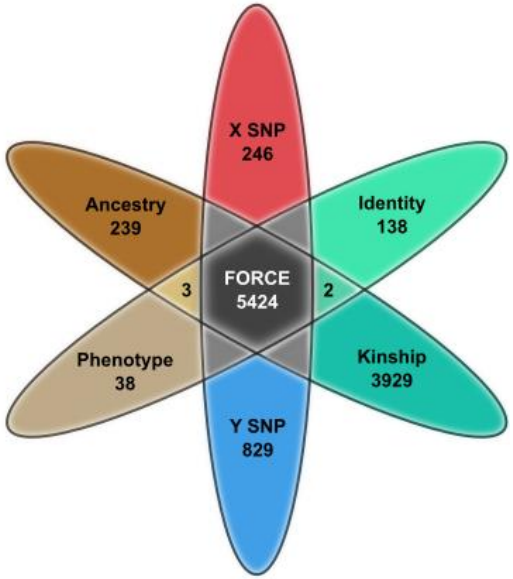
The FORCE panel

Interlaboratory study



September 22, 2022

Background & aim

- There is a need for standardization of forensic SNP markers.
- Different laboratories have different preferences when it comes to
  - Library preparation methods/techniques
  - Sequencing instruments
  - Bioinformatic tools and know-how
- Is it possible to “customize” the complete workflow (“from DNA to genotype”) for a ~5,000 SNPs marker panel?
  - One common set of SNPs, to be analyzed with different methods/assays
  - FORCE SNP panel, publication *Tillmar et al., 2021*.

FORCE interlab study

- 15 participating labs and at least 3 different methods/assays to be compared. The aim is to find:
  - Possibilities and challenges with the approach “one common SNP marker set, to be analyzed with different methods/assays”.
  - Possibilities and challenges with the specific FORCE SNP marker set.
  - Possibilities and challenges with MyBaits, QIAseq and AmpliSeq, for the analysis of ~5,000 SNPs.
  - Possibilities and challenges with interpretation (kinship, phenotypic/ancestry predictions) with data from ~5,000 SNPs.

FORCE interlab study

- Part 1 – Genotyping (DNA -> SNP profile)
  - 12 (+ 5 optional) samples will be included in the study.
  - Participating labs “select” their method of choice
    - myBaits, QIAseq or AmpliSeq (or other if available)
  - Bioinformatics and genotype calling will be performed at NBFM (for uniformity)
    - Bioinformatics and genotype calling can be performed, in parallel, at each lab (OPTIONAL)
- Part 2 – Interpretation (SNP profiles -> interpretation)
  - Kinship case
  - DVI case scenario
  - Ancestry/phenotype predictions

Part 1 – Wet exercise

General instructions for Part 1

- Analyze the samples and use the assay settings/parameters “as you normally would”, i.e. follow the specified protocol given by each company’s assay protocol, with your lab’s adjustments.
  - If you don’t have any earlier experience with the selected assay, suggestions of protocol settings/adjustments can be provide by:
    - QIAseq: Andreas Tillmar (andreas.tillmar@rmv.se)
    - MyBaits: Charla Marshall (charla.k.marshall.ctr@health.mil)
    - AmpliSeq: Daniel Kling (daniel.kling@rmv.se)
  - Any questions may also be answered by the contact persons given above.
- Information about samples and DNA input is given in the next slide.
- Include at least one negative control that follow the workflow all the way (incl sequencing).
- The number of samples to be sequenced together may vary depending on the sample/library quality, use recommendations given by the assay protocol, and/or desired read depth. Each lab will make their own decision, but Andreas/Charla/Daniel can give recommendation based on their experiences.
- FASTQ files (demultiplexed) should be transferred to andreas.tillmar@rmv.se using NextCloud (see below), together with an Excel summarizing run information (An Excel form will be provided later).

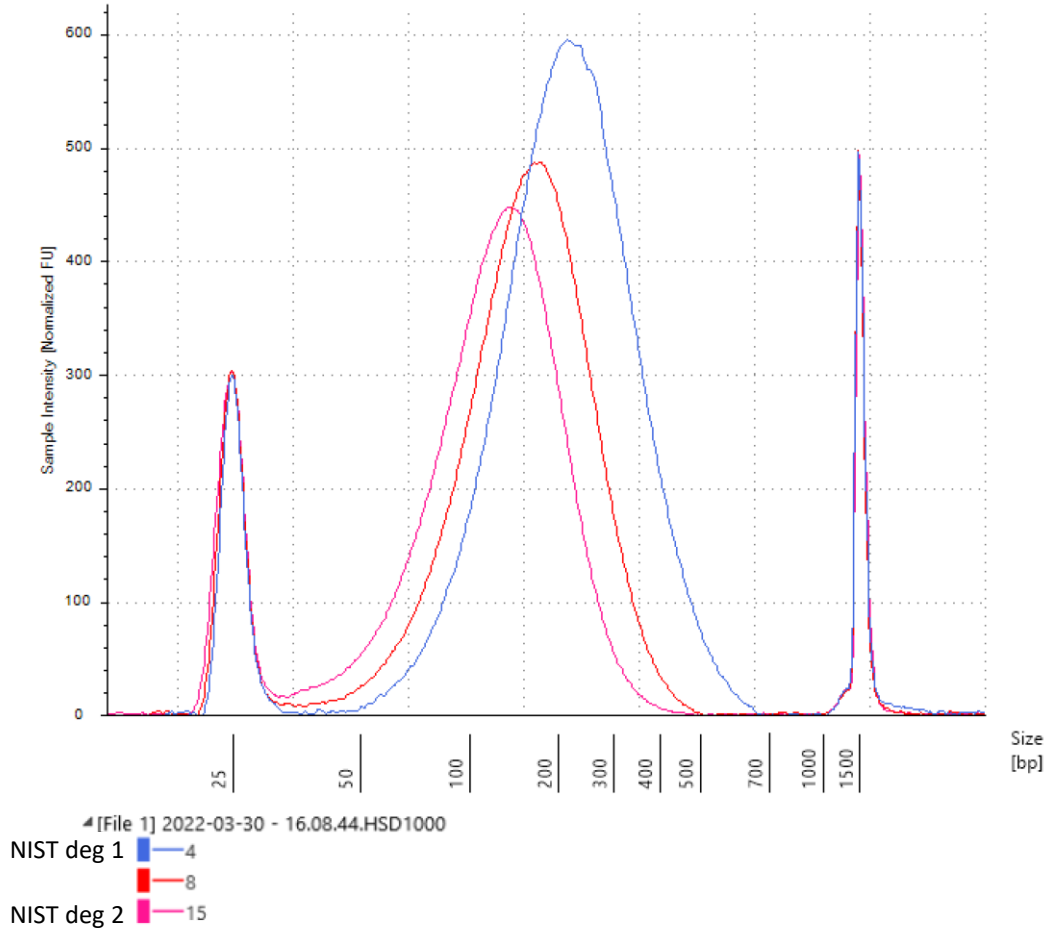
Samples

For the “up to” samples, each lab decides on volume/amount DNA to be used, based on their own preferences and choice of assay.

Sample label	Sample	Conc (pg/µl)	To be analyzed (µl)	Volume provided (µl)
FORCE Interlab Sample A	NA12877	5000	up to 50	50
FORCE Interlab Sample B	NA12878	5000	up to 50	50
FORCE Interlab Sample C	2800M_10ng	1667	6	12
FORCE Interlab Sample D	2800M_1ng	167	6	12
FORCE Interlab Sample E	2800M_300pg	50	6	12
FORCE Interlab Sample F	2800M_100pg	17	6	12
FORCE Interlab Sample G	2800M_30pg	5	6	12
FORCE Interlab Sample H	NIST deg 1 (4 minutes)	2400	up to 10	10
FORCE Interlab Sample I	NIST deg 2 (15 minutes)	1000	up to 10	10
FORCE Interlab Sample J	NIST 2391d-A	1600	up to 15	15
FORCE Interlab Sample K	NIST 2391d-B	1600	up to 15	15
FORCE Interlab Sample L	2800M with humic acid	1000	up to 25	25

+ OPTIONAL: 5 additional samples, representing relevant samples for each participating lab

NIST deg 1 and NIST deg 2, fragment size distribution



Submit/send FASTQ files to Linköping

- *NextCloud* will be used for FASTQ file sharing (unless your organization doesn't have any own file transfers service that you prefer).
- Andreas Tillmar will set up one unique folder for each lab. This folder will only be accessible for the lab's representative and Andreas Tillmar
- *NextCloud* uses a two-factor authentication process.
- Details and login instructions will be provided in November

Part 2 – Interpretation exercise

What?

- A kinship case
  - Are individual A and individual B first cousin once removed or unrelated?
- A DVI case scenario
  - Four bodies have been found from which partial SNP profiles have been established. It is believed that these bodies comes from a group of individuals that disappeared in 1960s
  - Reference samples from relatives exist, with complete SNP profiles
  - Establish the identity of the found bodies
- Ancestry/phenotype predictions
  - SNP profiles exist from two crime scene case samples. No information exists about these unknown donors. Predict eye, hair, skin color and biogeographical ancestry.

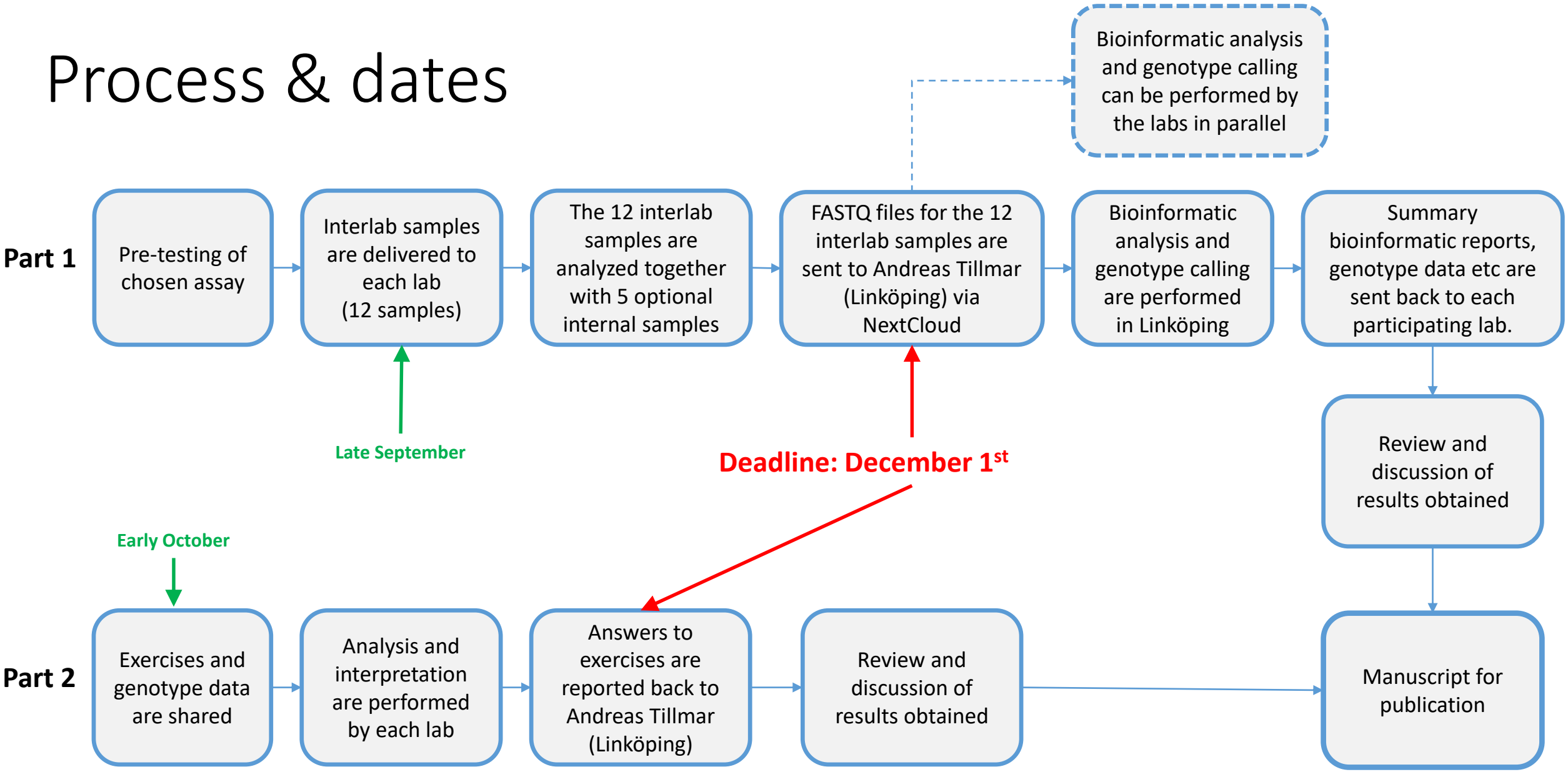
How?

- “Artificial” SNP profiles will be provided to each participating lab.
- Interpret and evaluate:
  - A: Use your own software and/or reference data.

  or
  - B: A software solution will be available (FamLink2 (*Kling et al*)) together with reference data.
- Results and conclusions will be reported back till Andreas Tillmar.
- More detailed instructions will come in early October.

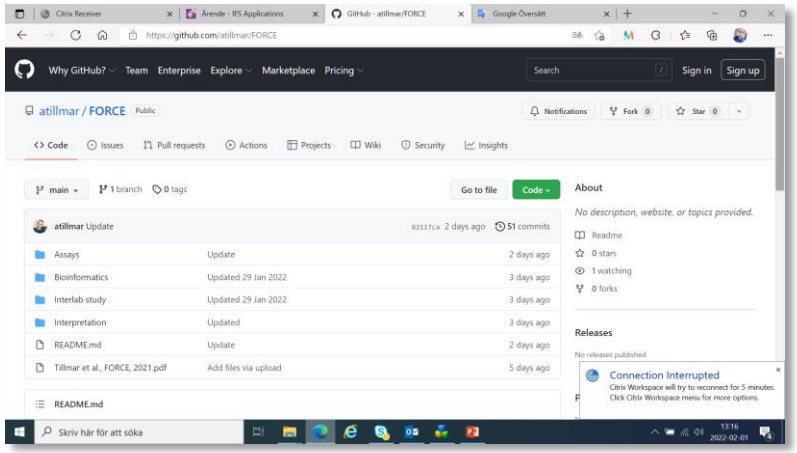
Process, timeline and other

Process & dates



A website for sharing information relevant for the interlab study

- https://github.com/atillmar/FORCE
  - Here you will find most of the information related to the testing, assays, downstream prediction tools and reference data



- This site will NOT be used to share any genotype data!
- A Q&A section is included on this site!

Question?

- Just send us an email and we are happy to help!

andreas.tillmar@rmv.se