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Protocols

LUX™ Primers
Instrument Protocols

For Using LUX™ Primers on the:

ABI PRISM® 7700
Bio-Rad iCycler™
Corbett Rotor-Gene 3000™
DNA Engine Opticon® 2
ABI PRISM® 7900
Roche LightCycler®
ABI PRISM® 7000
Cepheid Smart Cycler®
Stratagene Mx3000P™

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Instrument Protocols and Settings for LUX™ Primers

Specific protocols have been developed for using LUX™ Primers on real-time qPCR instruments. These protocols all use Platinum® Quantitative PCR SuperMix-UDG, available from Invitrogen (Cat. nos. 11730-017 and 11730-025).

For real-time quantitative RT-PCR, we recommend the SuperScript™ III Platinum® One-Step qRT-PCR Kit (Cat. nos. 11732-020 and 11732-088). See the kit manual for a protocol using LUX™ Primers.

Trademark Information

ABI PRISM® 7700

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the ABI PRISM® 7700. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 50 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer	10 uM	1 µl	50 µl	200 nM
Unlabeled primer	10 uM	1 µl	50 µl	200 nM
ROX Reference Dye	50X	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
Unlabeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
LUX™ labeled primer (2)	10 uM	1 µl	50 µl	200 nM
Unlabeled primer (2)	10 uM	1 µl	50 µl	200 nM
$MgCl_2$	50 mM	3 µl	150 µl	3 mM*
ROX Reference Dye	50X	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the ABI PRISM® 7700.

Recommended

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction	50	2 min	1		Yes
UDG inactivation/template denaturation	95	2 min	1		Yes
Denaturation	95	15 sec	40–50		Yes
Hybridization /Elongation	60	30 sec			Yes
Melting curve analysis	60–95			19 min	In the ramp

Alternative

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		Yes
UDG inactivation/template denaturation	95	2 min	1		Yes
Denaturation	95	15 sec	40–50		Yes
Hybridization	55	30 sec			Yes
Elongation	72	30 sec			Yes
Melting curve analysis	60–95			19 min	In the ramp

*Uracil DNA glycosylase-mediated carry-over prevention

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Protocol

1. Program the ABI PRISM® 7700 as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl of the master mix to each tube, strip well, or well of the qPCR plate.
4. Add 10 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/strip/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the ABI PRISM® 7700 user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Choose the **Single Reporter** plate format when opening a new plate to read.
- To set up the plate, define all of the wells that contain reactions and then select the appropriate **Dye Layer** (**FAM** or **JOE**) for each **Sample**, **Standard**, or **NTC**.
- Note that as long as you select a well with at least one dye, you can always change the dye layer parameter after analysis. Unselected wells will not be read.
- You can manually choose the cycling **Threshold** setting in the **Amplification Plot** after a reading.
- By default, the baseline **Start Cycle** is 6 and the **End Cycle** is 15, but you should change these to reflect the actual baseline of your specific amplification profile.
- To analyze the melting curve, go to the **File menu** and select **Export > Multicomponent** to create a multicomponent file. Then open the multicomponent file using the **Dissociation Curve** software program from ABI.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017	100 reactions
	11730-025	500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020	100 reactions
	11732-088	500 reactions

Roche LightCycler®

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the Roche LightCycler®. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 20 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (20 µl)	34 rxns (20 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	10 µl	340 µl	1X
LUX™ labeled primer	10 uM	1 µl	34 µl	500 nM
Unlabeled primer	10 uM	1 µl	34 µl	500 nM
Platinum® *Taq* DNA Polymerase	5 U/µl	0.12 µl	4 µl	1.2 U*
BSA (nonacetylated, ultrapure)	5 mg/ml	1 µl	34 µl	250 ng/µl
Autoclaved distilled water	—	to 18 µl	to 612 µl	—

*Final concentration includes Platinum® *Taq* from Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the LightCycler®.

Recommended

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		No
UDG inactivation/template denaturation	95	2 min	1		No
Denaturation	94	5 sec	40–50		No
Hybridization	55	10 sec			Single acquire
Elongation	72	10 sec			No
Melting curve	95	0 sec			
	55	15 sec			
	95	0 sec		0.1°C/sec	Continuous
	40	0 sec			

*Uracil DNA glycosylase-mediated carry-over prevention.

Protocol

1. Program the LightCycler® as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 18 µl of the master mix to each capillary.
4. Add 2 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the capillary.
5. Gently mix and make sure that all components are at the bottom of the tube. Centrifuge briefly.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

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Tips and Recommendations for Programming the Instrument

Follow the instructions in the LightCycler™ user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- The LightCycler can only be used with FAM-labeled LUX™ Primers.
- FAM is acquired in the F1 channel.
- Multiplex reactions are not available on the LightCycler™.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017 11730-025	100 reactions 500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020 11732-088	100 reactions 500 reactions

Bio-Rad iCycler™

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the Bio-Rad iCycler™. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 50 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer	10 uM	1 µl	50 µl	200 nM
Unlabeled primer	10 uM	1 µl	50 µl	200 nM
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

Cycling Program

See the following page for tips and recommendations on programming the iCycler™.

Recommended

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
Cycle 1: UDG reaction*	50	2 min	1		Yes
Cycle 2: UDG inactivation/template denaturation	95	2 min	1		Yes
Cycle 3: 1 Denaturation	95	10 sec	40–50		At 50°C: yellow camera
2 Hybridization	50	30 sec			
3 Elongation	72	30 sec			
Cycle 4: Hold	95	1 min			
Cycle 5: Hold	55	1 min			
Cycle 6: Melting curve	55	10 sec	80	0.5°C/cycle after 2 cycles	Green camera

*Uracil DNA glycosylase-mediated carry-over prevention.

Protocol

1. Program the Bio-Rad iCycler™ as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl of the master mix to each tube or plate well.
4. Add 10 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

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Tips and Recommendations for Programming the Instrument

Follow the instructions in the Bio-Rad iCycler™ user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- We recommend selecting **Experimental Plate** under **Select well factor source:**. Do not use the external well factor plate for calibration.
- Make sure that the first two cycles are programmed as separate cycles and not as two steps within a single cycle. The iCycler will automatically calibrate at or above 90°C immediately preceding the first cycle containing a temperature above 90°C, and this will kill the uracil DNA glycosylase.
- The iCycler™ must be calibrated for the specific dye used (FAM or JOE). Even though the software will list all the dyes while setting up a plate, this does not mean that the instrument has been calibrated for these dyes. To view the dyes for which the instrument is calibrated, go to the **C:\Program Files\Bio-Rad\iCycler\Ini** folder and open the **RME.ini** file in a text editor such as Notepad. In the file, you should see (for example):

  [FAM-490]

  490/20X_!_530/30M=4.021013e+003

  This indicates that the instrument has been calibrated for FAM.

  Refer to the iCycler™ manual or call Bio-Rad technical service for help with calibrating the dyes.
- For monoplex reactions, we recommend using just one dye type per plate as the data will look better for analysis.
- Multiplex reactions are currently not recommended on the iCycler™.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017 11730-025	100 reactions 500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020 11732-088	100 reactions 500 reactions

ABI PRISM® 7000

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the ABI PRISM® 7000. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 50 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer	10 uM	1 µl	50 µl	200 nM
Unlabeled primer	10 uM	1 µl	50 µl	200 nM
ROX Reference Dye	50X	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
Unlabeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
LUX™ labeled primer (2)	10 uM	0.5 µl	25 µl	200 nM
Unlabeled primer (2)	10 uM	0.5 µl	25 µl	200 nM
$MgCl_2$	50 mM	3 µl	150 µl	3 mM*
ROX Reference Dye	50X	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the ABI PRISM® 7700.

Recommended

Action	Temp (°C)	Time	Cycles
UDG reaction*	50	2 min	1
UDG inactivation/template denaturation	95	2 min	1
Denaturation	95	15 sec	40–50
Hybridization /Elongation	60	30 sec	
Hold	60	30 sec	
Melting curve analysis (select **Dissociation Curve** checkbox)	60		

*Uracil DNA glycosylase-mediated carry-over prevention

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Cycling Program, continued

Alternative

Action	Temp (°C)	Time	Cycles
UDG reaction*	50	2 min	1
UDG inactivation/template denaturation	95	2 min	1
Denaturation	95	15 sec	40–50
Hybridization	55	30 sec	
Elongation	72	30 sec	
Hold	60	30 sec	
Melting curve analysis (select **Dissociation Curve** checkbox)	60		

*Uracil DNA glycosylase-mediated carry-over prevention

Protocol

1. Program the ABI PRISM® 7000 as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl of the master mix to each tube, strip well, or well of the qPCR plate.
4. Add 10 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/strip/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the ABI PRISM® 7000 user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Check the light bulb intensity of the instrument monthly, as instructed in the user manual. The intensity value should be >100. If the intensity is too low, data acquisition will be adversely affected.
- When using both FAM- and JOE-labeled primers on the same plate but in different wells (i.e., in monoplex), you should select **both** FAM and JOE as the dye for all wells. In general, for monoplex reactions, we recommend using just one dye per plate because the analysis will look better.
- Use the following filters for each dye type: FAM—Filter A, JOE—Filter B, ROX—Filter D.
- To improve the quality of the LUX™ signal, you can increase the amount of ROX to 2.5 µl per 50-µl reaction.
- You can manually choose the cycling **Threshold** setting in the **Amplification Plot** after a reading.
- By default, the baseline **Start Cycle** is 6 and the **End Cycle** is 15, but you should change these to reflect the actual baseline of your specific amplification profile.
- The default parameters for analyzing the melting curve are acceptable.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017	100 reactions
	11730-025	500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020	100 reactions
	11732-088	500 reactions

Corbett Rotor-Gene 3000™

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the Corbett Rotor-Gene 3000™. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. Note that the 50-µl final reaction volume is suitable for 36-position rotors, and the 25-µl final reaction volume is suitable for 72-position rotors.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	Single rxn (25 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	12.5 µl	1X
LUX™ labeled primer	10 uM	1 µl	0.5 µl	200 nM
Unlabeled primer	10 uM	1 µl	0.5 µl	200 nM
Autoclaved distilled water	—	to 40 µl	to 20 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (50 µl)	Single rxn (25 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	12.5 µl	1X
LUX™ labeled primer (1)	10 uM	0.5 µl	0.25 µl	100 nM
Unlabeled primer (1)	10 uM	0.5 µl	0.25 µl	100 nM
LUX™ labeled primer (2)	10 uM	1 µl	0.5 µl	200 nM
Unlabeled primer (2)	10 uM	1 µl	0.5 µl	200 nM
$MgCl_2$	50 mM	3 µl	1.5 µl	3 mM*
Autoclaved distilled water	—	to 40 µl	to 20 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the Corbett Rotor-Gene 3000™.

Recommended

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		No
UDG inactivation/template denaturation	95	2 min	1		No
Denaturation	95	5 sec	40–50		No
Hybridization /Elongation	60	10 sec			Yes
Melting curve analysis	60–95			1°C/step (default: wait 60 sec on first step and 5 sec/step after)	Yes

Alternative

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		No
UDG inactivation/template denaturation	95	2 min	1		No
Denaturation	95	5 sec	40–50		No
Hybridization	55	10 sec			Yes
Elongation	72	10 sec			No
Melting curve analysis	60–95			1°C/step (default: wait 60 sec on first step and 5 sec/step after)	Yes

*Uracil DNA glycosylase-mediated carry-over prevention.

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Protocol

1. Program the Corbett Rotor-Gene 3000™ as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. The 50-µl final reaction volume is suitable for 36-position rotors, and the 25-µl final reaction volume is suitable for 72-position rotors. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl (36-position rotor) or 20 µl (72-position rotor) of the master mix to each tube.
4. Add 10 µl (36-position rotor) or 5 µl (72-position rotor) of sample template to each reaction vessel and cap the tube. The sample template may be 100 pg to 1 µg of genomic DNA or cDNA generated from 1 pg to 1 µg of total RNA.
5. Gently mix and make sure that all components are at the bottom of the tube.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the Corbett Rotor-Gene 3000™ user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Select the checkbox to automatically perform calibration for FAM or JOE dye before each run at 60°C.
- For analysis, select **Linear Scale** and then **Autoscale**. The default parameter should be **Dynamic Tube/α Slope Correct**. Then select **Autofind Threshold**.
- Optionally, you can select **Ignore First Cycle**.
- The default parameters are acceptable for analyzing the melting curve.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017	100 reactions
	11730-025	500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020	100 reactions
	11732-088	500 reactions

Cepheid Smart Cycler®

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the Cepheid Smart Cycler®. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 20 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (20 µl)	50 rxns (20 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	12.5 µl	625 µl	1X
LUX™ labeled primer	10 uM	0.5 µl	25 µl	200 nM
Unlabeled primer	10 uM	0.5 µl	25 µl	200 nM
Autoclaved distilled water	—	to 18 µl	to 900 µl	—

Cycling Program

See the following page for tips and recommendations on programming the Smart Cycler®.

Action	Temp (°C)	Time	Cycles	Acquisition
UDG reaction*	50	2 min	1	No
UDG inactivation/template denaturation	95	2 min	1	No
Denaturation	95	15 sec	40–50	No
Hybridization	50	30 sec		Yes
Elongation	72	30 sec		No
Melting curve analysis	60–95			Yes

*Uracil DNA glycosylase-mediated carry-over prevention.

Protocol

1. Program the Cepheid Smart Cycler® as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 18 µl of the master mix to each tube (use the 25-µl reaction tubes specific for the SmartCycler®).
4. Add 2 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube.
5. Gently mix and make sure that all components are at the bottom of the tube. Centrifuge briefly.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the Cepheid Smart Cycler® user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Use the 25-µl reaction tubes specific for this instrument.
- Multiplex reactions are currently not recommended on the Smart Cycler®.
- For each run, keep all channels on.
- Use the default parameters for analysis.

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Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017 11730-025	100 reactions 500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020 11732-088	100 reactions 500 reactions

DNA Engine Opticon® 2

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on MJ Research's DNA Engine Opticon® 2. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 50 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer	10 uM	1 µl	50 µl	200 nM
Unlabeled primer	10 uM	1 µl	50 µl	200 nM
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
Unlabeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
LUX™ labeled primer (2)	10 uM	1 µl	50 µl	200 nM
Unlabeled primer (2)	10 uM	1 µl	50 µl	200 nM
$MgCl_2$	50 mM	3 µl	150 µl	3 mM*
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the DNA Engine Opticon® 2.

Recommended

Action	Temp (°C)	Time	Ramp time	Acquisition
1 UDG reaction*	50	2 min	2°C/sec	No
2 UDG inactivation/template denaturation	95	2 min	2°C/sec	No
3 Denaturation	95	15 sec	2°C/sec	No
4 Hybridization /Elongation	60	30 sec	2°C/sec	No
5 Plate read			0.2°C/sec	1 sec + plate read
6 Go to line 3 for 49 times				No
7 Incubation	95	1 sec	2°C/sec	No
8 Melting curve	60–95	1 sec		Read every 0.2°C
9 Incubation	25	30 min		

*Uracil DNA glycosylase-mediated carry-over prevention.

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Cycling Program, continued

Alternative

Action	Temp (°C)	Time	Ramp time	Acquisition
1 UDG reaction*	50	2 min	2°C/sec	No
2 UDG inactivation/template denaturation	95	2 min	2°C/sec	No
3 Denaturation	95	15 sec	2°C/sec	No
4 Hybridization	60	30 sec	2°C/sec	No
5 Elongation	72	30 sec	2°C/sec	No
6 Plate read			0.2°C/sec	1 sec + plate read
7 Go to line 3 for 49 times				No
8 Incubation	95	1 sec	2°C/sec	No
9 Melting curve	60–95	1 sec		Read every 0.2°C
10 Incubation	25	30 min		

*Uracil DNA glycosylase-mediated carry-over prevention.

Protocol

1. Program the DNA Engine Opticon® 2as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl of the master mix to each tube or qPCR plate well (white tubes/plates are preferred).
4. Add 10 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the DNA Engine Opticon® 2 user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Be sure to select all wells in which there is a reaction. The reaction type can be changed after acquisition.
- For monoplex reactions: Select **Single** and then describe the plate: 50 µl, MJ white plate, Dye 1: FAM and Dye 2: FAM.
- For multiplex reactions: Select **Both** and then describe the plate: 50 µl, MJ white plate, Dye 1: FAM and Dye 2: JOE.
- For Quantitation analysis: Select **Autoscale**, **Smooth**, **Subtract baseline**, and **Average over cycle range** (cycle range: 1–50), and select the threshold manually.
- For Melting Curve analysis: Display **-dI/dT** and select **Subtract baseline**, **Global minimum**, and a temperature range of 60–60. Peak location boundaries should be 75–90.
- The JOE reading is done in the TET channel. You may need to recalibrate the machine to clean up the data. The recalibration protocol is included in the Opticon 2 manual.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017 11730-025	100 reactions 500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020 11732-088	100 reactions 500 reactions

Stratagene Mx3000P™

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the Mx3000P™. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 50 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer	10 uM	1 µl	50 µl	200 nM
Unlabeled primer	10 uM	1 µl	50 µl	200 nM
ROX Reference Dye (diluted 1/10)	Dilute 1/10	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (50 µl)	50 rxns (50 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	25 µl	1250 µl	1X
LUX™ labeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
Unlabeled primer (1)	10 uM	0.5 µl	25 µl	100 nM
LUX™ labeled primer (2)	10 uM	0.5 µl	25 µl	100 nM
Unlabeled primer (2)	10 uM	0.5 µl	25 µl	100 nM
$MgCl_2$	50 mM	3 µl	150 µl	3 mM*
ROX Reference Dye (diluted 1/10)	Dilute 1/10	1 µl	50 µl	1X
Autoclaved distilled water	—	to 40 µl	to 2000 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the Mx3000P™.

Action	Temp (°C)	Time	Cycles	Acquisition
UDG reaction*	50	2 min	1	No
UDG inactivation/template denaturation	95	2 min	1	No
Denaturation	95	15 sec	40–50	No
Hybridization	60	30 sec		Yes (at the end)
Elongation	72	30 sec		No
Denaturation	95	1 min		
Melting curve analysis	55–95	30 sec at 55°C and at 95°C		Yes (all)

*Uracil DNA glycosylase-mediated carry-over prevention.

Continued on the next page

Protocol

1. Program the Mx3000P™ as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 40 µl of the master mix to each tube or plate well.
4. Add 10 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the Stratagene Mx3000P™ user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- For a good signal with JOE-labeled primers, change the acquisition gain for the JOE filter from 1 X to 4X or 8X.
- The ROX Reference Dye should be diluted up to 1/10 prior to addition to the master mix to enable accurate pipetting.
- For quantitative analysis, select **Adaptive Baseline Algorithm**, **Amplified Base Threshold**, and **3pt Moving Average** as the default parameters. If there is too much background, adjust the baseline manually.

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017	100 reactions
	11730-025	500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020	100 reactions
	11732-088	500 reactions

ABI PRISM® 7900

The following real-time quantitative PCR settings and protocol are designed for using LUX™ Primers on the ABI PRISM® 7900. See the LUX™ Fluorogenic Primers manual (available at www.invitrogen.com/lux) for more information and additional protocols.

Master Mix

Prepare a Master Mix as described below, then add template to each reaction as described in the protocol on the following page. The standard reaction volume is 10 µl.

Monoplex reaction

Component	Initial conc.	Single rxn (10 µl)	50 rxns (10 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	5 µl	250 µl	1X
LUX™ labeled primer	10 uM	0.2 µl	10 µl	200 nM
Unlabeled primer	10 uM	0.2 µl	10 µl	200 nM
ROX Reference Dye	50X	0.2 µl	10 µl	1X
Autoclaved distilled water	—	to 8 µl	to 400 µl	—

Multiplex reaction

Component	Initial conc.	Single rxn (10 µl)	50 rxns (10 µl)	Final conc.
Platinum® qPCR SuperMix-UDG	2X	5 µl	250 µl	1X
LUX™ labeled primer (1)	10 uM	0.1 µl	5 µl	100 nM
Unlabeled primer (1)	10 uM	0.1 µl	5 µl	100 nM
LUX™ labeled primer (2)	10 uM	0.2 µl	10 µl	200 nM
Unlabeled primer (2)	10 uM	0.2 µl	10 µl	200 nM
$MgCl_2$	50 mM	0.6 µl	30 µl	3 mM*
ROX Reference Dye	50X	0.2 µl	10 µl	1X
Platinum® *Taq* DNA Polymerase	5 U	0.12 µl	6 µl	0.6 U**
Autoclaved distilled water	—	to 8 µl	to 400 µl	—

*6 mM total Mg^{++}, including $MgCl_2$ in Platinum® qPCR SuperMix-UDG

**1.2 U total Platinum® *Taq* DNA Polymerase, including 0.6 U in Platinum® qPCR SuperMix-UDG

Cycling Program

See the following page for tips and recommendations on programming the ABI PRISM® 7900.

Recommended Cycling Program

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		Yes
UDG inactivation/template denaturation	95	2 min	1		Yes
Denaturation	95	15 sec	40–50		Yes
Hybridization /Elongation	60	30 sec			Yes
Melting curve analysis (click on **Add Dissociation Stage** button)	95–60–95				Yes

*Uracil DNA glycosylase-mediated carry-over prevention

Continued on the next page

Cycling Program, continued

Alternative

Action	Temp (°C)	Time	Cycles	Ramp time	Acquisition
UDG reaction*	50	2 min	1		Yes
UDG inactivation/template denaturation	95	2 min	1		Yes
Denaturation	95	15 sec	40–50		Yes
Hybridization	55	30 sec			Yes
Elongation	72	30 sec			Yes
Melting curve analysis (click on **Add Dissociation Stage** button)	95–60–95				Yes

*Uracil DNA glycosylase-mediated carry-over prevention.

Protocol

1. Program the ABI PRISM® 7900 as shown in the cycling program. Optimal cycling temperatures and times may vary for different target sequences and primer sets.
2. Prepare a master mix of all components except template as specified. **Note**: Preparation of a master mix is **crucial** in quantitative applications to reduce pipetting errors.
3. For each reaction, add 8 µl of the master mix to each tube, strip well, or well of the qPCR plate.
4. Add 2 µl of sample template (100 pg to 1 µg of genomic DNA, or cDNA generated from 1 pg to 1 µg of total RNA) to each reaction vessel and cap or seal the tube/strip/plate.
5. Gently mix and make sure that all components are at the bottom of the tube/well. Centrifuge briefly if needed.
6. Place reactions in the thermal cycler programmed as described above. Collect and analyze the results.

Tips and Recommendations for Programming the Instrument

Follow the instructions in the ABI PRISM® 7900 user manual to program the instrument and analyze results. The following are tips and recommendations for programming the instrument:

- Choose the **Absolute Quantification** plate format when opening a new plate to read. Also make sure to select the appropriate **Container** from the drop-down menu.
- To set up the plate, click the **Add Detector** button and select the appropriate detectors for the plate (e.g., FAM, JOE, any custom detector settings that you create). Click the **Copy to Plate Document** button, then click **Done**.
- Select all of the wells that contain a particular marker and then check the **Use** box on the **Setup** menu. You can then assign a **Task** for each well (**Sample**, **Standard,** or **NTC**).
- Click on the **Instrument** tab to make any changes to the **Thermal Profile**. Click **Add Dissociation Stage** to do melt curve analysis of your reactions. The default parameters are acceptable.
- You can choose the cycling **Threshold** and **Baseline** settings in the **Amplification Plot** manually or automatically.
- The individual reaction volume can be reduced to 5 µl if necessary (e.g., if you are using 384-well plates).

Additional Products

Product	Catalog number	Size
Platinum® Quantitative PCR SuperMix-UDG (includes separate tubes of ROX Reference Dye and $MgCl_2$)	11730-017	100 reactions
	11730-025	500 reactions
SuperScript™ III Platinum® One-Step qRT-PCR Kit	11732-020	100 reactions
	11732-088	500 reactions

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