

Cell Based High Throughput Screen for α -synuclein

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Goals

- Identify FDA approved drugs and small molecules that block α -synuclein.
- Create a compendium of information to be mined to identify new therapeutics for PD.

Drug Database

- 900 unique US FDA approved drugs
- Complex queries can be performed
- Drug information
 - Chemical composition
 - Indications
 - Toxicity
 - Adverse Reactions
 - Manufacturer

Advantages of FDA drugs

- Immediately available
- Bioactive, bioavailable molecules
- Known chemical structure
- Potential known mechanism of action
- Known target protein/organ
- Biochemistry, pharmacology and toxicology
- Known indications
- Known contraindications, toxicity

α -synuclein

- α -synuclein found in Lewy bodies of PD.
- Over expression of human wt α -synuclein in mice results in nonfibrillar inclusions, loss of dopaminergic termini, and motor functions.

- Human α -synuclein promoter (10.7kb).
- The promoter is in pGL3 driving photinus pyralis luciferase.
- Co-transfect with pRL renilla luciferase.
- Transfect human neuroblastoma cell lines (SH-SY5Y and IMR-32).

- The bioassay for human α -synuclein promoter in the luciferase system works well.
- Advantages: highly specific, no endogenous activity, broad dynamic range.
- Disadvantages: requires substrate (luciferin), O_2 , and ATP, injection of substrate and read time.
- Ideal system for screening a 1000 not 10,000's compounds.

- Steady-Glo luciferase assay system used for screening 10000's compounds.
- Advantages: designed for HTS cell based luciferase assays, long-lived luminescence, homogeneous, only single reagent addition, half life 5 hours, several thousand compounds per hour.
- Disadvantages: requires substrate (luciferin) and O₂ and ATP.
- Dual-Glo system under development.