Adeno-associated viral vectors and stereotaxic delivery

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Adeno-Associated Virus

- Parvovirus family
- Linear ssDNA of either polarity
- Infects dividing and non-dividing cells
- Persists in episomal state in host nucleus
- Can infect large number of cell types
- ~4.7 kb wild type genome
AAV Life Cycle
Packaging Components of rAAV

1. Transfer vector: Inverted Terminal Repeats (ITR) flanking gene of interest
2. AAV helper plasmid expressing rep and cap proteins
3. Adenovirus helper plasmid expresses adenoviral proteins necessary for packaging
AAV as a Viral Vector

**Strengths**
- Low immune response
- Infects dividing and non-dividing cells
- Non-integrating
- Persistent expression
- Serotypes differentially infect cell types
- Various routes of delivery
- High titers

**Weaknesses**
- Not ideal for dividing cells, DNA is lost through cell division
- Small genome allowing gene cassette of ~4.4kb
- Serotypes differentially infect cell types
AAV Serotypes

• AAV is subdivided into serotypes which exhibit different tissue tropism

• Serotypes are not tissue specific because they can transduce many different cell types

• Serotypes have higher affinities for certain cell types due to differences in capsid proteins
## AAV Serotypes Target Different Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Optimal Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>AAV8, AAV9, AAVDJ</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>AAV1, AAV6-9</td>
</tr>
<tr>
<td>CNS</td>
<td>AAV1, AAV2, AAV5, AAV8, AAV9, AAVDJ</td>
</tr>
<tr>
<td>Photoreceptor Cells</td>
<td>AAV5</td>
</tr>
<tr>
<td>Lung</td>
<td>AAV9</td>
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<tr>
<td>Heart</td>
<td>AAV8</td>
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<tr>
<td>Pancreas</td>
<td>AAV8</td>
</tr>
<tr>
<td>Kidneys</td>
<td>AAV2</td>
</tr>
</tbody>
</table>
rAAV Production

- Triple transfection
- Wait 48-72 hours
- Lyse cells by freeze/thaw or sonication
- CsCl gradient, iodixanol gradient or heparin column
- Dialyze fractions
- Titer by Taqman qPCR
- Titers $>10^{12}$ vg/ml
Stereotaxic Delivery

- Good spatiotemporal control
- Any brain region or subpopulation of cells
- Inject at any postnatal date

Common Uses

- Fluorescent labeling of cell populations
- Neuronal track tracing
- Viral mediated gene knockdown or over-expression
- Cell specific targeting using cre transgenic mice
Stereotaxic injection protocol

- Anesthetize animal
- Secure rodent to stereotax, continue anesthesia
- Make incision and locate bregma
- Drill hole in skull at desired coordinate
- Load syringe with viral suspension
- Lower needle to proper coordinate
- Infuse viral particles at .1-.5 ul/min
Intracerebral injection for protein over-expression

Serotype: AAV9
Protein Expressed: Girk1-2a-mCherry and Girk2-2a-GFP
Amount injected: $10^9$ vg
Manufacturer: VVC

Ford et al., unpublished
Cell specific targeting using cre transgenic

Serotype: AAVDJ
Protein expressed: ArchT-YFP
Amount injected: $10^9$ vg
Manufacturer: VVC

McGinley et al, unpublished
Intraventricular Injection

Serotype: AAV2
Protein Expressed: GFP
Amount injected: $10^8$ vg
Manufacturer: UNC

Fu et al., Molecular Therapy, 2003
Intravenous Injection

Serotype: AAV9
Protein expressed: GFP
Amount injected: $4 \times 10^{11}$
Manufacturer: UPenn

Foust et al., Nature Biotechnology, 2009
Intranasal Injection

Serotype: AAV2
Protein Expressed: dsRed
Amount injected: $10^{10}$ vg
Manufacturer: VVC

Borisovska et al., unpublished
Neuronal Track Tracing

Serotype: AAV2
Proteins expressed: GFP and mCherry
Amount injected: ~10^8 vg
Manufacturer: UPenn

Deniz Kusefoglu et al., unpublished
Vollum Viral Core Services

• Free consultation

• Cloning, production and titering of AAV, lenti- and retro- viral particles

• Stereotaxic delivery of viral particles

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