TriLink BIOTECHNOLOGIES
part of Maravai LifeSciences

Connect Collaborate GROW

TriLink Solutions for NGS Library Prep (CleanTag®) and PCR Applications (CleanAmp®)

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CleanTag

CleanTag® Small RNA Library Prep Kit
Catalog# L-3206

Introduction

- Used to discover and profile miRNAs and other non-coding small RNAs
- Leverages the 5’phosphate and 3’hydroxyl group structure of small non-coding RNAs
- Requires no prior knowledge of sequence required (unlike Microarray or qPCR)
- Enables RNA ligase to add single stranded adapters to the unknown RNA so that it can be sequenced
- Compatible with most NGS platforms – Illumina and Ion Torrent

CleanTag® Small RNA Library Kit Highlights

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Highlights & Benefits

- Adapter-dimer control
  - ➢Unique, patented chemical technology prevents adapter-dimer formation
    - ➢Samples are more efficiently tagged and read
      - ➢more mappable reads
      - ➢Fewer failed amplifications
- Library Detection at ultra low input
  - ➢Wide range of usable total RNA input: 1 ng to 1,000 ng
- High throughput, fast workflow
  - ➢Only 6 hours from RNA input to purified library ready for sequencing
  - ➢Gel purification not necessary
  - ➢Sample is ready for automated bead purification (if desired)

NGS Research Area

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Somatic mutations in the genome

mRNA expression (transcriptomics)

Long non-coding RNA

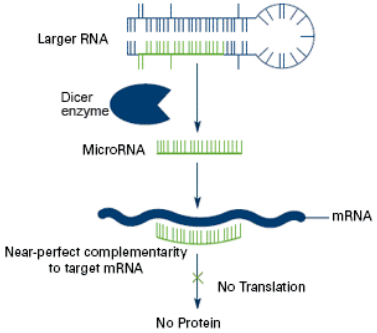
Small RNA and miRNA

Emerging uses:

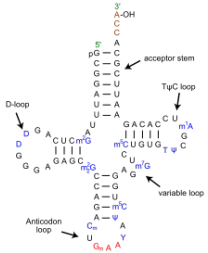
- Therapeutics and vaccines – gene edits
- Microbiome analysis
- Metagenomics
- Exosome and Extracellular Vesicle analysis
- Liquid biopsy

The expanded RNA universe now includes: miRNA and various small RNAs…

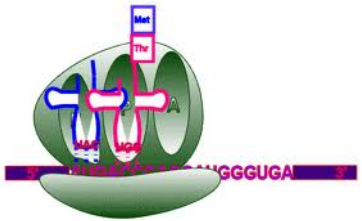
microRNAs (22-24 nt)



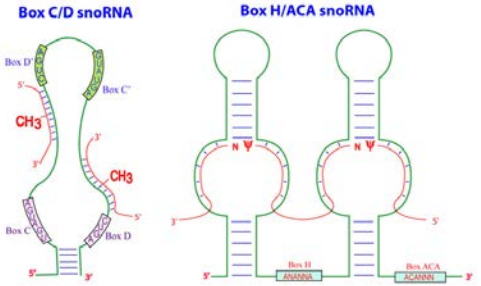
tRNA (73-94 nt)



tRF (tRNA fragments) (20-35 nt)

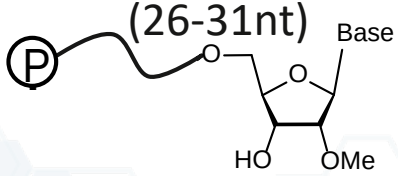


snoRNAs (small nucleolar RNA) (60-300 nt)



Y-RNA small-RNYs (s-RNYs) (24-34 nt)

piRNA (26-31nt)



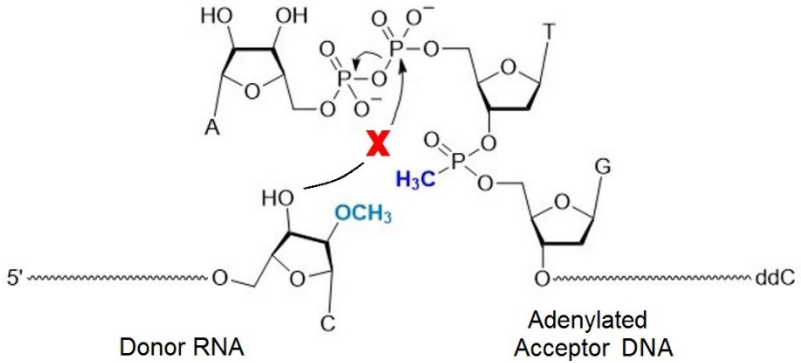
Sno-RNA derived RNA: snRNA (20-24 nt)

CleanTag® Technology

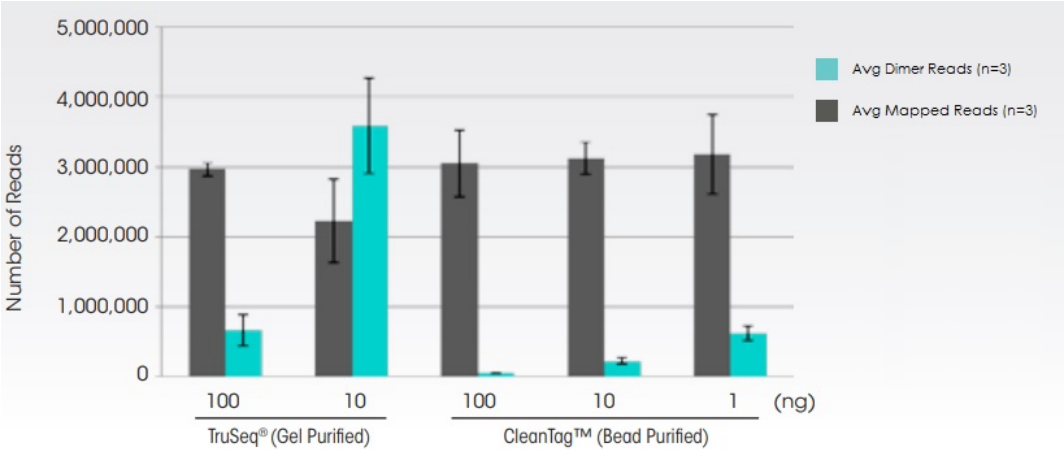
- CleanTag® uses modified adapters to reduce undesirable ligation of adapter to adapter

CleanTag™ 5'-Adapter
PO RNA, 5'-GUU CAG AGU UCU ACA GUC CGA CGA UC -3'

CleanTag™ 3'-Adapter
PO DNA, 5'-(rApp)-TGG AAT TCT CGG GTG CCA AGG (ddC)-3'



Low RNA input library results



CleanTag bead-purified libraries provide higher mapped-reads and reduced dimer-reads when compared to Illumina TruSeq

CleanTag maintains high mapped reads, low adapter dimer at much reduced input RNA

Index Primers are used with CleanTag® Kit

SKU	Unit Size	Price	Qty
L-3206-24	24 reactions	$1,887.00	- 1 +

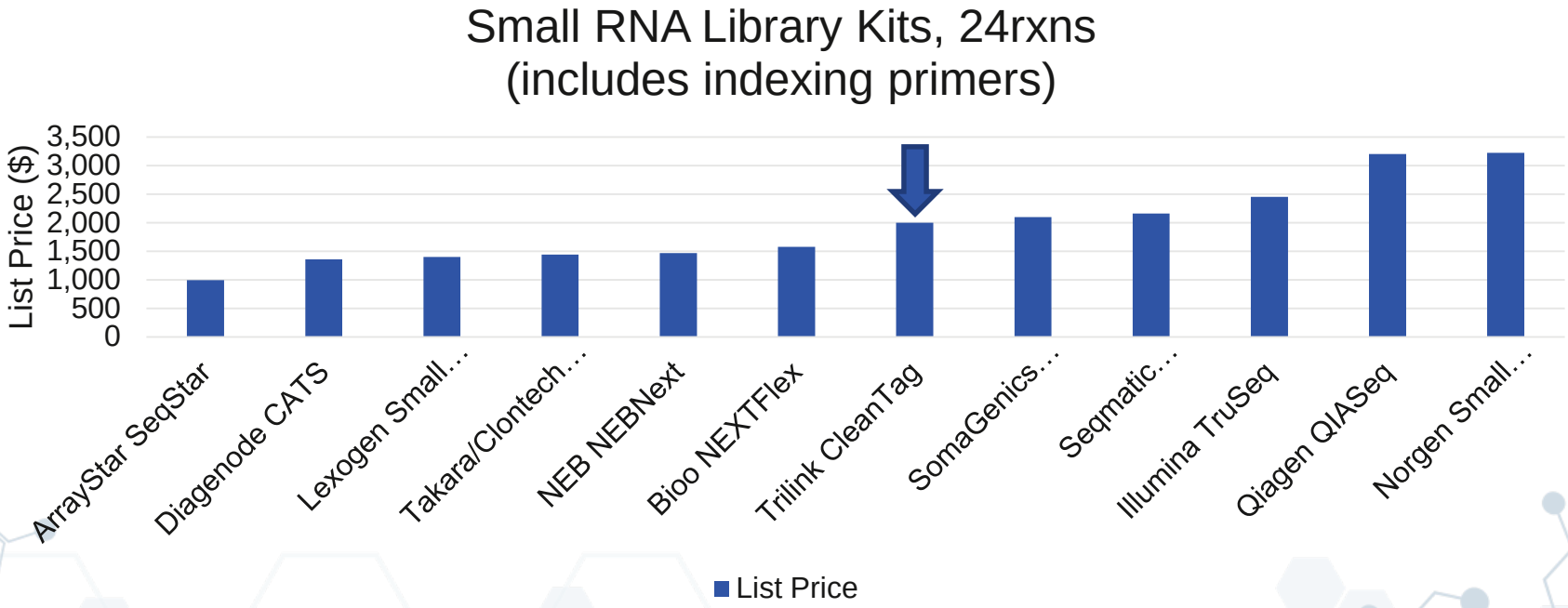
Index Primers for Illumina® Sequencing Technology

SKU	Unit Size	Price	Qty
L-3204-24	Set 1, Primers 1-12	$111.00	- 0 +
L-3205-24	Set 2, Primers 13-24	$111.00	- 0 +
L-3207-24	Set 3, Primers 25-36	$111.00	- 0 +
L-3208-24	Set 4, Primers 37-48	$111.00	- 0 +

Barcode Convert Primers for Ion Torrent™ Sequencing Technology

SKU	Unit Size	Price	Qty
L-3210-24	Set 1, Primers 1-12	$111.00	- 0 +
L-3211-24	Set 2, Primers 13-24	$111.00	- 0 +

The Library Kit Competition



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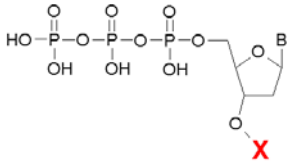
CleanAmp

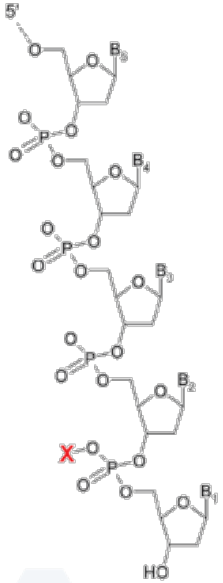
CleanAmp®: a Universal Hot Start

- Two CleanAmp technologies that enable Hot-Start PCR
  - Modified dNTPs
  - Modified custom oligo primers
- Can be used in all PCR applications
  - qPCR
  - High G/C sequences
  - RT-PCR
- Compatible with all *Taq* polymerase enzymes
- High fidelity and high-yielding reactions
- Multiplexing
- IP invented and owned by TriLink
  - Affordable licensing terms

Modified dNTP

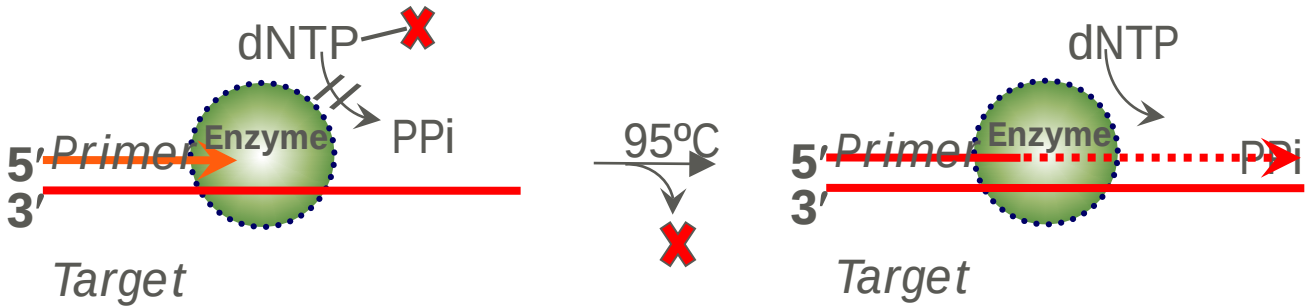


Modified PCR primer

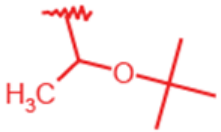


X = a reversibly attached blocking group that prevents polymerase extension until removed

How do Hot-Start CleanAmp® dNTPs work?



X = **Thermolabile Structure**

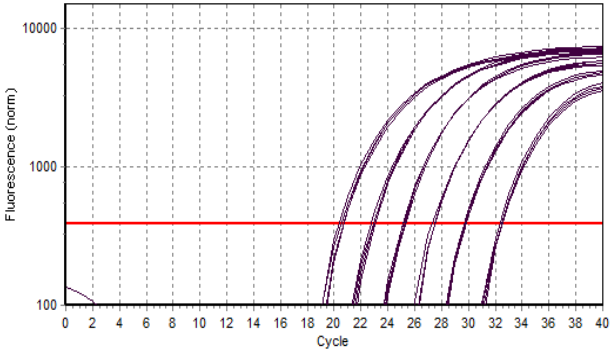


3'-TBE
(3'-tert-butoxy-ethoxy)

Application of CleanAmp® dNTPs to Fast Thermal Cycling Protocols

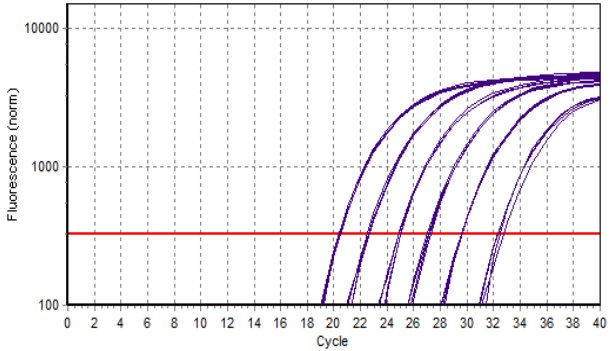
CleanAmp®

Standard Cycling
(1 hour 26 min)



Y = -3.403LOG(X) + 30.46
R^2 = 0.997; Efficiency = 0.97

Fast Thermal Cycling
(24 min)



Y = -3.359LOG(X) + 30.59
R^2 = 0.999; Efficiency = 0.98

PCR amplification of mouse gDNA (0.32-1000 ng), with CleanAmp™ dNTPs using Standard or Fast formulation and cycling conditions

CleanAmp® dNTPs

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Product	Catalog	Size
CleanAmp® dNTP Mix	N-9506-2	2 µmoles
10 mM each mixed dATP, dCTP, dGTP, dTTP	N-9506-10	10 µmoles
CleanAmp® dNTP Set	N-9507-2	2 µmoles
50 mM each vial dATP, dCTP, dGTP, dTTP	N-9507-10	10 µmoles
CleanAmp® dUTP Set	N-9508-2	2 µmoles
50 mM each vial dATP, dCTP, dGTP, dUTP	N-9508-10	10 µmoles
CleanAmp® 7-deaza-dGTP Mix	N-9504-2	2 µmoles
10 mM each mixed dATP, dCTP, dGTP*, dTTP (*=dGTP:7-deaza-dGTP;1:3)	N-9504-10	10 µmoles
CleanAmp® 7-Deaza-dGTP, 50 mM solution	N-9515-2	2 µmoles
	N-9515-10	10 µmoles
CleanAmp® dUTP, 50 mM solution	N-9524-2	2 µmoles
	N-9524-10	10 µmoles

- 2 µmoles is sufficient for 400 PCR reactions
- Please inquire for bulk pricing

CleanAmp® 2X Master Mixes – Four types

Product	Catalog	Size
CleanAmp® PCR 2X Master Mix	L-5101-100	100 rxns
CleanAmp® GC-Rich PCR 2X Master Mix[a]	L-5102-100	100 rxns
CleanAmp® Multiplex PCR 2X Master Mix[b]	L-5103-100	100 rxns
CleanAmp® One-Step RT-PCR 2X Master Mix[c]	L-5104-100 L-5104-25	100 rxns 25 rxns

Notes:

- All Master Mixes contain *Taq* DNA Polymerase and CleanAmp dNTPs

a. Optimized for targets over 60% GC content; with CleanAmp® 7-deaza-dGTP

b. Optimized reaction buffer suited for multiplex amplification

c. Also contains a reverse transcriptase and optimized one-step RT-PCR buffer

CleanAmp® Primers: Turbo and Precision

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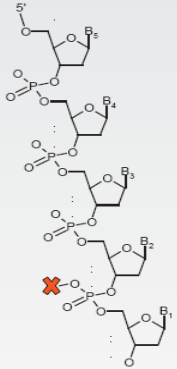
Turbo Primers

- Improve amplicon yield
- Reduce off-target formation

Applications:

- Fast cycling
- Multiplex PCR

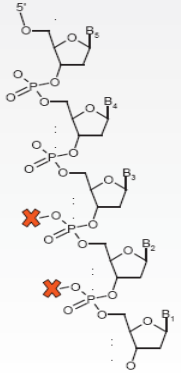


Precision Primers

- Improve specificity and limit of detection
- Greatest reduction in off-target formation

Applications

- Standard cycling
- One-step RT-PCR (singleplex and multiplex)
- Ligation PCR

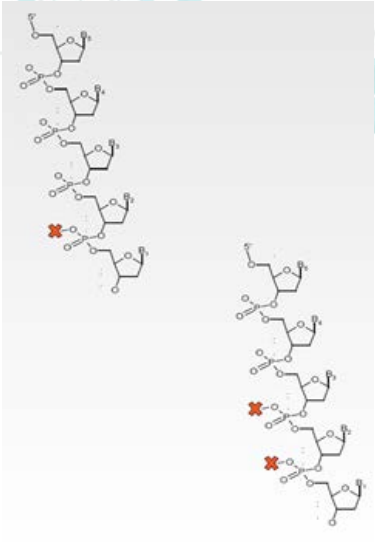


CleanAmp® Primers – Precision and Turbo

Product	Catalog #	Typical Yield
CleanAmp Turbo Primer	OT-4300	≥50 OD
CleanAmp Precision Primer	OP-4300	≥50 OD

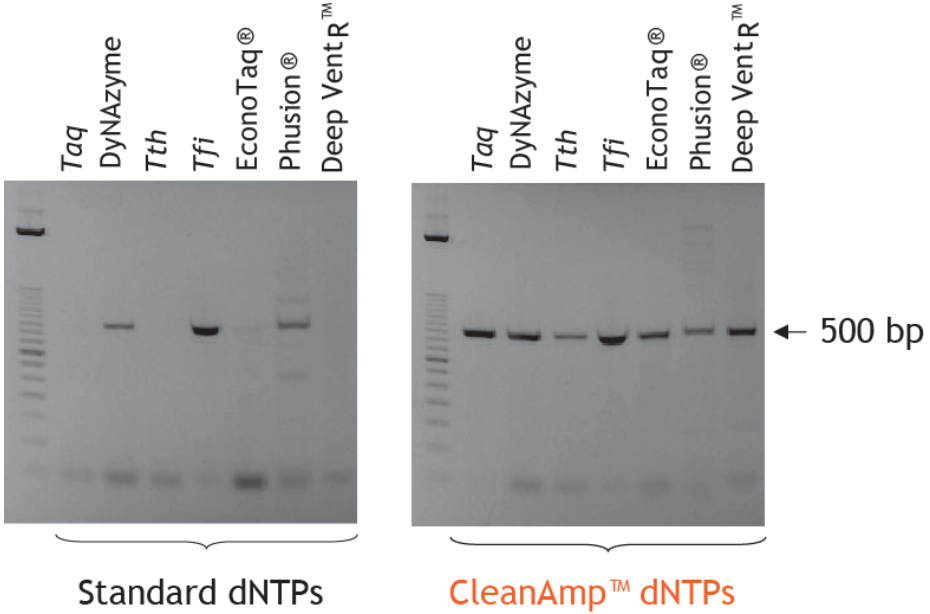
- CleanAmp® Turbo Primers may be use in fast PCR applications
- Primers are supplied cartridge purified
- RP-HPLC purification optional
- QC: PAGE, MS and RP-HPLC to ensure high quality

CleanAmp Turbo Primer



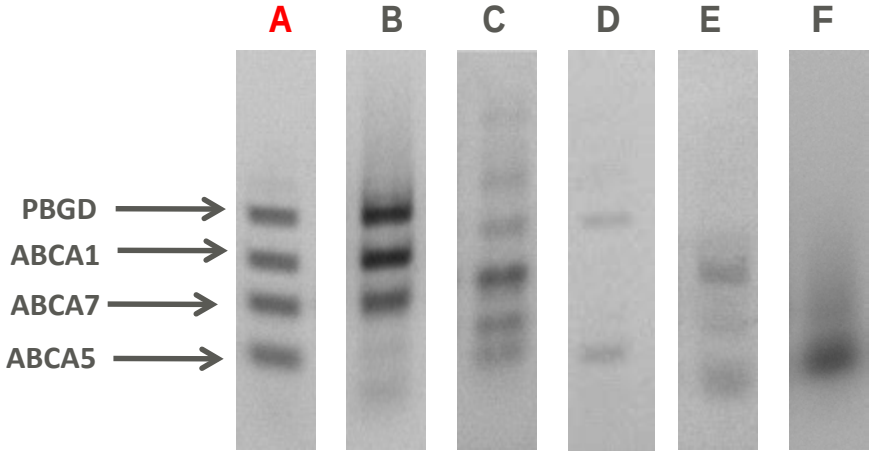
CleanAmp Precision Primer

CleanAmp™ dNTPs Improve Performance of many different polymerases

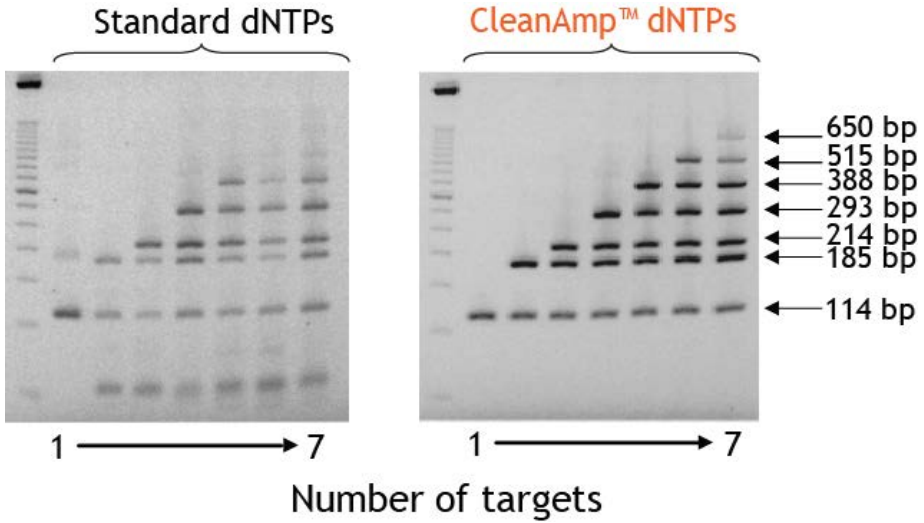


PCR amplification of Lambda gDNA (500 copies), with either standard or CleanAmp™ dNTPs.

Multiplex PCR Yield and Specificity is Improved by CleanAmp® dNTPs

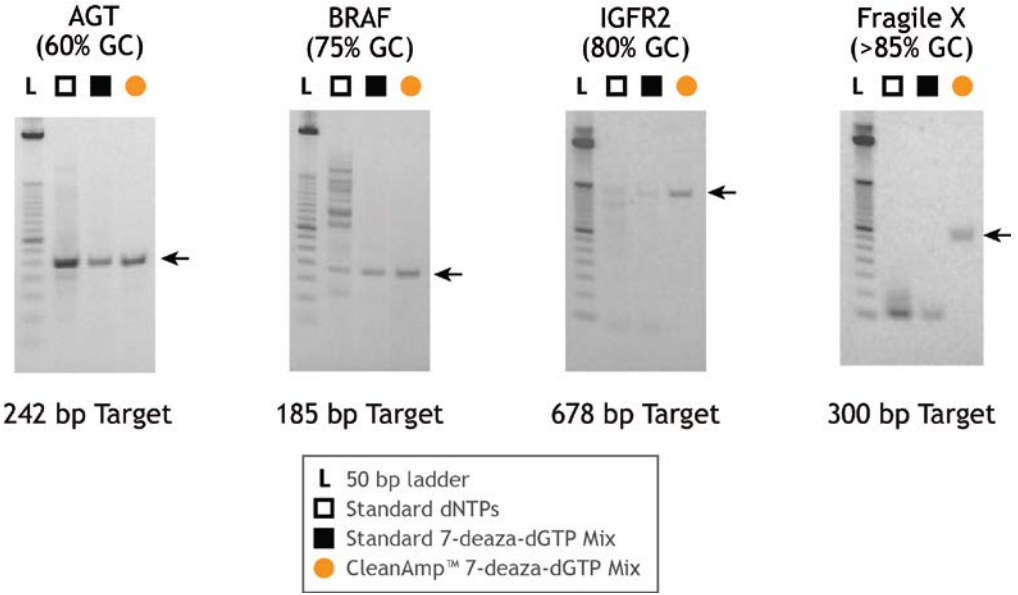


A CleanAmp® RT-PCR Master Mix
B Commercial Kit 1
C Commercial Kit 2
D Commercial Kit 3
E Commercial Kit 4
F Commercial Kit 5



PCR amplification of 7 targets in mouse gDNA (20 ng) using either standard or CleanAmp™ dNTPs and *Taq* DNA polymerase

Improved Amplification Yield/Specificity for High GC-Content Targets using CleanAmp 7-Deaza-GTP

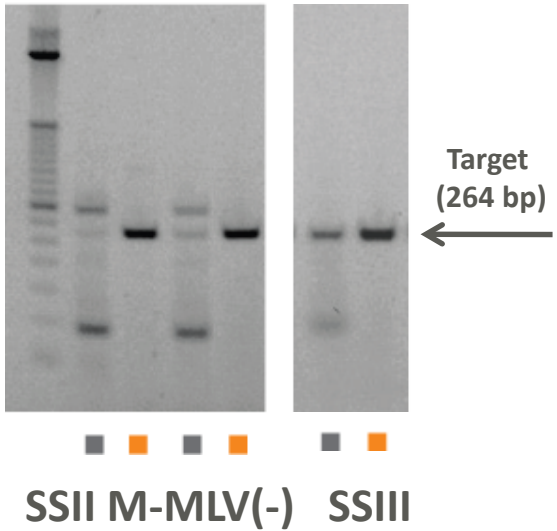


PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl2), Primers (0.1-0.2 µM), 0.2 mM dNTPs, where reactions with 7-deaza-dGTP used a 3:1 ratio of 7-deaza-dGTP:dGTP, 5 ng Human gDNA, 1.25 U Taq DNA polymerase, 25 µL.
Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), X°C (1 sec), 72°C (1 min)] 35 cycles; 72°C (7 min)
Where X (annealing temperature) varied depending on the target (ACE 66°C; BRAF 64°C; B4GN4 57°C; GNAQ 66°C)

CleanAmp® dNTPs Improve the Performance of Commercially Available of Reverse Transcriptases (RT-PCR)

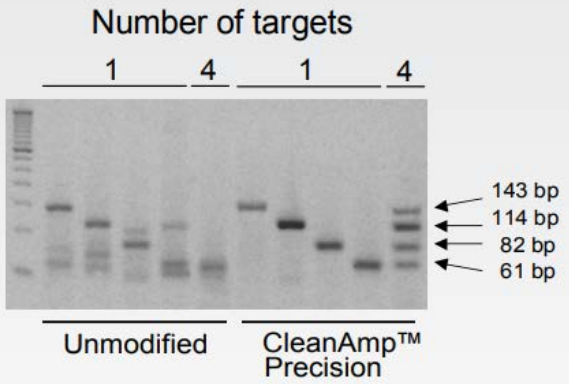
■ Standard dNTPs
■ CleanAmp® dNTPs

SSII: Superscript® II
M-MLV(-): M-MLV (RNase H-)
SSIII: Superscript® III



RT-PCR amplification of total trachea RNA (100 ng), with either standard or CleanAmp® dNTPs and at 42°C or 50°C.

CleanAmp® Precision Primers: Multiplex One-Step RT-PCR



CleanAmp™ Precision Primers demonstrate superior amplicon yield in multiplex one-step RT-PCR relative to unmodified primers

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl), 1.5 mM $MgCl_2$, ABCA7, ABCA6,ABCA5,ABCC10 Precision Primers (0.5 µM), oligo$(dT)_{18}$ primer (1 µM), 0.16 mM dNTPs, 0.5 µg Human trachea total RNA, 50 U/50 µL MMLV reverse transcriptase, 0.6 U *Taq* DNA polymerase, 50 µL.

Thermal cycling conditions: 42°C (30 min); 95°C (10 min); [95°C (15 sec), 60°C (1 min)] 45X; 72°C (5 min).

What are some popular Hot Start PCR Options?

- AmpliTaq Gold™ DNA Polymerase, **chemically modified Taq (**Roche)
- Deep Vent™ DNA Polymerases, Pyrococcus species GB-D (NEB)
- HotStarTaq DNA Polymerase, modified recombinant 94 kDa Taq DNA polymerase, **chemically mediated hot start**, Q-Solution additive for GC rich templates. QIAGEN
- HotStart Taq a.k.a. FastStart Taq. **chemically modified Taq**, Sold by NEB, Qiagen, Life Technologies, Sigma, Promega
- Phusion Hot Start Flex DNA Polymerase, Pyrococcus-like enzyme fusion with a processivity-enhancing domain. Includes **aptamer-based hot start**. Standard HF Buffer or GC Buffer for GC-rich templates
- For longer and high fidelity PCR
  - Taq mixed with a thermostable, proofreading polymerase (up to 5 kb)
  - Expand™ High FidelityPLUS PCR, PwoSuperYield DNA Polymerase, Roche (long PCR)
- Alternative recombinant thermostable polymerases (20-40kb)
  - Pfu and Pfu Turbo, Pfu (Pyrococcus furiosus) DNA Polymerase
  - Platinum® Pfx DNA Polymerase, Thermococcus kodakoraensis KOD, **antibody based hot-start**, PCRx Enhancer, Invitrogen
  - KOD FX and KOD Plus, Thermococcus kodakaraensis polymerase uses **2 anti-KOD DNA polymerase antibodies for Hot Start** PCR, Toyobo
  - Q5® High-Fidelity DNA Polymerase, high fidelity amplification, fusion polymerase with Sso7d DNA binding domain, NEB
- One-Fusion high-speed-fidelity Polymerase, artificial polymerase enzyme (not Hot Start), GeneOn (>10 kb)
- PfuUltra II Fusion HS DNA Polymerase, PfuUltra DNA polymerase fusion with double-stranded DNA binding protein, **hotstart antibody**, Agilent (19 kb)
- LongAmp® Taq DNA Polymerase, a blend of Taq and Deep Vent™ DNA Polymerases (NEB)

Competitor's Hot Start Shortcomings

- Expensive
- Not general or all-purpose; assay specific optimization
- Not compatible with all thermostable polymerases
- Additional number(s) of components in Master Mix
  - Residual components may interfere with some applications

CleanAmp® Customer Targets

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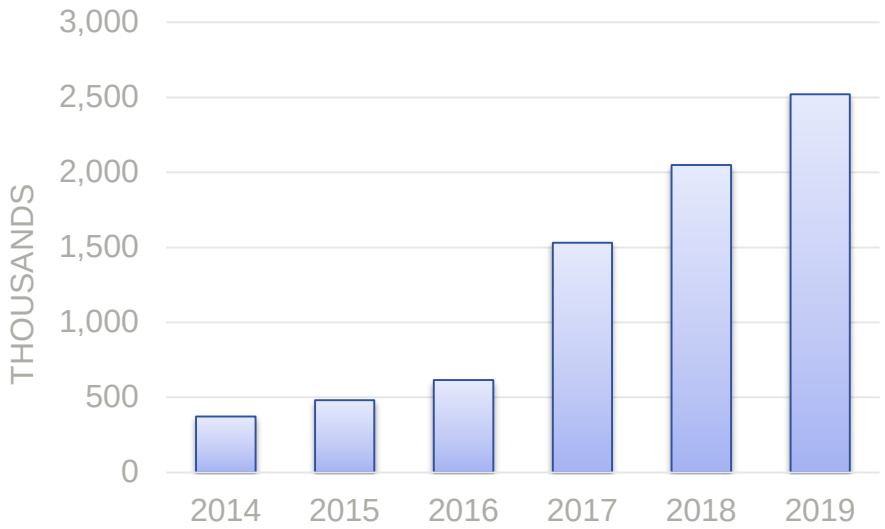
- IVD kit manufacturers
- Manufacturers of research and kit reagents
- Assay development companies
- Environmental testing labs
- Medical test labs
- Medical research centers
- University research labs
- Therapeutic and/or diagnostic biotechnology companies

Annual CleanAmp® Sales



- Main Asian Client: LifeRiver

CleanAmp® Conclusions

- CleanAmp® dNTPs provide exceptional results compared to other Hot Start options
- CleanAmp® dNTPs improve PCR performance relative to standard dNTPs for various targets of different lengths
- CleanAmp® dNTPs are compatible with, and improve PCR specificity of, many thermostable DNA polymerases
- CleanAmp® dNTPs can be employed in fast PCR cycling protocols
- CleanAmp® dNTPs enhance specificity and reaction efficiency in multiplex PCR allowing amplification of up to seven targets.
- CleanAmp® 7-deaza-dGTP improves the amplification of GC-rich targets and can be used with a variety of thermostable DNA polymerases
- CleanAmp® 7-deaza-dGTP improves sequencing of GC-rich targets with higher quality and longer reads
- polymerases.
- CleanAmp® dNTPs in One-Step RT-PCR 2X Master Mix outperforms other commercial RT-PCR kits and master mixes
- CleanAmp® dNTPs improve one-step RT-PCR specificity by introducing Hot Start control to both the RT and the PCR steps