AMPure DNA 选择与纯化

The following size selection protocol is for libraries with 200 bp inserts only. For libraries with different size fragment inserts, refer to the Table below for the appropriate volumes of beads to be added. The size selection protocol is based on a starting volume of 100 µl. Size selection conditions were optimized with AMPure XP beads; however, SPRIselect beads can be used following the same conditions.

以下尺寸选择方案仅适用于插入200 bp的文库。对于具有不同尺寸片段插入片段的文库，请参阅下表，了解要添加的相应体积的磁珠。大小选择方案基于100μl的起始体积。使用AMPure XP磁珠优化尺寸选择条件；但是，SPRIselect微珠可以在相同的条件下使用。

To select a different insert size than 200 bp, please use the volumes in this table:

要选择与200 bp不同的插入尺寸，请使用下表中的体积：

Table 1.1: Recommended conditions for bead based size selection.

表 1.1：基于磁珠的尺寸选择的推荐条件。

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| LIBRARY  PARAMETERS | APPROXIMATE  INSERT SIZE | 150 bp | 200 bp | 250 bp | 300-400 bp | 400-500 bp | 500-700 bp |
| Total Library Size  (insert + adaptor) | 270 bp | 320 bp | 400 bp | 400-500 bp | 500-600 bp | 600-800 bp |
| VOLUME TO  BE ADDED (μl) | 1st Bead Selection | 65 | 55 | 45 | 40 | 35 | 30 |
| 2nd Bead Selection | 25 | 25 | 25 | 20 | 15 | 15 |

3A.1. Vortex SPRIselect beads to resuspend. AMPure XP beads can be used as well. If using AMPure XP beads, please allow the beads to warm to room temperature for at least 30 minutes before use.

3A.1. 涡旋SPRI要重悬的珠子。AMPure XP珠子也可以使用。如果使用AMPure XP珠子，请在使用前让珠子升温至室温至少30分钟。

3A.2. Add 13.5 µl of dH2O to the ligation reaction for a 100 µl total volume.

3A.2. 向连接反应中加入13.5μl dH2O，总体积为100μl。

3A.3. Add 55 µl (0.55X) of resuspended SPRIselect beads to the 100 µl ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

3A.3. 向100μl连接反应中加入55μl（0.55X）重悬微珠。上下移液至少10次，搅拌均匀。在最后一次混合过程中，小心将所有液体从吸头中排出。也可以使用高涡旋3-5秒。如果在混合后离心样品，请务必在珠子开始沉淀之前停止离心。

3A.4. Incubate samples on bench top for at least 5 minutes at room temperature.

3A.4. 在室温下在工作台上孵育样品至少5分钟。

3A.5. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

3A.5. 将试管/板放在适当的磁性支架上，将磁珠与上清液分离。如有必要，在放置在磁性支架上之前，快速旋转样品以从管或板孔的侧面收集液体。

3A.6. After 5 minutes (or when the solution is clear), carefully transfer the supernatant containing your DNA to a new tube **(Caution: do not discard the supernatant)**. Discard the beads that contain the unwanted large fragments.

3A.6. 5分钟后（或溶液澄清时），**小心地将含有DNA的上清液转移到新管中（注意：不要丢弃上清液）。丢弃含有不需要的大碎片的珠子。**

3A.7. Add 25 µl (0.25X) resuspended SPRIselect beads to the supernatant and **mix at least 10 times.** Be careful to expel all of the liquid from the tip during the last mix. Then incubate samples on the bench top for at least 5 minutes at room temperature.

3A.7. 向上清液中加入25μl（0.25X）重悬SPRIselect珠，**混合至少10次**。在最后一次混合过程中，小心将所有液体从尖端排出。然后将样品在室温下在工作台上孵育至少5分钟。

3A.8. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

3A.8. 将试管/板放在适当的磁架上，将磁珠与上清液分离。如有必要，在放置在磁性支架上之前，快速离心样品以从管或板孔的侧面收集液体。

3A.9. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets **(Caution: do not discard beads)**.

3A.9. 5分钟后（或溶液澄清时），小心地除去并丢弃含有不需要的DNA的上清液。注意不要干扰含有所需DNA靶标的珠子**（注意：不要丢弃珠子）。**

3A.10. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

3A.10. 在磁架中向试管/板中加入200μl新鲜制备的80%乙醇。在室温下孵育30秒，然后小心地取出并弃去上清液。注意不要干扰含有DNA靶标的珠子。

3A.11. Repeat Step 3A.10 once. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

3A.11. 重复步骤3A.10一次。第二次洗涤后，请务必去除所有可见的液体。如有必要，短暂旋转试管/板，放回磁铁上，用p10移液器吸头去除痕量乙醇。

3A.12. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

3A.12. 将珠子风干最多5分钟，同时将管子/板放在磁性支架上，盖子打开。

**Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.**

**注意：不要过度干燥珠子。这可能导致DNA靶标的回收率降低。当珠子仍呈深棕色且有光泽时，但当所有可见液体都已蒸发时，洗脱样品。当珠子变成浅棕色并开始破裂时，它们太干了。**

3A.13. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads into 17 µl of 10 mM Tris-HCl or 0.1X TE.

3A.13. 从磁性支架上取下管子/板。将珠子中的DNA靶标洗脱到17μl 10mM Tris-HCl或0.1X TE中。

3A.14. Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

3A.14. 在涡旋混合器上或上下移液10次充分混合。在室温下孵育至少2分钟。如有必要，快速旋转样品以从管或板孔的侧面收集液体，然后再放回磁架上。

3A.15. Place the tube/plate on a magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 µl to a new PCR tube for amplification.

3A.15. 将管子/板放在磁性支架上。5分钟后（或当溶液澄清时），将15μl转移到新的PCR管中进行扩增。

**Safe Stopping Point: It is safe to store the library at -20°C.**

安全停止点：将库存放在 -20°C 下是安全的。

**3B. Cleanup of Adaptor-ligated DNA without Size Selection**

**无需大小选择即可纯化接头连接的 DNA**

3B.1. Vortex SPRIselect beads to resuspend (AMPure XP beads can be used as well). If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use.

3B.1. Vortex SPR选择要重悬的珠子（也可以使用AMPure XP珠子）。如果使用AMPure XP磁珠，请在使用前让磁珠加热至室温至少30分钟。

3B.2. Add 86.5 µl (1X) resuspended SPRIselect beads to the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

3B.2. 向接头连接反应中加入86.5μl（1X）重悬SPRIselect微珠。上下移液至少10次，搅拌均匀。在最后一次混合过程中，小心将所有液体从吸头中排出。也可以使用高涡旋3-5秒。如果在混合后离心样品，请务必在珠子开始沉淀之前停止离心。

3B.3. Incubate samples on bench top for at least 5 minutes at room temperature.

3B.3. 在室温下将样品在工作台上孵育至少5分钟。

3B.4. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

3B.4. 将试管/板放在适当的磁架上，将磁珠与上清液分离。如有必要，在放置在磁性支架上之前，快速旋转样品以从管或板孔的侧面收集液体。

3B.5. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets **(Caution: do not discard the beads)**.

3B.5. 5分钟后（或溶液澄清时），小心地取出并丢弃上清液。注意不要打扰含有DNA靶标的珠子（注意：不要丢弃珠子）。

3B.6. Add 200 µl of 80% freshly prepared ethanol to the tube/ plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

3B.6.在磁架中向管/板中加入200μl新鲜制备的80%乙醇。在室温下孵育30秒，然后小心地取出并弃去上清液。注意不要干扰含有DNA靶标的珠子。

3B.7. Repeat Step 3B.6 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

3B.7. 重复步骤3B.6一次，共洗涤两次。第二次洗涤后，请务必去除所有可见的液体。如有必要，短暂旋转试管/板，放回磁铁上，用 p10 移液器吸头去除痕量乙醇。

3B.8. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

3B.8. 将试管/板放在磁性支架上并打开盖子时，将珠子风干最多 5 分钟。

**Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.**

**注意：不要过度干燥珠子。这可能导致DNA靶标的回收率降低。当珠子仍呈深棕色且有光泽时，但当所有可见液体都已蒸发时，洗脱样品。当珠子变成浅棕色并开始破裂时，它们太干了。**

3B.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 µl of 10 mM Tris-HCl or 0.1X TE.

3B.9. 从磁性支架上取下管子/板。通过加入17 μl 10 mM Tris-HCl或0.1X TE从珠子中洗脱DNA靶标。

3B.10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

3B.10. 上下移液10次，或在涡旋混合器上充分混合。在室温下孵育至少2分钟。如有必要，快速旋转样品以从管或板孔的侧面收集液体，然后再放回磁架上。

3B.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 µl to a new PCR tube.

3B.11. 将管子/板放在磁性支架上。5分钟后（或当溶液澄清时），将15μl转移到新的PCR管中。

**Samples can be stored at –20°C.**

样品可在–20°C下储存。