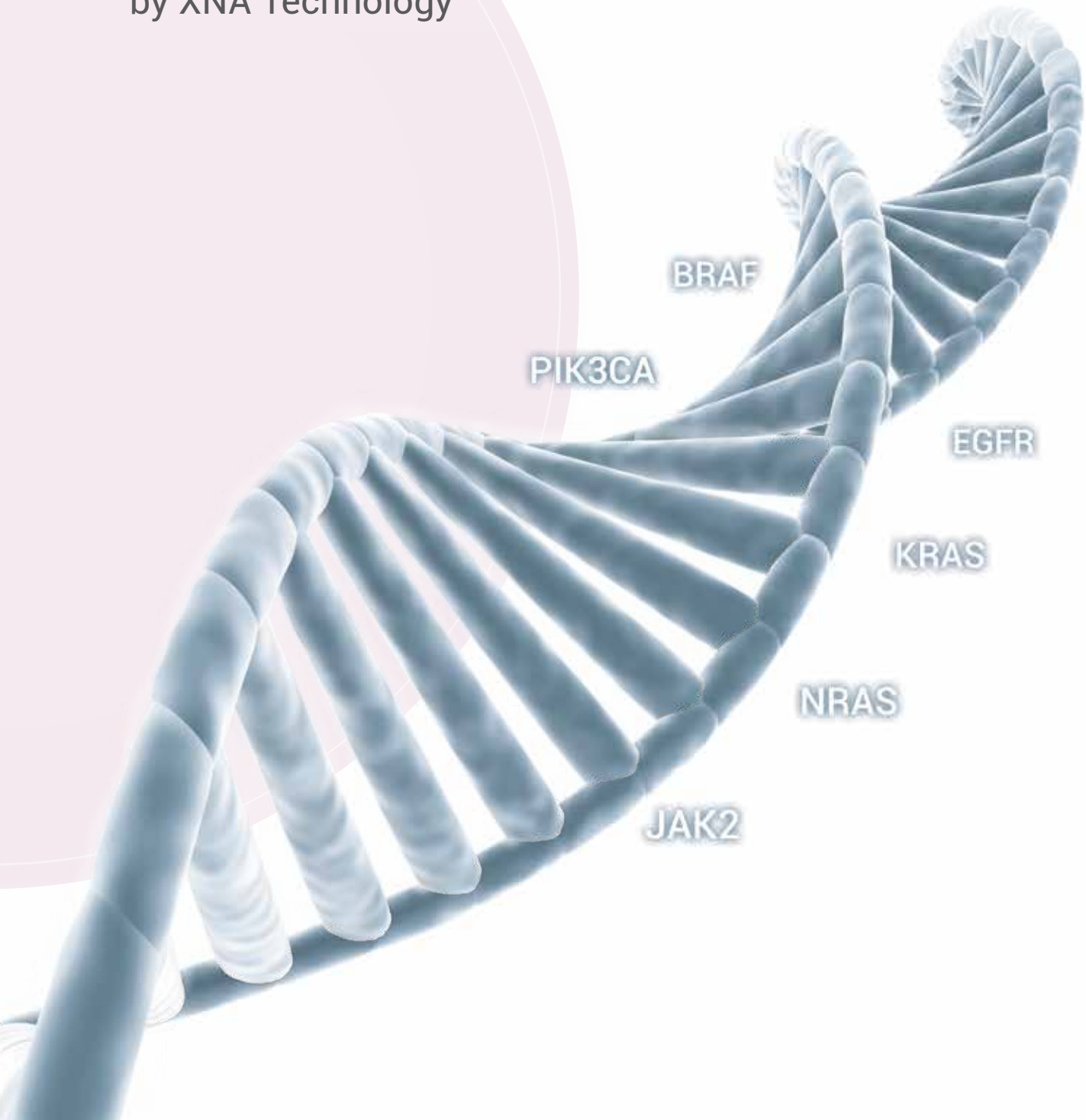
DIACARTA

IMPROVED SENSITIVITY FOR SINGLE GENE MUTATION DETECTION

QClamp® Gene Mutation Detection Tests Powered by XNA Technology



GENE MUTATION DETECTION AND CANCER

Cancer diagnostics is critical to accurately diagnose cancer for optimal therapy to save or extend cancer patients' lives. With development of different types of biomarkers, including genetic biomarkers, cancer patients can get much more detailed profiling based on these biomarkers, rather just cancer types, and personalized medicine can be applied for targeted therapy.

One great example is targeted therapies using companion diagnostics to identify EGFR mutations among non-small cell lung cancer (NSCLC) at different stages of the therapy and apply different tyrosine inhibitor drugs.

Figure 1. EGFR Mutation Development Upon Targeted Therapy Using Tyrosine Kinase Inhibitors (TKI)

- **Responded to the 1st and 2nd generation of TKIs:**
  The first generation of TKI (Erlotinib and Gefitinib) and second generation of TKI (Afatinib, Dacomitinib) are effective in NSCLC patients with EGFR mutations in Exon 19 deletion and L858R

EXON 18	EXON 19	EXON 20	EXON 21
G719X	Exon 19 del/Ins		L858R L861Q

- **Resistant to the 1st and 2nd generation of TKIs, but responded to the 3rd generation of TKIs:**
  Some of the patients developed resistance to the drugs after 8-14 months period due to the development of a different mutation, T790M in Exon 20. In this stage, a different drug, such as Osimertinib could be used to inhibit the growth of the NSCLC

EXON 18	EXON 19	EXON 20	EXON 21
G719X	Exon 19 del/Ins	+ T790M	L858R L861Q

- **Resistant to the 3rd generation of TKIs:**
  Another mutation, C797S, can be developed in a proportion of patients, which leads to patients' resistance to Osimertinib after a period of time. At this stage, the patients have to use another drug

EXON 18	EXON 19	EXON 20	EXON 21
G719X	Exon 19 del/Ins	T790M + C797S	L858R L861Q

- **CONCLUSIONS**
  Cancer recurrence has to be closely monitored with the EGFR mutations for doctors to prescribe the right medicine for the right patients.

CURRENT GENE MUTATION DETECTION TECHNOLOGIES AND WHY XNA TECHNOLOGY IS THE OPTIMAL CHOICE

Sanger Sequencing

- Advantage: accurate result and is therefore the gold standard
- Disadvantages: low sensitivity (20% to 25% Variant Allele Frequency, VAF)

Pyrosequencing Assays

- Advantages: better sensitivity and throughput than Sanger sequencing, early form of NGS
- Disadvantages: low sensitivity: 5% to 8% VAF

NGS Sequencing

- Advantages: high-throughput and good sensitivity - 1% to 5% VAF, or even better
- Disadvantages: costly and time consuming (7 to 10 days)

Digital Droplet PCR (ddPCR)

- Advantages: high sensitivity and claimed to be 0.001% VAF
- Disadvantages: much less sensitivity observed in testing than claimed and suffers false-positive results

qPCR Analysis

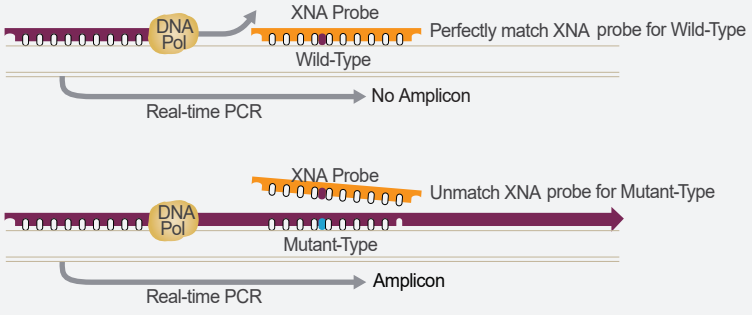
- Sensitivity can reach 1% VAF for some targets. Rapid and little hands-on work
- Multiple methods for qPCR and a lot of variations in sensitivity. Some of them are only 10% VAF

XNA Molecular Clamps

- **Sensitivity: 0.1% to 0.5% VAF** much more sensitive than regular qPCR and other qPCR-derived techniques
- Achieve ultra-sensitivity by only amplifying mutant DNA and block wild-type sequences

Application of XNA Technology to qPCR Increases Detection Sensitivity

XNA, xenonucleic acids, are innovative new nucleic acid molecular oligomers that hybridize by Watson-Crick base pairing to target DNA sequences yet have a modified chemical backbone. XNA oligomers are highly effective at hybridizing to targeted normal DNA sequences and can be employed as molecular clamps in quantitative real-time polymerase chain reactions (PCR) or as highly specific molecular probes for detection of nucleic acid target sequences. The XNA tightly binds to the wildtype sequence that is 100% complementary in sequence and blocks DNA polymerase from DNA elongation; only the mutant target sequence gets amplified because the XNA:mutant DNA duplex is not stable due to mismatch and fall off from the template in PCR reactions.



INTRODUCE QCLAMP® SINGLE GENE MUTATION DETECTION TESTS

XNA TECHNOLOGY | CE/IVD CERTIFIED

QClamp® Gene Mutation Detection Tests are highly sensitive qPCR-based assays for tissue biopsy (FFPE) and liquid biopsy (ctDNA) applications. QClamp® assays detect reliably 0.1% to 0.5% mutant DNA out of wild-type DNA for targeted mutations, providing lower detection limit compared with competition due to strict enrichment of mutant sequences while suppressing amplification of wild-type sequences.

QClamp® assays provide a rapid, reproducible and affordable solution which employs a simple workflow and PCR machines that are commonly used in research and clinical labs. The tests can be performed on standard real-time qPCR instruments such as ABI QuantStudio 5, Roche LightCycler® 480 and Bio-Rad CFX384.

Significance in the Detection of Driver Gene Mutation

Driver gene mutations are critical for the development of cancer. Driver genes include oncogenes, tumor suppressor genes, and genes responsible for DNA repair. Detection of these gene mutations can help diagnose cancer and provide strategies for cancer therapy as companion diagnostics.

Leverage XNA Technology

QClamp® single gene mutation detection kits leverage XNA technology and use XNA clamps in qPCR reactions to detect mutations in single genes including EGFR, KRAS, NRAS, BRAF, JAK2 and PIK3CA. Because mutations in these genes are well characterized and some are related to targeted therapies, detection of these mutations provide important information for pathologists and oncologists regarding the patients' profile and treatment strategies.

The QClamp® detection kits use the XNA sequence binding to and blocking the wild-type targeted sequence from amplification in the qPCR reaction, thereby selectively amplifying the mutant sequence. Regular qPCR reactions will not be able to sensitively identify the mutants.

Limit of Detection (LOD)

The LOD for the qPCR assay is determined by running the QClamp® assay using a serial dilution of mutant DNA in wild-type background at different total DNA inputs and several mutation frequencies for each target. To determine if a sample is positive (contains mutation) or negative (does not contain mutation) for a particular target sequence, the ΔCq of the sample at this target sequence and the validated standard ΔCq for this target sequence for a positive and negative control will need to be compared.

Cq difference (ΔCq) = Sample Assay Cq - Internal Control Assay (beta-actin) Cq

If further sequencing is required, the qPCR reactions can be sequenced directly by Sanger sequencing using target-specific primers.

QClamp® Single Gene Mutation Detection Tests Covered Mutations

Genes	Covered Mutations
KRAS	Codons 12, 13, 59, 61, 117 and 146
NRAS	Codons 12, 13, 59, 61, 117 and 146
EGFR	Codons 719, 861, Ex19del, Ex20insASV and S768I, T790M and L858R mutations
BRAF	Codon 600
JAK2	Codon 617
PIK3CA	Codons 542, 545 and 1047

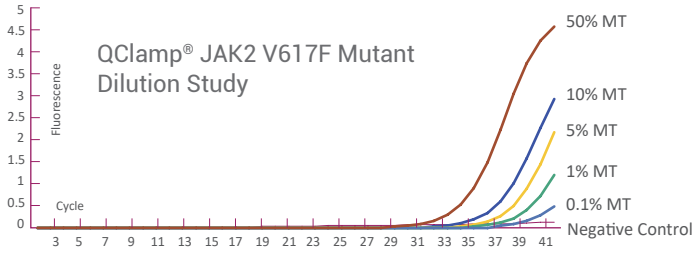
Correct calls of different percentage reference mutant inputs in the varying amount of the wild-type gDNA using Bio-Rad CFX 384

Bio-Rad CFX384	DNA Input, ng/well 7.5 % Correct Call	DNA Input, ng/well 5 % Correct Call	DNA Input, ng/well 2.5 % Correct Call
1% mutation	100%	100%	100%
0.5% mutation	100%	100%	100%
0.1% mutation	0%	0%	0%
1% mutation	100%	100%	100%
0.5% mutation	100%	100%	100%
0.1% mutation	0%	0%	0%
1% mutation	100%	100%	100%
0.5% mutation	100%	100%	100%
0.1% mutation	0%	0%	67%
1% mutation	100%	100%	100%
0.5% mutation	100%	100%	100%
0.1% mutation	33%	67%	67%
1% mutation	100%	100%	100%
0.5% mutation	0%	67%	67%
0.1% mutation	0%	0%	0%
1% mutation	100%	100%	100%
0.5% mutation	100%	100%	67%
0.1% mutation	0%	33%	0%

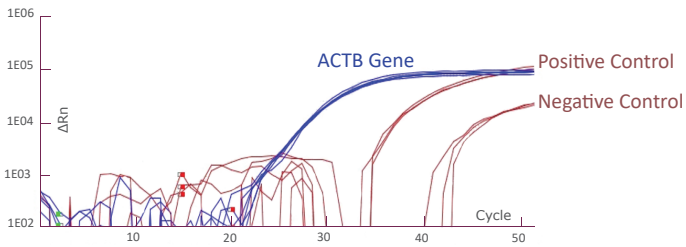
STREAMLINED WORKFLOW

1. **DNA Isolation & Quantification**
   Extract DNA from FFPE or plasma using a commercial DNA extraction kit followed by measuring the concentration using fluorometric analysis
2. **Set up qPCR**
   Mix the assay reagents, load into PCR plate, add controls and extracted DNA ~ 30-60 minutes
3. **Enter Amplification Parameters**
   Enter amplification parameters on qPCR instrument, load PCR plate and start the run ~ 2.5 hours
4. **Data Analysis**
   Determine the presence or absence of mutations according to the Cq value cutoffs ~ 15 minutes

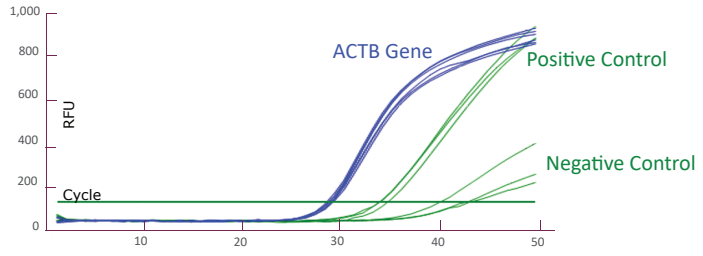
Below 0.1% Detection Sensitivity of Mutant DNA



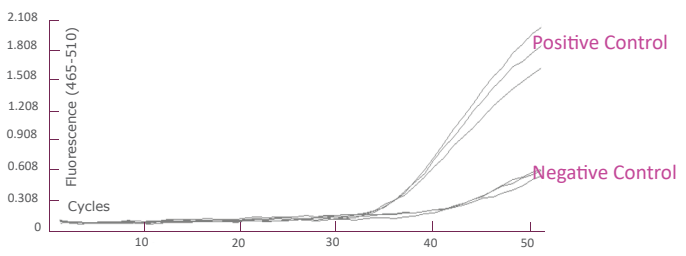
Amplification Plot from ABI QuantStudio 5 (JAK2)



Amplification Plot from Bio-Rad CFX384 (JAK2)



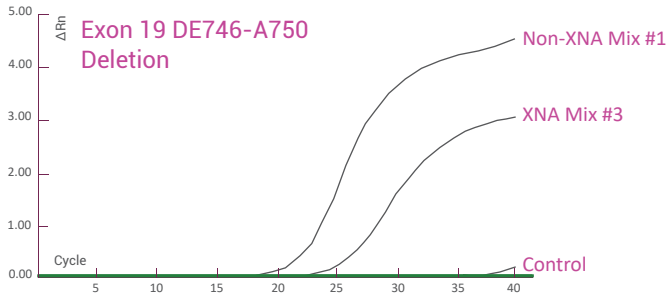
Amplification Plot from Roche LightCycler® 480 (JAK2)

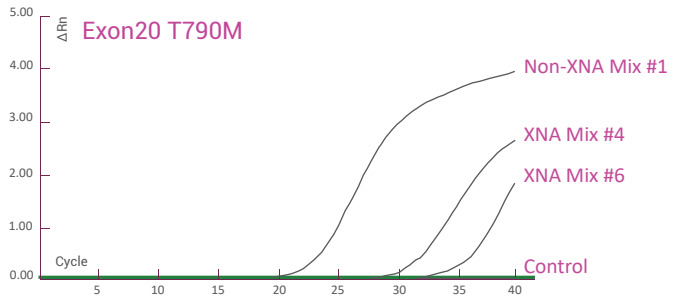


UTILIZING QCLAMP® ON CLINICAL SAMPLE MUTATION DETECTION

Clinical FFPE Sample

Human tumor FFPE samples for EGFR gene mutation detection. EGFR mutations (Exon 19 deletion and T790M) in human tumor FFPE samples are detected using QClamp® EGFR detection kit. Abundant wild-type DNA is detected at early qPCR cycles when no XNA is present. With XNA, only mutant DNA is detected at late qPCR cycles and wild-type DNA amplification is blocked.

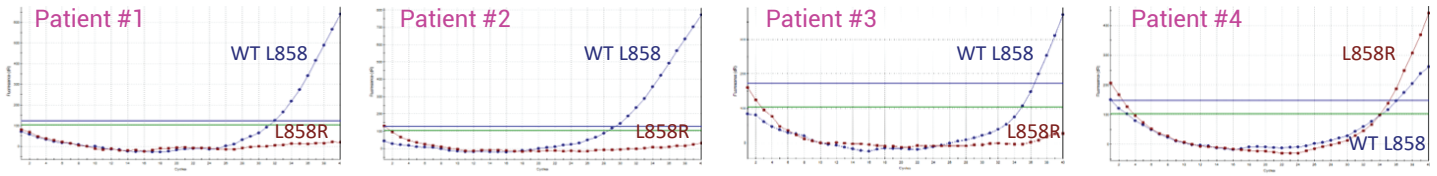




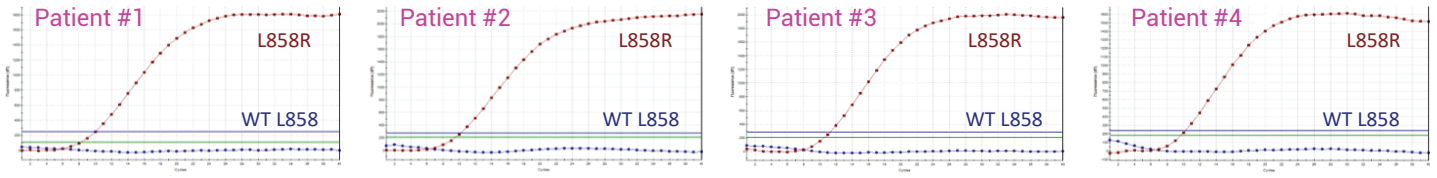
Clinical Plasma Sample

Detecting L858R in Plasma from 4 NSCLC Patients with and without XNA. In the presence of XNA, only wild-type sequence is detected and mutant DNA is only detected in the presence of XNA.

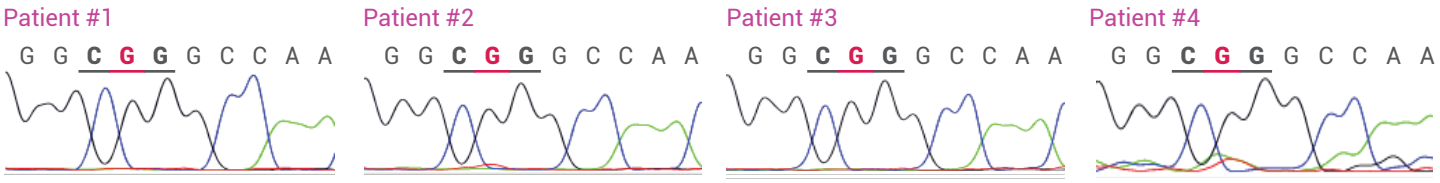
Without XNA Technology - Mainly Wild-Type (WT) L858 Detected



With XNA Technology - WT Blocked, only L858R Mutant Detected



L858R Detected by Sanger Sequencing after XNA Blocking (WT = CTG; L858R = CGG)



FEATURES AND BENEFITS OF QCLAMP® ASSAYS

ULTRA-SENSITIVE
Detect reliably 0.1% to 0.5% VAF mutant DNA out of wild-type DNA for targeted mutations

SAMPLE READY
Suitable for Plasma and FFPE Samples

LOW INPUT DNA
Minimum 5ng input DNA per reaction. Less than 2 tubes of blood (10mL each) needed for cfDNA

COMPREHENSIVE COVERAGE
Covering all relevant somatic mutations in KRAS, NRAS, EGFR, BRAF, JAK2 and PIK3CA oncogenes

FAST RESULTS
Less than 4 hours of assay run time

GREAT VERSATILITY
Validated on the most common qPCR machines with minimized variability

PRODUCT SPECIFICATION			
Product Name	QClamp® Gene Mutation Detection Tests	**Pack Size**	30 Samples
Intended Use	For In Vitro Diagnostic Use (CE/IVD) or For Research Use	Detection Chemistry	TaqMan
Sample Type	FFPE and Plasma	**Turnaround Time**	Less than 4 Hours
Input DNA	5-10 ng/Reaction	**Stability**	Stable for 12 Months at -25 °C to -15 °C
Validated Instruments	Roche LightCycler® 480, Bio-Rad CFX384 and ABI QuantStudio 5		

ORDERING INFORMATION

Product Name	Pack Size	Catalog Number (CE/IVD)	Catalog Number (Research-Use-Only)
QClamp® KRAS Mutation Detection Test	30 Samples	DC-10-3010	DC-10-3010R
QClamp® NRAS Mutation Detection Test	30 Samples	DC-10-3020	DC-10-3020R
QClamp® EGFR Mutation Detection Test	30 Samples	DC-10-0012	DC-10-0012R
QClamp® BRAF Mutation Detection Test	30 Samples	DC-10-0197	DC-10-0197R
QClamp® JAK2 Mutation Detection Test	30 Samples	DC-10-0166	DC-10-0166R
QClamp® PIK3CA Mutation Detection Test	30 Samples	DC-10-0172	DC-10-0172R

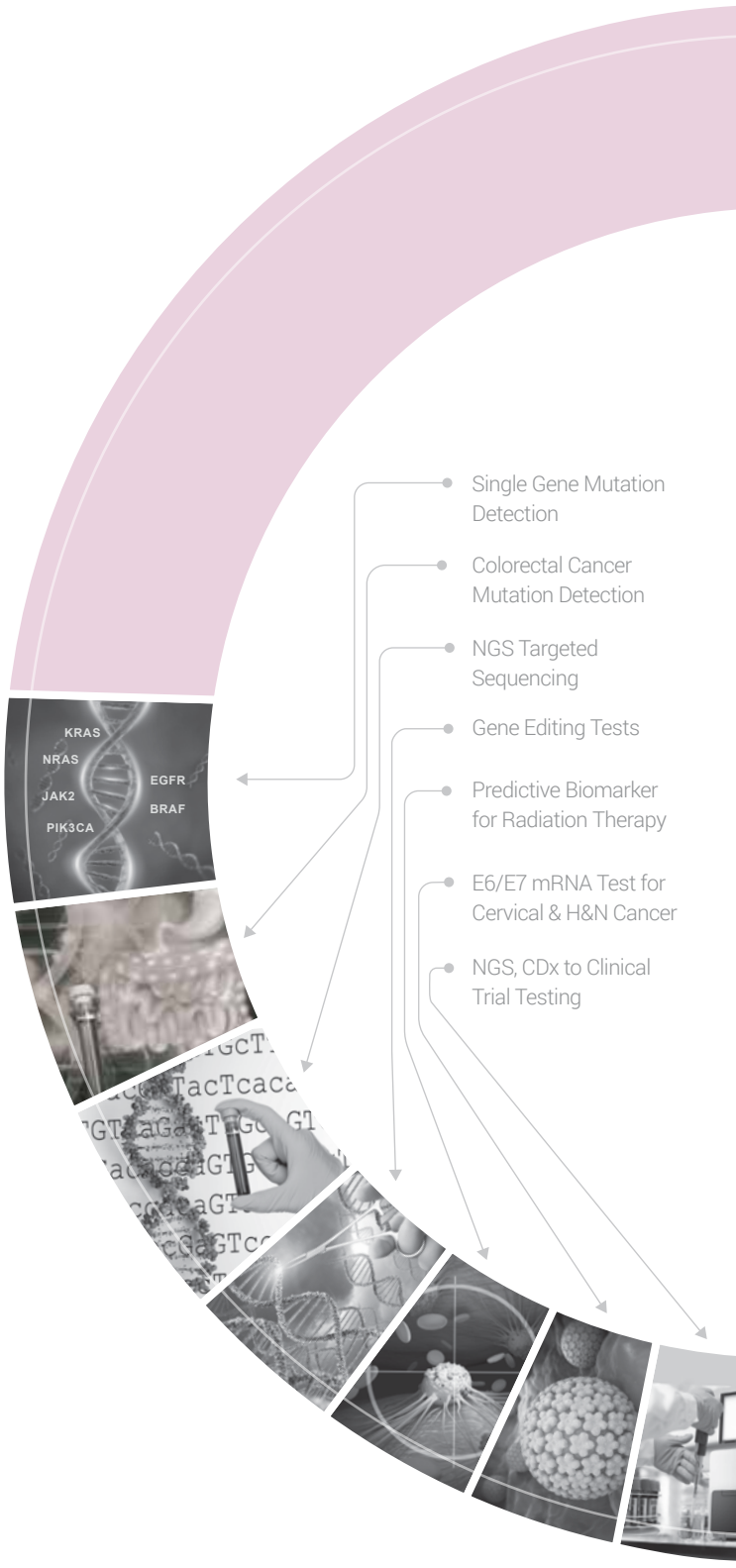
References

1. Powell, M.J., et. al., High Sensitivity Detection of Tumor Gene Mutations. BAOJ Cancer Sciences, 2015, (1), 1 http://bioaccent.org/cancer-sciences/cancer-sciences1.php

2. Myers, M.B., et. al., Breast Cancer Heterogeneity Examined by High-Sensitivity Quantification of PIK3CA, BRAF, KRAS, and HRAS Mutations in Normal Brest and Ductal Carcinomas. Neoplasia 2016 in press.

3. Yan et al. Genetic Alteration and Mutation Profiling of Circulating Cell-Free Tumor DNA (cfDNA) for Diagnosis and Targeted Therapy of Gastrointestinal Stromal tumors. Chin J Cancer (2016) 35 : 68 DOI 10.1186 /s40880-016-013-1

Redefining Precision Molecular Diagnostics through Cancer Gene Mutation Detection



DIACARTA, INC.
2600 Hilltop Drive
Richmond, CA 94806
United States
information@diacarta.com
1-800-246-8878
www.diacarta.com