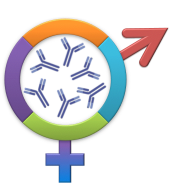
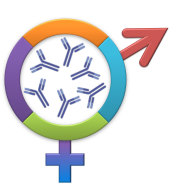
Title	  This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 741874 **SOP 08- Blood sampling, DNA isolation and whole genome SNP genotyping**
Document type	SOP
Version N.	1.0
Date	October 7th 2019

	NAME	**DATE**
PREPARED BY	Vibeke Olsen	05152019
REVIEWED BY	Åslaug Helland	
APPROVED BY		
PURPOSE: *Describe blood sampling procedure, DNA isolation and SNP analysis in G_DEFINER*		

Acronyms:	

Related SOPs:	

Appendices:	

Title	 GENDER·NET Plus Promoting gender equality in H2020 and the ERA G-DEFINER This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 741874 **SOP 08- Blood sampling, DNA isolation and whole genome SNP genotyping**
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1. Scope and applicability

This SOP applies to thecollection of blood specimens at baseline (before treatment start) for SNP analysis, further isolation of DNA and Whole genome SNP genotyping analysis in the G_DEFINER study. The main purpose for this sampling protocol is to ensure that blood samples from patients included in G_DEFINER are processed in a consistent manner at all study sites. In addition describe the methods for DNA isolation and SNP genotyping.

2. Glossary/definitions

OUH: Oslo University Hospital

3. Responsibilities

OUH: Åslaug Helland, responsible PI at OUH, responsible for this SOP
OUH: Vibeke Olsen, Research engineer, SOP writer
PIs at G_DEFINER study sites: responsible for implementing SOP at own study site

4. Procedure forcollection of blood specimens for SNP analysis

In G-DEFINER, blood specimens will be collected at baseline (**before** treatment start) for Whole genome SNP genotyping analysis. Collection and processing will be done according to this SOP. All samples will initially be biobanked at the sampling hospital, and transferred to the department of Cancer Genetics at the Norwegian Radiumhospital, OUHupon agreement.

4.1. Equipment list

- 1 x 10ml EDTA tubes (BD Vacutainer K2EDTA tube, lavender lid, Catalogue #367525)
- Centrifuge with swing out bucket rotor
- Sterile screw-cap cryotubes for plasma/buffy coat aliquots (1,5 or 2 ml) labelled according to CRF
- Filter pipette tips/Pasteur pipette
- General lab equipment

4.2. Sample collection

- Gently invert the EDTA tubes 8-10 times immediately following sample collection. Do NOT shake.
- Store the vacutainertubes upright at room temperature (RT) until centrifugation.
- The blood samples should be centrifuged as soon as possible within 2.0 hours of blood collection, and processed into aliquots **immediately**due to cfDNA quality.
- See section 4.7 for instruction on dates registration in the study database.

4.3. Plasma and Buffy Coat preparation from the EDTA tube

- Centrifuge the sample in a centrifuge with swing out bucket rotor at 1000 g for 10 min with the brake on 2, at RT.
- Collect the plasma layer with aPasteur pipette without disturbing the buffy coat layer. Leave approx. 0.5 cm of the plasma layer to avoid contamination of the underlying buffy coat layer (with lymphocyte DNA).
- Aliquot plasma in 2-3 1.0 ml aliquots in labelled cryotubes.

4.4. Collecting the buffy coat:

- Aliquot the buffy coat fraction to a labelled cryotube and freeze immediately at -80 for future isolation of germline DNA. All specimens should remain at -80°C prior to shipping. The samples should not be thawed prior to shipping.
- See section 4.7 for instruction on dates registration in the study database.

4.5. DNA Isolation (by OUH):

- DNA will be isolated from the buffy coat fraction using the QIAamp DNA Mini Kit (Qiagen) according to manufacturer'smanual, and stored at -20°C. Any remaining DNA from SNP analysis will be stored for 10 years from end of study.
- DNA concentrations will be measured by NanoDrop™, and a fluorescence-based method (like Qubit™).
- See section 4.7 for instruction on dates registration in the study database.

4.6. Whole genome SNP genotyping analysis:

- Whole genome SNP genotyping will be carried out on Infinium HumanOmni2.5-8 v1.4 BeadChip microarrays (Illumina) by Genomic Core facility at OUH.
- See section 4.7 for instruction on dates registration in the study database.

4.7. Registration of the dates in the database

- Register in the study database the following dates: date of blood collection, date of freezing, date of defrost, date site lab received blood sample, date of RNA/DNA isolation, date of analysis.
- The paper DCFs "CRF 07 - Study specific blood and stool samples" may be used to collect data for a later entry in the study database.

5. Dissemination

The SOP will be disseminated according to the G-DEFINER SOP 01 - Development, Approval and Review documents , and the PI at each site will ensure implementation of the SOP

6. References

7. Document history

Version No.	Date	Reviewer	Details of changes
0.1	May 15th2019	ÅH	First version
0.2	June 5th 2019	RM	Graphical adjustments, modified n. According to the SOP sequence
0.3	Aug.21th 2019	VO	Addition of storage conditions (4.5)
1.0	October 7th 2019	RM	Added section 4.7: Registration of dates on the database