

DamID flowchart

Transfect / Infect cells¹ with Dam-fusion protein vectors²

- Transfections: Use Dam and Dam-fusion protein vectors, include controls (use vector without Dam, take along untransfected cells). Transfect cells with vectors and select for stable integration.
- Lentiviral infections: Use virus to integrate Dam-fusion expression cassettes.
- ▶ *Important:* For DamID Dam-fusion proteins should be expressed at very low levels.

Isolate genomic DNA

Amplify methylated gDNA by methylation specific PCR

- Isolate methylated fragment by digestion with DpnI restriction enzyme.
- Ligate adaptors to methylated fragments.
- Amplify methylated fragments by PCR (Visualize PCR product on agarose gel).
- ▶ *Important:* Include negative controls (leave out DpnI and take along unmethylated gDNA), these controls should not give a PCR product.

Label and hybridize amplified fragments to microarray

- Label amplified DNA fragments.
- Depending on the array type the DNA can be reduced in size by DNase digestion.
- ▶ *Important:* Always cohybridize Dam-fusion against “Dam only” to correct for unspecific Dam binding and local differences in chromatin accessibility.

Image and Data analysis

- Ratio of the fluorescent signal of Dam-Fusion/Dam denotes the level of binding of the Dam-fusion protein to the probed sequence.

¹ DamID has successfully been used in whole flies, and in fly, human and mouse cell lines, please see www.nki.nl/nkidep/vansteensel for detailed protocols.

² Please see www.nki.nl/nkidep/vansteensel for available vectors.