

Gene expression analysis & ePCR

Optimized research from sample to result

Christophe Leroy, PhD

TSS Molecular Biology

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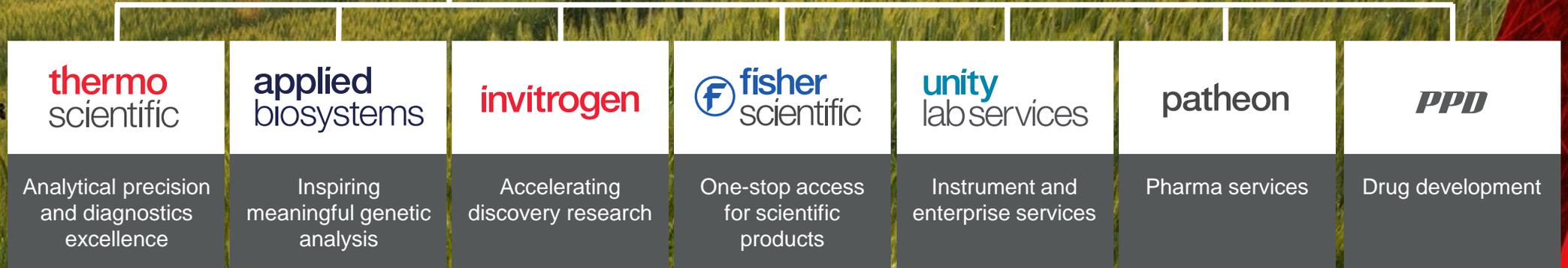
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Our leading scientific products, services and workflow solutions

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serving science



Overview of gene expression analysis workflows



RNA extraction

When you need high-quality, pure, intact RNA



ePCR & genotyping

When a yes or no answer is required about larger rearrangements, like homozygosity or heterozygosity, or in the presence of insertions or deletions



RNA quantification

When you run a gene expression experiment and do not get results, quantifying the RNA in your sample is useful for troubleshooting.



qPCR

When flexibility is required around number of samples, number of targets, and content selection for gene expression research



Reverse transcription

When there is a need to reverse-transcribe the most difficult RNA samples, such as those degraded during purification



Invitrogen™ Egel POWER SNAP / PLUS

When there's a need to load ePCR or to clone DNA

Gene expression analysis: RT-PCR workflow

RNA isolation and quantification



PureLink or MagMAX
RNA extraction kits



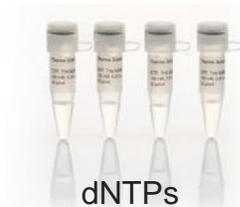
Invitrogen™ Qubit™ 4
Fluorometer or
Thermo Scientific™
NanoDrop™ One^C
spectrophotometer



Reverse transcription



SuperScript IV products



dNTPs



Random hexamers



DNA amplification



Invitrogen™ Platinum™ II
Taq products



Thermal cyclers
and PCR plastics



Nucleic acid electrophoresis



Invitrogen™ E-Gel™
Power Snap
Electrophoresis System



Invitrogen™ E-Gel™
Power Snap Plus
Electrophoresis System

Sample preparation

Selecting an RNA extraction method

Organic



Spin columns



Magnetic beads



Crude lysate



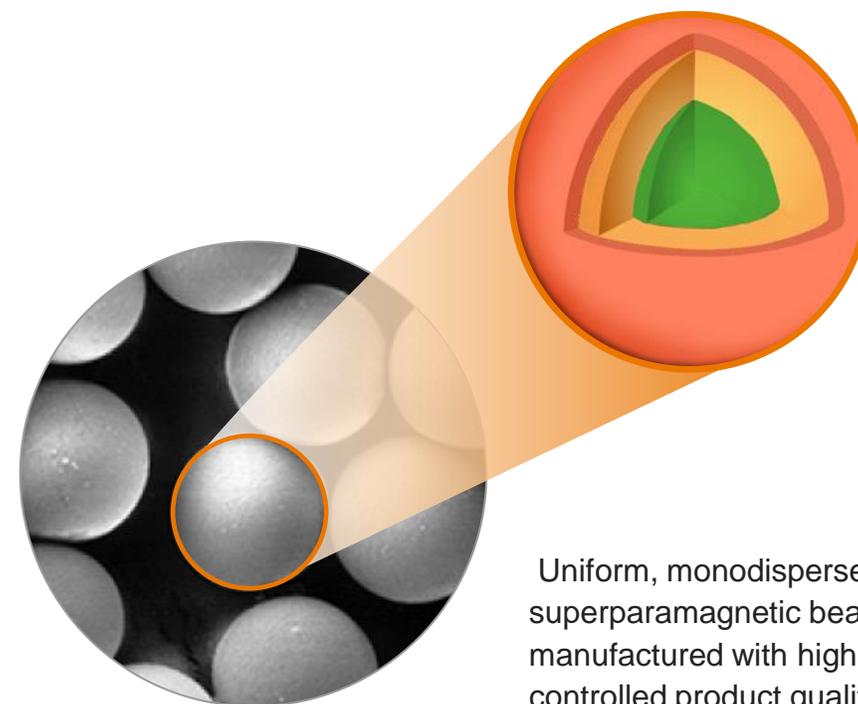
	Invitrogen™ TRIzol™ Reagent	Thermo Scientific™ GeneJET™ kits, Invitrogen™ PureLink™ kits	Applied Biosystems™ MagMAX™ kits	Invitrogen™ Cells-to-C _T ™ kits Invitrogen™ Superscript IV™ CellsDirect
Samples	Tissues, cells, plants, blood, and more	Cells, tissues, blood, plants, and more	Cells, blood, tissues, fixed slides, plants, and more	Cells only
Purity	Medium	High	Highest	Good enough
Ease of use	Hard	Easy	Automated	Easy
Hazardous	Yes	No	No	No
Throughput	Low	Medium	High—can be automated	High
Recommended for	Extraction of RNA from fatty tissues such as brain or kidney	Most medium-volume RNA extraction	Highest-purity RNA and/or high numbers of samples	qPCR from cells

Magnetic particle (bead) technology for extraction

Advantages of magnetic bead-based extraction

- **Binding efficiency**—the large surface area of a magnetic bead and thorough exposure to the target during mixing enables superior binding and washing efficiency
- **Improved elution**—efficient elution in small volumes
- **Greater sample range**—efficient removal of inhibitors from a wide range of samples with different viscosities
- **Versatility**—suitable for low- to high-throughput applications
- **Consistent yields**—less variation between runs, compared to columns; more reproducible
- **Faster process without centrifugation**
- **Less loss/greater capture**—get more of your nucleic acid/protein from your sample

Magnetic particle

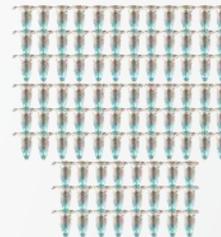


Uniform, monodisperse superparamagnetic beads, manufactured with highly controlled product qualities to help ensure the highest degree of reproducibility

Versatility through various throughput levels

KingFisher instruments provide throughput options that suit a wide variety of research needs

	Automation “light”	Semi-automation	Full automation
			
	Thermo Scientific™ KingFisher™ Duo Prime system*	Thermo Scientific™ KingFisher™ APEX system**	Thermo Scientific™ KingFisher™ Presto system**
Instrument size	Stand-alone compact benchtop	Stand-alone benchtop	Benchtop + robotic liquid handler
Throughput level	Low to medium ●	Medium to high ● ●	Ultrahigh ● ● ●
Samples per run/plate format	6–12 per run	96 or 24 per run	96 or 24 per run



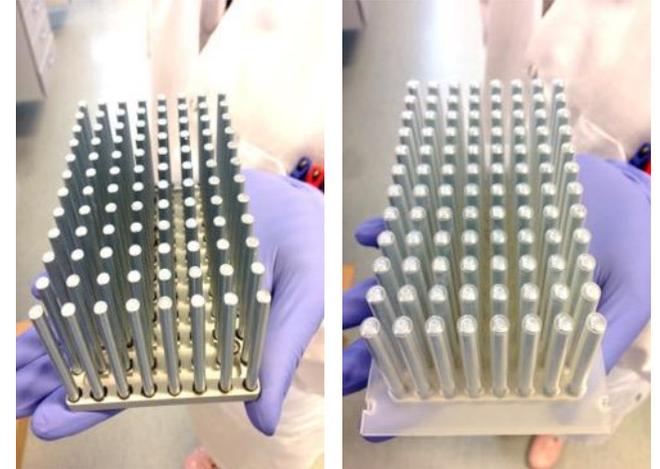
* For Research Use Only.

** For Research Use Only. Not for use in diagnostic procedures.

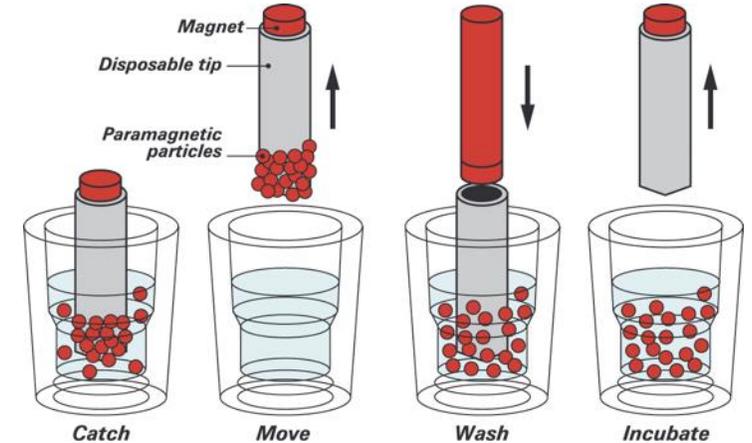
Thermo Scientific™ KingFisher™ instruments move the magnetic beads

Benefits

- ✓ Only the magnetic beads are transferred
- ✓ Contaminants are left behind
- ✓ The result is high-quality, concentrated nucleic acids and proteins
- ✓ Cross-contamination is eliminated



KingFisher magnetic rods and rods covered with plastic



Bead-based nucleic acid isolation solutions and plates

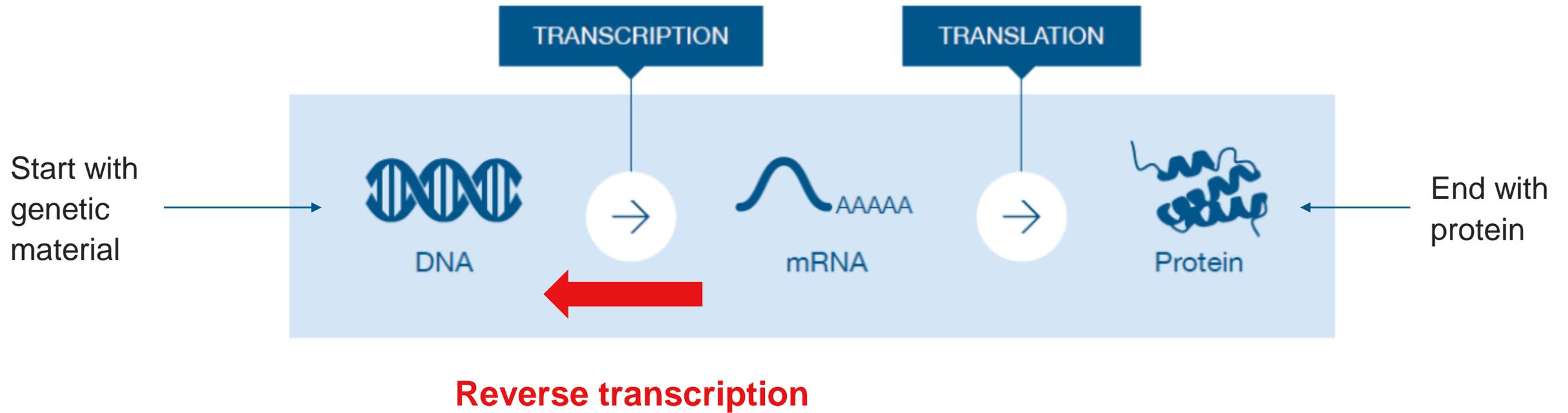
Sample type	Product	Protocol time	Input capacity	Maximum yield	Quantity	Cat. No.
Most sample types, including cells, blood, tissue, and yeast	Applied Biosystems™ MagMAX™ <i>mirVana</i> ™ Total RNA Isolation Kit	40 min	10 mg tissue, 5 x 10 ⁶ cells	Varies	96 preps	A27828
FFPE samples	Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit	4 hr total (1 hr hands-on)	Up to two 20 µm sections	Varies by tissue type	96 preps	A31881
Multiple, including urine, fecal, soil, saliva, stool, swabs	Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Kit	1 hr total time for 96 samples, 50 min for 12 samples	100 mg stool 400 µL of saliva and urine	Up to 500 µg of nucleic acid from 100 mg of stool	96 preps	A42357
Multiple, including viruses and gram-negative bacteria from blood, swabs, urine, and viral transport media (VTM)	Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	15 min hands-on time and <30 min extraction time with KingFisher instruments	200 µL to 2 mL of biofluids	≥50 viral copies	100 and 1,000 prep kits	A42352 (100 preps) A48310 (1,000 preps)
Multiple, including whole blood, saliva, buccal swabs, buffy coat, blood cards, tissue culture cells	Thermo Scientific™ KingFisher™ Ready DNA Ultra 2.0 Prefilled Plates	96 samples in 51 min on KingFisher Flex system, or 12 samples in 45 min on the KingFisher Duo Prime system	50–400 µL in 96-well plate >400 µL–2 mL in 24-well plate	1.5–4 µg (50 µL of blood) 2–12 µg (1 buccal swab)	24 or 96 rxn kit	A36586 (Flex) and A36584 (Duo Prime)

Questions



Reverse transcription

What is reverse transcription?



Synthesis of complementary DNA (cDNA) from an RNA template

Reverse transcriptase attributes

DNA polymerase activity

RNase H activity

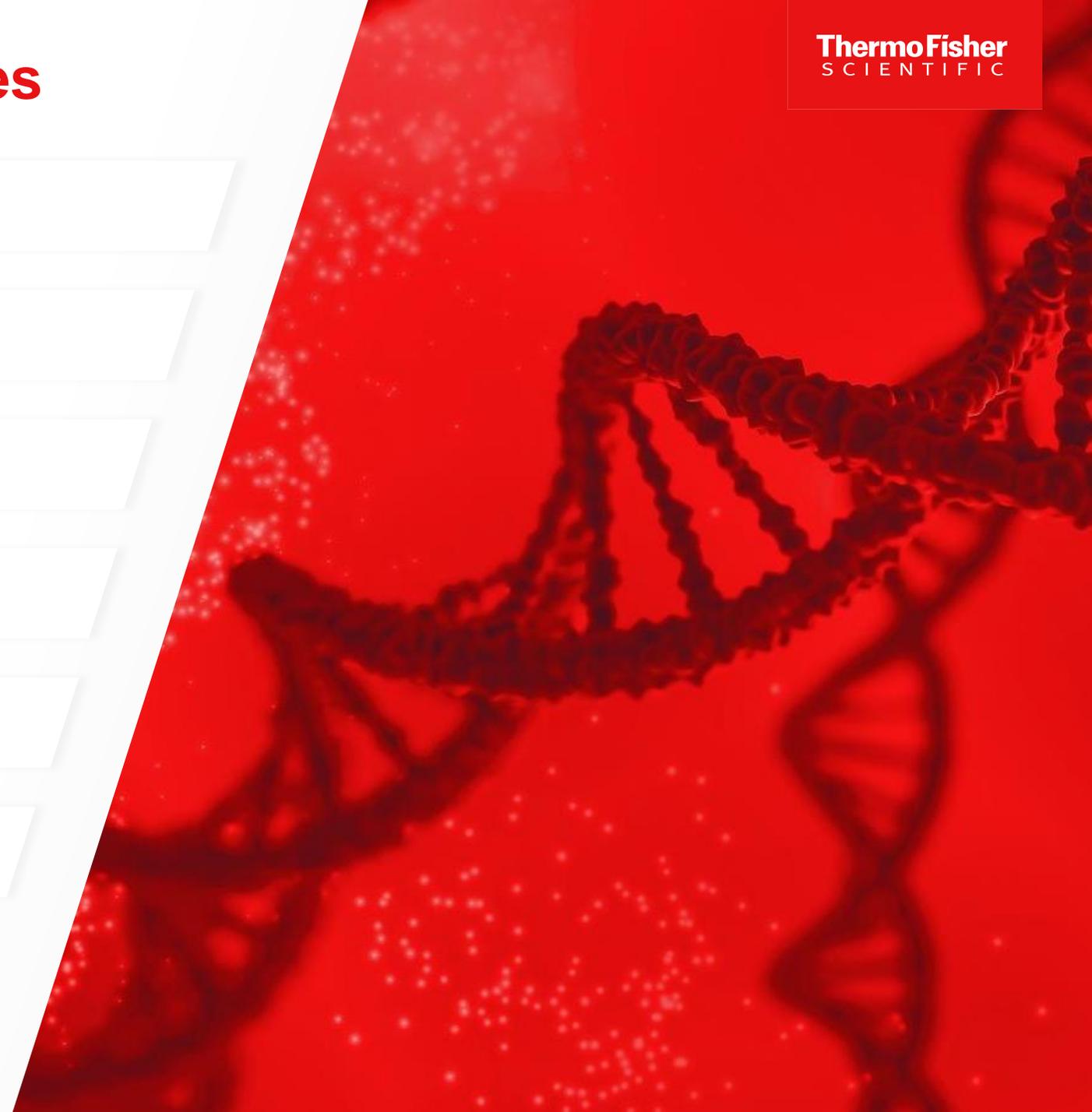
Thermostability

Sensitivity

Processivity

Terminal transferase activity

Fidelity



Discovery of reverse transcriptase

Discovered by
Howard Temin and David Baltimore

- Moloney murine leukemia virus (MMLV)
- Jointly awarded the Nobel Prize in 1975

Reverse transcriptases have been found
in many organisms

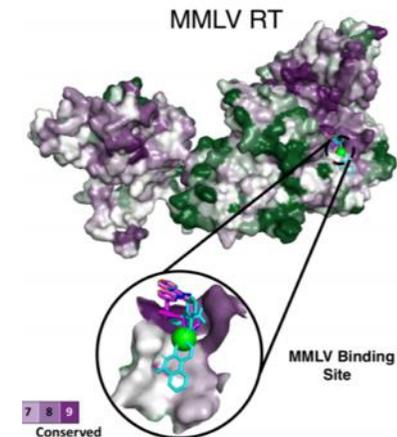
- HIV-1 reverse transcriptase
- AMV reverse transcriptase from avian myeloblastosis virus
- M-MLV reverse transcriptase from Moloney murine leukemia virus



Howard Temin



David Baltimore



Reverse transcriptase evolution

1

Wild-type RTs (MMLV RT and AMV RT)

- RNase H⁺
- Active at 37°C–42°C
- Good cDNA yields
- Efficient transcription up to 7 kb
- Good sensitivity

2

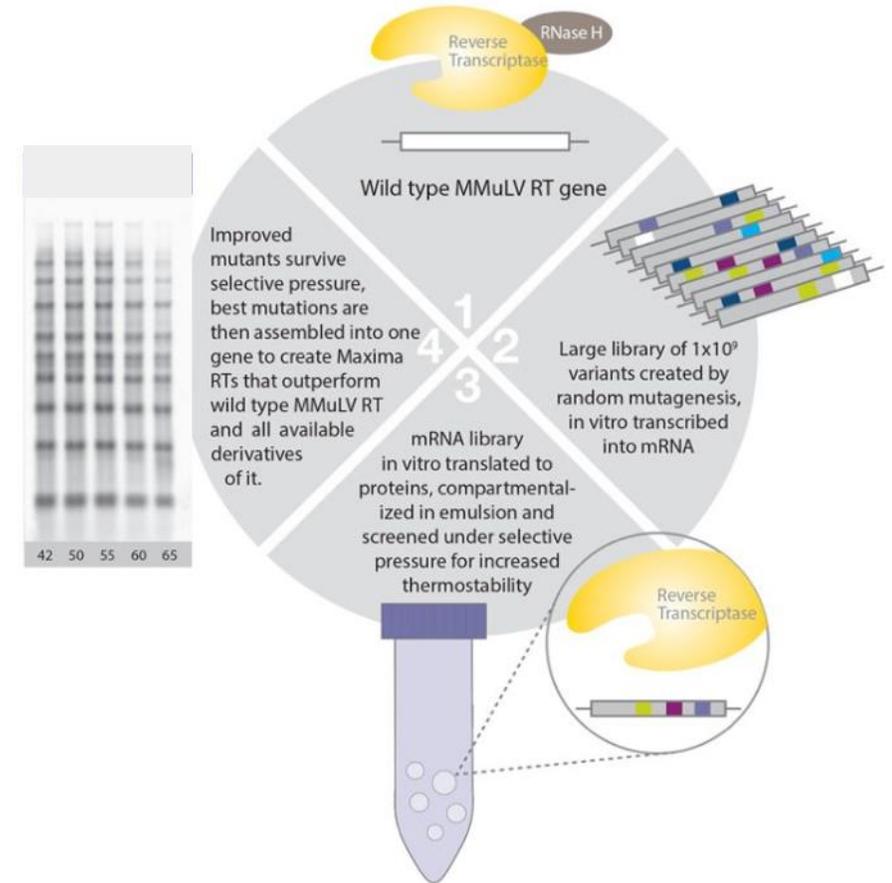
RNase H⁻ RT mutants

- RNase H⁻
- Active at 37°C–42°C
- Better cDNA yields
- Efficient transcription up to 13 kb
- Better sensitivity

3

Engineered RTs

- RNase H⁻ and RNase H⁺
- Active even at 65°C
- Best cDNA yields
- Efficient transcription up to 20 kb
- Best sensitivity



High processivity improves RT speed

RT Research Use Only, gDNA removal in 2 minutes

Traditional reverse transcription workflow with DNase I (105 min)



SuperScript IV VILO Master Mix workflow with ezDNase enzyme (27 min)



■ DNase treatment ■ DNase inactivation ■ Primer annealing ■ Reverse transcription ■ Reverse transcription termination

Invitrogen™ SuperScript™ IV VILO™ Master Mix provides a fast and easy protocol

Reverse transcriptase reagent formats

Invitrogen™ product formats

Stand-alone enzyme

- Cost-effective
- Comes with buffer
- Works with reagents from other sources

SuperScript™ IV Reverse Transcriptase



1st-strand cDNA synthesis

- Recommended for applications other than RT-qPCR
- Flexible format for optimization

SuperScript™ IV First-Strand Synthesis System



Master mix

- Convenient
- Time-saving
- Recommended for two-step RT-qPCR

SuperScript™ IV VILO™ Master Mix



One-step RT-PCR

- Endpoint PCR
- Virus detection
- Specific primers
- Fewer targets
- Fast protocol

SuperScript™ IV One-Step RT-PCR System



Direct RT

- Fast protocol
- No RNA isolation
- Cell lysis + RT
- Limited numbers of sample types

SuperScript™ IV CellsDirect cDNA Synthesis Kit



Questions

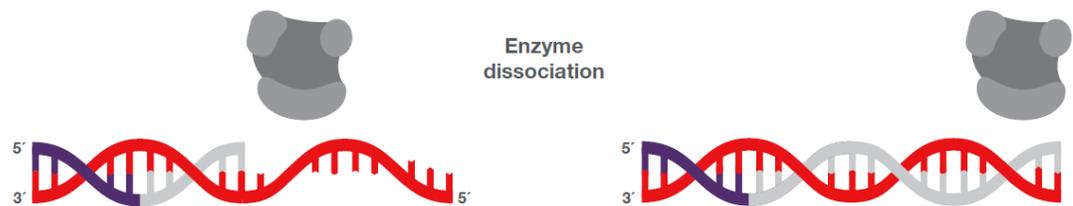


ePCR & Genotyping

Properties of DNA polymerases important for PCR

Processivity

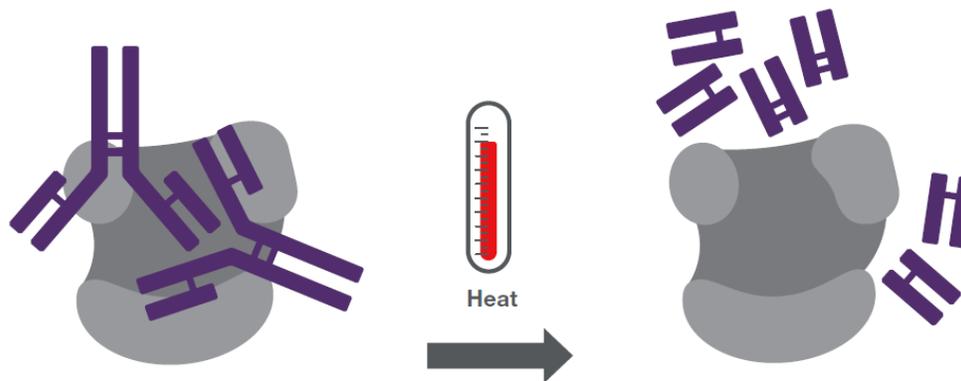
Measure of enzyme's nucleotide incorporation



- ➔ Speed
- ➔ Inhibitor tolerance
- ➔ Amplification length

Hot start

Enzyme activation at high temperature



- ➔ Specificity
- ➔ Hot-start PCR

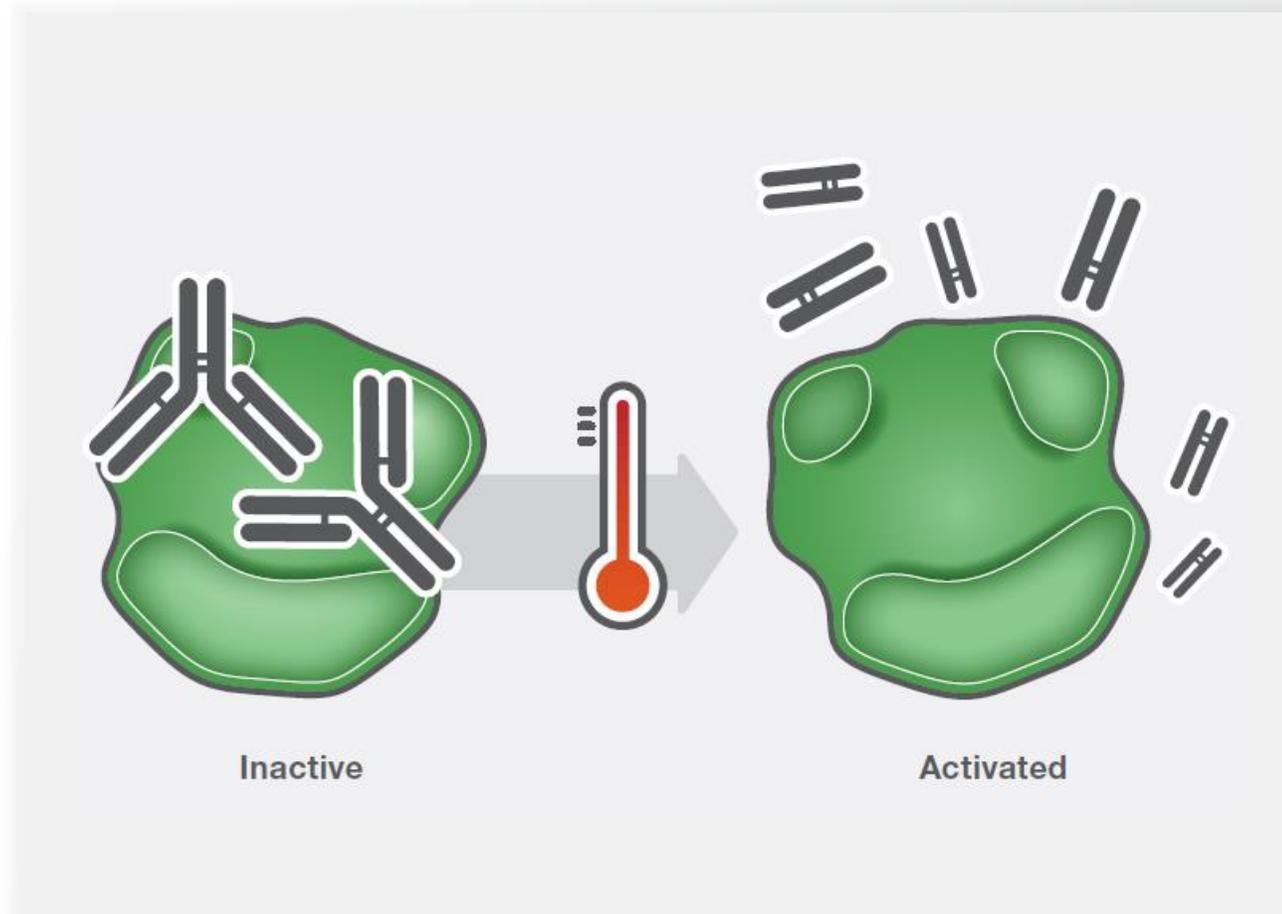
Key benefits of Platinum hot-start technology

Benefits

- ➔ Greater yield of the target amplicon
- ➔ Prevention of nonspecific amplification and primer degradation
- ➔ Convenience of room-temperature setup

How

- ➔ Proprietary antibodies bound to Invitrogen™ Platinum™ enzymes inhibit enzyme activity until the initial PCR denaturation step

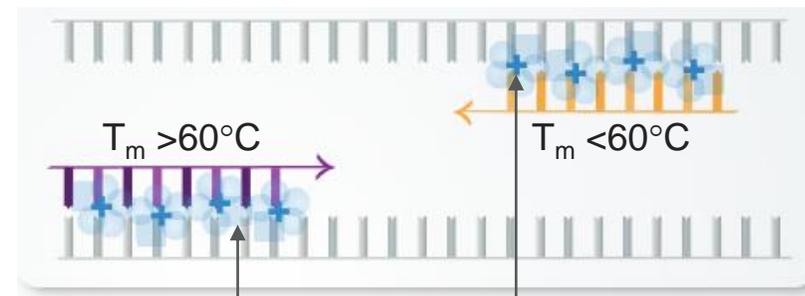


Skip primer annealing temperature calculation



Obstacle

Spending too much time optimizing annealing temperatures of each primer set



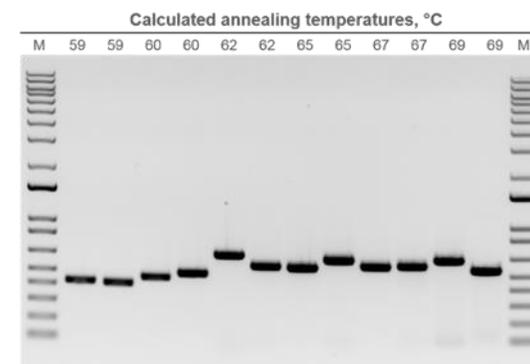
Isostabilizing component of the Platinum II buffers



Solution

Formulation that enables a universal annealing temperature for primers

- Enabled by an isostabilizing component of the Invitrogen™ Platinum™ II buffers
- Less PCR optimization required when working with multiple primer sets



One annealing temperature (60°C) for all primers

Platinum™ II *Taq* Hot-Start DNA Polymerase

Highlights:

- **Innovative buffer**—enables universal annealing protocol by isostabilizing primer-template duplex structures
- **Engineered *Taq* DNA polymerase**—allows fast cycling and resistance to common inhibitors
- **Platinum hot-start technology**—ensures superior specificity, sensitivity, and yields; allows for room temperature reaction setup
- **Optional green buffer**—reduces pipetting error with direct gel loading



thermofisher.com/platinumiiitaq



T_m calculator no longer needed!

Invitrogen™ Platinum™ SuperFi™ II DNA Polymerase

ThermoFisher
SCIENTIFIC

Highlights

- Exceptional >300x *Taq* fidelity
- Universal annealing temperature for different primer sets
- Co-cycling of PCR targets of different lengths
- Improved success in long-range PCR
- Efficient amplification of difficult targets
 - GC-rich PCR (supplementary enhancer not required)
 - High tolerance to common inhibitors
- High yield, specificity, and sensitivity



thermofisher.com/platinumsuperfi



T_m calculator no longer needed!

Key benefits of Phusion Plus DNA Polymerase



No more calculation of annealing temperature (T_m)—uses **universal annealing temperature** for all primers



Fewer **PCR runs**—can co-cycle targets of different lengths



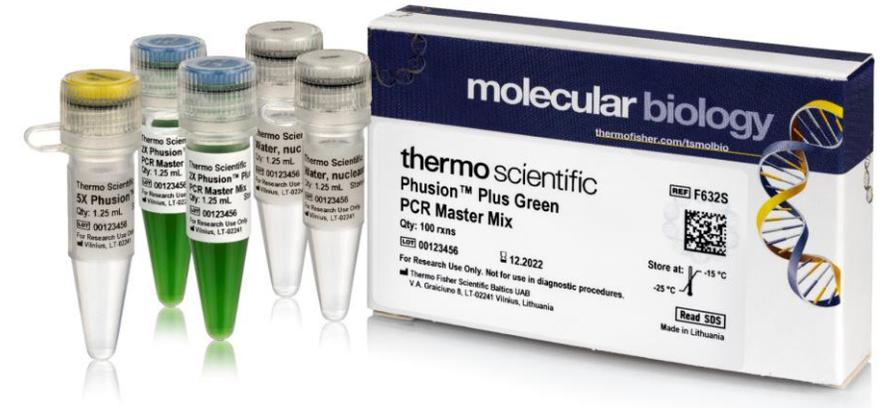
Improved PCR **sequence accuracy**—enzyme fidelity increases to >100x that of *Taq* enzyme



Efficient **GC-rich amplification**—new GC enhancer included



Suitable for **lab automation** setup—assembled reactions stable at room temperature for 24 hr



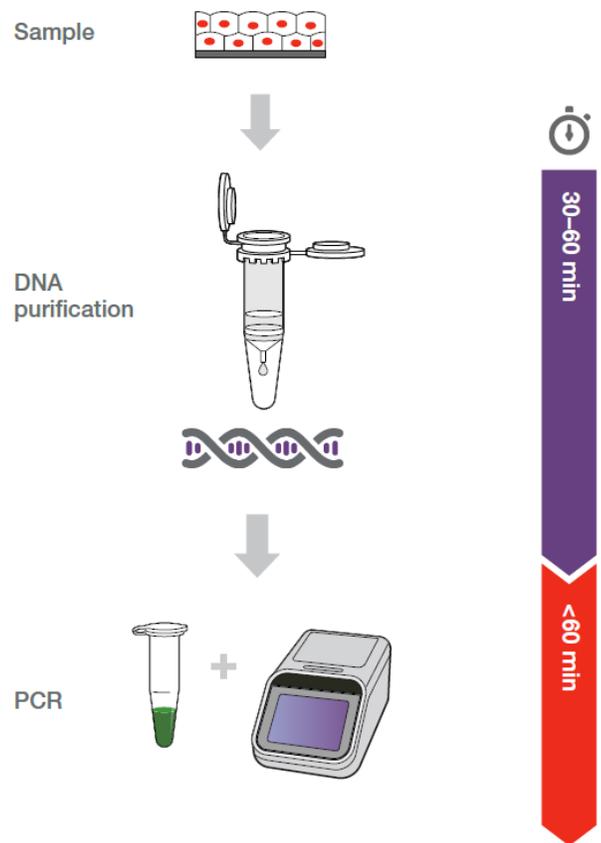
thermofisher.com/phusionplus



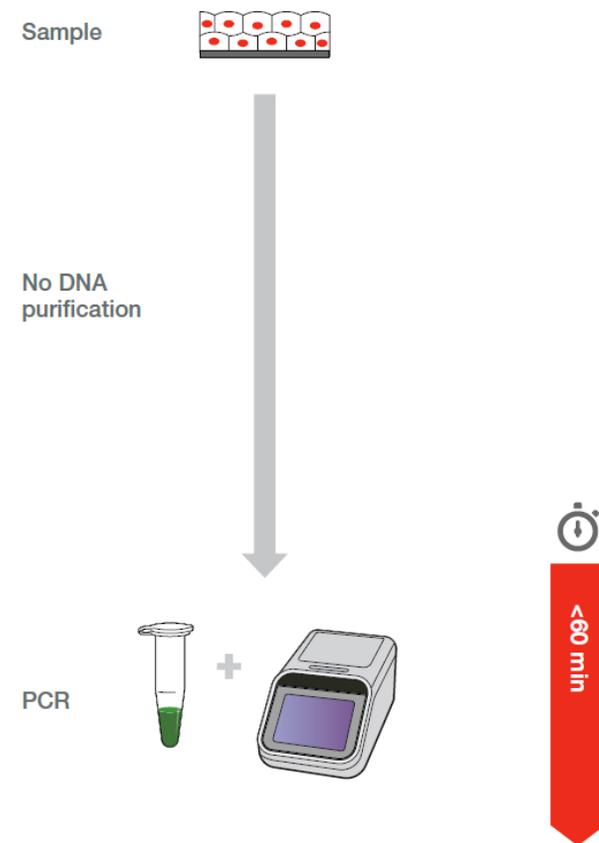
T_m calculator no longer needed!

Conventional PCR vs. Direct PCR Workflow

Conventional approach



Direct PCR approach



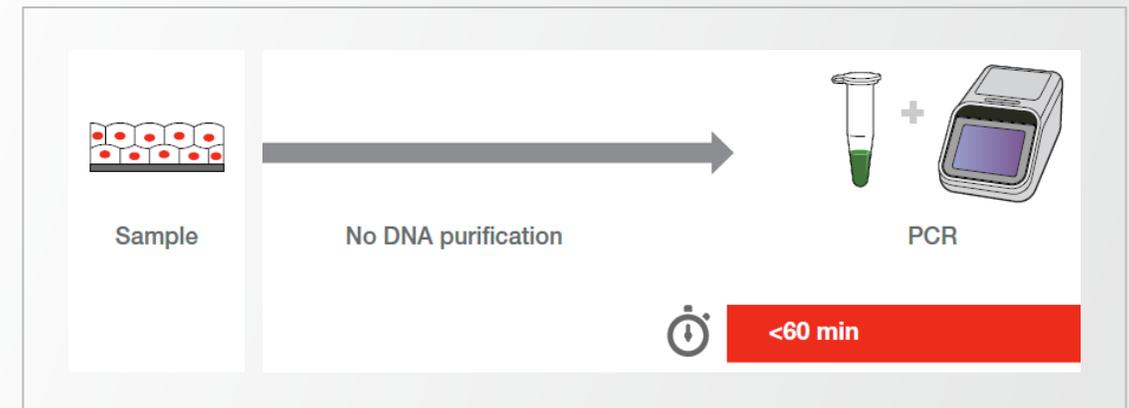
Platinum Direct PCR Universal Master Mix

Highlights

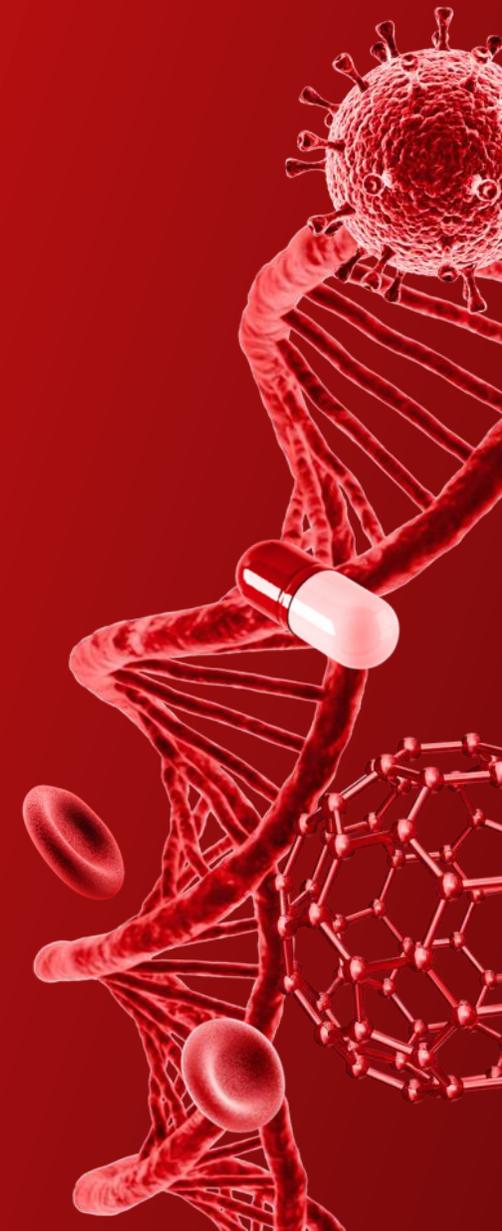
- Direct amplification from variety of samples
 - DNA purification not required prior to PCR
- Universal annealing temperature for different primer sets
- Co-cycling of targets of different lengths
- High yield, specificity, and sensitivity
- Efficient amplification of GC-rich sequences
- Multiplex PCR up to 5 targets



thermofisher.com/platinum-direct-pcr



Questions



Electrophoresis

Invitrogen™ E-Gel™ electrophoresis workflows

Platform

E-Gel™ agarose gels

Accessories

Imaging

Low throughput



For 11–22 wells



SYBR™ Safe/SYBR™ Gold stain

E-Gel Power Snap system

High throughput



For 48/96 wells



SYBR Safe stain

E-Gel Power Snap Plus system

Questions



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