Document Number: **SASoM/METHOD/082.v4**

Title: **AMPure PCR purification for GeCKOv2 Next Generation Sequencing (NGS).**

Version: **v4**

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SOP History		
Number	Date	Reason for Change
v1	05/08/2015	Original
v2	05/08/2017	Update
V3	05/08/2019	Update
V4	05/08/2021	Update

1.0 Purpose –

This SOP describes the current procedure for AMPure PCR purification for GeCKOv2 NGS in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the staff in the SASoM involved in AMPure PCR purification for GeCKOv2 NGS.

3.0 Responsibilities –

All staff are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

This protocol describes the purification of 100µL PCR products for each of the 14 samples resulting from the GeCKOv2 screen. Each PCR reaction should be processed separately.

Materials:

- Agencourt AMPure XP 5.0mL (cat #A63880)
- 96 Well Microwell storage plates, 0.8mL (cat # 11867642)
- Magnetic stand-96 Applied Biosystems Fisher Scientific (Lab 249 or cat #10579773)

Method:

1. Transfer all PCR products into a 96-well plate (Microwell storage plate).

2. Gently shake the Agencourt AMPure XP bottle to resuspend any magnetic particles that may have settled. Add 180 µL Agencourt AMPure XP for every 100uL of PCR reaction volume, see chart below:

PCR Reaction Volume (µL)	AMPure XP Volume (µL)
10	18
20	36
50	90
100	180

The volume of Agencourt AMPure XP for a given reaction can be derived from the following equation: (Volume of Agencourt AMPure XP per reaction) = 1.8 x (Reaction Volume)

3. Mix AMPure XP and PCR product thoroughly by pipette mixing 10 times. Let the mixed samples incubate for 5 minutes at room temperature for maximum recovery. This step binds PCR products 100bp and larger to the magnetic beads. Pipette mixing is preferable as it tends to be more reproducible. The colour of the mixture should appear homogenous after mixing.

4. Place the reaction plate onto a Magnetic stand-96 Applied Biosystems (Fisher Scientific) for 2 minutes to separate beads from the solution. Wait for the solution to clear before proceeding to the next step.

5. Aspirate the cleared solution from the reaction plate and discard. This step must be performed while the reaction plate is situated on the magnetic stand. Do not disturb the ring of separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

6. Dispense 200µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature. Aspirate out the ethanol and discard. Repeat for a total of two washes.

It is important to perform these steps with the reaction plate situated on magnetic stand. Do not disturb the separated magnetic beads. Be sure to remove all of the ethanol from the bottom of the well as it is a known PCR inhibitor.

NOTE: Leaving the plate to air-dry for ≤ 5 min at room temperature is optional to ensure all traces of Ethanol are removed but take care not to over dry the bead ring (bead ring appears cracked) as this will significantly decrease elution efficiency.

7. Take the 96-well microwell storage plate from the magnet platform and place it on the bench, add 40µL of elution buffer (reagent grade water) to each well of the reaction plate and pipette mix 10 times. The liquid level will be high enough to contact the magnetic beads at a 40µL elution volume. A greater volume of elution buffer can be used, but using less than 40µL will require extra mixing (to ensure the liquid comes into contact with the beads) and may not be sufficient to elute the entire PCR product. Elution is quite rapid and it is not necessary for the beads to go back into solution for it to occur.

8. Place the 96-well microwell storage plate onto the magnetic stand for 1 minute to separate beads from the solution.

9. Transfer the elutant from each well in 14 wells into a new plate. For long-term freezer storage, Agencourt recommends transferring Agencourt AMPure XP purified samples into a new plate to prevent beads from shattering.

10. Proceed to quantitate samples using first the QubiT protocol (SASoM-METHOD-079) and then the Bioanalyser protocol (SASoM-METHOD-083)

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training -
All users must be fully trained in cell culture. All users must be fully trained in the handling of virus cultures.

8.0 Related documents –
8.1 Risk assessments:
RA/BIOL/001 - Handling of bacteria

RA/BIOL/004 - Tissue culture

RA/GM/002 - Viral-mediated gene delivery into mammalian cells

8.2 SOPs:
SASoM/METHOD/075 - GeCKO lentiCRISPRv2 library DNA amplification in Lucigen bacteria.

SASoM/METHOD/077 - GeCKO genomic DNA purification Qiagen Midi kit.

SASoM/METHOD/078 - Transduction of GECKO Library in MiaPaCa2 cells.

SASoM/METHOD/079-DNA quantification using QubiT fluorometer 2.0 for GeCKO library.

SASoM/METHOD/080- PCR for GeCKOv2 library preparation for Next generation sequencing.

SASoM/METHOD/081-sgRNA Target Guide Sequence Cloning into pLentiCRISPRv2.

SASoM/METHOD/082-AMPure PCR purification for GeCKOv2 NGS.

SASoM/METHOD/083-Quality control of GeCKOv2 for NGS using Agilent Bioanalyzer system.

9.0 Approval and sign off –

Author:

Name: Paul Reynolds

Position: Principal Investigator

Signature:  Date: 07/10/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE