Recombinase Polymerase Amplification (RPA) on Paper

Aim of the Experiment

Preparation of RPA mixture on paper for storage and usage in automated process on PCR Chip.

Materials

- nuclease-free H_2O (nf H_2O, Sigma Aldrich, USA)
- 200 mM $MgCl_2$ (Carl Roth, Germany)
- 280 mM MgAc (Carl Roth, Germany)
- Liquid N_2
- 10 μM Primer A
- 10 μM Primer B
- Template DNA
- RNase Inhibitor, Murine (NEB, 40000 U/ml, Germany)
- Reaction buffer (10x RNAPol Reaction Buffer, NEB, Germany)
- T7 polymerase (T7 RNA Polymerase, NEB, Germany)
- Ribonucleotide Solution Mix (rNTP, NEB, Germany)
- DNaseI (RNAase-free) (NEB, Germany)
- DNaseI buffer (NEB, Germany)
- RPA Kit (TwistDx, UK)
- PCR cleanup kit (NEB, Germany)
- Polydimethylpolysiloxane (PDMS, Dow Corning, USA)
- Heat block at 37 °C / portable PCR Device at 37 °C
- Metal plate

- Dessicator
- Nuclease-free filter paper (glass microfiber paper 934-AH RTU, Whatman, GE healthcare, Germany)
- Sonicator

Procedure

1. Put metal plate into -80 °C freezer for pre-cooling. Take care that it only has little contact with the surface.
2. Mix 15 μl of TwistDx RPA Rehydration Buffer with 2.5 μl of Primer A and 2.5 μl Primer B.
3. Resuspend RPA Pellet.
4. Apply resuspended solution to paperstrip that has been prepared by wax-printing to show a small circle (ca. 3 mm in diameter).
5. Carefully put the paperstrip into liquid N_2. Take care that you do not destroy the droplet that has formed on the paperstrip. Leave for several minutes.
6. Put the metal plate from the -80 °C freezer into the dessicator. Quickly apply vacuum in order to get rid of the condensed water that has crystallized on it.
7. Apply the paperstrip from the liquid N_2 to the dessicator. Leave it in the dessicator for 30 minutes.
8. Store the paperstrip in a petridish or seal in PDMS chip via sonication. Wrap with parafilm to prevent humidity to flow in.

Reaction

1. Mix 2 μl of your sample with 29.5 μl Rehydration Buffer, 14.5 μl nuclease-free water and 2.5 μl MgAc either on the PDMS chip or by pipetting. Apply to paperstrip.
2. Incubate for 40 min at 37 °C.
3. (Optional) For testing, use PCR clean-up kit and load on gel.

4. ADD GEL HERE