TaqMan® Sample-to-SNP™ Kit

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *Applied Biosystems TaqMan® Sample-to-SNP™ Kit Protocol* (PN 4402136). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare the samples

1 Lyse the samples

a. Obtain the samples for lysis according to Table 1 on page 2.

b. Thoroughly mix the **Lysis Solution**.

c. Add one volume of **Lysis Solution** to each 1.5-mL microcentrifuge tube or well of the plate containing the sample. See Table 1 on page 2 for volumes based on sample type and sample quantity.

d. Pipette up and down to mix the **Lysis Solution** and the sample in each tube or plate well.

e. Cap the tubes or seal the plate with an adhesive cover, then centrifuge the tubes or plate briefly.

2 Incubate the samples

Incubate the samples according to sample type as shown in Table 1 on page 2. For samples incubated at 95 °C, cool at room temperature for 30 seconds before stabilizing the DNA (step 3).

3 Stabilize the DNA

a. Thoroughly mix the **DNA Stabilizing Solution**.

b. Open the tube or uncover the plate.

c. Add one volume of **DNA Stabilizing Solution** to each tube or well of the plate containing sample. See Table 1 on page 2 for volumes based on sample type.

d. Pipette up and down to mix the solution and the sample in each tube or plate well.

e. Cap the tubes or seal the plate with an adhesive cover, then centrifuge the tubes or plate briefly.

4 (Optional) Store the sample lysates

You can store the sample lysate at 4 °C. For longer storage, you can store the sample lysate at –20 °C. Before use, mix the sample lysate.

5 Preamplify the samples or perform fast genotyping

Note: If the amount of sample is limited, preamplification may be necessary. Applied Biosystems recommends a test study without preamplification to determine if the fluorescence signal is sufficient for good allelic discrimination.

If you:

- Need to preamplify the samples, go to "Preamplify the samples" on page 3.
- Do *not* need to preamplify the samples, go to "Perform fast genotyping" on page 4.

Table 1 Preparation of sample lysate according to sample type

Sample type	Sample input	Volume of Lysis Solution (µL)	Incubation Temperature for 3 minutes (°C)	Volume of DNA Stabilizing Solution (µL)	Notes
Blood (freshly drawn, EDTA, citrate, heparin)	2 µL	20	Room temperature	20	—
Blood, cells, saliva (blood cards, FTA paper)	3-mm punch	50	95	50	—
Cell culture suspension	2 µL	20	Room temperature	20	—
Buccal swab	1	400	95	400	1. Twist the swab from the cap. 2. Rotate and firmly brush the swab using 20 strokes throughout the inside cheek.‡ 3. Use a 1.5-mL screw-capped tube and immerse the swab into the Lysis Solution. 4. Rotate the swab 5 times. 5. Lift the swab above the Lysis Solution, then press the swab against the side of the tube to squeeze out its contents. 6. Dispose of the swab. 7. Continue preparing the sample (see "Incubate the samples" on page 1).
Rat or mouse tail	1 to 2 mm	50	95	50	—
Tissue	1 to 2 mm	50	95	50	—
Hair with follicle	2 to 3 follicles	50	95	50	Ensure that the hair and follicles are immersed in Lysis Solution.
Leaf punch or needle	3-mm leaf punch or 2- to 3-mm needle	50	95	50	—
Seed chip	2- to 3-mm seed chip or 2 to 5 mg pulverized seed	50	95	50	—
Formalin-fixed paraffin-embedded tissue (FFPE)	2 to 3 pieces of a 10-µm section	200	95	200	• Before the lysis step, you can deparafinize the FFPE tissue using a standard protocol. • Ensure that the FFPE is immersed in Lysis Solution.

‡ The swab may be air-dried, re-capped, then stored at room temperature.

Preamplify the samples

1 Prepare the preamplification mix

a. Thoroughly mix the TaqMan® PreAmp Master Mix (PN 4391128) by swirling the bottle.

b. Thaw any frozen TaqMan® assay reagents by placing them on ice. Vortex then centrifuge the tubes briefly.

c. In a 1.5-mL microcentrifuge tube, combine equal volumes of 20× TaqMan® SNP Genotyping assays of up to 100 assays, then dilute the combined assays in 1× TE buffer to a final concentration of 0.2×.

d. For each sample, combine in a PCR tube the components as shown in the table below. Multiply the volume for one reaction component in the table below by the total number of reactions, then add that volume of the component to the tube.

Preamplification of sample

Component for preamplification	Volume for one 10-µL reaction (µL)	Volume for one 50 -µL reaction (µL)
TaqMan® PreAmp Master Mix, 2×	5	25
0.2× Pooled assay mix	2.5	12.5
Sample lysate	1.2	6
DNase-free water	1.3	6.5
Total	10	50

2 Set up the run method

Set up the run method using the following conditions:

- Thermal-cycling conditions:

Stage	Step	Temp	Time
Holding	DNA polymerase activation	95 °C	10 min
Cycling (14 cycles)	Denature	95 °C	15 sec
	Anneal/Extend	60 °C	4 min

- Run speed: **9600 emulation** or **standard**
- Reaction volume: **10** µL or **50** µL

3 Load and run the plate

Load the reaction plate into the thermal cycler, then start the run.

4 Dilute the preamplified samples

After the run, dilute the preamplified products 1 to 20 in 1× TE Buffer.

Go to "Perform fast genotyping" on page 4.

5 (Optional) Stopping point

For long-term storage, you can store the preamplified sample (undiluted or diluted) at –20 °C.

Perform fast genotyping

1 Prepare the PCR mix

Note: To prepare for fast genotyping, refer to "Before you begin fast genotyping" in the *TaqMan® Sample-to-SNP™ Kit Protocol* (PN 4402136).

a. Thoroughly mix the **TaqMan® GTXpress™ Master Mix** before use. Avoid creating bubbles.

b. Thaw on ice, vortex, then centrifuge TaqMan® assay reagents, sample lysate, or genomic DNA.

c. In an appropriate tube, combine the reaction mix components shown in the table below. Determine the reaction volume appropriate to the instrument and plate. Multiply the volume for one reaction component in the table below by the total number of reactions, then add that volume of the reaction component to the tube.

PCR reaction mix components

Component	Volume for 5-µL PCR reaction (µL/well)	Volume for 10-µL PCR reaction (µL/well)	Volume for 25-µL PCR reaction (µL/well)
TaqMan® GTXpress™ Master Mix (2×)	2.50	5.0	12.50
TaqMan® genotyping assay mix (20×)‡ §	0.25	0.5	1.25
DNase-free water	1.25	2.5	6.25
Total	4.0	8.0	20.0

‡ For ease of use, dilute 40× and 80× Assay Mixes to 20× working solutions with 1× TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Use DNase-free water.

§ If you use Custom TaqMan® Probes and Sequence Detection Primers rather than a TaqMan® genotyping assay, Applied Biosystems recommends 900 nM for primers and 200 nM for probes.

d. Cap, vortex the tubes, then briefly spin down the contents to eliminate air bubbles.

e. Into each well of a reaction plate, pipette the PCR reaction mix volume (4, 8, or 20 µL) appropriate to your plate.

f. Observe the purple tracking dye in each well to ensure uniform filling.

g. Seal the plate with a MicroAmp™ clear adhesive film.

h. Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles.

i. Remove the clear adhesive film from the plate, then pipette one control or diluted DNA sample into the appropriate well(s).

j. Add sample lysate, diluted preamplification product, or DNA control to each well according to the volume of the PCR reaction volume:

Volume of sample lysate, diluted preamplification product, or DNA control (µL/PCR reaction)		
5-µL reaction	**10-µL reaction**	**25-µL reaction**
1.0	2.0	5.0

k. Cover the plate with MicroAmp™ Optical Adhesive Film or MicroAmp™ Optical Caps.

l. Centrifuge the plate briefly to spin down the contents and eliminate air bubbles.

m. Use a MicroAmp™ Optical Film Compression Pad when you use a MicroAmp™ Optical Adhesive Film. Ensure that the gray, nonreflective side of the pad faces down on the plate. Also use a compression pad with a MicroAmp™ Optical 96-well plate on the 7900HT Real-Time PCR System.

2 Perform the PCR

a. Set up the following run conditions:

IMPORTANT! These conditions are optimized for use only with TaqMan® genotyping assays on the PCR systems specified in the table below and with the instruments and reaction plates specified in the *TaqMan® Sample-to-SNP™ Kit Protocol*, Appendix A.

Stage	Step	Temp	Time (StepOne™, StepOne Plus™,7900)	Time (Fast 7500)	Time (7300, 7500)
Holding	DNA polymerase activation	95 °C	20 sec	20 sec	20 sec
Cycling (40 cycles)	Denature	95 °C	3 sec	3 sec	15 sec
	Anneal/Extend	60 °C	20 sec‡	30 sec	60 sec

‡ Use the minimum extension time available on your instrument but no less than 20 seconds.

- Run speed: **Fast** or **Standard**
- Reaction volume: **5**, **10**, or **25** µL

b. Load the reaction plate into the thermal cycler, then start the run.

3 Read the plate

After PCR amplification, you perform an endpoint plate read on a real-time PCR instrument.

IMPORTANT! For all real-time PCR instruments, reagardless of default temperature, use a post-read temperature of 25 °C when using the **TaqMan® GTXpress™ Master Mix**.

The SDS software uses the fluorescence measurements from each well made during the plate read, then plots R_n (signal) values. The software determines which alleles are in each sample for later allelic discrimination analysis. Refer to the allelic discrimination section of the appropriate instrument user guide for instructions on how to use the system software to perform the plate read and analysis.

4 Analyze the results

a. If allelic discrimination is not possible because of low fluorescence, return the plate to the thermal cycler, then perform another 10 PCR cycles using the following thermal-cycling conditions change according to run conditions in previous table:

Stage	Step	Temp	Time (StepOne™, StepOne Plus™,7900)	Time (Fast 7500)	Time (7300, 7500)
Cycling 10 cycles	Denature	95 °C	3 sec	3 sec	15 sec
	Anneal/Extend	60 °C	20 sec‡	30 sec	60 sec

‡ Use the minimum extension time available on your instrument but no less than 20 seconds.

b. Perform allelic discrimination analysis again to see if the results improve. For optimal results, never exceed a total of 50 cycles.

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