Restriction Digestion: circa 1h

Information:2 Applications:
-- to check if Plasmid has desired length
-- preparation for Ligation of Plasmid and Insert (from PCR)

Before: have a plasmid, for Ligation: do PCR of insert
After: run a gel to check length, or proceed with ligation

If done for ligation:
Run digestion of inserts for 2h instead of 30 mins, afterwards run all product on gel, cut out, clean. Then nanodrop
Vector: Run digestion for 2h instead of 30 mins, then do CIP treatment (45mins at 37°C, CIP at -20°C in Gimli), then also do gel and cleanup. Then nanodrop.

Use this website to check buffer for enzyme combination: https://nebcloner.neb.com/#!/redigest

Protocol with Example:

Restriction Enzymes:
DO NOT TAKE THEM OUT OF -20°C UNTIL PROTOCOL EXPLICITLY SAYS TO DO SO.
- EcorV Fast digest
- ScaI Fast digest
- XbaI Fast digest

Restriction Enzyme buffers :
- NEB 3.1 buffer
- NEB Cutsmart buffer

Other chemicals:
- ddH20
- plasmids

Objects:
- Iceblock at -20°C
- Eppendorf rack
- 5 Eppendorfs

Calculations:
For 20µl total amount:
ddH20 13µl
Plasmid 1µgramm in total (4µl of 73ng/µl stock)
buffer 2µl
enzyme 1µl

Procedure:

Preparation:
Preheat the thing that heats eppendorfs to 37°C.
Autoclaved water will do.
Label your eppendorfs

Steps:
Add ddH20, Buffer, Plasmid/DNA. Add Enzyme last (always at -20°C). Incubate (time and temp according to Enzyme and Length, as well as if it's just to visualize or if it is for ligation).

Start the gel preparation while it digests!

Run a gel and/or proceed with ligation.