Ampli1™ KITS

SINGLE CELL **PRECISION**

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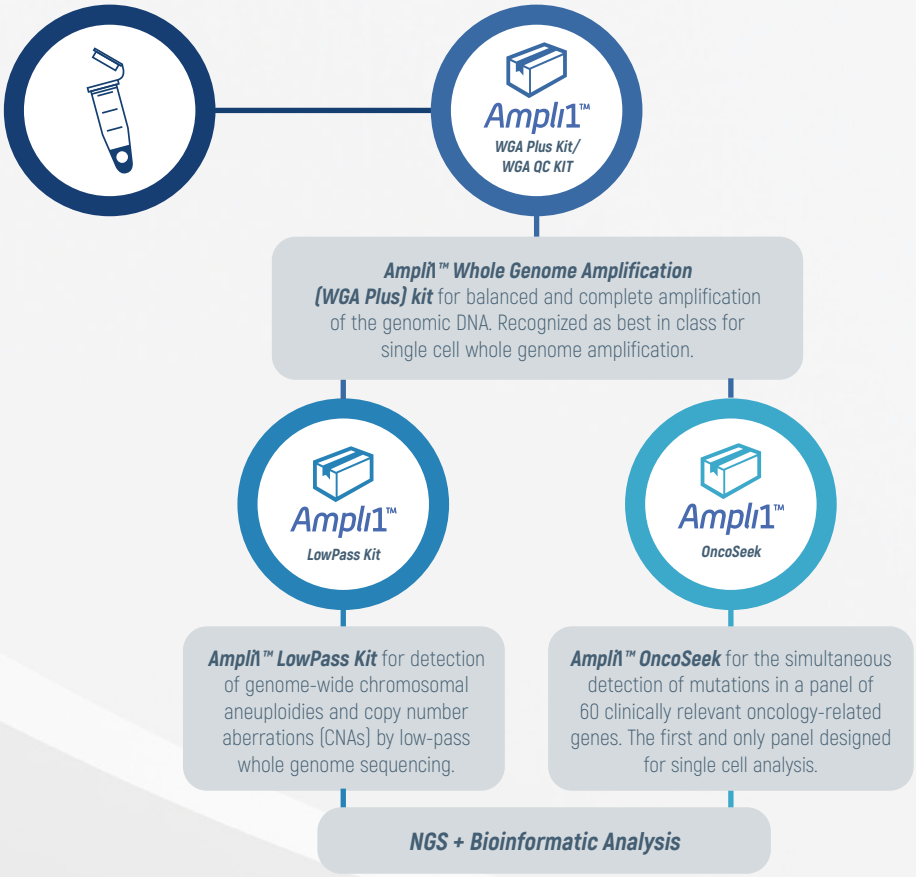
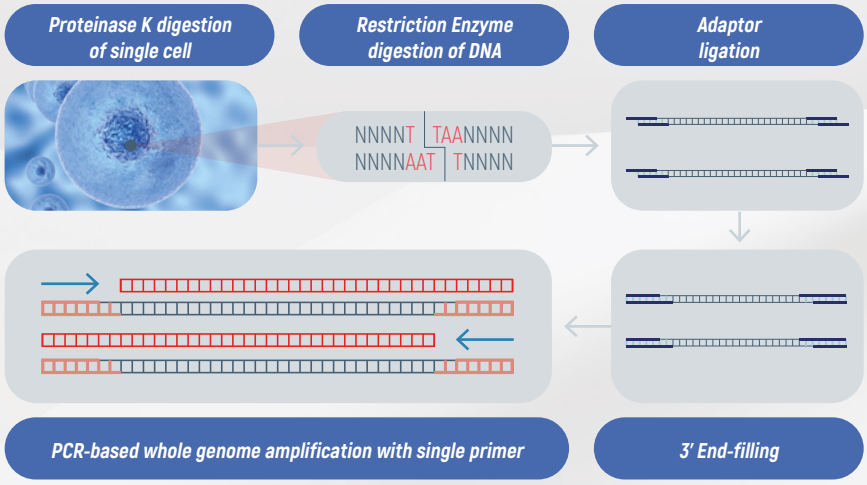
Ampli1™ WORKFLOW: SINGLE CELL DNA ANALYSIS

Single-cell analysis allows the study of cell-to-cell variation within a cell population and is a prerequisite for in-depth understanding of tumor heterogeneity and to study diseases and drug development. Successful analysis of single cell genome starts with an appropriate Whole Genome Amplification (WGA) step and ends with an optimized sequencing process and bioinformatic analysis matching the characteristics of the adopted WGA. ***Ampli1™* kits** provide a complete range of products covering the entire workflow and optimized for different kinds of genetic analysis on the amplified DNA.



Ampli1™ WGA PLUS KIT: BEST-IN-CLASS FOR SINGLE CELL WHOLE GENOME AMPLIFICATION

The ***Ampli1*™ Whole Genome Amplification (WGA Plus) kit** has been developed and optimized for balanced and complete amplification of the genomic DNA content of a single cell.
Following cell lysis, DNA is **digested with a restriction enzyme** and adaptors are ligated onto the DNA fragments.
Amplification is mediated by a **single highly specific PCR primer**.



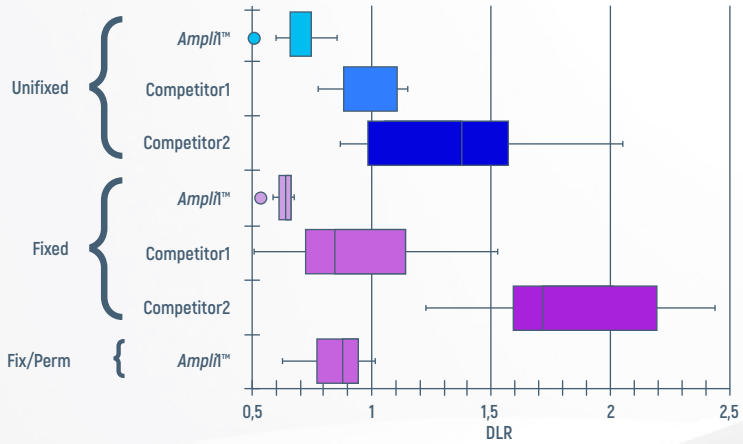
Ampli1™ WGA METHOD: LOW ALLELIC DROPOUT (ADO) RATE

Most WGA methods use random priming and uncontrolled amplification, causing frequent allelic dropout due to preferential (unbalanced) amplification of one of the two alleles. ***Ampli1*™ WGA** provides a robust and reproducible amplification efficiency on every locus and on each one of the two alleles reducing preferential amplification and achieving the **lower ADO rate** compared to other methods.

%ADO Measured					
Papers WGA	***2014 Blinder V et al WGS***	***2015 Huang L et al WGS***	***2016 Babayan A et al WES***	***2017 Borgstorm E et al WES***	***2016 Normand E et al STR***
***Ampli1*™**	**2%**		**9%**	**7-9%**	**6%**
Competitor1			24%	65-98%	49%
Competitor2		21-28%		18-47%	
Competitor3		76%			69%
Competitor4		33-38%	100%	93-95%	

Binder V et al, Hum Mutat. 2014 - Huang L et al, Annu Rev Genomics Hum Genet. 2015 - Babayan A et al, Oncotarget. 2016 - Borgström E et al, PLoS One. 2017 - Normand E et al, Prenat Diagn. 2016

Ampli1™ WGA METHOD: ROBUST AMPLIFICATION FROM BOTH FRESH AND FIXED CELLS



Ampli1™ WGA Method performs best **both with fresh and fixed cells** in terms of absolute DLRS value and of standard deviation compared to other WGA methods. Derivative Log Ratio Spread (DLRS) are indicative of the noise of the system. **Lower values allow higher resolution analysis of Copy Number Variation (CNV).**

Normand E et al, Prenat Diagn. 2016, "Comparison of three whole genome amplification methods for detection of genomic aberrations in single cells"

NEW *Ampli1*™ WGA PLUS FEATURES:

LOWEST ALLELIC DROP OUT

BEST IN CLASS FOR SINGLE CELL WGA

COMPATIBLE WITH LIQUID HANDLERS

NEW 4 HOURS PROTOCOL!

REPRODUCIBLE AMPLIFICATION EFFICENCY

USER FRIENDLY PROCEDURE

Ampli1™ WGA QC KIT: QUALITY CONTROL OF WGA PRODUCT

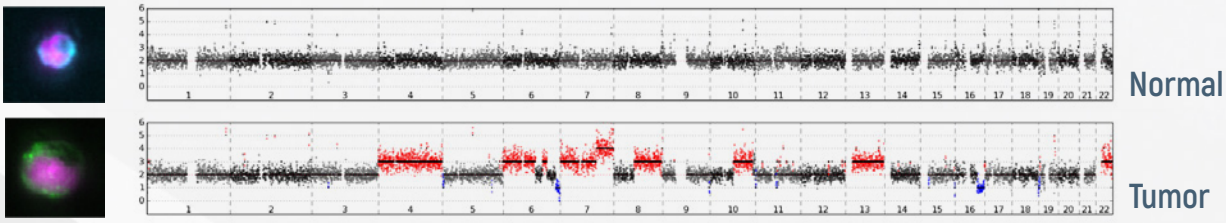
The *Ampli1*™ WGA QC Kit provides a useful quality control check of the WGA procedure by utilizing a PCR-based assay to establish DNA integrity. Cells with good quality DNA typically produce 3 or 4 PCR bands, while cells with degraded DNA will show fewer QC bands. The number of bands defines a "**Genome Integrity Index**" (GII) for predicting success of various downstream analytical methods. *(Polzer B et al., 2014 EMBO Molecular Medicine)*

AMPLI1™ KITS ARE FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES
The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

Ampli1™ LOWPASS: SPECIFIC, SENSITIVE AND QUANTITATIVE DETECTION OF COPY-NUMBER ABERRATIONS (CNAs) IN SINGLE CELL

The Library preparation kit is used to detect chromosomal aneuploidies and CNAs by lowpass whole genome sequencing. It is designed to work with the *Ampli1*™ WGA kit.

CK / CD45 / DAPI



Copy-Number Alteration patterns in cancer:

- *Prognostic factors*
- *Markers of immune evasion*
- *Predictive factors of chemoresistance*

Ferrarini et. al, PLOS One 2018 https://doi.org/10.1371/journal.pone.0193689

For Illumina and IonTorrent Platforms

Ampli1™ ONCOSEEK PANEL: ACCURATE DETECTION OF SOMATIC VARIANTS AND FOCAL CNAS FROM SINGLE CELL

The *Ampli1*™ OncoSeek Panel enables the preparation of high-quality targeted NGS libraries for the simultaneous detection of single nucleotide variants (SNVs), insertions/deletions (indels) and copy-number amplifications (CNAs) in a panel of 60 clinically relevant oncology-related genes. It is designed to work with the *Ampli1*™ WGA product.

ABL1	CDK6	FBXW7	HRAS	MLH1	PTPN11
AKT1	CDKN2A	FGFR1	IDH1	MPL	RG1
ALK	CSF1R	FGFR2	IDH2	MSH6	RET
APC	CTNNB1	FGFR3	JAK2	MYC	SMAD4
AR	DDR2	FGFR4	JAK3	MYCN	SMARCB1
ATM	DNMT3A	FLT3	KDR	NOTCH1	SMO
BRAF	EGFR	GNA11	KIT	NRAS	SRC
CCND1	ERBB2	GNAQ	KRAS	PDGFRA	TP53
CDH1	ERBB4	GNAS	MAP2K1	PIK3CA	TSC1
CDK4	EZH2	HNF1A	MET	PTEN	HVL

SNV, InDel
SNV, InDel, copy-number amplification
copy-number amplification

- *First and only panel designed **for single cell analysis**.*
- *Coverage **of 60 oncology-relevant genes** including more than 2500 mutation hotspots and CNAs for a subset of 19 genes.*
- ***Very Low Allelic Dropout Rate** (ADO=12.7% ± 4.2%)*

For Illumina Platforms

Ampli1™ WORKFLOW: CNV AND TARGET SEQUENCING FROM THE SAME SINGLE CELL

Mutational and copy number status of APC gene in a patient with metastatic cancer of unknown primary

In CTC1 and CTC2 a APC mutation is detected by *Ampli1*™ OncoSeek in homozygous state (Fig1), which is consistent with the loss of one of the 2 alleles detected by *Ampli1*™ LowPass (Fig2).

The analysis shows a deletion of APC gene in CTC3 consistently detected by both *Ampli1*™ LowPass and OncoSeek (*Laprovitera et al., 2021 Front. Cell Dev. Biol.):*

- *Ampli1*™ LowPass detected a genome segment of chromosome 5 with Copy Number = 0
- *Ampli1*™ OncoSeek shows no coverage on all APC gene amplicons in agreement with the presence of a deletion.

gene	effect	hgvsp	CTC1	CTC2	CTC3
APC	frameshft_variant	p.T1556Nfs*3	90,22	99,23	0,00

Fig1: Ampli1™ OncoSeek somatic mutation detection in 3 CTCs of a patient with metastatic cancer of unknown primary. An APC mutation is detected only in CTC1 and CTC2.

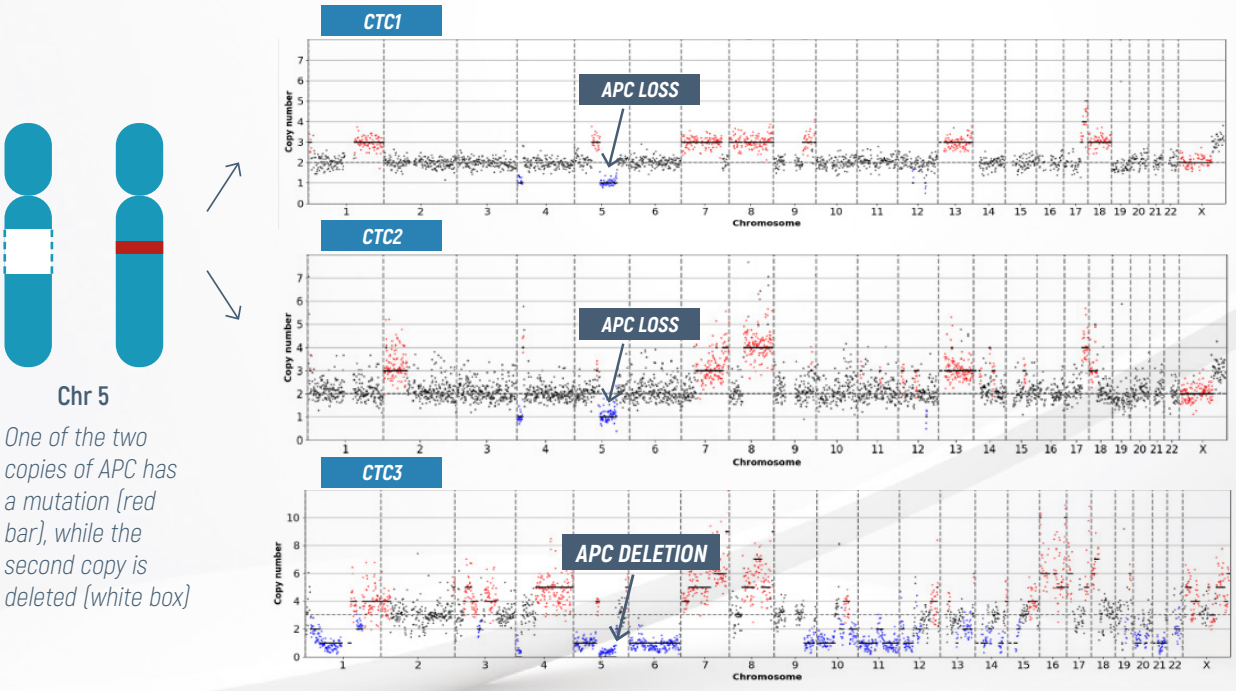


Fig2: Ampli1™ LowPass genome-wide copy number profiles of CTC1, CTC2, CTC3 from the same patient represented in Fig.1

Ampli1™

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