Use of BacMam technology to express full-length LRRK2 and to profile inhibitors using cell-based and biochemical assays

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ABSTRACT

Studies of LRRK2 function and inhibition have been hampered by a lack of tools to express full-length LRRK2 in diverse cell types as well as high-throughput biochemical and cell-based assays. To address these needs, we have developed BacMam particles to express both tagged and untagged LRRK2 (wild-type and mutants). Compared to standard lipid transfection, BacMam technology has enabled higher levels of expression of LRRK2 in both well plate cell lines (e.g. U-2 OS, HEK293T) and cells more physiologically relevant to neuroscience such as neuroblastoma SH-SY5Y and primary astrocytes. Furthermore, the ability to express high levels of LRRK2 in these systems has enabled modular high-throughput assays for compounds that bind to full-length LRRK2. Utilizing BacMam expression and LanthaScreen® technology, biochemical assays to measure inhibitor binding and cellular assays to measure inhibitor effects on kinase activity were developed. In addition, application of BacMam technology has enabled expression levels sufficient to produce highly purified, full-length LRRK2.

Figure 1. Introduction to BacMam Technology

The BacMam technology (A) is based on use of a modified baculovirus to efficiently deliver and express genes in mammalian cells. Baculoviruses are non-replicating in mammalian cells and thus have an excellent safety profile combined with being well-tolerated by cells. BacMam reagents have been used in cell-based assays, live cell imaging, stem cell biology and many other applications. BacMam reagents are used in your normal workflow as any other reagent for cell-based research; take the reagent from the fridge, add it to cells, incubate and assay (B). Transfection is efficient and reproducible in most cell lines, including primary and stem cells. The BacMam platform enables easy transduction of large quantities of cells in batch mode; transduced cells can be stored frozen in aliquots for later use, providing assay-ready cells when you need them - with no loss of activity. Cells can be assayed within hours of thawing and plating.

Figure 2. LRRK2 expression levels are higher with the BacMam system than lipid transfection

Expression levels for LRRK2 were compared for the BacMam system and for a lipid transfection method. Much higher expression was observed for the BacMam system.

Figure 3. BacMam-expressed GFP-LRRK2 recapitulates inhibitor dependent relocalization

Figure 4. Principle of BacMam-enabled LanthaScreen® LRRK2 [pSer935] Cellular Assay

Using Time-Resolved Förster Resonance Energy Transfer (TR-FRET) technology, we developed a high-throughput compatible homogeneous cellular assay for monitoring the LRRK2 phosphorylation at Ser935. LRRK2-GFP fusion proteins are transiently expressed in a variety of cell backgrounds via BacMam. Cells are plated in 384-well assay plates. Phosphorylation at Ser935 in these cells is detected using a terbium labeled pSer935 antibody that generates TR-FRET signals between terbium and GFP.

Figure 5. Validation of BacMam-enabled LanthaScreen® LRRK2 [pSer935] Cellular Assay

Confirmation by Western blotting

C. IC₅₀ values for known inhibitors

-70% Inhibition >-80% Inhibition Average F-factor

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<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
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Figure 6. Kinase Binding Assay in lysates with full-length LRRK2

BacMam LRRK2-GFP reagent was used to transduce HEK293T cells and generate a lysate containing full-length LRRK2. Kinase Binding Assays were then performed comparing the full length LRRK2 lysate and purified truncated LRRK2. Optimal lysate and trimer concentration were determined. 5nM truncated kinase (PV4873) or 3nM full length kinase (A14177), 2µM Eu-anti GFP antibody (A14173) and Tracer 236 (PV5592) were used to assay binding of several kinase inhibitors to LRRK2. While many inhibitors appear to bind with similar affinity to truncated and full-length LRRK2, others (notably GW5074) appear to have significantly altered affinity.

Figure 7. Production of purified and active full-length LRRK2 1,2

Affinity tagged LRRK2 was expressed with the BacMam system, purified, and analyzed by Coomassie-stained SDS-PAGE (A). Purified, full length LRRK2 was active in a LanthaScreen activity assay using LRRKtide as the substrate (B). The activity assay using full-length LRRK2 was validated with the relatively specific inhibitor LRRK2-B1-1 (C). We have successfully purified full-length WT, D1994A, G2019S and R1441C.

CONCLUSIONS

An extensive set of tools have been developed to enable the discovery and characterization of inhibitors of LRRK2. These tools allow for HTS compatible screens in multiple formats for full-length LRRK2 and its mutants in both biochemical and cellular environments.

References

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