

**ROCHE GC-RICH PCR SYSTEM
MEDICAL SEQUENCING GROUP**

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Revision Date: 11/21/06

Version 1.0

PURPOSE: This protocol should be used for amplification of GC-rich genomic regions.

MATERIALS AND EQUIPMENT:

GC-Rich (3)	Thermal Cycler
ddH ₂ O	Centrifuge
dNTP (80mM)	Ice
Primer (1.2μM)	Cooler
DNA (5ng/ul)	Pipettes of appropriate size
5x GC-Rich (2)	Filtered pipette tips of appropriate size
GC-Rich Enzyme (1)	Gloves
384 well microtiter plates	Mask
384 well dental dams	

PROCEDURE:

1. General procedures and information
 - 1.1. Wipe all pipettes with ethanol wipes prior to use in this procedure.
 - 1.2. Clean bench with 10% bleach before and after use.
 - 1.3. Wear a clean lab coat and gloves at all times
 - 1.4. Do not allow other personnel to be in the area where reactions are being done without also following clean procedure.
 - 1.5. During times when a hood cannot be accessed, a disposable mask must be worn while working with DNA, WGA and PCR reactions.
 - 1.6. Filtered tips must be used during ALL medical sequencing activities.
2. Thaw following reagents and store on ice.
3. Use the guidelines below for reaction setup.
 - 3.1. **Mix 1**

GC-Rich (3)	1.0 * _____ = _____
Water (5)	2.4 * _____ = _____
dNTP (80 mM)	0.1 * _____ = _____
Primer (1.2 uM)	3.3 * _____ = _____
DNA (5 ng/ul)	1.0 * _____ = _____
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	7.8
 - 3.2. **Mix 2**

5x GC-Rich (2)	2.0 * _____ = _____
GC-Rich Enzyme (1)	0.2 * _____ = _____
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	2.2
4. Combine contents of each mix separately and place on ice.
5. Pipette Mix 1 and 2 into a 384 well microtiter plate. **Note:** Total volume for each well will be 10μl. This step may also be done via automation depending on DNA sample size.
6. Quick spin at 1500rpm for 30 seconds in a centrifuge. Seal with a 384 well dental dam before placing into the thermal cycler.

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7. Run reactions on the following cycle:
 - 1= 95 3:00
 - 2=95 0:30
 - 3=60 0:30
 - 4=72 0:45
 - 5=Goto 2, 9 x's
 - 6=95 0:30
 - 7=60 0:30
 - 8=72 0:45 + 0:05 for each additional cycle
 - 9=Goto 6, 19 x's
 - 10=72 7:00
8. After cycling is complete, refer to the appropriate Medical Sequencing Exo/SAP protocol to proceed.