**Compiled by Pei-Ching(Tessa) Tsai, M.S.**

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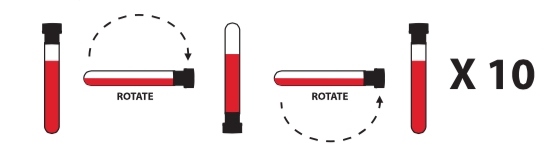
**SCAMP-Seq protocol**

**Purification of circulating free DNA from whole blood**

**Materials:**

* Cell-Free DNA BCT ® 100-Tube Box (10ml), Streck (Cat. no. 218962)
* QIAamp® Circulating Nucleic Acid Kit, QIAGEN (Cat. no. 55114)

**Preparation:**

1. Collect blood specimen in Cell-Free DNA BCT ® tube and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate test results. One inversion is a complete turn of the wrist, 180 degrees, and back per the figure below:
2. For ctDNA and genomic DNA analysis: blood samples collected are stable for 14 days when stored between 6 °C-37 °C.
3. For circulating epithelial cells (tumor cells): blood samples collected are stable for 4 days when stored between 15 °C-30 °C.
4. Set up heat blocks @ 56°C
5. Set up water bath @ 60°C
6. Ensure that Buffer ACB, Buffer ACW1, and Buffer ACW2 have been prepared according to the instructions in the QIAamp Circulating Nucleic Acid Handbook page 14 (2nd edition January 2011).
7. Add 67.5ul of carrier RNA (**cRNA**, 0.2ug/ul in Buffer AVE) to 10.6ml Buffer ACL. This mixture is enough for ~13 samples. \*\*\*Please note the ratio of cRNA/ACL will need to be altered and optimized according to the volume of plasma used. \*\*\* *For larger plasma volumes, see QIAamp Circulating Nucleic Acid Handbook “Protocol: Purification of Circulating Nucleic Acids from 2ml, 3ml, 4ml or 5ml Serum or Plasma”, page 24-26.***Procedure:**

*Purification of circulating free DNA from 1 ml plasma:*

1. **To separate plasma, centrifuge whole blood at 300 x g for 20 minutes at room temperature.**
2. **Remove the upper plasma layer and transfer to a clean tube. Collect the buffy coat layer or the red blood cell layer for cellular genomic DNA isolation** (please refer to page 4 below for the isolation protocol).
3. **Centrifuge the plasma at 1500 x g for 10 minutes.**
4. **Transfer the supernatant to a new tube.**
5. **Repeat step 3 and 4 once more, to ensure the removal of contaminating cells in the plasma.**

**For long term storage: you can freeze the plasma at -80°C.**

**Save ~1ml of the RBC layer for gDNA isolation later.**

1. **Pipet 100 μl of QIAGEN Proteinase K into a 50 ml conical tube.**
2. **Add 1ml of plasma to the 50ml tube.**
3. **Add 0.8 ml Buffer ACL (containing 1.0 μg carrier RNA). Close the cap and mix by pulse-vortexing for 30 s.**

Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

Note: Do not interrupt the procedure at this time. Proceed immediately to step 9 to start the lysis incubation.

1. **Incubate at 60°C for 30 min.**
2. **Add 1.8 ml Buffer ACB to the lysate in the tube. Mix thoroughly by pulse-vortexing for 15–30 s.**
3. **Place the conical containing the lysate–Buffer ACB mixture on ice for 5 min.**
4. **Connect each QIAamp Mini column to a VacConnector and place them on top of the QIAvac 24 Plus VacValves. Insert a 20 ml tube extender on top of each QIAamp Mini column (picture of assembly on page 19)**

Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage. For all vacuum steps (Step 13-16), keep the lid of the column open.

Note: Keep the collection tube for the dry spin in step 17.

1. **Carefully apply the lysate–Buffer ACB mixture from step 11 into the tube extender with the QIAamp Mini column. Open the VacValves. When all the lysate has run through the column, close each VacValve and release the pressure. Carefully remove and discard the tube extender.**

Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

1. **Apply 600 μl Buffer ACW1 to the QIAamp Mini column and open the VacValve. As the last of the Buffer ACW1 runs through the QIAamp Mini column, close the VacValve and release the pressure.**
2. **Apply 750 μl Buffer ACW2 to the QIAamp Mini column and open the VacValve. After all of the Buffer ACW2 has been drawn through the QIAamp Mini column, close the VacValve and release the pressure.**
3. **Apply 750 μl of ethanol (96–100%) to the QIAamp Mini column and open the VacValve. After all of the ethanol passes through the spin column, close the VacValve and release the pressure.**
4. **Close the lid of the QIAamp Mini column, remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube and centrifuge at full speed (16,000 xg) for 3 min to dry the column.**
5. **Place the QIAamp Mini Column in a new 2 ml collection tube. Open the lid and incubate the assembly at 56°C for 10 min to dry the membrane completely.**
6. **Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 18. Carefully apply 35 μl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.**

Important: Ensure that the elution buffer AVE is equilibrated to room temperature (15–25°C). If elution is done in small volumes (<50 μl) the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications.

1. **Centrifuge at full speed (16,000 x g) for 1 min to elute the nucleic acids.**

Note: In some cases where the cfDNA concentrations in the plasma are high, a second or third elution from the column can be performed.

1. **Measure your yield on the Qubit and run the Bioanalyzer to assay the quality of your purification.**

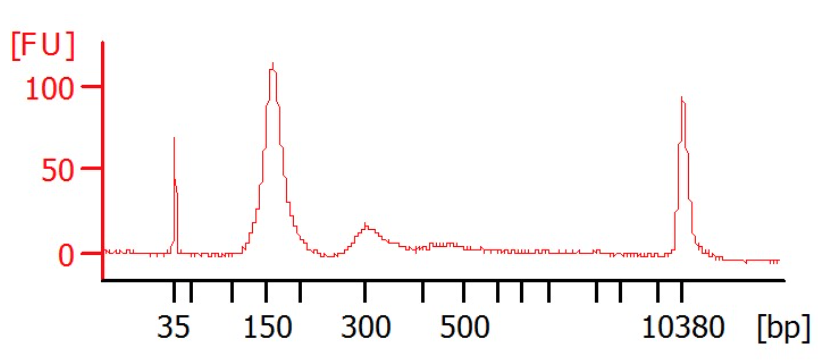


Figure 1. High sensitivity bioanalyzer trace with purified circulating free DNA (cfDNA). cfDNA is concentrated around 150-500bp.

1. **Eluates are ready to continue with the barcoded library construction or storage.** **The purified cfDNA can be stored short term at 4°C, but storage at -20°C is recommended.**

**Purification of cellular genomic DNA from whole blood**

**Materials:**

* Red blood cell layer isolated from streck tube in page2, step 2
* QIAamp DNA Micro Kit, QIAGEN (Cat. no. 56304); Handbook 2nd edition May 2010.

**Preparation:**

1. Set up heat blocks @ 56°C
2. Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate. Shake it before starting the procedure.
3. Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Shake it before starting the procedure.
4. Check whether precipitate has formed in Buffer ATL and AL. If necessary, dissolve by heating to 70°C with gentle agitation.

**Procedure:**

1. **Thaw blood sample at room temperature (15–25°C).**
2. **Pipet 100 μl of whole blood into a 1.5 ml microcentrifuge tube.**
3. **Add 400 μl of Buffer ATL.**
4. **Add 40 μl proteinase K.**
5. **Add 400 μl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s.**

To ensure efficient lysis it is essential that the sample, Buffer ATL, proteinase K, and Buffer AL are thoroughly mixed to yield a homogeneous solution.

Note: If the volume of blood is lower than 10 μl, addition of carrier RNA to Buffer AL is recommended (see Handbook page 15). Note: carrier RNA does not dissolve in Buffer AL, it must first be dissolved in Buffer AE and then added to Buffer AL.

A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during the incubation in step 6.

1. **Incubate at 56°C for 10 min.**

Note*: If samples are mixed during the incubation, DNA yields can be improved.*

1. **Pulse-spin the 1.5 ml tube to remove the condensation from inside the lid.**
2. **Add 400 μl of ethanol (96–100%), close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate for 3 min at room temperature.**

Note: *If room temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.*

1. **Pulse-spin the tube.**
2. **Carefully transfer the entire lysate to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 min. Discard the flow-through and place the QIAamp MinElute column in a clean 2 ml collection tube.** If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.
3. **Carefully open the QIAamp MinElute column and add 500 μl Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 min. Discard the flow-through and place the QIAamp MinElute column in a clean 2 ml collection tube.**
4. **Carefully open the QIAamp MinElute column and add 500 μl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 min. Discard the flow-through and place the QIAamp MinElute column in a clean 2 ml collection tube.**

Contact between the QIAamp MinElute column and the flow-through should be avoided.

1. **Centrifuge at full speed (16,000 x g) for 3 min to dry the membrane completely.**

This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

1. **Discard the flow-through and place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided). Carefully open the lid of the QIAamp MinElute column and apply 35 μl Buffer AE or distilled water to the center of the membrane.**

Remember that the volume of eluate will be up to 5 μl less than the volume of elution solution applied to the column.

1. **Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (16,000 x g) for 1 min.**

Incubating the QIAamp MinElute column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

1. **Elution step can be repeated 2-3 times to increase DNA recovery, depending on the amount of starting material used.**
2. **Quality of recovered DNA can be assayed through agarose gel electrophoresis.**

**Barcoded library construction**

**Materials:**

* KAPA Hyper Prep Kit Illumina® platforms (Cat. no. KR0961-v1.14)

**Procedure:**

***Sonication -For genomic DNA ONLY***

1. **Load 870 ng of purified genomic DNA in a total volume of 87 μl into Covaris microtubes (use PCR-grade H2O).**
2. **Sonicate the DNA to 200bp using the following Covaris sytem settings: Peak Power 175, Duty Factor10%, Cycles/burst 200, Time 180sec, Temp 4-7C.**
3. **Pulse-spin and transfer 50ul of the DNA to a clean PCR tube to proceed with library preparation.**

***1. End Repair and A-Tailing***

**1.1 Assemble each End Repair & A-Tailing reaction as follows in a PCR tube or plate:**

|  |  |
| --- | --- |
| **Component** | **Volume (ul)** |
| Fragmented double-stranded DNA (500ng) or cfDNA (6-100ng) | 50 |
| End Repair & A-Tailing Buffer † | 7 |
| End Repair & A-Tailing Enzyme Mix † | 3 |
| **Total Volume** | **60** |

† The buffer and enzyme mix may be pre-mixed and added in a single pipetting step.

**1.2 Mix thoroughly and pulse-spin.**

**1.3 Incubate in a thermocycler with the following thermal profile:**

|  |  |  |
| --- | --- | --- |
| **Step** | **Temp** | **Time** |
| End Repair & A-Tailing | 20°C | 30 min |
| 65°C | 30 min |
| HOLD | 4°C | ∞ |

**1.4 Proceed immediately to the next step.**

***2. Adapter Ligation***

**2.1 Assemble each Adapter Ligation reaction as follows:**

|  |  |
| --- | --- |
| **Component** | **Volume (ul)** |
| End Repair & A-Tailing reaction product | 60 |
| Ligation Buffer | 30 |
| DNA Ligase | 10 |
| Diluted adapter in PCR-grate water† | 10 |
| **Total Volume** | **110** |

† Final adapter concentrations in the reaction will vary based on the amount of Input DNA. At this point, different barcoded adapters can be introduced for multiplexed sequencing. Values are outlined in the following table:

|  |  |  |
| --- | --- | --- |
| **Input DNA** | **Adapter stock concentration (uM)** | **Final adapter concentration in 110ul** |
| 50ng-1ug | 10uM | 680nM |
| 25ng | 340nM |
| 10ng | 136nM |
| 5ng | 68nM |
| 2.5ng | 34nM |
| 1ng | 14nM |

**2.2 Mix thoroughly and pulse-spin**

**2.3 Incubate at 20 °C for 15 min. And at 4 °C for 15 min.**

**2.4 Proceed immediately to the next step.**

***3. Post-ligation Cleanup***

NOTE: Make sure AMPure XP beads equilibrate to room temperature for at least 30min.

**3.1 Perform a 0.8X AMPure** **XP bead cleanup by combining the following:**

|  |  |
| --- | --- |
| **Component** | **Volume (ul)** |
| Adapter Ligation reaction product | 110 |
| Agencourt® AMPure XP reagent | 88 |
| **Total Volume** | **198** |

**3.2 Thoroughly resuspend the beads by pipetting up and down multiple times.** This cleanup can be performed in a U-bottom plate, PCR tube strip or a96 well plate. Instructions below reflect purification done in a plate.

**3.3 Incubate the mixture at room temperature for 15 min to allow binding of your DNA to the beads.**

**3.4 Place the plate on a magnet to capture the beads. Let the plate sit on the magnet until the liquid is clear (3-5 min).**

**3.5 Carefully pipet out the supernatant and discard it.**

**3.6 Keeping the plate on the magnet, add 200 μl of 80% ethanol.**

**3.7 Let the plate sit at room temperature for ≥30 sec.**

**3.8 Carefully remove and discard the ethanol.**

**3.9 Repeat Step 3.6-3.8 one more time, for a total of two washes. On the last wash, try to remove all residual ethanol without disturbing the beads.**

**3.10 Air dry the beads at room temperature, until all of the ethanol has evaporated (~8- 10mins).** Caution: over-drying the beads may result in yield loss.

**3.11 Remove the plate from the magnet.**

**3.12 Resuspend the beads in 23 μl of elution buffer (10 mM Tris-HCl, pH 8.0) or H2O to elute your DNA.**

**3.15 Incubate the plate at room temperature for 1-2 min.**

**3.16 Place the plate on the magnet to capture the beads. Let it sit until the liquid is clear.**

**3.17 Transfer approximately 20ul clear supernatant to a new tube.**

***4. Library Amplification***

**4.1 Assemble each library amplification reaction as follows:**

|  |  |
| --- | --- |
| **Component** | **Volume (ul)** |
| 2X KAPA HiFi HotStart ReadyMix | 25 |
| 10X Library Amplification Primer Mix | 5 |
| Adapter-ligated DNA | 20 |
| **Total Volume** | **50** |

**4.2 Mix thoroughly and centrifuge briefly.**

**4.3 Amplify using the following cycling protocol:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temp** | **Time** | **Cycles** |
| Initial denaturation | 98°C | 45 sec | 1 |
| Denaturation | 98°C | 15 sec | Minimum number required for optimal amplification (Table below) |
| Annealing† | 60°C | 30 sec |
| Extension | 72°C | 30 sec |
| Final extension | 72°C | 1 min | 1 |
| HOLD | 4°C | ∞ | 1 |
| **Input DNA** | **Number of cycles required to generate :** | | |
| **100ng** | **1ug** | |
| 1ug | 0-1 | 2-3 | |
| 500ng | 0-1 | 3-4 | |
| 250ng | 1-3 | 4-6 | |
| 100ng | 3-4 | 6-7 | |
| 50ng | 4-5 | 6-7 | |
| 25ng | 5-7 | 8-10 | |
| 10ng | 8-11 | 11-14 | |
| 5ng | 10-13 | 13-16 | |
| 2.5ng | 12-14 | 15-17 | |
| 1ng | 14-16 | 17-19 | |

**4.4 Store the plate at 4 °C or -20 °C for up to 72 hours, or proceed directly to Step**

***5. Post- amplification Cleanup***

**5.1 Perform a 1X AMPure XP cleanup by combining the following:**

|  |  |
| --- | --- |
| **Component** | **Volume (ul)** |
| Library Amplification reaction product | 50 |
| Agencourt® AMPure® XP reagent | 50 |
| **Total Volume** | **100** |

**5.2 Thoroughly resuspend the beads by pipetting up and down multiple times.**

**5.3 Incubate the mixture at room temperature for 15 min to allow binding of your DNA to the beads.**

**5.4 Place the plate on a magnet to capture the beads. Let the plate sit on the magnet until the liquid is clear (3-5 min).**

**5.5 Carefully pipet out the supernatant and discard it.**

**5.6 Keeping the plate on the magnet, add 200 μl of 80% ethanol.**

**5.7 Let the plate sit at room temperature for ≥30 sec.**

**5.8 Carefully remove and discard the ethanol.**

**5.9 Repeat Step 5.6-5.8. Try to remove all residual ethanol without disturbing the beads.**

**5.10 Dry the beads at room temperature, until all of the ethanol has evaporated (~ 8-10mins).** Caution: over-drying the beads may result in dramatic yield loss.

**5.11 Remove the plate from the magnet.**

**5.12 Resuspend the beads in 25-55 μl of elution buffer (10 mM Tris-HCl, pH 8.0) or PCR-grade water. Always use PCR-grade water if proceeding to targeted capture.**

**5.15 Incubate the plate at room temperature for 2 min.**

**5.16 Place the plate on the magnet to capture the beads. Incubate until the liquid is clear.**

**5.17 Transfer approximately 20-50ul of the supernatant to a new tube and proceed with library QC, target capture or sequencing, as appropriate. Store purified, amplified libraries at 4 °C (up to 1 week), or at -20 °C.**

**References**

* KAPA Hyper Prep Kit Illumina® platforms Technical Data Sheet
* Baslan, T., et al. (2016). "Corrigendum: Genome-wide copy number analysis of single cells." Nat Protoc **11**(3): 616.

**Copy Number Analysis Protocol**

**Procedure:**

1. **Dilute the samples to 10 nM, pool different libraries if applying multiplex sequencing, and send the samples for sequencing.**

**NimbleGen Roche Capture Protocol**

***Capture***

1. **Add 5 μL COT Human DNA (1 mg/mL) to a new 1.5 mL tube**
2. **Add 1-2 μg of pooled libraries**
3. **Add 10 μL NextFlexFwd (FA) oligo (100 μM)**
4. **Add 10 μL of pooled Barcodes (unannealed, L, 100 μM each: make a master mix for easier pipetting)**
5. **Vortex gently and pulse-spin**
6. **Open lid and dry on speed vac (high heat) ~ 0.5-1.5 hours. Drying times depend on the total sample volume. When done, the sample is reduced to a dry bubble and it is often invisible.**
7. **Without disturbing the dried DNA pellet, add the following:**
   1. **7.5 μL 2x Hybridization Buffer**
   2. **3 μL Hybridization Component A**

**NOTE: Dried DNA will be very viscous, be sure to mix only after you’ve added the buffer and Component A**

1. **Vortex gently and pulse-spin**
2. **Incubate at 95°C for 10 minutes**
3. **Pulse-spin and transfer reaction (~10.5 μL) to a 0.2 mL PCR tube**
4. **Add 4.5 μL of the SeqCap EZ Choice Capture (either Exome(V2) or any custom Roche capture). Flick and pulse-spin the PCR tube**
5. **Incubate at 47°C on PCR block for 64-72 hours**
   1. **Set volume = 20 μL**
   2. **PCR program: “Exome/47C” [Set lid at 57°C]**

***Wash***

**Preparation**

1. Set water bath to 47**°**C
2. Equilibriate the SeqCapEZ Hybridization and Wash Kit (#05634261001, -20**°**C) and the Pure Capture Bead Kit (#06977952001, 4**°**C) to RT for at least 30 minutes prior to use.
3. Dilute the wash buffers
   1. Make sure no precipitates remain in the solution

|  |  |  |
| --- | --- | --- |
| Buffer Stock | Buffer Volume (μL) | H2O Volume (μL) |
| 10x Stringent Wash 4 | 40 | 360 |
| 10x Wash Buffer 1 | 30 | 270 |
| 10x Wash Buffer 2 | 20 | 180 |
| 10x Wash Buffer 3 | 20 | 180 |
| 2.5x Bead Wash Buffer | 200 | 300 |

1. Preincubate the 400 μL of diluted Stringent Wash 4 and 100 uL of diluted Wash Buffer 1 in the 47**°**C water bath
   1. Keep remaining buffer dilutions at RT

**Procedures**

1. **Place 100 μL of SeqCap EZ Capture Beads (Exact same as ones sold by Invitrogen #2017-08; Dynabeads M-270 Streptavidin 10mg/ml) in a 1.5 mL tube**
   1. **Mix beads thoroughly before use**
2. **Place the tube on the magnet**
3. **Remove and discard the supernatant**
4. **Add 200 μL of diluted Bead Wash Buffer and vortex for 10 seconds**
5. **Place tube on the magnet and discard the supernatant**
6. **Repeat steps 4 and 5 once more, for a total of 2 washes.**
7. **Repeat steps 4 and 5 once more, but with 100 μL of Bead Wash buffer**
   1. Remove the supernatant immediately before adding the DNA+library mixture. \*\*\* Do not let your beads dry \*\*\*
8. **Keep beads in the 1.5 mL tube and transfer the hybridization reaction from the PCR block to your washed beads. Pipet up and down to mix. Do this next to the PCR block.**
9. **Incubate the tube in the 47°C water bath for a total of 45 minutes, vortexing every 15 minutes** 
   1. **From here on, all steps must be done as fast as possible**

*After the 45 minute incubation, follow steps 10-16 next to the water bath*

1. **Add 100 μL of the heated Wash Buffer 1 directly to the beads containing your sample. Pipet to mix**
2. **Place on the magnet for 2-5 seconds (do this quickly!) and discard the supernatant**
3. **Add 200 μL of the heated Stringent Wash Buffer 4. Pipet to mix**
4. **Incubate in the 47°C water bath for 5 minutes**
5. **Place on the magnet for 2-5 seconds (do this quickly!) and discard the supernatant**
6. **Repeat steps 12-14 one more time, for a total of 2 washes**
7. **Add 200 μL of the diluted Wash Buffer 1 at RT and vortex for 2 minutes. Pulse-spin briefly**
8. **Place on the magnet and discard the supernatant**
9. **Add 200 μL of the diluted Wash Buffer 2 at RT and vortex for 1 minute. Pulse-spin briefly**
10. **Place on the magnet and discard the supernatant**
11. **Add 200 μL of diluted Wash Buffer 3 at RT and vortex for 30 seconds. Pulse-spin briefly**
12. **Place on the magnet and discard the supernatant**
13. **Add 94 μL of PCR-grade H2O to resuspend beads and continue with the PCR Amplification**
    1. **There is no need to elute the DNA off the beads. Both beads and the captured DNA will be used in the PCR amplification.**

***PCR Amplification***

1. **To the DNA+bead mixture add:**
   1. **100 μL of KAPA 2x HiFI HotStart Mix (#KM2602, KM2612, KM2605 at -20C),**
   2. **6 μL of BioScience primers (20 μM each primer)**
2. **Split reaction into two 0.2 mL PCR tubes (100 μL on each tube)**
3. **PCR program: “EXOME/KAPA\_12“ 🡨this number changes from experiment to experiment!! Try to use the least amount of PCR cycles possible**
   1. Set Lid to tracking
   2. 1 – 98**°**C\_45sec

***Suggested amplification cycles***

For T200: 12 cycles

For T1000 or T2000: 11cycles

For V.2 (whole exome): 11cycles

2 – 98**°**C\_15sec

3 – 60**°**C\_30sec

4 – 72**°**C\_30sec

5 – go to step 2 11more times

6 – 72**°**C\_1min

7 – 4**°**C\_forever

***PCR Clean-up***

1. **Combine the 2 PCR reactions into a 1.5 mL tube**
2. **Add 360 μL of the SeqCap EZ Purification Beads from the kit (Exact same ones as sold by Beckman Coulter, Ampure XP beads). Pipet up and down until mixture is homogeneous.** 
   1. **Make sure Purification Beads have equilibrated to RT for at least 30 minutes prior to use**
3. **Incubate 15 minutes at RT**
4. **Place on the magnet for 3 minutes and discard the supernatant**
5. **Wash in 600 μL of 80% ETOH. Let sit on the magnet for 30 seconds**
6. **Remove and discard the ETOH**
7. **Repeat 5-6 one more time, for a total of 2 washes**
8. **Let dry ~10 minutes at RT**
9. **Resuspend in 32 μL PCR-grade H2O (suspension volume can be adjusted up to 52 μL H2O)**
10. **Place the tube on the magnet until the solution clears**
11. **Collect 30 μL of the supernatant and transfer into a new 1.5 mL tube**
    1. **NOTE: it works best if you use your P20 and pipet 2X15ul**
12. **Measure yield on the Qubit and also run the qPCR**
13. **Dilute the samples to 10 nM and send the samples for sequencing.**

**References**

* Baslan, T., et al. (2016). "Corrigendum: Genome-wide copy number analysis of single cells." Nat Protoc **11**(3): 616.
* Leung, M. L., et al. (2016). "Highly multiplexed targeted DNA sequencing from single nuclei." Nat Protoc **11**(2): 214-235.
* **Acknoledgements**
* This protocol is supported by Dr. E. Sei, Dr. M. Leung, A. Casasent, C. Kim, and J. Waters.