egSEQ® Hybridisation Capture
Targeted Sequencing Panel

Hybridisation Capture Target Enrichment
Designed for Illumina® Next Generation Sequencing

User Guide

Version 1.04
29th July, 2022

For Research Use Only.
Not for in-vitro diagnostic use unless otherwise specified.

Table of Contents

Before You Start

This user guide describes an optimised protocol for hybridisation target enrichment using an egSEQ Hybridisation Capture targeted sequencing panel. It is suitable for use with the Illumina sequencing platforms. For MGI platforms, please consult the relevant manual, which can be provided by contacting us at support@eggenetics.com.

Please read these notes carefully before use and contact our customer care team if anything is unclear.

- Control and quantify the pre-capture library.

- Before the experiment, confirm whether the self-prepared reagents (such as absolute ethanol) meet the experimental conditions and are within shelf-life. These are listed in the *Other Required Materials* section below. Prepare additional batches as required.

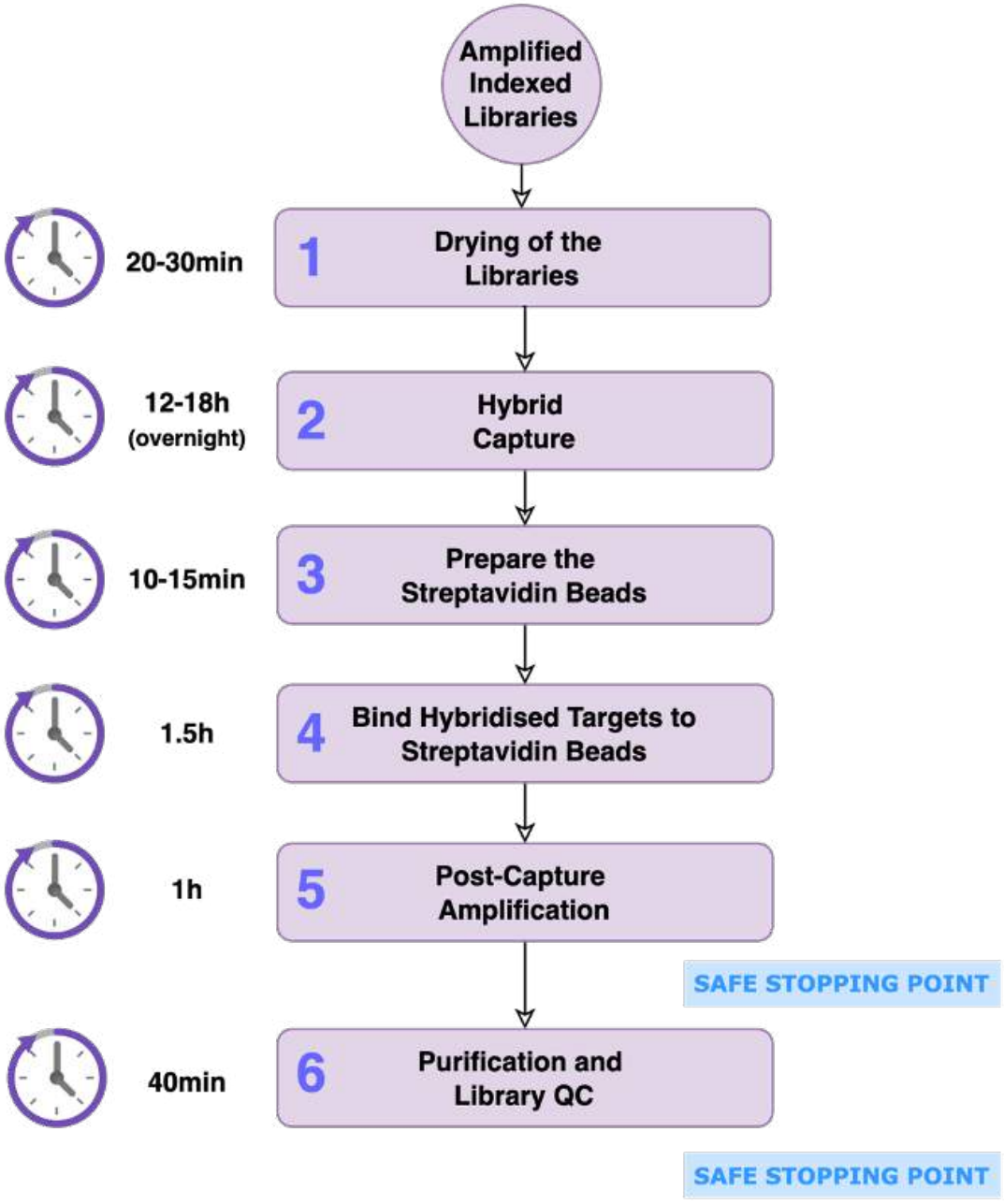
- Suitable stopping points are highlighted clearly in the instructions. Refrain from stopping at other stages of the experiment.

- Always use reagents and consumables that are certified sterile, DNase/RNase-Free. Wipe down work area and pipettes with an RNase- and DNA-cleaning product.

- The recommended time for hybridisation is 12 to 18 h. Please plan appropriately.

Overview of Workflow

There are 6 steps in the workflow, outlined below with estimated time to completion for each step.



Materials Provided

The constituents of a hybridisation-based capture experiment include:

- egSEQ Hyb & Wash Kit (consisting of three modules A, B & C)
- egSEQ Eco Universal Blocking Oligo
- egSEQ Target Probes

In addition, if not already present in the laboratory:

- egSEQ Cap Beads & Nuclease-Free Water

egSEQ Hyb & Wash Kit (All items included)

Item	Screw Cap	Components	Storage	Volume	
				16 rxn	96 rxn
egSEQ Hyb & Wash Kit *(Module A)*	Purple	Hyb Human Block	-20°C ± 5°C	88 µL	540 µL
		RNase Block		88 µL	540 µL
		Hyb Buffer		360 µL	2*1080 µL
egSEQ Hyb & Wash Kit *(Module B)*	Clear	Binding Buffer	15°C - 25°C	14 mL	84 mL
		Wash Buffer 1		4 mL	24 mL
		Wash Buffer 2		18 mL	108 mL
egSEQ Hyb & Wash Kit *(Module C)*	Orange	Post PCR Master Mix	-20°C ± 5°C	450 µL	2*1350 µL
		Post PCR Primer		32 µL	192 µL

egSEQ Adapter Blockers (Item based on your multiplexing requirements)

Item	Screw Cap	Blocking Capacity	Storage	Volume	
				16 rxn	96 rxn
egSEQ Eco Universal Blocking Oligo	Purple	up to 3 µg of library, i.e. up to 6-plex (500 ng per library)	-20°C ± 5°C	36 µL	200 µL
egSEQ Universal Blocking Oligo	Purple	up to 6 µg of library, i.e. up to 12-plex (500 ng per library)	-20°C ± 5°C	36 µL	200 µL

egSEQ Target Probes (Item based on your target)

Item	Screw Cap	Storage	Volume	
			16 rxn	96 rxn
Exome Core Target Probes	Red	< -70°C	36 µL	216 µL
Exome Inherit Target Probes	Red	< -70°C	36 µL	216 µL
Comprehensive Oncology Target Probes	Red	< -70°C	36 µL	216 µL
Haematology Target Probes	Red	< -70°C	36 µL	216 µL
Custom Target Probes	Red	< -70°C	…	…

egSEQ Cap Beads & Nuclease-Free Water (All items included)

Item	Screw Cap	Storage	Volume		
			1000 µL	5 mL	50 mL
egSEQ Cap Beads *	Clear	2°C - 8°C	1000 µL	5 mL	50 mL
Nuclease-Free Water	Clear	2°C - 8°C	1000 µL	5 mL	50 mL

* *egSEQ Cap Beads* are Streptavidin-coated magnetic beads for hybridisation-based capture and different from *egSEQ Pure Beads*. Dynabeads MyOne Streptavidin T1 by Thermo Fisher (Cat#65602) may be used as an alternative to *egSEQ Cap Beads*.

Other Required Materials

The materials below are recommended by Edinburgh Genetics. Please select appropriate materials based on experience and availability.

Reagents Required

Item	Recommendation	Supplier Catalogue #
Ethanol Absolute	-	-
Nuclease-Free Water	-	-
Magnetic Beads (for Purification) *	egSEQ Pure Beads	Edinburgh Genetics (EG1200)
	Agencourt AMPure XP Kit	Beckman Coulter (A63880)
Fragment Analyser Reagent	Agilent DNA 1000 Kit	Agilent (5067-1504)
Nucleic Acid Quantification Assay	Qubit dsDNA HS Assay Kit	Thermo Fisher (C47257)

* Not to be confused with *egSEQ Cap Beads*.

Equipment Required

Item	Recommendation	Supplier Catalogue #
96-well Magnetic Stand, 0.2 mL block	DynaMag-96 Side	Thermo Fisher (12331D)
Fragment Analyser	Agilent 2100 Bioanalyzer	Agilent (G2939AA)
Nucleic Acid Quantifier	Qubit® 4.0 Fluorometer	Thermo Fisher (Q33238)
Thermal Mixer, 0.2 mL block	Eppendorf ThermoMixer®	Eppendorf (5382000015)
Vortex Mixer	-	
Mini Centrifuge	-	
Ice Block	-	
Thermal Cycler	-	
Vertical Rotating Mixer, 0.2mL block	-	
Vacuum Concentrators & Pumps	SPD2010 Integrated SpeedVac	Thermo Fisher (SPD2010-220)

*Consumables Required**

Item	Recommendation	Supplier Catalogue #
Qubit tubes, 0.5 mL	Qubit® assay tubes	Thermo Fisher (Q32856)
PCR tubes, 0.2 mL	-	-
8-tubes strip, 0.2 mL	-	-
Pipette tips, 10 µL	-	-
Pipette tips, 200 µL	-	-

* References to PCR tube(s) in this guide also apply to plate(s). You may choose to use suitable PCR plate(s).

1. Step 1. Preparation

Reagents Required:
- Hyb Human Block
- RNase Block
- egSEQ Eco Universal Blocking Oligo
- egSEQ Target Probes
- Hyb Buffer
- Pre-Capture Libraries

Equipment Required:
- Thermal cycler
- Vortex mixer
- Mini centrifuge

Step-through

1.1. Thaw the *Hyb Human Block*, *RNase Block*, and *egSEQ Eco Universal Blocking Oligo* on ice. Once thawed, mix each of them thoroughly by quick vortexing to avoid any localised concentrations. Briefly spin down vortexed reagents before use.

1.2. Thaw the *egSEQ Target Probes* on ice. Vortex and spin down the reagent before use. Place back on ice.

1.3. Thaw the pre-capture library on ice. Mix thoroughly and centrifuge briefly.

1.4. Vortex the *Hyb Buffer* to mix well. If precipitate is present, heat at 37°C. Vortex and make sure all precipitate is dissolved.

2. Step 2. Drying & Hybridisation

Reagents Required:
- Nuclease-Free Water
- Pre-Capture Libraries
- Hyb Buffer
- Hyb Human Block
- egSEQ Eco Universal Blocking Oligo
- RNase Block
- Nuclease-Free Water
- egSEQ Target Probes

Equipment Required:
- Thermal cycler
- Vacuum centrifuge concentrator
- Mini centrifuge
- Vortex mixer

This section outlines the vacuum evaporation method for library concentration, which is recommended. If magnetic bead purification is preferred, go to Appendix 1.

Step-through

2.1. Add 750 ng of the library to the PCR tube. If multiplexing libraries in a single hybridisation-based capture reaction, add 500 ng per library. Vortex and spin down the sample before use.

2.2. Dry down the pre-capture library in a vacuum centrifuge concentrator, such as a SpeedVac. Open the cap of the PCR tube, and concentrate the libraries to dry.

Before concentrating the library, estimate the concentration time by using the same volume of water. Over-drying will cause a loss of the pre-capture library.

2.3. Prepare the Hybridisation Master Mix as indicated below. Mix well and centrifuge briefly.

Component	Volume per Reaction
Hyb Buffer	13 µL
Hyb Human Block	5 µL
egSEQ Eco Universal Blocking Oligo	2 µL
RNase Block	5 µL
Nuclease-Free Water	3 µL
egSEQ Target Probes	2 µL
Total	**30 µL**

Add *egSEQ Target Probes* last to the Hybridisation Master Mix or add *egSEQ Target Probes* after *RNase Block*.

2.4. Add 30 µL of Hybridisation Master Mix to the dried pre-capture library. Vortex for 30 sec to completely dissolve the dried DNA at the bottom of the tube, and spin briefly on a mini centrifuge.

2.5. Place the PCR tube on a thermal cycler, and start the program as indicated below.

Temperature	Time
Heat lid temperature 85°C	
80°C	5 minutes
50°C	Hold

2.6. Incubate for 12 to 18 h. Start next step (STEP 3) 30 min before the incubation finishes.

3. Step 3. Pre-Capture Preparation

Reagents Required:	**Equipment Required:**
• egSEQ Cap Beads • Ethanol Absolute • Wash Buffer 1 • Wash Buffer 2 • Binding Buffer	• Mini centrifuge • Magnetic stand • Vortex mixer

Follow steps in this section to prepare reagents required for target capture, proceed to Step 4 directly to prevent excessive heating time and evaporation.

Step-through

3.1. Vortex the *egSEQ Cap Beads* for 30 seconds to mix well, and equilibrate to room temperature for 30 min.

3.2. Prepare the 80% ethanol for post-capture purification. Place the 80% ethanol at room temperature.

3.3. Vortex the *Wash Buffer 1* for 3 seconds to mix well. If precipitate is present, heat the *Wash Buffer 1* at 37°C, until the precipitate is dissolved. Vortex for 3 sec to mix well, and spin briefly on a mini centrifuge.

3.4. Heat the *Wash Buffer 2* at 50°C.

3.5. Add 100 µL of *egSEQ Cap Beads* to a new PCR tube, and place the PCR tube containing *egSEQ Cap Beads* on a magnetic stand and allow them to fully separate from the supernatant (approximately 1 min). Remove and discard the clear supernatant.

Please use *egSEQ Cap Beads* or *Dynabeads MyOne Streptavidin T1* by Thermo Fisher (Cat#65602). Other magnetic beads like C1, M270, M280 or Purification Magnetic Bead are not suitable for capture.

3.6. Remove the PCR tube containing *egSEQ Cap Beads* from the magnetic stand. Add 180 µL of *Binding Buffer*, and vortex for 10 seconds or pipette to mix well.

3.7. Spin briefly on a mini centrifuge, and place the PCR tube containing *egSEQ Cap Beads* on a magnetic stand and allow the *egSEQ Cap Beads* to fully separate

from the supernatant (approximately 1 min). Remove and discard the clear supernatant.

3.8. Repeat Steps 3.6 and 3.7 two more times (three times in total).

3.9. Remove the PCR tube containing *egSEQ Cap Beads* from the magnetic stand. Add 180 µL of *Binding Buffer* and vortex for 10 seconds or pipette to mix well.

Proceed to Step 4 directly to prevent excessive heating time and evaporation.

4. Step 4: Target Capture

Reagents Required:	Equipment Required:
• Wash Buffer 1	• Thermal mixer
• Wash Buffer 2	• Thermal cycler
• 80% Ethanol (freshly prepared)	• Magnetic stand
• egSEQ Cap Beads	• Vertical rotating mixer
• Nuclease-Free Water	• Mini centrifuge

Step-through

4.1. Keep the PCR tube containing the Hybridisation Master Mix (from STEP 2) on a thermal cycler at 50°C and quickly transfer the 180 µL of *egSEQ Cap Beads* to the Hybridisation Master Mix. Pipette to mix well.

4.2. Incubate the PCR tube on a vertical rotating mixer, mixing gently (< 10 rpm) for 30 minutes at room temperature.

4.3. Remove the PCR tube from the mixer. Spin briefly on a mini centrifuge, place the tube containing *egSEQ Cap Beads* on a magnetic stand, and allow the them to fully separate from the supernatant (approximately 2 minutes). Remove and discard the clear supernatant.

4.4. Remove the PCR tube containing *egSEQ Cap Beads* from the magnetic stand. Add 150 µL of *Wash Buffer 1* and pipette to mix well. Then incubate the PCR tube on a vertical rotating mixer, mix gently (< 10 rpm) for 15 minutes at room temperature.

4.5. Remove the PCR tube from the vertical rotating mixer. Spin briefly on a mini centrifuge, place the PCR tube containing *egSEQ Cap Beads* on a magnetic stand, and allow them to fully separate from the supernatant (approximately 2 minutes). Remove and discard the clear supernatant.

4.6. Remove the PCR tube containing *egSEQ Cap Beads* from the magnetic stand. Add 150 µL of pre-heated *Wash Buffer 2* and pipette to mix well. Spin briefly on a mini centrifuge. Incubate the PCR tube at 50°C for 10 minutes on a thermal mixer.

4.7. Remove the PCR tube from the thermal mixer. Spin briefly on a mini centrifuge, place the PCR tube containing *egSEQ Cap Beads* on a magnetic stand,

and allow them to fully separate from the supernatant (approximately 2 minutes). Remove and discard the clear supernatant.

4.8. Repeat Step 4.6 and 4.7 two more times (three times in total).

4.9. Keep the PCR tube on a magnetic stand.

4.10. Add 200 µL of 80% ethanol. Incubate at room temperature for 30 seconds. Remove and discard the clear supernatant. After the final wash, remove all traces of supernatant using a 10 µL pipette.

4.11. Add 48 µL of Nuclease-Free Water. Remove the PCR tube containing *egSEQ Cap Beads* from the magnetic stand. Vortex for 10 seconds or pipette to mix well.

Do not discard the *egSEQ Cap Beads as the* captured libraries are contained here!

Proceed immediately to the next step.

5. Step 5: Post-PCR Amplification

Reagents Required:
- Post PCR Master Mix
- Post PCR Primer

Equipment Required:
- Thermal cycler
- Mini centrifuge
- Vortex mixer

Step-through

5.1. Thaw *Post PCR Master Mix* and *Post PCR Primer* on ice, then vortex for 3 seconds to mix well. Spin briefly on a mini centrifuge and place on ice.

5.2. *Post PCR Primer* is library type specific, ensure you are using the correct Post PCR Primer.

5.3. Prepare PCR Reaction Mixture as indicated below, and vortex for 3 seconds or pipette to mix well.

Component	Volume per Reaction
Libraries from STEP 4	24 µL
Post PCR Primer	1 µL
Post PCR Master Mix	25 µL
Total Volume	**50 µL**

Add only 24 µL out of the 48 µL of Libraries prepared in STEP 4.

5.4. Place PCR tube on the thermal cycler and start the program indicated below.

Temperature	Time	Cycles
Heat lid temperature 85°C		
95°C	1 min	1
98°C	20 sec	X Cycles
60°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	**Hold**	**1**

Pre-Capture Library input	X Cycles
750 ng	N
1.5 µg	N-1
3 µg	N-2
6 µg	N-3

For post-PCR cycles, please refer to the PCR cycle number N from the tube label of *egSEQ Target Probes*. The number of post-PCR cycles is related to total pre-capture library input.

5.5. Proceed to STEP 6 when the program finishes.

This is a safe stopping point.

6. Step 6: Library Purification

Reagents Required:	**Equipment Required:**
• egSEQ Pure Beads	• Magnetic stand
• 80% Ethanol (freshly prepared)	• Mini centrifuge
• Nuclease-Free Water	• Vortex mixer
	• Fragment analyser
	• Nucleic acid quantifier

Step-through

6.1. Vortex the *egSEQ Pure Beads* for 30 seconds to mix well. Equilibrate the egSEQ Pure Beads to room temperature for 30 min.

egSEQ Pure Beads or *Agencourt AMPure XP* are the recommended magnetic beads for purification in this protocol. If an alternative is preferred, please assess the volume of beads used for purification via pre-experiments.

6.2. Add 55 µL (1.1×) of *egSEQ Pure Beads* to each amplified sample. Pipette or vortex to mix well. Incubate at room temperature for 5 minutes.

6.3. Spin briefly on a mini centrifuge. Place the PCR tube containing both *egSEQ Cap Beads* and *egSEQ Pure Beads* on a magnetic stand and allow the magnetic beads to fully separate from the supernatant (approximately 3 minutes).

6.4. Keep the PCR tube containing magnetic beads on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 seconds.

6.5. Keep the PCR tube containing magnetic beads on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant. (May spin briefly on a mini centrifuge and remove all traces of supernatant using a 10 µL pipette.)

6.6. Keep the PCR tube containing beads on the magnetic stand at room temperature for 3 to 5 minutes to dry the bead pellet.

Do not dry the bead pellet to the point that the pellet appears cracked during any of the bead drying steps in the protocol. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

6.7. Add 25 µL of Nuclease-Free Water. Remove the PCR tube containing beads from the magnetic stand. Vortex for 10 seconds or pipette to mix well. Incubate at room temperature for 2 minutes.

6.8. Spin briefly on a mini centrifuge. Place the PCR tube containing beads on a magnetic stand and allow the beads to fully separate from the supernatant.

6.9. Transfer 23 µL of clear supernatant containing the captured library to a clean PCR tube. Captured library can be stored at -20°C for up to 1 month.

6.10. Quantify each captured library using a nucleic acid quantification reagent such as Qubit dsDNA High Sensitivity Assay Kit.

6.11. Analyse the fragment size of captured library using a suitable fragment analyser such as Agilent 2100 Bioanalyzer.

The experiment ends here!

Appendix 1: Library Concentration (Magnetic Bead Purification) (Optional)

Reagents Required:	Equipment Required:
• Pre-Capture Libraries	• Magnetic stand
• egSEQ Pure Beads	• Thermal cycler
• 80% Ethanol (freshly prepared)	• Vortex mixer
• Nuclear-Free Water	• Mini centrifuge

Step-through

1. Add 750 ng of library to the PCR tube. If multiplexing libraries in a single hybridisation-capture reaction, add 500 ng of library each. Vortex and spin down the sample before use.

2. Add 1.8× volume of *egSEQ Pure Beads*. Pipette or vortex to mix well. Incubate at room temperature for 5 minutes.

3. Spin briefly on a mini centrifuge. Place the PCR tube containing *egSEQ Pure Beads* on a magnetic stand and allow the beads to fully separate from the supernatant (approximately 3 minutes).

4. Keep the PCR tube containing *egSEQ Pure Beads* on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube, incubate at room temperature for 30 seconds.

5. Keep the PCR tube containing *egSEQ Pure Beads* on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 seconds. Remove and discard the clear supernatant. Spin briefly on a mini centrifuge. Place the PCR tube containing *egSEQ Pure Beads* on a magnetic stand and allow the beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 µL pipette.

6. Keep the PCR tube containing *egSEQ Pure Beads* on the magnetic stand at room temperature for 3 to 5 minutes to dry the bead pellet.

7. Prepare Hybridisation Master Mix as indicated below

Component	Volume per Reaction
Hyb Buffer	13 µL
Hyb Human Block	5 µL
egSEQ Eco Universal Blocking Oligo	2 µL
RNase Block	5 µL
Nuclease-Free Water	3 µL
egSEQ Target Probes	2 µL
Total Volume	**30 µL**

8. Add 30 µL of Hybridisation Master Mix to the PCR tube containing *egSEQ Pure Beads*. Vortex for 10 seconds or pipette to mix well. Incubate at room temperature for 3 minutes.

9. Spin briefly on a mini centrifuge. Place the PCR tube containing *egSEQ Pure Beads* on a magnetic stand and allow the beads to fully separate from the supernatant (approximately 3 minutes).

10. Transfer 28 µL of clear supernatant containing the pre-capture library and Hybridisation Master Mix to a clean PCR tube. Spin briefly on a mini centrifuge.

11. Place the PCR tube on the thermal cycler, and start the program as indicated below:

Temperature	Time
Heat lid temperature to 85°C	
80°C	5 minutes
50°C	**Hold**

12. Incubate for 12 to 18 h. Start next step (STEP 3) 30 minutes before the incubation finishes.

Technical Support

support@eggenetics.com
(44) 131 261 6686

eggenetics.com

EDINBURGH GENETICS

For Research Use Only.
Not for in-vitro diagnostic use unless otherwise specified.