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Platinum® Multiplex PCR Master Mix

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For detailed instructions and troubleshooting information, refer to the *Platinum® Multiplex PCR Master Mix User Guide* (Part no. 4463722). You can download a PDF version of the protocol at **www.appliedbiosystems.com**.

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Platinum® Multiplex PCR Master Mix User Guide* (Part no. 4463722). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1 Prepare for PCR

a. Choose a thermal cycler and a reaction plate or tubes.

b. Calculate the number of reactions required to perform each assay, including no-template controls (NTC). Add an excess of 10% volume to account for pipetting loss.

Note: You can run multiple PCR reactions on one plate, but include controls for each run.

2 Design, verify, and synthesize the primers

IMPORTANT! Avoid GC-rich targets if possible, otherwise keep amplicon sizes <300 bp and avoid simultaneous amplification of targets with >70% or <25% GC content. GC Enhancer is provided for targets with >65% GC content.

a. Choose an amplicon site, and then choose primer pairs that do not amplify pseudogenes or other related genes using one of these software options:

- Primer Express® software
- Primer3Plus software (available at **www.bioinformatics.nl**) with the following variables:

Variable	Definition
Primer size	21-30 nt
Primer T_m	59-65 °C
Primer GC%	40-60%
Concentration of monovalent cations	50 mM
Annealing oligo concentration	50 nM
Mispriming/Repeat Library	Based on your assay
Remaining variables	Default or User-defined

b. Perform in-silico PCR at **http://genome.ucsc.edu** and check the following:

- The PCR product is unique.
- The size of the product differentiates from adjacent amplicons.
- The T_m for each primer is between 59-65 °C.
- No homology exists at the primer targeting sites.

Note: Retrieve this information on the **Details** page after performing **BLAT**.

c. Synthesize primers with a reliable oligo vendor and verify concentrations with a spectrophotometer after receipt.

d. Experimentally verify the primers in singleplex PCR and by electrophoresis before combining for multiplex PCR.

Note: Only use primers that generate a single, clean band of correct size for multiplex PCR.

e. Store primers in small aliquots at -20 °C and avoid repeated freezing and thawing.

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3 Prepare the PCR reaction

a. Combine all primers for the multiplex reaction in one tube and adjust the reaction volume to a final concentration of 0.5 µM per primer with 0.1× TE buffer.

b. Store aliquots of primer mix at -20 °C for up to a year or 4 °C for up to two months.

c. Thaw primer mixes, templates, GC enhancer (optional), and master mix.

d. Mix all tubes by inverting ~10 times and spinning briefly. Place all tubes on ice.

IMPORTANT! Avoid generating bubbles when mixing.

e. Prepare the PCR reaction mix as follows, using your number of calculated reactions (on page 1):

Component	Volume per 50–µL reaction	Final Concentration
Platinum® Multiplex PCR Master Mix, 2×	25	1×
10× Primer mix (0.5 µM each)	5–10†	50–100 nM per primer
GC Enhancer (Optional)	0–10‡	0–20%
PCR-grade water	Variable- Bring final volume to 50 µL minus template DNA volume	—

† Use 10 µl of primer mix (final concentration: 100 nM of each primer) when less than 0.1 µg of DNA is used. Lowering the primer concentration reduces potential secondary products.

‡ For targets with 65 to 75% GC, use 6 µL of the GC Enhancer. For targets with >75% GC, start with 10 µL. If increased specificity is required, add 1 to 2 µL GC Enhancer.

f. Combine all components in an appropriate tube, mix well by inverting, and centrifuge briefly.

g. Dispense appropriate volumes of the PCR mix to the bottom of the wells of your reaction plate or PCR tubes.

h. Adjust the final reaction volume to 50 µL with 0.1–0.2 µg of DNA template or with PCR-grade water (for the NTC).

i. Seal the plate or cap the tubes, mix well by inverting, and briefly centrifuge.

4 Run the PCR reaction

a. Configure the run method per your instrument user manual.

b. Use the following parameters based on your analysis method:

Stage	Step	Temperature	Time
Holding	Activation of Platinum® Multiplex PCR Master Mix	95 °C	2 min
Cycles (30–40)† (25–40)‡	Denature	95 °C	30 sec
	Anneal	60 °C	90 sec
	Extend	72 °C	60 sec/kb of the largest amplicon
Holding	Final Extension	72 °C† or 60 °C‡	10 min† or 30 min‡
Holding	Final Hold	4 °C	∞

† For agarose gel electrophoresis, Lab901, or Agilent 2100 Bioanalyzer

‡ For capillary electrophoresis

c. Load your reaction plate and start the run.

5 Analyze the PCR products

Analyze your results with one of the following methods:

- Lab901 or Agilent® 2100 Bioanalyzer™ instrument, or capillary electrophoresis. Refer to your instrument user manual for instructions.
- Agarose gel electrophoresis:
  - **a.** Choose the appropriate agarose concentration according to the total number of amplicons, the size difference between adjacent amplicons and the amplicon size range in each reaction.
  - **b.** Load and run gels according to the manufacturer's instructions to achieve maximum separation of adjacent amplicons.
  - **c.** Take pictures of gels with a UV transilluminator.

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