution for a total of 1680 data points.

QuantideX® qPCR BCR-ABL minor Kit

	Replicates tested	Log Reduction	Median LOD (%ratio)
Human RNA	90	LR4.61	0.0025%
Cell Lines	80	LR5.31	0.0005%

Table 1: LOD of the QuantideX® qPCR BCR-ABL minor Kit as determined by CLSI EP17-A2 guidelines by testing Human RNA and cell line dilutions spanning multiple lots, batch runs, days, operators, and instruments.

Precise: Minimal variability across the entire dynamic range

QuantideX® qPCR BCR-ABL IS Kit

Target MR	Mean MR	Std Dev	Limits of Agreement (95%)
1	0.697	0.092	1.53
2	1.634	0.069	1.37
3	2.658	0.053	1.28
3.5	3.185	0.077	1.43
4	3.675	0.092	1.53

Table 2: Precision evaluated using 5 different levels of positive specimens, tested by 3 operators over 20 runs each. Each level was tested 90 times for a total of 450 data points.

QuantideX® qPCR BCR-ABL minor Kit

Target LR	Mean LR	Std Dev
1	0.98	0.12
2	1.95	0.17
3	2.96	0.12
4	3.98	0.17

Table 3: Assay precision determined by testing 4 different log reduction (LR) levels in human RNA, using 2 operators, and 8 runs for a total of 192 data points.

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