

PRODUCTION PROTOCOL FOR SMALL SCALE  
GENOMIC AMPLIFICATION USING NEB PHI29

Production Author: Sacha Scott

Revision Date: 10/10/05

Version 1.3

**PURPOSE:** This protocol describes the genomic amplification process for Mutational Profiling tumor samples using NEB phi29.

**MATERIALS AND EQUIPMENT:**

10mM dNTP's	Pipettes of appropriate size
10X NEB phi29 buffer	Filtered pipette tips
10X BSA	Centrifuge
ddH <sub>2</sub> O	1.5% agarose gel
NEB phi29 polymerase	Marker VI
100uM random heptamers	1X TAE buffer
Tumor DNA template @ 5ng/uL	Gel rig and power source
384 well dental dams	384 well microtiter plate
1.7mL microfuge tubes	Hood
Thermal Cycler	Disposable mask
Ethanol wipes	

**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.** Prepare phi29 amplification cocktail.

- 2.1. Determine the amount of reagents needed for the number of reactions to be done using the following recipe. Include a human genomic positive control and a negative control in the calculations for total reactions needed. Note: The NEB phi29 polymerase must be stored at -20° C. All other reagents may be stored in the 4° C.

3.0uL 10mM dNTP's x number of reactions  
3.0uL NEB 10X phi29 buffer x number of reactions  
6.0uL 10X BSA x number of reactions  
13.0uL ddH<sub>2</sub>O x number of reactions  
1.0uL NEB phi29 polymerase x number of reactions  
2.0uL 100uM random heptamers x number of reactions  
2.0uL Tumor DNA template @ 5ng/uL

Note: DNA templates can be retrieved in 20uL aliquots from the -80° refrigerator. Place DNA in the 4° refrigerator as a working stock. DO NOT RE-FREEZE DNA.

- 2.2. Before adding reagents, vortex NEB 10X phi29 buffer to resuspend any precipitate visible in the tube. Note: It is imperative to vortex tube until ALL precipitate is in solution. If the precipitate does not resuspend after vortexing, do not use. Throw the tube in the trash and begin with a new tube of NEB 10X

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phi29 buffer.

- 2.3. In a 1.7mL microfuge tube add all reagents except DNA in the order listed above. Mix the cocktail gently by inverting the tube. Place tube on ice or in the 4° until needed. Note: Cocktail must be used the same day it is made.
- 2.4. Using an electronic repeating pipette, aliquot 28uL of phi29 amplification cocktail into the wells of a new 384 well microtiter plate.
- 2.5. Add 2uL of template DNA to each well containing the cocktail.
- 2.6. Quick spin, seal the tray with a dental dam and place in the thermal cycler as soon as possible. Change volume to 30ul before phi29 cycler program begins. Cycling conditions are as follows:
  - 30° C for 18 hours
  - 65° C for 5 minutes
  - 4° C forever
- 2.7. Run a gel on amplification product
  - 2.7.1. Retrieve 1.5% agarose gels from the Mutational Profiling 4° C.
  - 2.7.2. Prepare gel to be loaded from a 384 well source tray by choosing the appropriate Biomek Gel program. The Biomek will aliquot the following to the source tray:
    - 2.0uL xylene cyanol
    - 2.0uL PCR product
  - 2.7.3. Load samples into gel. The marker lanes should be loaded with 5uL of Marker VI.
  - 2.7.4. Run gel at 140Volts for 50 minutes.
  - 2.7.5. Take a picture of each gel, making sure the marker is exposed consistently from gel to gel using a 0.8 second exposure.
- 2.8. Label 1.7mL microfuge tubes with the DNA name/number, date and wga. When cycling program is complete, quick spin the tray and prepare a 1:10 dilution of the template in the labeled 1.7mL tube by putting 270uL ddH<sub>2</sub>O in each tube then adding the 30uL amplification product.

### 3. PCR with WGA product

- 3.1. Store WGA product in the 4° C until needed for PCR
- 3.2. See Mutational Profiling Production Protocols to proceed with PCR.

#### Revisions:

08/30/05 – Version 1.1

1. Changed BSA requirement in Equipment and Materials section from 100X BSA to 10X BSA.
2. Removed dilution step for making 10X BSA. BSA will now be made by Materials Core.
3. Changed ddH<sub>2</sub>O brew input to 11uL from 12uL.
4. Changed dna brew input (@5ng/uL) to 2uL from 1uL.

09/12/05 – Version 1.2

1. Changed 100uM random heptamer input from 4uL to 2uL.

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2. Added step 2.2 which outlines the process for vortexing NEB 10X phi29 buffer prior to use.

10/05/05 - Version 1.3

1. Changed the following step in the Prepare phi29 amplification cocktail section:
  - Determine the amount of reagents needed for the number of reactions to be done using the following recipe. Include a human genomic positive control and a negative control in the calculations for total reactions needed.
2. Changed ddH2O addition in phi29 ampification cocktail from 11.0uL to 13.0uL.
3. Changed the cocktail aliquot into 384 well microtiter plate from 30uL to 28uL.
4. Changed addition of dna template to reaction from 1uL to 2uL.
5. Changed volume adjustment on cycler program from 25uL to 30uL.
6. Added the following line to the General procedures and information section:
  - 3.3.** Clean bench with 10% bleach before and after use.
7. Added the following step in the Prepare phi29 amplification cocktail section:
  - 3.3.1.** Prepare gel to be loaded from a 384 well source tray by choosing the appropriate Biomek Gel program. The Biomek will aliquot the following to the source tray:
    - 2.0uL xylene cyanol
    - 2.0uL PCR product