人蛋白质组芯片试验原理

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试验流程与原理：

1. 芯片封闭：用 BSA 封闭芯片上未结合蛋白质的位点。

2. 血清孵育：血清用 BSA 按比例稀释并加入到芯片的活性表面，使血清中抗体与目的蛋白质特异性结合。

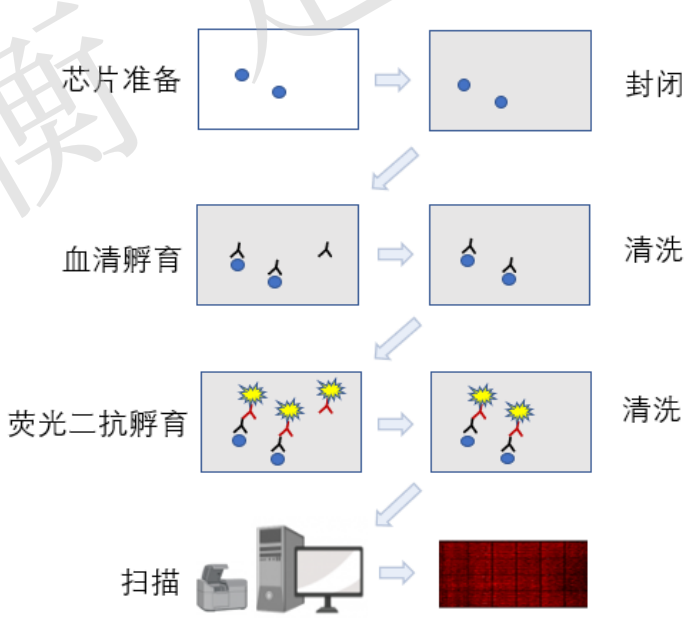
3. 清洗：去除非特异性结合的血清抗体。

4. 荧光二抗孵育：荧光二抗用 BSA 按比例稀释并加入到芯片的活性表面，使荧光二抗与血清抗体特异性结合。

5. 清洗：去除非特异性结合的荧光二抗。

6. 荧光检测：使用荧光扫描仪扫描芯片，获得荧光信号数据。

5. 数据分析：将数据归一化并进行统计分析。



附：芯片生产过程

1. HuProt™蛋白质库的建立：

HuProt™蛋白质库克隆来自公共 ORF 库或独立合成，使用 Gateway 重组克隆系统（Invitrogen，CA），将人 ORF 从入门克隆穿梭至酵母高拷贝表达载体（pEGH-A），该载体在半乳糖诱导型 GAL1 启动子的控制下产生 GST-His6 融合蛋白。将质粒回收到大肠杆菌中，并通过限制性核酸内切酶消化进行验证。将具有正确大小插入片段的质粒转化到酵母中进行蛋白质纯化。

Creation of HuProt™ Library：

HuProt™ library clones were derived from public ORF libraries or independently synthesized; entry clones are from the laboratories of Heng Zhu and Seth Blackshaw (The Johns Hopkins University). Using the Gateway recombinant cloning system (Invitrogen, CA), human ORFs were shuttled from the entry clones to a yeast high-copy expression vector (pEGH-A) that produces GST-His6 fusion proteins under the control of the galactose-inducible GAL1 promoter. Plasmids were rescued into *E. coli* and verified by restriction endonuclease digestion. Plasmids with inserts of correct size were transformed into yeast for protein purification (Hu S *et al*, 2009; Jeong J et al, 2012)

2. HuProt™中克隆验证和整理：

为了检查并确认 HuProt™文库中每个人类 ORF，对入门克隆和衍生自它们的酵母表达载体进行了双向 Sanger 测序。使用 Blast +将 ORF 序列与多个公共数据库（UniProt，CCDS，RefSeq 和 Ensembl）进行比对，如果克隆覆盖了已知蛋白的整个序列，则该克隆被认为是全长（F），如果部分匹配则被视为截短（TRUNC）克隆。由于源克隆的 ORF 包括含有未翻译区域、未注释的剪接变体和单核苷酸多态性，因此将克隆分为几类，有完全匹配到已知的蛋白质编码转录组，也有潜在蛋白质编码 ORF。

Validating and Curating Clones used in HuProt™. To check and confirm the identity of each human ORF in the HuProt™ libary, bidirectional Sanger sequencing was conducted on both the entry clones and the yeast expression vectors that were derived from them (Venkataraman A *et al*, 2018). Blast+ was used to align the ORF sequence to multiple public databases (UniProt, CCDS, RefSeq, and Ensembl) to generate an integrated alignment score for each clone. If a clone covered the entire sequence of a known protein, the clone is considered full length (F), whereas partial matches were regarded as indicative of truncated (TRUNC) clones. Because the source clones included ORFs containing untranslated regions, unannotated splice variants, and single-nucleotide polymorphisms, the clones were categorized into groups ranging from perfect matches to the known protein-coding transcriptome, to as-yet potential protein-coding ORFs that are not yet reviewed.

3. HuProt™文库中的蛋白质纯化：

从由编码人 ORF 的表达载体转化的酵母中纯化蛋白质，人蛋白质作为 GST-His6 的融合蛋白。

Protein Purification from the HuProt™ Library：

Proteins were purified from yeast transformed with expression vectors encoding the human ORFs. Human proteins were purified as GST-His6 fusion proteins from yeast using a previously described high-throughput purification protocol (Hu S *et al*, 2009; Zhu et *al*., 2001). Using a 96-well format, the samples are purified from yeast extracts using glutathione-agarose beads. 0.1% Triton is included in the lysis buffer and washers to ensure that the purified proteins are free of lipids.

4. 蛋白质芯片点制与检测：

用 Arrayjet UltraMarathon 打印机（英国 Arrayjet）将纯化的人类蛋白质以 384 孔格式排列并打印在 PATH 载玻片（GraceBio，美国）上，分成不同的“Block”。在抗 GST 分析中显示> 95％的斑点的前景/背景信号（F / B）值大于（含）1.5 则阵列可用。 HuProt™包含许多与第二检测试剂反应的对照，包括滴定的 GST 蛋白质、组蛋白、小鼠和兔抗生物素、小鼠 IgM 和用于链霉亲和素检测的生物素标记对照等。每个“Block”还包含一行质控点，Alex Fluor 555/647 作为标志。

Protein Microarray Production & Testing：

The purified human proteins were arrayed in a 384-well format and printed on PATH slides (GraceBio, USA), using an Arrayjet UltraMarathon printer (Arrayjet, UK) to create a block format. Arrays that show >95% of the spots with a foreground/background signal (F/B) ratio of at least 1.5 in an anti-GST assay are classified usable. A number of controls that are reactive with secondary detection reagents are included on HuProt™. Controls include titrated GST protein, histones, mouse and rabbit anti-biotin, mouse IgM, and biotin-tagged control for streptavidin detection. Each block also contains a row of control spots, including Alex Fluor 555/647 as landmarks.