Double Restriction Digest Protocol (NEB)

Good tool to use when doing a restriction digest:
http://nebcloner.neb.com/#!/redigest

Materials:

- DNA
- NEBBuffer
- Restriction enzymes
- Nuclease-free water
- Eppendorf tubes
- Incubator

Protocol:

1. Set up the reaction as follows in an Eppendorf tube: (50 µL reaction)
    - 1 µg DNA (concentration needs to be known beforehand)
    - 5 µL 10X NEBuffer*
    - 1 µL Restriction enzyme 1*
    - 1 µL Restriction enzyme 2*
    - Nuclease-free water up to 50 µL
2. Incubate at 37 ℃ for 1 hour.

* Check the constructs in Benchling to decide which restriction enzymes should be used, then decide which buffer to use using either the NEBcloner tool or the performance chart for NEB restriction enzymes. You want a buffer in which both enzymes have 100% activity.

*If using PstI and EcoRI, add 3 µl of EcoRI in order to reach optimal cleaving.