**Haplobank**

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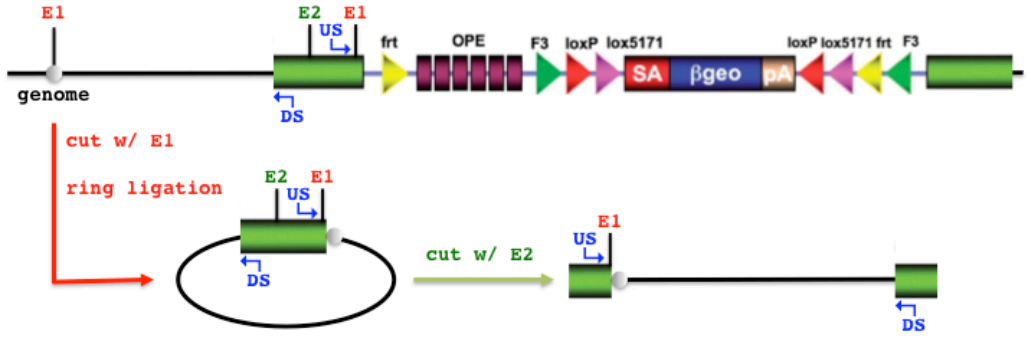
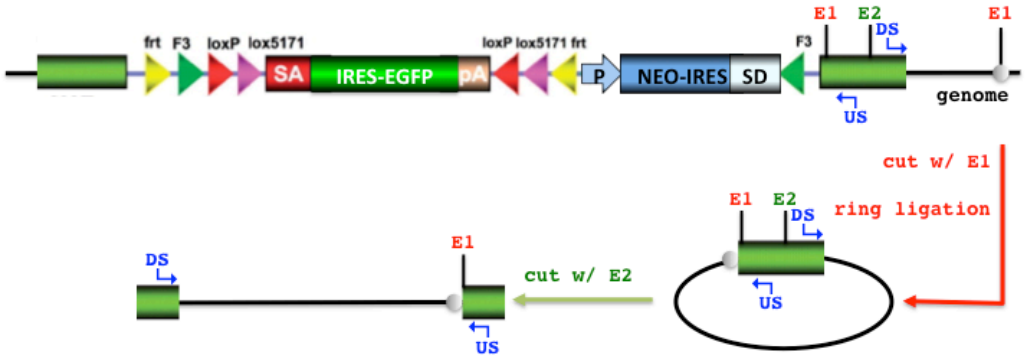
# Inverse PCR: PCR Strategy to Map Genomic Integration Sites of Gene Trap Vectors

Enzyme1 (E1) is used to fragment the genome. As the recognition sequence for E1 is also present in the terminal repeat of the gene trap vector, it is possible to retrieve the exact integration site of the gene trap cassette within the genome by circularizing E1-digested gDNA (ring ligation) and subsequently amplifying the genomic region by inverse PCR (iPCR) using primers "US" and "DS". To improve iPCR efficiency, a linearization step using E2 was introduced, which re-opens the rings generated previously. Moreover, each integration site can be mapped by using two different enzymes E1. We would recommend you to split your samples and use both enzymes E1 on each sample in parallel.
Please keep in mind the "directionality" of the mapping strategies for different mutagens, as this affects the assignment of a particular mutation to the sense and anti-sense strand in the genome, respectively.

Mapping – 5' strategy (Lenti, Retro)




Mapping – 3' strategy (Tol2-GT, Tol2-pA)



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- **Mutagenesis systems**

| | Enzyme1 (E1) | Enzyme2 (E2) | Mapping Strategy |
|---|---|---|---|
| ☐ Lenti | NlaIII, TaqI | PacI | 5' |
| ☐ Retro | NlaIII, MseI | SbfI | 5' |
| ☐ Tol2-GT | NlaIII, TaqI | PacI | 3' |
| ☐ Tol2-pA | NlaIII, TaqI | PacI | 3' |

- **universal iPCR primer sequences**
  DS GAGCCAGAACCAGAAGGAACTTGAC
  US GTGACTGGAGTTCAGACGTGTGCTCTTC

- **Reagents and Buffers**
  RNAse A (Qiagen, 19101, 100mg/ml)
  optional: a phase lock system
  Roti-Phenol/Chloroform/Isoamyl alcohol (Roth, A156.2)
  mixture of Chloroform:Isoamyl alcohol 24:1 (Sigma, Merck)
  Isopropanol, 70% Ethanol and TE Buffer
  Picogreen, Invitrogen
  Restriction enzymes and buffers, New England Biolabs
  T4 DNA ligase and buffer, Roche Applied Science
  PCR kit (polymerase and buffer)
  QIAquick PCR Purification Kit (Qiagen) or Sera Mag Speedbeads (Thermo, 45152105080350) & Binding Buffer
  illustra ExoProStar 1-Step Kit (GE)

- **prepare Genomic DNA lysis buffer (GDLB) and autoclave it**
  10mM Tris-HCl pH 8.0
  5mM EDTA
  100mM NaCl
  1.0% SDS
  1 mg/ml Proteinase K

## iPCR Protocol

1. **Genomic DNA prep**
   - lyse cell pellet according to pellet size in 0,5-5mL GDLB
   - inc. 55˚C, o/n
   - add 1:1000 RNAse A (Qiagen, 100mg/ml) for ~1h, 37˚C
   - 2x Phenol/Chloroform/Isoamyl alcohol extractions (using a phase lock system)
   - 1x Chloroform/Isoamy alcohol extraction (using a phase lock system)
   - Isopropanol precipitation of gDNA

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- wash gDNA pellet w/ 70% EtOH
- resuspend in 0,05-1mL TE Buffer
- quantify DNA using Picogreen

**2. Digestion of samples w/ enzymes E1 in parallel (per sample two seperate reactions!)**

| | |
|---|---|
| gDNA 100ng/µl | 10 µl |
| 10x CutSmart | 8 µl |
| Enzyme1 | 3 µl |
| dH2O | 59 µl |
| | 80 µl |

inc. 37˚C or 65˚C (TaqI), o/n

**3. Purify restriction digests (Qiagen PCR kit or Mag Speedbeads), elute in 50µl in total**

**4. Ring ligation**

| | |
|---|---|
| E1-gDNA | 50 µl |
| 10x LB | 30 µl |
| T4 DNA Ligase | 2 µl |
| dH2O | 218 µl |
| | 300 µl |

inc. 16˚C, o/n

**5. Heat-inactivate T4 DNA Ligase at 65˚C for 15min**

**6. Linearize samples w/ enzyme E2 at 37˚C for 2h**

| | |
|---|---|
| RL-E1-gDNA | 300 µl |
| Enzyme2 | 1 µl |

**7. Purify restriction digest (Qiagen PCR kit or Mag Speedbeads), elute in 50µl in total**

**8. iPCR reaction**

| | |
|---|---|
| E2-RL-E1-gDNA from 7. | 10 µl |
| primer US 100 uM | 0.1µl |
| primer DS 100 uM | 0.1µl |
| 10 mM dNTP mix | 1 µl |
| 10x pol. buffer | 5 µl |
| 20x polymerase | 3 µl |
| dH2O | 30.8µl |
| | 50 µl |

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Biorad C1000 cycle parameters

```
95deg, 3'
95deg, 15"|
61deg, 25"| 37x
72deg, 75"|
72deg, 5'
```

Analyze 20µl on an agarose gel, purify PCR products using the QIAquick kit or the ExoStar kit and use them for Sanger Sequencing.