

**ADVANTAGE TC2 PCR - CLONTECH  
MEDICAL SEQUENCING GROUP**

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Version 1.0

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

**MATERIALS AND EQUIPMENT:**

|                            |   |
|----------------------------|---|
| 5x Buffer                  | Thermal Cycler                            |
| GC-Melt                    | Centrifuge                                |
| DNA (10ng/ul)              | Ice                                       |
| Primer (1.2µM)             | Cooler                                    |
| 50X Mix dNTP (10mM each)   | Pipettes of appropriate size              |
| GS2 Polymerase Mix         | Filtered pipette tips of appropriate size |
| ddH <sub>2</sub> O         | 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. Remove all the following components from the freezer and place at room temperature to thaw completely.
3. Use the guide below for reaction setup.

|                          | 10ul  |         |
|--------------------------|-------|---------|
| 5x Buffer                | 2.0 * | = _____ |
| GC-Melt                  | 2.0 * | = _____ |
| DNA (10ng/ul)            | 2.0 * | = _____ |
| Primer (1.2 uM)          | 3.3 * | = _____ |
| 50X Mix dNTP (10mM each) | 0.2 * | = _____ |
| GC2 Polymerase Mix (50x) | 0.2 * | = _____ |
| Water                    | 0.3 * | = _____ |
| Total                    | 10    |         |

4. Combine all mixes before adding DNA.
5. Aliquot 10µl of reaction mix per well of a 384 well microtiter plate. **Note:** Automation may be used for this step depending on the number of DNA samples to be amplified.
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. Use the following thermal cycling procedure:

|                  |  |
|------------------|--|
| 1=94             | 3:00   |
| 2=94             | 0:30   |
| 3=60             | 0:15 (modified to allow for anneal of primers) |
| 4=68             | 1:30   |
| 5=Goto 2, 29 x's |  |
| 6=68             | 3:00   |
8. After cycling is complete, refer to the appropriate Medical Sequencing Exo/SAP protocol to proceed.