rhAmp™ SNP Genotyping System

Accurate, affordable genotyping with the next evolution of PCR

Improve the precision of your PCR-based SNP genotyping with rhAmp SNP Genotyping technology. This technology combines a unique two-enzyme system and RNA-DNA hybrid primers to precisely interrogate target SNPs (Figure 1). Using IDT's universal reporter chemistry, rhAmp SNP Genotyping offers a simple, high performance genotyping solution at an affordable price.

Superior discrimination versus traditional methods

Blocked primers minimize non-specific amplification. The 3′ end of rhAmp primers incorporate a blocking group that prevents extension unless de-blocking occurs by RNase H2 enzyme cleavage.

Precision primer activation by RNase H2. rhAmp primers contain an RNA base near the 3′ end. RNase H2 enzyme recognizes this RNA base only when hybridized to its perfect complement, initiating primer cleavage and activation.

Highly discriminatory Taq DNA polymerase. rhAmp Genotyping features a novel Taq Polymerase, uniquely modified with increased sensitivity toward allelic mismatches.

benefits

Generate the highest level of performance with greater than 99.5% call accuracy for over 90% of assays tested

Interrogate SNPs in difficult sequence regions with amplicon sizes as small as 40 bp

Validate markers affordably using the smallest pack size commercially available

Ensure confidence in your data with gBlocks® Gene Fragments as control templates

Discover more at
www.idtdna.com/rhAmp-Genotyping

rhAmp™ SNP Genotyping mechanism

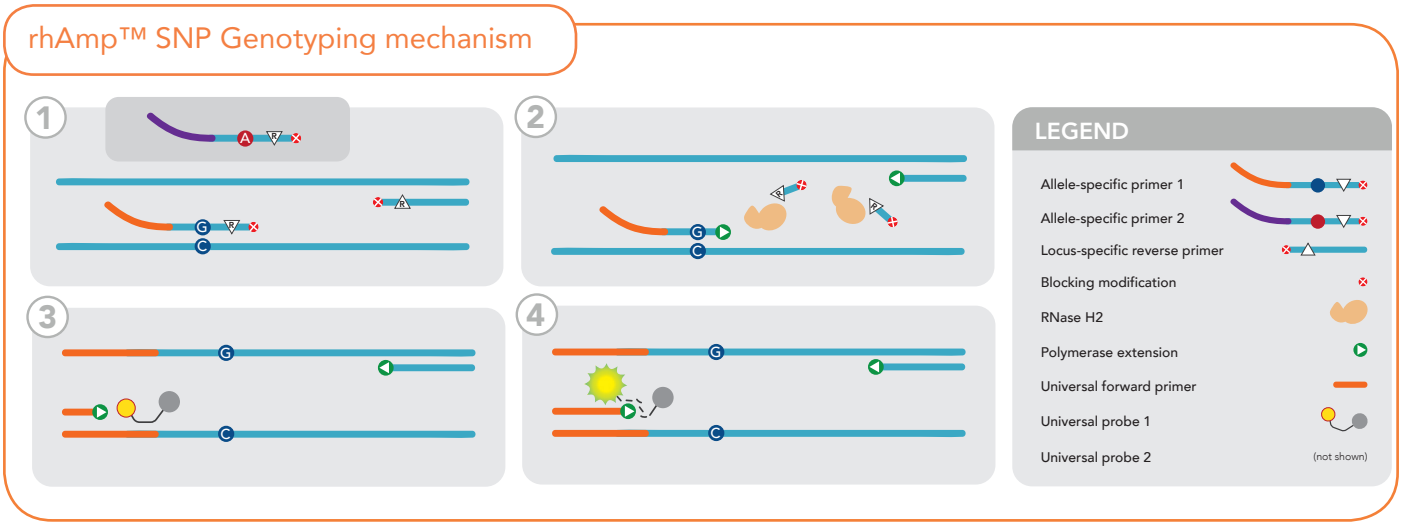

Figure 1. Schematic representation of a rhAmp™ SNP Genotyping PCR cycle. All components needed to measure both alleles are combined in a single reaction before cycling. (**1**) Both allele-specific primers query the SNP locus. (**2**) RNase H2 enzyme cleaves the primers that are perfectly annealed to the target sequence, removing the RNA base and 3′ blocking modification, which allows extension by the Taq Polymerase. (**3**) During the first two amplification cycles, a tail sequence is incorporated into the amplicon that is subsequently recognized by a probe-based, universal reporter system. (**4**) Polymerase extension leads to degradation of the probe and signal generation.

Complete workflow

The rhAmp SNP portfolio includes all components needed to successfully generate high quality genotyping data on any commonly available real-time PCR instrument.

- Predesigned assay collection for human genotyping
- Custom assay design tool for novel human SNPs or other species
- Master mix and universal reporter system with optional reference dye

Comprehensive collection of predesigned assays

The rhAmp predesigned assay library contains designs for >10 million human SNPs with minor allele frequencies >1%. rhAmp SNP Assays demonstrate consistently high performance with >99.5% call accuracy on more than 90% of tested assays. The assay library includes:

- Approximately 70,000 coding SNPs
- 330,000 common SNPs found in RefSeq
- Functionally validated assays targeting SNPs in genes involved in absorption, distribution, metabolism, and excretion (ADME) of pharmaceuticals

Custom assay design tool

The rhAmp Genotyping Design Tool can be used to design assays for SNPs not currently included in the human, predesigned assay library or for SNPs in any other species. The IDT custom assay pipeline drives a high design rate by accommodating short amplicons (as small as 40 bp) and can deliver designs in challenging sequence regions that other design tools cannot address. Custom assay design is easily performed by submitting target SNP sequences for any genome in FASTA format.

Fast, efficient, single-tube chemistry

The rhAmp SNP Genotyping System employs a single-tube assay setup and universal PCR cycling conditions to allow for easy automation (Figure 2). The system delivers accurate genotypes after only 90 minutes of cycling time. Reactions can be performed on a real-time qPCR instrument, or on a standard thermal cycler followed by analysis on a fluorescence detection instrument. A choice of reagent mixes is provided to accommodate different reference dye requirements of various qPCR instruments.

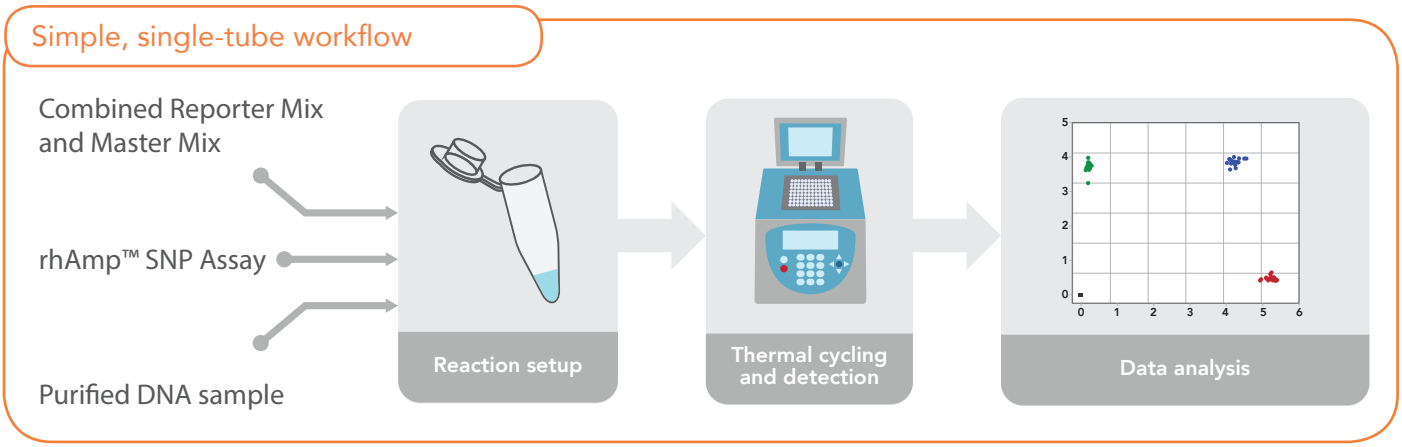

Figure 2. A simple, single-tube reaction chemistry supports streamlined lab processes. All reagents are combined in the initial reaction setup, which is then stable for up to 24 hr at room temperature before cycling. rhAmp SNP Genotyping is compatible with common qPCR platforms.

Greater signal-to-noise ratio for higher confidence calls

rhAmp SNP Assays deliver an increased signal-to-noise ratio over traditional 5'-nuclease chemistry (Figures 3–4). In rhAmp technology, RNA-DNA hybrid primers are blocked at the 3' end to prevent primer-dimer formation and non-specific amplification, thus eliminating unbalanced consumption of reaction components. Moreover, rhAmp universal reporter sequences are optimized to provide robust and consistent signals, leading to improved cluster separation for higher confidence genotyping calls (Figure 3).

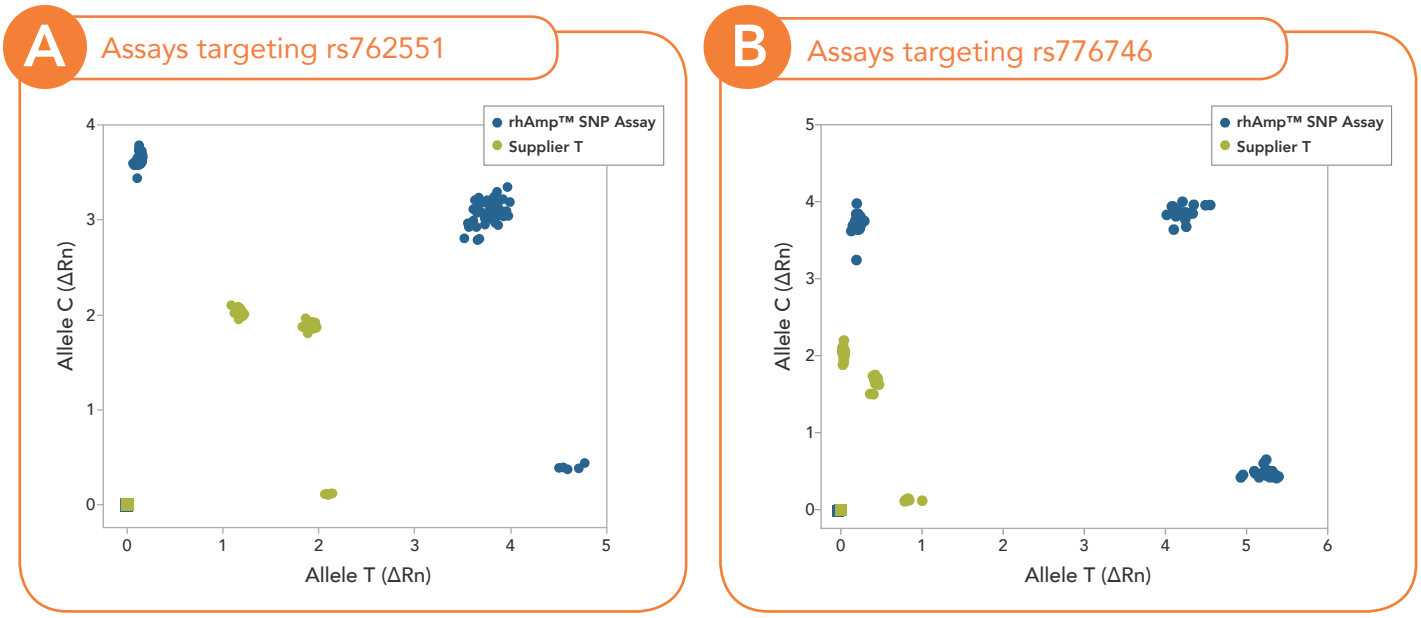

Figure 3. rhAmp™ SNP Genotyping achieves a higher fluorescent signal and improved cluster separation than assays from Supplier T. Allelic discrimination plots for SNPs located in ADME genes (**A**) *CYP1A2* (rs762551) and (**B**) *CYP3A5* (rs776746) show higher fluorescent signal for rhAmp ADME SNP Assays (blue circles) than 5'-nuclease assays from Supplier T (green circles). Human gDNA from 91 individuals (Coriell Institute) was analyzed using 3 ng of gDNA in 5 µL reactions. Analysis was performed using QuantStudio™ 7 Flex Real-Time PCR System software (Thermo Fisher). ADME = absorption, distribution, metabolism, and excretion.

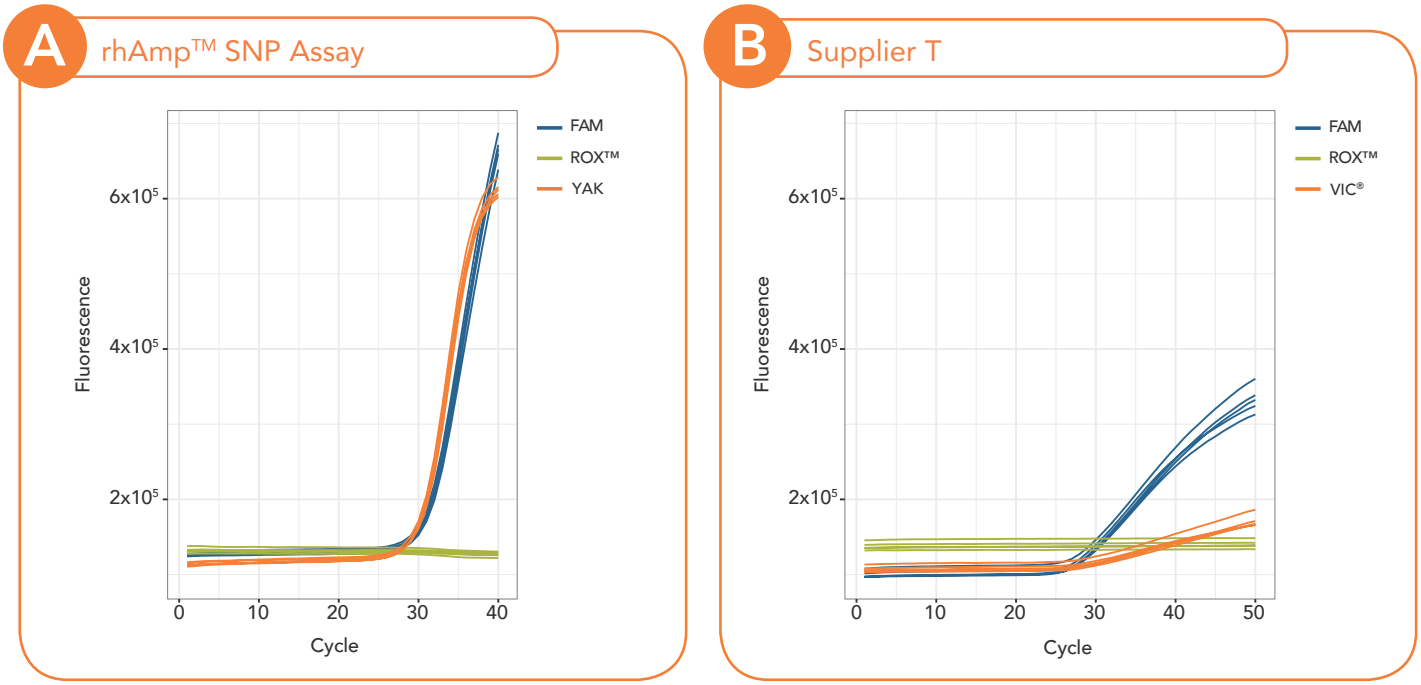

Figure 4. rhAmp™ SNP Assay demonstrates higher signal in fewer overall cycles than Supplier T. Multicomponent plots of genotyping assays targeting ADME gene *CYP3A5* (rs776746) demonstrate higher and more uniform endpoint fluorescence for the (**A**) rhAmp ADME SNP Assay compared with (**B**) 5'-nuclease genotyping assay from Supplier T. Human gDNA from 5 individuals with heterozygous genotype (Coriell Institute) was analyzed using 3 ng of gDNA in 5 µL reactions. Data was generated using the QuantStudio™ 7 Flex Real-Time PCR System (Thermo Fisher). Cycling conditions followed manufacturer's recomendations: 40 cycles for rhAmp SNP genotyping and 50 cycles for the 5'-nuclease genotyping assay from Supplier T. Yakima Yellow® (YAK) signal was detected using the VIC® channel without the need for recalibration.

Ordering information

The rhAmp SNP Genotyping System includes your choice of SNP assays coupled with a genotyping master mix and universal reporter mix. Master mix and reporter mixes are available in matching sizes for easy ordering. SNP and ADME Assays can be ordered in skirted tube or matrix rack format. Synthetic control templates can be easily ordered online during the checkout process.

rhAmp SNP Assays and rhAmp ADME SNP Assays

rhAmp SNP Assays are universal-reporter–based, SNP genotyping assays with RNase-H2–cleavable primers that are generated using our custom assay design tool for improved accuracy and specificity. rhAmp ADME SNP Assays targeting genes responsible for the absorption, distribution, metabolism, and excretion of pharmaceutical compounds are also experimentally validated.

Product	Package size	Reactions*	Concentration
rhAmp™ SNP Assay or rhAmp™ ADME SNP Assay	XS	100	20X
	S	750	20X
	M	2500	80X
	L	6000	80X

*Number of reactions is based on a 10 µL reaction volume.

rhAmp Genotyping Master Mix

This 2X master mix solution contains the required enzymes and components necessary for activation and amplification of rhAmp SNP Assays.

Product	Total volume (mL)	Unit size	Catalog #
rhAmp™ Genotyping Master Mix	0.5	1 x 0.5 mL	1076014
	5	1 x 5 mL	1076015
	10	2 x 5 mL	1076016
	25	5 x 5 mL	1076017
	50	1 x 50 mL	1076018

rhAmp Reporter Mix

This 40X universal reporter probe mix, available with or without the reference dye required by certain instruments, is for use with rhAmp Genotyping Master Mix and rhAmp SNP Assays.

Product	Unit size (µL)	Catalog #
rhAmp™ Reporter Mix w/Reference	25	1076020
	250	1076021
	500	1076022
	1250	1076023
	2500	1076024
rhAmp™ Reporter Mix	25	1076025
	250	1076026
	500	1076027
	1250	1076028
	2500	1076029

gBlocks® Gene Fragments (optional)

gBlocks Gene Fragments are double-stranded, sequence-verified, genomic blocks available for use as controls.

www.idtdna.com/rhAmp-Genotyping

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

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